Natural history, Staging and Prognosis in Systemic Amyloidosis

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Declaration

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

Abstract

Background

Systemic amyloidosis is a rare disorder of protein misfolding and aggregation ranging from indolent localised disease to rapidly progressive multi-organ failure. Systemic light-chain (AL) and transthyretin (ATTR) amyloidosis are the commonest amyloid types. Treatment options have improved significantly in recent years with further novel agents under evaluation.

Aims

To characterise the presentation and natural history of patients with ATTR amyloidosis to inform management in an era of novel disease modifying therapies.

To evaluate trends in patient and renal survival in renal AL amyloidosis and assess outcomes following kidney transplantation to inform clinical decision making and patient selection for transplantation.

Specific chapter aims include:

- Identify markers of disease progression in ATTR-cardiomyopathy (ATTR-CM)
- Characterise the natural history of early stage ATTR-CM
- Describe a rare group of patients with variant ATTR (ATTRv) amyloidosis with atypical cardiac radionucleotide uptake
- Report the healthcare utilization prior to diagnosis with ATTR amyloidosis
- Describe trends in patient and renal survival in renal AL amyloidosis

- Report kidney transplant outcomes in amyloidosis compared with other aetiologies of renal disease

Results and Conclusions

National Amyloidosis Centre (NAC) ATTR Stage is prognostic throughout follow up in ATTR-CM whilst increasing NT-proBNP and NYHA class are independently associated with mortality.

Patients with early stage ATTR-CM have comparable survival to the matched UK general population but significantly increased cardiovascular morbidity suggesting potential benefit from disease modifying treatment.

Variant ATTR cardiomyopathy associated with the p.Ser97Tyr TTR gene variant demonstrates relatively low cardiac radionucleotide uptake warranting special diagnostic consideration.

Healthcare utilization is high and varied prior to diagnosis in ATTR-CM highlighting opportunities for, and potential benefits of, early diagnosis and treatment.

Renal and patient survival in renal AL amyloidosis has progressively improved. Patient and allograft survival post kidney transplantation in selected individuals with AA and AL amyloidosis are comparable to patients with diabetic nephropathy.

Impact Statement

The work in this thesis focuses on the natural history, staging and prognosis of predominantly transthyretin and systemic AL amyloidosis, both rare conditions associated with significant morbidity and mortality. Recently there have been significant advances in diagnosis and treatment, changing the outlook for patients, and raising questions regarding optimal care.

Transthyretin cardiomyopathy diagnoses have markedly increased following the publication of validated non-biopsy diagnostic criteria, and increased disease awareness associated with the development of novel disease modifying therapies.[1-5] At the commencement of this thesis numerous novel therapies were undergoing phase III clinical trials in ATTR-CM having demonstrated efficacy in neuropathic forms of ATTRv amyloidosis. There were however no validated markers of disease progression or treatment response.[3,5] Clinical trial end-points included non-validated parameters such as 6-minute walk test (6MWT).[6] Chapter 3 demonstrates that NAC ATTR stage remains prognostic throughout the disease course, and that an increase in stage was associated with mortality. Building on this, Chapter 4 highlights increasing NT-proBNP and New York Heart Association (NYHA) heart failure class as independent predictors of mortality supporting further study into their utility as markers of treatment response.

Transthyretin amyloid deposits are a common finding at autopsy in the elderly, although the proportion with clinical disease in life is uncertain.[7,8] Diagnoses of ATTR-CM have risen exponentially with an increasing proportion diagnosed with early stage disease.[1] Chapter 5 assesses the natural history of patients with low

levels of NT-proBNP and a minimal diuretic requirement, demonstrating comparable survival to age matched controls but significant additional cardiovascular morbidity suggesting a potential benefit to early commencement of disease modifying therapy.

Chapter 6 describes a group of patients with ATTRv secondary to the rare p.Ser97Tyr TTR gene variant who have relatively low levels of radionucleotide cardiac uptake for their degree of cardiac amyloidosis. These patients often do not meet the non-biopsy diagnostic criteria and warrant specific diagnostic consideration to avoid misdiagnosis and inappropriate treatment.[2]

Chapter 7 outlines the pre-diagnostic healthcare utilization of patients with ATTR amyloidosis demonstrating high usage across a broad range of specialties highlighting opportunities for earlier diagnosis, and potentially improved patient outcomes and reduced service usage in a future era of disease modifying therapy.

Patient and renal survival in renal AL amyloidosis were historically poor, however increasingly efficacious chemotherapeutic options at diagnosis and relapse have improved outcomes significantly in recent years.[9-12] Chapter 8 investigates renal and patient survival across the last two decades in patients with renal AL amyloidosis demonstrating improvements in both with time, more noticeable in patients with less advanced disease at diagnosis. This highlights the importance of early diagnosis and a potential future role for specific anti-amyloid therapy for those with more advanced disease. Chapter 9 investigates patient and allograft survival following kidney transplantation for end stage renal disease due to renal amyloidosis demonstrating comparable outcomes to matched patients with diabetic nephropathy. This work supports nephrologists in decision making regarding prognosis, dialysis,

and transplantation in systemic amyloidosis, and supports the value of kidney transplantation in selected patients.

Ethical Statement

All patients were managed in accordance with the Declaration of Helsinki and provided written informed consent for anonymous publication of their data. These studies were approved by the local research ethics committee.

Research Paper Declaration Form

The following chapters include work published at the time of thesis submission:

Chapter 1:

Advances in Diagnosis and Treatment of Cardiac and Renal Amyloidosis. Steven Law, Marianna Fontana, Julian D Gillmore. 2021. Cardiology Clinics; doi: 10.1016/j.ccl.2021.04.010.

When to suspect and how to approach a diagnosis of amyloidosis. Steven Law, Julian D Gillmore. 2022. The American Journal of Medicine; doi: 10.1016/j.amjmed.2022.01.004

Both articles written by myself under the supervision of collaborating authors.

Chapter 3:

Disease progression in cardiac transthyretin amyloidosis is indicated by serial calculation of National Amyloidosis Centre transthyretin amyloidosis stage. Steven Law, Aviva Petrie, Liza Chacko, Oliver C Cohen, Sriram Ravichandran, Janet A Gilbertson, Dorota Rowczenio, Ashutosh Wechalekar, Ana Martinez-Naharro, Helen J Lackmann, Carol J Whelan, David F Hutt, Philip N Hawkins, Marianna Fontana and Julian D Gillmore. 2020. ESC Heart failure; doi 10.1002/ehf2/12989

Myself, Julian Gillmore and Marianna Fontana were responsible for conceiving the study. I compiled the data, performed all the statistical analysis under the guidance

of Aviva Petrie, and produced the manuscript under the supervision of Julian Gillmore and Marianna Fontana. Other co-authors assisted in data collection.

Chapter 4:

Change in N-terminal pro-B-type natriuretic peptide at 1 year predicts mortality in wild-type transthyretin amyloid cardiomyopathy. Steven Law, Aviva Petrie, Liza Chacko, Oliver C Cohen, Sriram Ravichandran, Janet A Gilbertson, Dorota Rowczenio, Ashutosh D Wechalekar, Ana Martinez-Naharro, Helen J Lackmann, Carol J Whelan, David F Hutt, Philip N Hawkins, Marianna Fontana and Julian D Gillmore. 2022. Heart; doi:10.1136/heartjnl-2021-319063

Myself, Julian Gillmore and Marianna Fontana were responsible for conceiving the study. I compiled the data, performed all the statistical analysis under the guidance of Aviva Petrie, and produced the manuscript under the supervision of Julian Gillmore and Marianna Fontana. Other co-authors assisted in data collection.

Chapter 5:

Characteristics and natural history of early-stage cardiac transthyretin amyloidosis. Steven Law, Melanie Bezard, Aviva Petrie, Liza Chacko, Oliver C Cohen, Sriram Ravichandran, Olabisi Ogunbiyi, Mounira Kharoubi, Sashiananthan Ganeshananthan, Sharmananthan Ganeshananthan, Janet A Gilbertson, Dorota Rowczenio, Ashutosh Wechalekar, Ana Martinez-Naharro, Helen J Lackmann, Carol

J Whelan, David F Hutt, Philip N Hawkins, Thibaud Damy, Marianna Fontana and Julian D Gillmore. 2022. European Heart Journal; doi:10.1093/eurheartj/ehac259

Myself, Julian Gillmore and Marianna Fontana were responsible for conceiving the study. I compiled the data, performed all the majority of the statistical analysis under the guidance of Aviva Petrie, and produced the manuscript under the supervision of Julian Gillmore. Aviva Petrie was solely responsible for the validation model as detailed in the methods. Other co-authors assisted in data collection.

Chapter 8:

Renal Transplant Outcomes in Amyloidosis. Steven Law, Oliver Cohen, Helen J Lachmann, Tamer Rezk, Janet A Gilbertson, Dorota Rowczenio, Ashutosh D Wechalekar, Philip N Hawkins, Reza Motallebzadeh and Julian D Gillmore. 2021. Nephrology Dialysis Transplantation; doi:10.1093/ndt/gfaa293

Myself, Julian Gillmore and Reza Motallebzadeh were responsible for conceiving the study. I compiled the data, performed all the statistical analysis under the guidance of Aviva Petrie, and produced the manuscript under the supervision of Reza Motallebzadeh and Julian Gillmore. Other co-authors assisted in data collection.

Permission has been obtained for use of all the above articles within my thesis.

See Appendix 1 detailed form signed by myself and supervisor Julian Gillmore.

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Abbreviations

AL – light chain amyloidosis

ATTR – transthyretin amyloidosis

ATTR-CM – transthyretin cardiomyopathy

ATTRv – variant ATTR amyloidosis

NAC – National Amyloidosis Centre

NT-proBNP – N-terminal pro B-type natriuretic peptide

NYHA - New York Heart Association

6MWT – six-minute walk test

SAA – serum amyloid A

AA – systemic amyloid A amyloidosis

TTR – transthyretin

CMR – cardiac magnetic resonance

LECT2 - leucocyte chemotactic factor 2

ASCT - autologous stem cell transplantation

AApoAI - apolipoprotein AI

NS – nervous system

ATTRwt – wild-type transthyretin amyloidosis

ATTR-PN – transthyretin neuropathy

ATTR-mixed – transthyretin cardiomyopathy and neuropathy

eGFR – estimated glomerular filtration rate

Tc-DPD - 99mTechnetium labelled 3,3-diphosphono-1,2-propanodicarboxylic acid

SAP – ^{123}Iodine-labelled serum amyloid P

GGT – gamma-glutamyl transferase

LVEF – left ventricular ejection fraction

IVSd – interventricular septal thickness at end diastole

LGE – late gadolinium enhancement

ECV – extracellular volume

NSF – nephrogenic systemic fibrosis

CKD – chronic kidney disease

CI – confidence interval

DPD – 3,3-diphosphono-1,2-propanodicarboxylic acid

PYP – pyrophosphate

HMDP - hydroxymethylene diphosphonate

uPCR – urinary protein creatinine ratio

uACR – urinary albumin creatinine ratio

AFib – fibrinogen Aα-chain

ALP – alkaline phosphatase

AST – aspartate transaminase

ALT – alanine transaminase

IHC – immunohistochemistry

LDMS - laser capture microdissection and tandem mass spectrometry

HDM-ASCT - high dose melphalan with autologous stem cell transplantation

ATTR-ACT - Safety and Efficacy of Tafamidis in Patients with Transthyretin Cardiomyopathy

NSAID - non-steroidal anti-inflammatory

BMI – body mass index

NIS-LL – neuropathic impairment score of the lower limbs

ESRD – end stage renal disease

NHSBT - National Health Service Blood and Transplant

CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration

DNA – deoxyribonucleic acid

AApoAIV – apolipoprotein AIV

CR – complete response

VGPR – very good partial response

PR – partial response

NR – no response

dFLC – difference between involved (amyloidogenic) and uninvolved serum free light chains

NHS – National Health Service

KM – Kaplan-Meier

ROC – receiver operatory characteristic

IQR – interquartile range

PPM – permanent pacemaker

MRA - mineralocorticoid antagonist

ARB - angiotensin receptor blocker

TIA – transient ischemic attack

PND – peripheral nerve disability score

HES - hospital episodes statistics

A&E - accident and emergency

MDT - multidisciplinary team

BJP - Bence jones protein

RRT – renal replacement therapy

ONS - Office of National Statistics

LVPW – left ventricular posterior wall thickness

IF – immunofixation

MCM – maximal conservative management

HD – haemodialysis

PD – peritoneal dialysis.

ADPKD - autosomal dominant polycystic kidney disease

DN – diabetic nephropathy

HLA – human leukocyte antigen

cRF – calculated reaction frequency

DGF – delayed graft function

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Chapter One: Introduction

This chapter is based on the following publications[13,14]:

Advances in Diagnosis and Treatment of Cardiac and Renal Amyloidosis. Steven Law, Marianna Fontana, Julian D Gillmore. 2021. Cardiology Clinics; doi: 10.1016/j.ccl.2021.04.010.

When to suspect and how to approach a diagnosis of amyloidosis. Steven Law, Julian D Gillmore. 2022. The American Journal of Medicine; doi: 10.1016/j.amjmed.2022.01.004

Permission has been obtained for use of both articles within this thesis.

The amyloidoses are a spectrum of diseases with a common final pathological pathway of extracellular amyloid fibril formation, disruption of tissue structure, and organ dysfunction.[15,16] Clinical presentation is heterogeneous depending on the organ systems involved. Patients present to a range of medical and surgical specialties and a high index of suspicion is required for diagnosis.[15] Following confirmation of amyloid deposition, determining the amyloid fibril type is crucial to guide treatment and inform prognosis. Treatment focuses on reducing the concentration of amyloid precursor protein and has improved substantially for many amyloid types in recent years.[3,5,11,17] There remains no approved treatments to directly accelerate amyloid removal, and advanced disease at diagnosis continues to carry a poor prognosis.[18,19]

Pathogenesis

The key pathological step in amyloid deposition is protein misfolding and aggregation, leading to the extracellular deposition of amyloid fibrils, disruption of local cellular structure, impairment of organ function, and symptomatic amyloidosis.[15,20] Over 30 'amyloidogenic' proteins have been identified in humans.[21] The propensity for protein misfolding increases in the presence of: abnormal protein structure (e.g. abnormal fibrinogen Aα-chain protein in hereditary fibrinogen Aα-chain amyloidosis), excessive concentration of a structurally normal protein (e.g. serum amyloid A [SAA] protein in systemic serum amyloid A amyloidosis [AA amyloidosis]), or for unknown reasons associated with the aging process (e.g. transthyretin [TTR] protein in wild-type transthyretin amyloidosis [ATTR]).[15,21,22] The fibrillary precursor protein defines the amyloid type and dictates the clinical phenotype, treatment, and prognosis.

Epidemiology

The prevalence of systemic amyloidosis is increasing annually.[23,24] On review of 11,006 patients referred to the UK National Amyloidosis Centre (NAC) between 1987 and 2019, referrals have increased six-fold.[1] Trends in the diagnosis of amyloidosis have changed significantly in recent years. Systemic light chain (AL) amyloidosis remains the most diagnosed amyloid type, while diagnoses of systemic AA amyloidosis have fallen substantially following the widespread use of biological

therapy in the treatment of chronic inflammatory conditions. There has been a rapid rise in diagnoses of ATTR amyloidosis.[1] This reflects increased awareness, advances in cardiac magnetic resonance (CMR) imaging and bone scintigraphy, alongside the widespread adoption of validated non-biopsy diagnostic criteria.[2,25,26] The true prevalence of transthyretin cardiomyopathy (ATTR-CM) remains unknown. It is noteworthy that autopsy studies have demonstrated ATTR amyloid deposits in the hearts of up to 25% of males >80 years of age, although the majority were not diagnosed in life with cardiomyopathy or symptoms of heart failure.[7] It remains unclear to what extent ATTR-CM may have been missed or whether ATTR amyloid was of no clinical consequence in a substantial proportion of such individuals. What is clear however, is that ATTR-CM is far more common than previously suspected accounting for >10% of heart failure with preserved ejection fraction.[7,8]

Types of amyloidosis

Features of the commonest amyloid types are outlined in Table 1.1. Clinical phenotype is dictated by the amyloid type, although there remains variability within the same amyloid type. For example, systemic AL amyloidosis most commonly presents with heart failure due to deposition of amyloid in the myocardium, and or proteinuric kidney disease due to amyloid deposition in the glomerulus, but can present with liver failure, peripheral neuropathy, autonomic neuropathy, carpal tunnel syndrome or macroglossia amongst other presentations.

Table 1.1: Summary of the commonest amyloid types and their fibrillary precursor protein, underlying cause, clinical phenotype, and treatment

Amyloid type	**Fibrillary precursor protein**	**Underlying cause**	**Commonest organs involved**	**Treatment**
AL	Monoclonal immunoglobulin light chain	B cell dyscrasia	Kidneys, heart, liver, NS, soft tissues, gastrointestinal system	Chemotherapy and/or ASCT
Wild-type ATTR	Wild-type transthyretin	Unknown, associated with aging	Heart, soft tissues	TTR stabiliser, gene silencers
Hereditary ATTR	Variant transthyretin	TTR gene variant	Heart, peripheral NS, autonomic NS, soft tissues	TTR stabilisers, gene silencers
Systemic AA	Serum amyloid A	Chronic inflammation	Kidneys, liver, spleen, heart (<1%)	Control of inflammation
LECT2	LECT2	Unknown	Kidneys, liver	Supportive
Fibrinogen Aα-chain	Variant fibrinogen	Fibrinogen gene variant	Kidneys, liver	Supportive
AApoA1	Variant ApoA1	AApoA1 gene variant	Kidneys, liver, heart	Supportive
Lysozyme	Variant lysozyme	Lysozyme gene variant	Liver, kidneys, gastrointestinal tract, skin, lacrimal and salivary glands[27]	Supportive
Gelsolin	Variant gelsolin	Gelsolin gene variant	Kidneys, NS, cranial nerves, kidneys	Supportive

ASCT - autologous stem cell transplantation; LECT2 - Leucocyte chemotactic factor 2; AApoAI - Apolipoprotein AI; NS – nervous system.

Systemic light chain amyloidosis

Systemic AL amyloidosis occurs due to the production of abnormal amyloidogenic monoclonal light chains from an underlying clonal dyscrasia. The median age of diagnosis is 63 years with 1.3% diagnosed under the age of 34.[28] Multiorgan involvement is common with renal involvement present in 58% at diagnosis, cardiac involvement in 71%, and cardiorenal involvement in 38%.[28,29] Gastrointestinal, liver, soft tissue, and both peripheral and autonomic nervous systems may also be affected. Clinical presentation is dependent on organ involvement, with proteinuria, renal impairment and rapidly progressive heart failure commonest alongside non-specific symptoms of weight loss, weakness and fatigue. Patients can present via almost any medical specialty, and a high index of suspicion is key to early diagnosis before significant organ damage has occurred.

Transthyretin amyloidosis

Transthyretin amyloidosis occurs when the normal transthyretin tetramer is cleaved or dissociates into amyloidogenic monomers. It may be hereditary, associated with a *TTR* gene variant encoding structurally abnormal TTR protein (ATTRv), or wild-type (ATTRwt) in which the *TTR* gene is normal but, for unknown reasons associated with aging, monomeric or cleaved TTR protein misfolds into fibrils.[30]

Wild-type transthyretin amyloidosis

Wild-type ATTR amyloidosis commonly affects elderly males with a median age at diagnosis of 79 years and >90% male predominance.[31] Presentation is typically with

heart failure due to a restrictive cardiomyopathy (ATTR-CM) often preceded by a history, sometimes dating back many years, of soft tissue or synovial amyloid infiltration such as carpal tunnel syndrome, spinal stenosis or osteoarthritis.

Hereditary transthyretin amyloidosis

Hereditary, or variant, ATTR (ATTRv) amyloidosis commonly presents with either neuropathy (ATTR-PN), ATTR-CM or both (ATTR-mixed) depending, in part, on the specific TTR variant (Table 1.2). The p.V142I TTR variant is commonly associated with a dominant cardiac phenotype, while p.T80A is typically associated with a mixed phenotype, and the p.V50M TTR variant typically causes ATTR-PN when disease onset is under age 50 years, and ATTR-mixed when over 50 years.[32] Hereditary ATTR amyloidosis has an autosomal dominant pattern of inheritance but disease penetrance is incomplete.

Table 1.2: Clinical manifestations by common TTR variants.

TTR variant	Epidemiology	Age of onset	Features at presentation
Val122Ile	Almost isolated to patients of African origin (4% of African Americans)	77[19]	Heart failure and cardiomyopathy with neuropathy in 10%
p.T80A	Commonly patients of Irish heritage (1% of County Donegal)	Mid 60's	80% peripheral neuropathy, 95% autonomic neuropathy, 53% heart failure, 100% cardiac uptake on DPD scintigraphy[33]
Val30Met	Foci in Portugal, Japan and Sweden amongst others[a]	30-60's[a]	Commonly peripheral and autonomic neuropathy, with variable cardiac conduction disease and cardiomyopathy[a,34]

[a] The Val30Met TTR mutation is present in several geographic foci, the best studied being in Portugal, Japan, and Sweden. Age of onset and clinical phenotype vary significantly between foci.

Systemic AA amyloidosis

Systemic AA amyloidosis typically presents with proteinuria and renal dysfunction occurring in the setting of prolonged elevation in SAA protein concentration. SAA protein, an acute phase reactant, is elevated in chronic inflammatory conditions such as chronic inflammatory arthritis (60% of systemic AA amyloidosis cases), chronic infections (15%), periodic fever syndromes (9%), and inflammatory bowel disease (5%).[22] Renal and splenic infiltration by amyloid are common at diagnosis while cardiac amyloidosis is rare (<1%). The increasingly widespread use of biological therapies, allowing better control of many inflammatory conditions, has been associated with a reduction in incidence of systemic AA amyloidosis.[1]

Clinical presentation

Due to the insidious, non-specific and diverse nature of symptoms, alongside its rarity, the diagnosis of amyloidosis is frequently delayed until advanced amyloidotic organ dysfunction is present.[35-37] Patients with amyloidosis present to a range of specialties including cardiology, haematology, nephrology, gastroenterology, neurology, orthopaedics and hand surgery amongst others. The likelihood of systemic amyloidosis increases significantly in the presence of multi system dysfunction and active enquiry and investigation are required for prompt diagnosis.

Certain populations are at risk of developing amyloidosis and benefit from routine monitoring for suggestive signs and symptoms. Patients at risk of systemic AL include those with clonal disorders such as monoclonal gammopathy of uncertain significance, multiple myeloma, Waldenstrom's macroglobulinaemia and chronic lymphocytic leukaemia. Patients at risk of systemic AA amyloidosis include those

with chronic inflammatory conditions such as inflammatory arthropathies, periodic fever syndromes, and inflammatory bowel disease, and either recurrent or chronic infections such as bronchiectasis and tuberculosis. Routine enquiry for symptoms and assessment of renal function, urine dipstick, liver function and NT-proBNP in at risk groups improve the likelihood of diagnosing amyloidosis early. Patients with a family history of hereditary amyloidosis may be offered predictive genetic testing, and carriers should undergo age-appropriate work up depending on the amyloid type.

Cardiac amyloidosis commonly presents with heart failure or arrhythmias, with a restrictive cardiomyopathy by echocardiography. In cardiac AL amyloidosis, heart failure symptoms progress rapidly over months, while in ATTR-CM symptoms develop more gradually over years, typically with less severe heart failure and lower NT-proBNP concentration for the degree of cardiac amyloid infiltration. Atrial fibrillation occurs in up to 70% of patients with ATTR-CM and 26% with cardiac AL amyloidosis and is poorly tolerated.[38] Intra-cardiac thrombus and thromboembolic stroke are common regardless of CHA_2DS_2-VASc score.[39] Conduction disease requiring pacemaker insertion is particularly common in ATTR-CM.[39] Hypotension develops as the disease progresses, often occurring disproportionately following the introduction or escalation of angiotensin convertase enzyme inhibition or beta-blockade.

Renal amyloidosis presents with a combination of reduction in estimated glomerular filtration rate (eGFR) and proteinuria, depending on the location of amyloid deposits within the kidney. Systemic AL and AA amyloidosis commonly present with nephrotic range proteinuria associated with extensive glomerular amyloid deposition. LECT2 amyloidosis typically presents with minimal or no

proteinuria as amyloid deposition is predominantly interstitial or vascular. Significant proteinuria is rare in ATTRwt-CM despite frequent loss of eGFR; loss of eGFR likely reflects poor renal perfusion from poor cardiac output, hypotension and diuretic therapy. The presence of proteinuria in the context of cardiac amyloidosis usually indicates systemic AL amyloidosis.

Peripheral and autonomic neuropathy commonly occur in AL, hereditary ATTR, and hereditary AApoAI amyloidosis. Peripheral neuropathy presents as painful paraesthesia with small fibre involvement progressing to numbness and weakness as large fibres become affected.[40] It is important to distinguish amyloid neuropathy due to direct nerve infiltration, from compression neuropathy (i.e. carpal tunnel syndrome) for which decompression surgery may be indicated. Autonomic dysfunction may present with erectile dysfunction, postural hypotension, diarrhoea, constipation, nausea, early satiety, and weight loss. The identification of neuropathy is important since its presence affects treatment options. In ATTRv, gene silencing therapy is only currently licenced in the presence of neuropathy, whilst in systemic AL amyloidosis, certain potentially neurotoxic chemotherapeutic agents may be avoided.

Soft tissue involvement is common in AL amyloidosis with macroglossia and periorbital bruising pathognomonic features of the disease. Macroglossia presents as a painful dry tongue, increased tongue biting, and dental indentation. Carpal tunnel syndrome is strongly associated with both systemic AL and ATTR amyloidosis. A history of carpal tunnel syndrome is present in ~50% patients with ATTRwt-CM, preceding diagnosis of cardiomyopathy by a median of ~8 years.[41]

Approach to diagnosis

The diagnosis of systemic amyloidosis requires a stepwise approach, starting with a high index of suspicion.[15] A comprehensive history is crucial to determine the extent of organ involvement. This is followed by basic investigations to support the initial clinical suspicion and assess organ function, followed by more focussed advanced investigations (Table 1.3). Subsequent histological demonstration of amyloid deposits and determination of the amyloid fibril protein are usually required to confirm the diagnosis, followed by investigations to identify underlying causes. ATTR-CM is the exception to the requirement for histological confirmation, as the diagnosis can often be made using a validated ‘non-biopsy’ diagnostic algorithm (discussed below).[2]

Table 1.3: Non-invasive investigations for systemic amyloidosis.

System	Investigation	Suggestive findings
Cardiac	Cardiac biomarkers	Persistently elevated serum NT-proBNP and troponin
	Electrocardiogram	Heart block, atrial arrhythmia, small QRS complexes, poor R wave progression, pseudo infarction pattern[42]
	Echocardiogram	Biventricular hypertrophy, small left ventricular cavity, reduced global longitudinal strain with apical sparing, pericardial effusion, diastolic dysfunction[42]
	Tc-DPD scintigraphy	Cardiac uptake >99% sensitive for ATTR-CM; also present in ~30% of cardiac AL amyloidosis[43]
	CMR	Elevated T1, increased extracellular volume, late gadolinium enhancement in a subendocardial, diffuse or transmural pattern[44-46]
Renal	Creatinine and eGFR	Frequently abnormal but may be normal
	Urinary proteinuria	Nephrotic range common in AL and AA amyloidosis Minimal in LECT2 and hereditary amyloidosis
Peripheral NS	Nerve conduction studies	Small fibre neuropathy in early disease, progressing to a large fibre axonal sensorimotor lower limb predominant neuropathy[47] Carpal tunnel syndrome
Autonomic NS	Postural blood pressure	Postural blood pressure drop, especially in AL and ATTRv
Liver	Liver function tests	Raised GGT and alkaline phosphatase Raised bilirubin in advanced disease
	US abdomen	Hepatomegaly and or splenomegaly
Special	SAP scintigraphy	Identifies amyloid deposits in the liver, spleen, kidneys, adrenal glands and bones[48] Does not provide information on the gastrointestinal tract, nervous system, or myocardium
	Genotyping	Consider in all cases of ATTR amyloidosis, and in the presence of a suggestive family history

NS – nervous system; Tc-DPD - 99mTechnetium labelled 3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy; CMR – cardiac magnetic resonance imaging; eGFR – estimate glomerular filtration rate; SAP – ^{123}Iodine-labelled serum amyloid P scintigraphy; GGT – gamma-glutamyl transferase; US - ultrasound.

Assessment of organ involvement

Cardiac assessment

Assessment of a patient with suspected cardiac amyloidosis involves a combination of investigations including electrocardiography, cardiac biomarkers, a variety of cardiac imaging modalities and in certain cases endomyocardial biopsy. Confirming the presence of cardiac amyloidosis is the first step to diagnosis followed by confirmation of amyloid type. Cardiac imaging findings do differ between amyloid types however none are sufficiently sensitive to confirm amyloid type alone and further investigation is imperative.[49-51]

Electrocardiogram

Electrocardiographic changes in cardiac amyloidosis include low voltages in the limb leads, pseudo-infarction pattern, atrioventricular blocks and atrial arrhythmias amongst others, although these are of low diagnostic sensitivity.[52]

Cardiac biomarkers

N-terminal pro B-type natriuretic peptide and troponin are typically elevated in cardiac amyloidosis although their sensitivity in early disease remains to be determined. Both have a poor specificity for cardiac amyloidosis and further investigations are required to confirm a cardiac amyloidosis diagnosis. Both have a low specificity for cardiac amyloidosis although Troponin T has demonstrated some utility in distinguishing cardiac amyloidosis from other causes of left ventricular

hypertrophy and change in NT-proBNP is a prognostic marker of disease progression and treatment response in cardiac AL amyloidosis.[18,19,53-56]

Echocardiography

Echocardiographic findings in cardiac amyloidosis include biventricular wall thickening, reduced ventricular chamber volumes, bi-atrial enlargement, pericardial effusion, thickened valves, granular speckled myocardial appearance, and diastolic dysfunction (Figure 1.1 A).[34,42,57] In early disease left ventricular ejection fraction (LVEF) is typically preserved while longitudinal myocardial function is impaired and this assists the differentiation of cardiac amyloidosis from other hypertrophic cardiomyopathy phenotypes (Figure 1.1 B and C).[58,59] An interventricular wall thickness at end diastole (IVSd) of >12mm in the absence of an alternative cause, is classically used to define cardiac amyloidosis in the medical literature, although CMR, which is significantly more sensitive and specific than echocardiography, has shown that there may be significant cardiac amyloidosis, particularly AL type, despite lesser degrees of wall thickening.[60]

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging is the gold standard imaging modality for cardiac amyloidosis, providing assessment of structure, function and myocardial tissue characterisation.[61] Amyloid deposition expands the myocardial extracellular space resulting in diffuse late gadolinium enhancement (LGE) contrasting other causes of left ventricular hypertrophy (Figure 1.1 I).[25] LGE alone has a sensitivity and specificity of 85% and 92% for cardiac amyloidosis; this improves with the

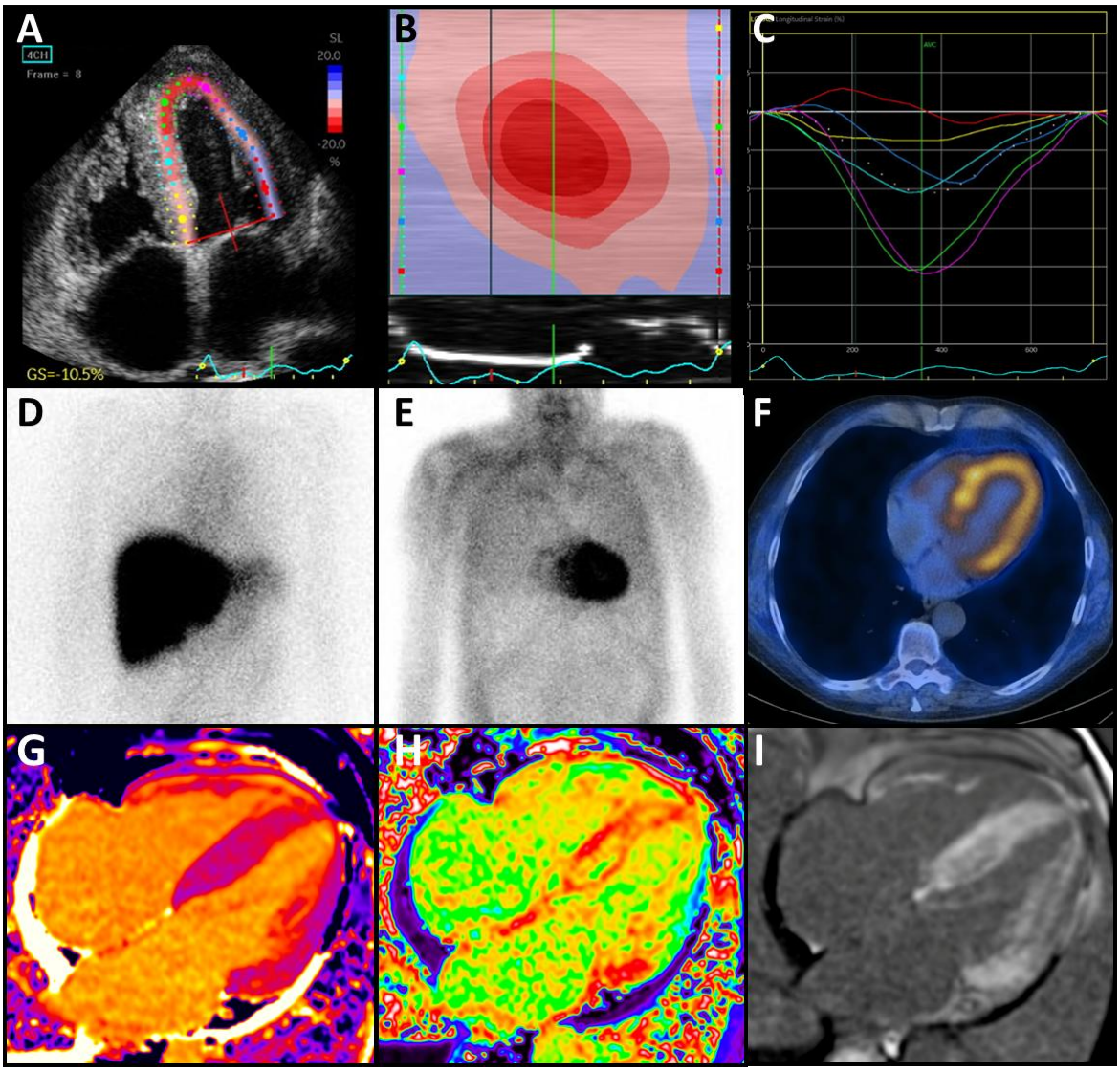
addition of T1 mapping and extracellular volume (ECV) measurements (Figure 1.1 G and H).[62,63] Transition from subendocardial to transmural LGE, increasing ECV, and increasing T1 value all predict mortality in cardiac amyloidosis.[45,64] Serial CMR imaging allows quantification of cardiac amyloid burden and monitoring of treatment response with regression of amyloid deposition demonstrable in both cardiac AL amyloidosis and ATTR-CM following treatment.[65,66] One potential concern of CMR imaging the risk of nephrogenic systemic fibrosis (NSF) in patients with advanced chronic kidney disease (CKD); however, a review of 4,931 patients with CKD stage IV or V, who received group II gadolinium-based contrast agents, demonstrated a pooled incidence of NSF of 0% (95% confidence interval [CI]: 0-0.07).[67] Non-gadolinium enhanced CMR with T1 mapping offers an alternative although less sensitive and specific imaging option.[63]

Bone scintigraphy

Technetium-labelled bisphosphates (3,3-diphosphono-1,2-propanedicarboxylic acid [DPD], pyrophosphate [PYP], and hydroxymethylene diphosphonate [HMDP]) localise to ATTR amyloid deposits in the myocardium, producing cardiac uptake on bone scintigraphy (Figure 1.1 E and F).[26,68] Bone scintigraphy has a sensitivity of >99%, and a specificity of 68% in ATTR-CM, and uptake can occur before the development of clinical symptoms, serum biomarker, echocardiographic, or CMR abnormalities.[2] The poor specificity of cardiac uptake for ATTR-CM reflects uptake in other forms of cardiac amyloidosis, particularly AL amyloidosis in which there is cardiac uptake on DPD scintigraphy in ~30% of patients including Perugini grade 2 or 3 uptake in up to 10% of cases, but also cardiac AApoAI amyloidosis which is

associated with low grade (Perugini grade 1) uptake. [43,69] The diagnosis of ATTR-CM cannot therefore be confirmed by bone scintigraphy alone.[2]

Figure 1.1: Imaging features of systemic amyloidosis.



Echocardiography: A) four chamber view with biventricular hypertrophy, B and C) 'bullseye' pattern of apical sparing of longitudinal strain and reduced global longitudinal strain as assessed by echocardiography. Nuclear medicine imaging: D) SAP scintigraphy demonstrating liver amyloid deposition, E) Tc-DPD scintigraphy with Perugini grade 3 cardiac uptake, F) single-photon emission computer tomography demonstrating biventricular cardiac uptake. Cardiac magnetic resonance imaging: G) diffusely elevated T1 values at 1149ms, H) diffusely elevated extracellular volume of 63%, I) diffuse transmural late gadolinium enhancement.

Renal assessment

Renal amyloidosis presents with variable proteinuria and renal impairment. Basic investigations include serum creatinine and quantification of proteinuria. Historically, 24 hour urinary collection was required to quantify proteinuria; recent studies suggests spot urinary protein creatinine ratio (uPCR) or spot urinary albumin creatinine ratio (uACR) may be sufficiently accurate although results are mixed and this has not yet translated into amyloidosis guidelines.[70,71] Renal dysfunction and proteinuria in the presence of a monoclonal protein has a wide differential diagnosis and renal biopsy is crucial to confirm amyloid and/or exclude the ever increasing list of alternative associated pathological lesions. The pattern of amyloid deposition provides clinical clues to the amyloid type: for example, LECT2 amyloidosis predominantly affects the interstitial compartments whereas fibrinogen Aα-chain (AFib) amyloidosis is associated with striking glomerular enlargement.[72,73] Despite historical concerns, recent studies suggests renal biopsy in amyloidosis does not carry an increased risk of bleeding compared to other causes of renal dysfunction.[74]

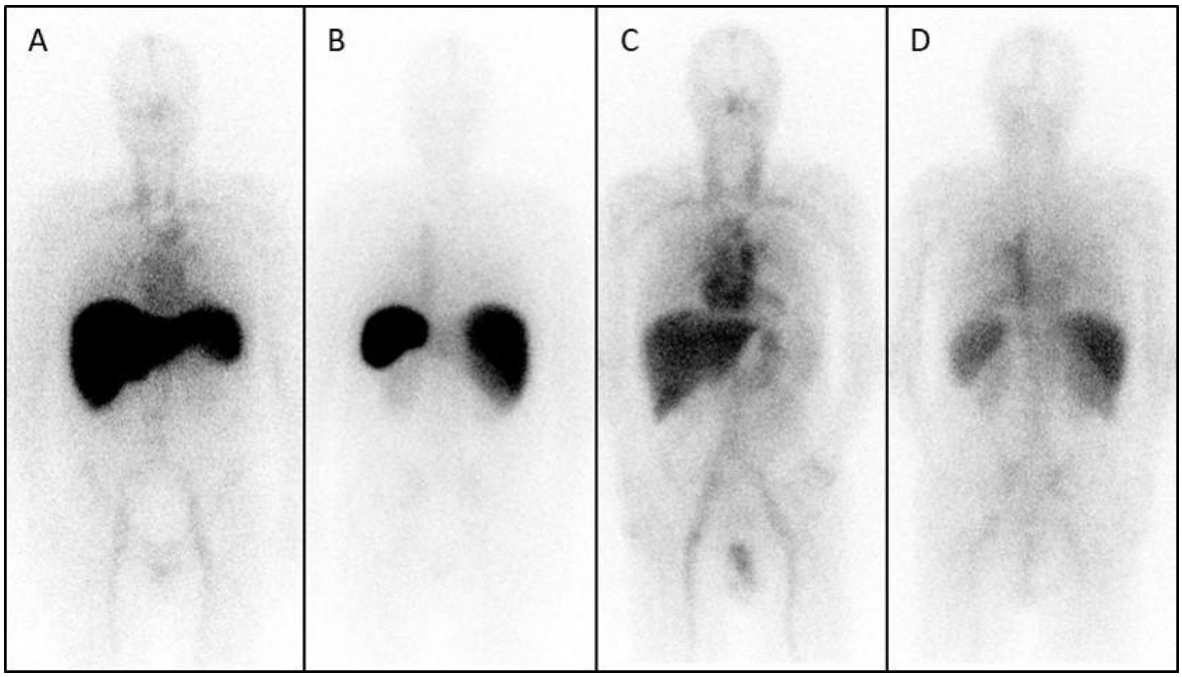
Liver assessment

Liver amyloidosis typically presents with asymptomatic elevations of alkaline phosphatase (ALP) and GGT. In more advanced disease hyperbilirubinaemia and the associated clinical sequelae may develop alongside elevations in aspartate transaminase (AST) and alanine transaminase (ALT).

Serum amyloid P scintigraphy

^{123}Iodine-labelled serum amyloid P scintigraphy allows identification of amyloid deposits in the spleen, liver, kidneys, adrenal glands, and bones (examples in Figure 1.1D and Figure 1.2). It does not provide accurate assessment of cardiac, gastrointestinal or nerve involvement by amyloidosis.[48] The sensitivity of SAP scintigraphy for detecting renal amyloid deposition reduces as the eGFR falls.

Figure 1.2: ^{123}Iodine-labelled serum amyloid P scintigraphy in systemic amyloidosis



Whole body anterior (A,C) and posterior (B,D) scintigraphy following injection of 123I-labelled serum amyloid P component (SAP). Panels A and B: moderate amyloid load in the liver, spleen, and kidneys in a patient with multiple myeloma. Panel C and D: no visceral amyloid deposits; uptake in the visceral organs match the myocardial blood pool.

Nervous system assessment

Standard nerve conduction studies may be normal in early amyloid neuropathy although small fibre dysfunction may be present on more advanced testing.[47] As amyloid neuropathy progresses an axonal neuropathy typically develops which tends to be more sensory than motor, and predominantly lower limb over upper limb.[47] Postural blood pressure is frequently abnormal with autonomic neuropathy.

Assessment of underlying cause

Haematology investigations

All patients with a suspicion of amyloidosis require urgent haematological investigations to identify patients likely to have systemic AL amyloidosis who may require urgent treatment. Due to the often subtle nature of the clonal dyscrasia underlying AL amyloidosis, a combination of serum and urine immunofixation electrophoresis and the serum free light chain assay are essential in order to achieve a sensitivity of >98% in detecting the causative clonal dyscrasia.[75] In health, serum free kappa light chains are produced at twice the rate of lambda light chains, and both are removed by the kidneys. In renal impairment serum free light chain concentrations are raised, with kappa increasing disproportionately to lambda, thus increasing the expected serum kappa to lambda ratio.[76]

Genetic testing

The commonest hereditary causes of cardiac amyloidosis are due to variants in the *TTR* gene. *TTR* gene variants are inherited in an autosomal dominant manner and

display incomplete penetrance. The clinical phenotype is dictated by the specific TTR gene variant (Table 1.2). Rare hereditary amyloidosis, such as hereditary AApoAI and AFib amyloidosis, should be considered in the presence of a family history, atypical clinical presentation, absence of a plasma cell dyscrasia or inflammatory condition, and in the case of certain histological morphologies (e.g. isolated and extensive glomerular amyloid in AFib amyloidosis). A suspicion of hereditary amyloidosis may be investigated by sequencing of known hereditary amyloidosis genes alongside specific immunohistochemistry (IHC) and / or laser capture microdissection and tandem mass spectrometry (LDMS) of obtained tissue to confirm the amyloidogenic precursor protein.

Confirmation of diagnosis

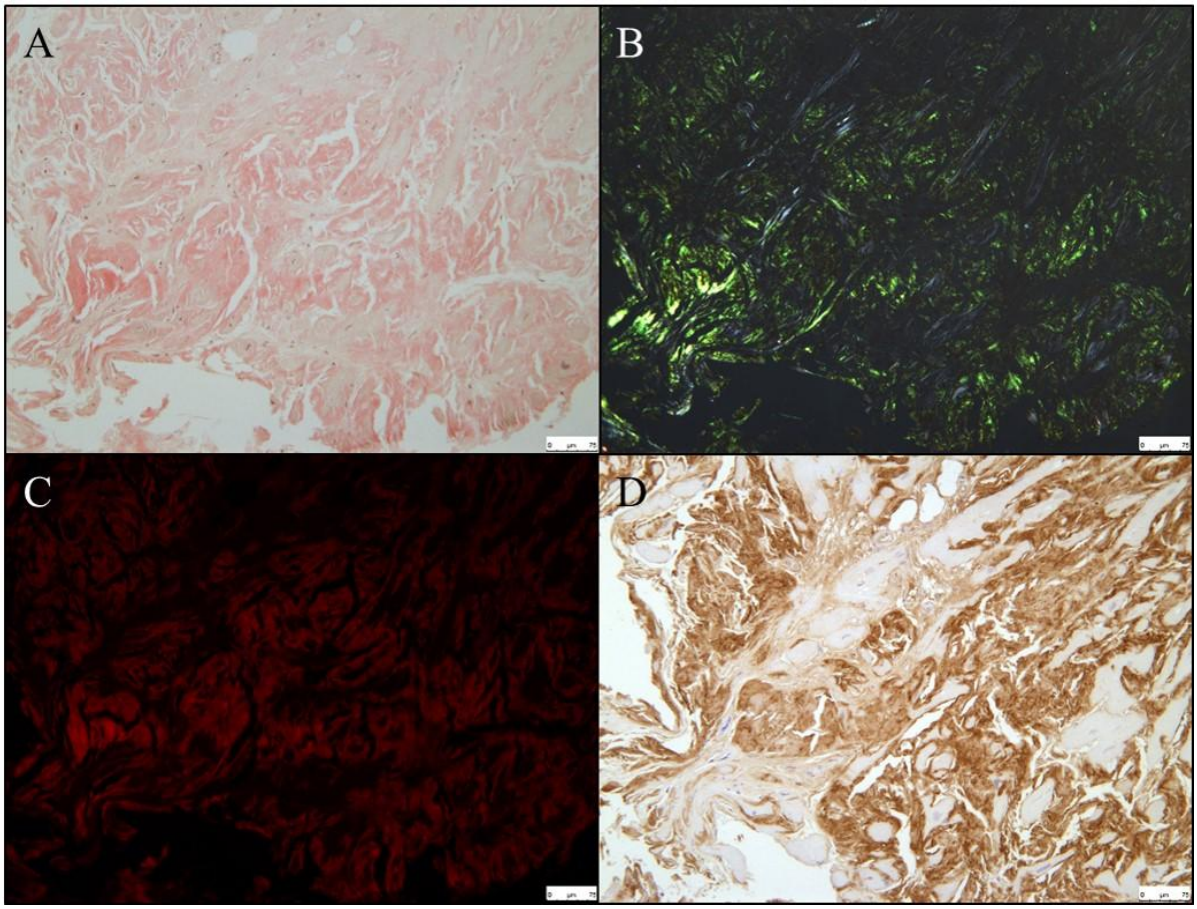
Histological demonstration of amyloid deposits and confirmation of the amyloid fibril protein are typically required to confirm the diagnosis. ATTR-CM is the exception where the diagnosis can often be made without histology using a validated 'non-biopsy' diagnostic algorithm.[2]

Histology

Histological identification and typing of amyloid deposition in an affected organ is the gold-standard diagnostic investigation for amyloidosis. When a target organ biopsy is deemed high risk, screening biopsies such as a fat aspiration, bone marrow trephine or gastrointestinal biopsy may identify amyloid deposition with varying sensitivities.[77] Amyloid appears as an acellular, eosinophilic material on light microscopy, with

randomly orientated non-branching fibrils of approximately 10 nm in diameter on electron microscopy.[15] Amyloid deposition is confirmed by observing apple green birefringence following Congo red staining when viewed under cross polarized light (Figure 1.3 A, B and C).[16,78] Following this, amyloid type may be determined by IHC using a panel of antibodies although there is no immunospecific staining up to 30% of cases (Figure 1.3 D).[78,79] LDMS of amyloidotic tissue is able to identify the presence and type of amyloid in > 95% of cases including > 80% of those in which immunohistochemistry is indeterminate, and requires only a small quantity of amyloidotic tissue.[79,80]

Figure 1.3: Endomyocardial transthyretin amyloid deposition



Endomyocardial histology demonstrating: (A) congophilia within myocytes following congo red staining; (B) apple green birefringence when viewed under polarised light; (C) congophilia under fluorescent light; (D) positive immunohistochemistry with transthyretin antibody.

Non-biopsy diagnosis of ATTR-CM

A diagnosis of ATTR-CM can be made without histology if all of the following validated non-biopsy diagnostic criteria are met: heart failure, a suggestive or characteristic amyloid echocardiogram or CMR, a Perugini grade 2 or 3 DPD/PYP scan, a normal serum free light chain ratio, absence of a serum paraprotein by electrophoresis and immunofixation, and absence of urinary Bence Jones protein by urine immunofixation.[2] Diagnosis of ATTR-CM should be followed by sequencing of the *TTR* gene to distinguish between ATTRv-CM and ATTRwt-CM.

Disease Staging and Prognosis

Systemic AL amyloidosis

Biomarker based staging systems allow stratification of patients into prognostic groups in both cardiac AL and ATTR amyloidosis. The latest Mayo classification stratifies patients with AL amyloidosis into four stages based on serum troponin T, NT-proBNP, and the difference between the involved and uninvolved serum free light chain concentration; producing four stages with median survivals of 94, 40, 14, and 6 months respectively.[56] In addition, a diagnostic NT-proBNP > 8500ng/L and systolic blood pressure < 100mmHg identify an especially poor prognostic group.[18]

The need for dialysis in AL amyloidosis with renal involvement is predicted by diagnostic proteinuria >5g/24hrs and eGFR<50mls/min/1.73m^2; patients with neither criterion have a 1% risk of dialysis requirement at 2 years while patients with one criterion have a 12% risk, and those with both 48%. Cardiorenal involvement in systemic AL amyloidosis is associated with a median survival of 18.5 months;

diagnostic NT-proBNP > 8500ng/L and eGFR < 30ml/min/m^2 predict mortality and dialysis requirement.[29]

Transthyretin amyloidosis

Two staging systems predict survival from diagnosis in the absence of disease modifying therapy for patients with ATTR-CM. The NAC ATTR staging system stratifies ATTRwt-CM and ATTRv-CM patients into three prognostic groups at diagnosis; stage I: NT-proBNP ≤ 3000ng/L and eGFR ≥ 45ml/min/1.73m^2, stage III: NT-proBNP > 3000ng/L and eGFR < 45ml/min/1.73m^2, with the remainder stage II. Median survival in the absence of disease modifying therapy was 69, 47 and 24 months for stage I, II and III respectively. At present the NAC ATTR staging system is validated at diagnosis only, and it remains to be seen whether it has utility during follow up as a marker of disease progression.

Grogan et al presented a staging system for ATTRwt-CM stratifying patients into three groups at diagnosis; stage I: troponin T <0.05ng/ml and NT-proBNP <3000pg/ml, stage III troponin T >0.05ng/ml and NT-proBNP >3000pg/ml, with the remainder stage II. Four-year patient survival estimates in the absence of disease modifying therapy were 57%, 42% and 18% for stage I, II and III respectively.

Treatment

General principles

The universal aim of treatment in systemic amyloidosis is to reduce ongoing amyloid formation, thereby altering the equilibrium between rate of amyloid formation and rate of natural amyloid clearance (regression) in favour of the latter, thus leading to improvement in organ function. This is achieved by reducing the concentration of the circulating amyloid precursor protein and treatment is therefore specific to the amyloid type. For example, systemic AL amyloidosis is treated with chemotherapy to reduce the concentration of circulating amyloidogenic monoclonal immunoglobulin light chain, while ATTR amyloidosis is treated with novel drugs which reduce the concentration of circulating amyloidogenic TTR protein such as 'gene silencers' or TTR stabilisers.[3,4] At present there are no approved treatments to actively accelerate removal of existing amyloid deposits. Depletion of circulating serum amyloid P, a circulating protein and key constituent of amyloid fibrils, followed by anti-serum amyloid P antibody administration resulting in recruitment of macrophages into amyloid, was shown to accelerate amyloid clearance, but such antibody therapies remain some way from clinical practice.[81] Gradual natural amyloid regression and improvement in organ function can occur over months to years, provided that the reduction in circulating precursor protein concentration is profound and sustained.

Systemic light chain amyloidosis

Treatment of systemic AL amyloidosis is targeted at the underlying plasma cell dyscrasia, aiming to reduce the concentration of amyloidogenic monoclonal immunoglobulin light chains as rapidly and durably as possible. Treatment includes

consideration of high dose melphalan with autologous stem cell transplantation (HDM-ASCT) in eligible patients, and or combination chemotherapy.[82] HDM-ASCT may also be considered later in a patient's disease course if they improve following initial chemotherapy.[83] Combination chemotherapy is directed at the underlying haematological disorder with frequent use of bortezomib based regimens first line in plasma cell disorders, and lymphoma regimens in lymphoproliferative disorders. Throughout and following treatment, haematologic response is assessed and monitored by measuring the change in amyloidogenic serum free light chain concentration. Amyloidotic organ response is assessed by change in surrogate markers of organ function such as NT-proBNP in cardiac amyloidosis and proteinuria and eGFR in renal amyloidosis. These biomarkers are predictive of outcomes, and should be serially monitored throughout the disease course. Haematologic relapse, particularly if accompanied by worsening amyloidotic organ function, typically requires further treatment.[29,55,60,84] Patient outcomes have improved dramatically since the introduction of proteasome inhibitors such as bortezomib, with multiple other highly effective new therapies such as daratumumab and ixazomib offering an ever greater array of treatment options in systemic AL amyloidosis.[28,85]

Advanced cardiac AL amyloidosis is associated with a high risk of sudden cardiac death with both electromechanical dissociation and ventricular arrhythmias demonstrated.[86] Prophylactic anti-arrhythmic therapy with amiodarone and cardiac monitoring during initial bortezomib dosing for Mayo stage ≥III patients is practiced by some clinicians, although evidence of clinical benefit is lacking. Prophylactic implantation of cardiac defibrillators have not been found to improve survival.[86]

Supportive treatment of cardiorenal amyloidosis includes loop diuretics and aldosterone antagonists to maintain euvolaemia, as serum potassium and blood

pressure allow. In the presence of significant cardiac amyloidosis hypertension is rare and both angiotensin and beta blockade are often poorly tolerated.

Transthyretin amyloidosis

In ATTR amyloidosis there are two key approaches to disease modifying therapy: stabilising the TTR tetramer, and reduction of TTR production with 'gene silencing' therapies. TTR becomes amyloidogenic when the normal tetramer dissociates or is selectively cleaved into monomers and oligomers.[30] Tafamidis, a TTR stabiliser, binds to the TTR tetramer, inhibiting dissociation *in vitro*, and in a phase 3 placebo controlled trial of patients with ATTR-CM, namely, Safety and Efficacy of Tafamidis in Patients With Transthyretin Cardiomyopathy (ATTR-ACT), was associated with a reduction in all-cause mortality and cardiovascular-related hospitalizations, and a slowing of decline in six-minute walk test (6MWT) distance and quality of life compared to placebo; it also slows progression of neuropathy in ATTRv-PN.[87] Sub-group analysis in ATTR-ACT highlighted patients with New York Heart Association (NYHA) class ≤ II heart failure as benefiting most from tafamidis, emphasizing the importance of early diagnosis and treatment.[4] The non-steroidal anti-inflammatory (NSAID) agent, diflunisal, also stabilises TTR *in vitro* and slows the progression of neuropathy in ATTRv, although evidence of benefit in ATTR-CM is limited, and the inherent fluid retentive and nephrotoxic properties associated with NSAIDs are unattractive in this cohort.[88,89] A phase III randomised placebo controlled trial of acoramidis, another small molecule TTR stabiliser, is ongoing after phase II trials demonstrated safety and near complete *in vitro* stabilisation of the TTR tetramer in both wild-type and variant ATTR amyloidosis (Table 1.4).[90]

Table 1.4: Clinical trials in ATTR-CM

	Trial	Phase	Mechanism	Administration	Primary outcome measures
Acoramidis	ATTRibute-CM, NCT03860935	III	TTR stabiliser	Oral, twice daily	Change in 6MWT, mortality
Patisiran	APOLLO-B, NCT03997383	III	RNA interference	Intravenous infusion thrice weekly	Change in 6MWT
Inotersen	NCT03702829, open label extension	II	Antisense oligonucleotide inhibitor	Subcutaneous injection weekly	Change in longitudinal left ventricular strain
Vutrisiran	HELIOS-B, NCT04153149	III	RNA interference	Subcutaneous injection 3 monthly	Composite of mortality and cardiovascular events
AKCEA-TTR LRx	CARIO-TTRansform, NCT04136171	III	Ligand conjugated antisense agent	Subcutaneous injection 4 weekly	Composite of cardiovascular mortality and events, change in 6MWT
Doxycycline and Tauroursodeoxycholic acid[a]	NCT01171859	III	Amyloid fibril disruption	Oral, Twice and thrice daily	Combination of change in modified BMI, NIS-LL, and NT-proBNP

6MWT: six-minute walk test, BMI: body mass index, NIS-LL: neuropathic impairment score of the lower limbs. [a] Only recruiting patients with ATTRwt-CM or ATTRv-CM due to Ile68Leu or Val122Ile TTR variants

Patisiran, a ribonucleic acid interference agent, and inotersen, an antisense oligonucleotide inhibitor, reduce hepatic production of TTR achieving a median TTR reduction of 81% and 79% respectively.[5] Both agents were shown in phase 3 trials to stabilise or improve neuropathic, functional and quality of life scores in ATTRv-PN.[3,5] Sub-group analysis of patients with ATTRv-CM in the patisiran phase 3 trial (APOLLO) showed improvements in NT-proBNP and post-hoc analysis of this cohort showed a reduction in mortality and cardiovascular related hospitalisations among those receiving patisiran compared to placebo.[91] A recent small series of ATTRv-CM patients treated with patisiran and diflunisal showed CMR evidence of cardiac amyloid regression.[66,91,92] Several such therapies are currently being specifically evaluated in ATTR-CM within phase 3 clinical trials, summarized in Table 1.4. Primary outcomes are listed although at the time of writing none of these have been validated as markers of treatment response, or disease progression (mortality excluded).

Historically liver transplantation was the only disease-modifying treatment for ATTRv, replacing the production of variant amyloidogenic TTR with structurally normal TTR. Liver transplantation improves survival in patients with ATTRv-PN associated with the V30M TTR variant, particularly when performed early in the disease course, but caution is advised in advanced disease or when significant pre-existing ATTRv-CM is present.[93,94] The role of liver transplantation in the era of gene silencers has diminished and remains to be determined.

Systemic serum amyloid A amyloidosis

Treatment of systemic AA amyloidosis focuses on controlling the underlying cause of inflammation and hence reducing the concentration of the amyloidogenic SAA protein. Mortality and time to end stage renal failure are associated with SAA concentration during follow up.[22] Increasingly widespread use of biologic therapies to control inflammatory joint and bowel disease has seen a fall in the incidence of systemic AA amyloidosis in the Western world.[1]

Kidney transplantation

Diagnostic delay is common in systemic amyloidosis and patients frequently present with advanced organ dysfunction. With improving treatment options in systemic AL and AA amyloidosis there is an increasing group of patients with controlled underlying disease but irreversible end stage renal disease (ESRD) who may benefit from kidney transplantation. Historically, kidney transplantation in systemic amyloidosis was associated with poor patient and graft outcomes, although more recent studies have suggested outcomes are improving. [95-98] Angel-Korman et al reported 49 patients with AL amyloidosis undergoing kidney transplantation demonstrating the association of a haematologic CR or VGPR and time of transplantation with improved allograft and patient survival.[98] Given the incurable and multisystem nature of amyloidosis it remains unclear which patients may benefit from kidney transplantation and how patient and graft outcomes compare with other aetiologies of end stage renal disease. Both clonal disorders and chronic inflammatory diseases are prone to relapse conferring a risk of recurrent amyloidosis in the kidney transplant. Kidney transplantation is associated with significant quality

of life and survival advantages over dialysis therapy in most eligible patients although it remains to be seen if this is the case for patients with systemic amyloidosis.

Treatment response and disease progression

Systemic AL amyloidosis

Assessment of response to clone directed therapy in systemic AL amyloidosis is well established and assessed in two ways:

1) The haematologic response: defined by the change in concentration of amyloidogenic light chain protein, serum paraprotein and Bence Jones protein.
2) Individual organ response: defined by the change in markers of organ involvement.

Higher degrees of haematologic response predict improved patient survival and organ function.[17,54,99] The International Symposium on Amyloid and Amyloidosis have published a consensus opinion paper on agreed organ response criteria including renal, cardiac, liver and peripheral nerve.[60] More recently, several other organ response criteria have been demonstrated to predict either organ or patient survival.[100,101]

Transthyretin amyloidosis

At commencement of this thesis there were no published, validated markers of disease progression or treatment response in ATTR-CM. This represents a key unmet need given the range of novel therapies undergoing phase III clinical trials and potentially to

be soon in widespread use. The ATTR-ACT trial, which showed a reduction in mortality with tafamidis compared to placebo, showed a slower rate of NT-proBNP increase in the intervention group compared to the placebo group.[4] The APOLLO-A and NEURO-TTR studies of patisiran and inotersen confirmed their benefit in neuropathic ATTRv; in patients with concomitant cardiac amyloidosis the NT-proBNP reduced significantly in those in the intervention arm compared to placebo.[3,5,91,92,102] These findings suggest NT-proBNP as a potential biomarker of treatment response although this has not been validated.

Chapter Two: Methods and materials

Declaration

Studies were conceived and designed by myself and supervisor Professor Julian Gillmore. Patient data was either extracted from the NAC database using Microsoft access or collected manually from clinical correspondence. Exceptions to this include: Chapter 5 'Characteristics and natural history of early-stage cardiac transthyretin amyloidosis' for which a data request was made to the French Mondor Amyloidosis Network with data subsequently collected by Dr Melanie Bezard; Chapter 7: 'Pre-diagnostic healthcare utilization in patients with transthyretin amyloidosis in the United Kingdom' data was extracted from the Hospital Episodes and Statistics database via linkage using patients National Health Service number, this was performed by Rosie McDonald at IQVIA following Confidentiality Advisory Group approval; and Chapter 9 'Renal transplant outcomes in amyloidosis' where data was requested from the UK Transplant Registry of the Organ Donation and Transplant Directorate of National Health Service Blood and Transplant (NHSBT) regarding transplantation specific level data for amyloidosis recipients and for non-amyloidosis control groups. Statistical analysis was performed by myself under the guidance of Dr Aviva Petrie of the Eastman Dental Institute at the University College London with the exception of Chapter 5 'Characteristics and natural history of early-stage cardiac transthyretin amyloidosis' in which the internal validation model was constructed solely by Dr Aviva Petrie. A range of investigations reported in this thesis were performed by colleagues at the NAC including:

- Blood pressure assessment, pulse assessment, urine sampling and phlebotomy by the nursing staff and health care assistant team

- Echocardiography by Babita Pawarova, Brooke Douglas, Sevda Ward and Claire Hubbard
- SAP and Tc-DPD scintigraphy by David Hutt, Florentina Grigore, Rosario Coronado and Emma Wilson
- Gene sequencing by Dorota Rowczenio, Anna Baginska and Hadija Trojer
- Histology and immunohistochemistry preparation and interpretation by Janet Gilbertson and Nicola Botcher under the guidance of pathology accredited amyloidosis consultants
- Laser capture microdissection and tandem mass spectrometry preparation by Janet Gilbertson and Nicola Botcher with subsequent result analysis and interpretation performed by Graham Taylor, Nigel Rendell, Julian Gillmore and Philip Hawkins

Patients

All patients were reviewed at the UK NAC following a referral with suspected or confirmed amyloidosis. The NAC is the only specialist referral centre for amyloidosis in the United Kingdom seeing patients from throughout the UK as well as a small proportion from outside the UK. All patients included in this thesis provided explicit informed consent. Patient data was stored in a Microsoft access database allowing extraction of data retrospectively. Survival and date of death data are continuously updated through data from the Office of National Statistics and updates from family members contacting the centre. Patients were seen at referral and typically followed up on a 6 to 24 month basis depending on clinical need.

Functional assessment

All patients underwent functional assessment using the NYHA classification (Table 2.1). Patients underwent a 6MWT up until March 2020 prior to the introduction of social distancing restrictions. Patients were advised to walk as far as they can in six minutes, on a flat surface corridor, varying their speed and resting as required.[103,104]

Table 2.1 New York Heart Association classification of heart failure

Class	Description
I	No limitation of physical activity. Ordinary physical activity does not cause symptoms
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, shortness of breath or chest pain.
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, shortness of breath or chest pain.
IV	Symptoms of heart failure at rest. Any physical activity causes further discomfort.

Renal assessment

Native renal and renal allograft function was evaluated at each visit by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) eGFR calculation, serum creatinine and 24-hour urinary protein measurement; urinary protein to creatinine ratio and albumin to creatinine ratio were increasingly assessed from 2020 onwards.[105] Renal histology performed externally was requested and reviewed at

the NAC for amyloid deposition and typing. Native kidney and kidney allografts were also assessed by ^{123}I-labelled SAP scintigraphy where indicated.

Cardiac assessment

All patients referred for evaluation underwent baseline cardiac investigations including a functional assessment, electrocardiogram and echocardiography; Tc-DPD scintigraphy and CMR were performed where indicated.

Cardiac biomarkers

NT-proBNP was measured with an electrochemiluminescence sandwich immunoassay on the Elecsys system 2010 (Roche Diagnostics) and high-sensitivity troponin T assay was performed with a second-generation assay after 16 December 2015, and prior to that, with a first-generation TnT assay.

Electrocardiography

All patients underwent electrocardiography which were performed by NAC nursing and healthcare staff and interpreted by amyloidosis physicians.

Echocardiography

Echocardiography was performed by echocardiographers with experience of cardiac amyloidosis on General Electric Vivid 7 machines using EchoPac software. All echocardiograms were read and reported by two independent experts in amyloid echocardiography.

Radionuclide (^{99m}Tc-DPD and ^{99m}Tc-HMDP) scintigraphy

Patients seen at the NAC were scanned after intravenous injection of ~700 MBq of ^{99m}Tc-DPD. Whole body planar and SPECT/CT images were acquired 3 hours post-injection in all patients. Images were acquired using a low energy, high-resolution collimator and a scan speed of 10 cm/min. Cardiac retention of all ^{99m}Tc-DPD scans was determined by two independent experienced readers according to the grading devised by Perugini et al as either grade 0: no cardiac uptake, grade 1: cardiac uptake which is less intense than bone signal, grade 2: cardiac uptake of similar or greater intensity than bone signal, or grade 3: cardiac uptake with significantly reduced or absent bone signal.[26] Patients reviewed at the Amyloidosis Mondor Network in France underwent ^{99m}Tc-HMDP scintigraphy with a heart-to-mediastinum ratio of ≥ 1.21 being considered equivalent to Perugini grade ≥ 2 cardiac by ^{99m}Tc-DPD scintigraphy as previously reported.[26,106]

Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging was performed with gadolinium based contrast agents. All patients underwent volumetric assessment, LGE images and T1 mapping. All CMR scans were scored by 2 independent experienced readers and categorized as follows: No cardiac amyloidosis - normal CMR or CMR indicating non-amyloid diagnosis (e.g. hypertensive heart disease or hypertrophic cardiomyopathy); Characteristic amyloid CMR – CMR displaying all of the following features: increased wall thickness, increased LV mass, altered gadolinium kinetics, diffuse subendocardial or transmural LGE, and elevated T1 mapping; Suggestive amyloid CMR – all CMR scans not fulfilling the criteria for ‘No cardiac amyloidosis’ or

'Characteristic amyloid CMR' such that cardiac amyloidosis could not confidently be excluded or confirmed.

SAP scintigraphy

Visceral amyloid load and organ involvement was evaluated by whole body anterior and posterior scintigraphy after administration of ^{123}I-SAP component using an Elscint Superhelix gamma camera, as previously described.[48] Visceral amyloid load was stratified as follows: small – organ uptake but substantial blood pool signal, moderate – more intense organ uptake with reduced blood pool signal, and large – strong organ localization with little or no blood pool signal.[81] SAP scintigraphy was performed at diagnosis, and at one to three yearly intervals determined by clinical need. Images were interpreted by a panel of physicians with experience of interpreting over 20,000 scans.[107,108]

Histology, immunohistochemistry and proteomics

All histology samples were reviewed centrally at the NAC. All formalin fixed paraffin-embedded biopsies were stained with Congo red dye and viewed under crossed polarized light according to the method of Puchtler *et al*.[109] The amyloid fibril type was established by immunohistochemical staining of amyloid deposits using a panel of monospecific antibodies reactive with serum amyloid A (SAA) protein, kappa and lambda immunoglobulin light chains, transthyretin, and where necessary, antibodies against AApoAI, apolipoprotein A-IV (AApoAIV) and AFib, and/or by microdissection of amyloid deposits and proteomic analysis, as previously described.[78,79]

Genetic sequencing

All patients with ATTR amyloidosis underwent TTR genotyping. Deoxyribonucleic acid (DNA) was extracted from whole blood, amplified by polymerase-chain-reaction assay, and the whole coding region of the transthyretin gene was sequenced, as previously described.[110] Where clinically indicated the AApoAI, AApoAIV, AFib, lysozyme and gelsolin genes were also sequenced.

Non-biopsy diagnostic criteria for transthyretin cardiomyopathy

The validated non-biopsy criteria for ATTR-CM were defined as all of the following: echocardiogram or CMR suggestive of amyloid cardiomyopathy, Perugini grade ≥ 2 radionuclide scan, and absence of a monoclonal protein by serum free light chain assay and by serum and urine immunofixation.[2]

Amyloidosis staging

Patients with ATTR-CM were stratified into NAC Stage at diagnosis based on their diagnostic NT-proBNP and eGFR (Table 2.2).[19] Patients with cardiac AL amyloidosis were stratified at diagnosis according to the original Mayo stage with the addition of the 8500ng/L NT-proBNP cut-off (Table 2.2).[18] Patients with renal AL amyloidosis were stratified at diagnosis based on their eGFR and proteinuria quantification (Table 2.2).[111]

Assessment of haematologic response

Haematologic response following treatment for systemic AL amyloidosis is classified as a complete response (CR), very good partial response (VGPR), partial response

(PR) and no response (NR) based predominantly on the change in difference between involved (amyloidogenic) and uninvolved serum free light chain (dFLC) as outlined in Table 2.3.[54]

Table 2.2. Amyloidosis disease stage

Amyloid type	Stage	Criteria
ATTR-CM	NAC Stage I	NT-proBNP ≤ 3000ng/L and eGFR ≥ 45mls/min
	NAC Stage II	Either NT-proBNP > 3000ng/L or eGFR < 45mls/min
	NAC Stage III	NT-proBNP > 3000ng/L and eGFR < 45mls/min
Cardiac AL amyloidosis	Mayo I	NT-proBNP ≤ 332 ng/L and Troponin T ≤ 35ng/L
	Mayo II	Either NT-proBNP > 332ng/L or Troponin T > 35ng/L
	Mayo IIIa	NT-proBNP 333 - 8500 ng/L and Troponin T > 35ng/L
	Mayo IIIb	NT-proBNP > 8500 ng/L and Troponin T > 35ng/L
Renal AL amyloidosis	Stage I	Proteinuria ≤ 5g/24hr and eGFR ≥ 50mls/min
	Stage II	Either proteinuria > 5g/24hr or eGFR < 50mls/min
	Stage III	Proteinuria > 5g/24hr and eGFR < 50mls/min

Table 2.3: Haematologic response to treatment criteria

Haematologic response	Criteria
Complete response	Normalisation of serum free light chains with a negative serum and urinary electrophoresis and immunofixation
Very good partial response	dFLC <40mg/L
Partial response	Reduction in dFLC of >50%
No response	None of the above

Statistical analysis

Statistical analyses were performed using SPSS (IBM Corp, 2017), Stata (Stata Corp, 2019) and GraphPad Prism Version 5.03 and are detailed in the methods of each chapter.

Ethical statement

All patients were managed in accordance with the Declaration of Helsinki and provided informed consent for anonymous publication of their data. These studies were approved by the local research ethics committee.

Chapter Three: Disease progression in cardiac ATTR amyloidosis is indicated by serial calculation of National Amyloidosis Centre ATTR Stage

This chapter is based on the following publication[112]:

Disease progression in cardiac transthyretin amyloidosis is indicated by serial calculation of National Amyloidosis Centre transthyretin amyloidosis stage. Steven Law, Aviva Petria, Liza Chacko, Oliver C Cohen, Sriram Ravichandran, Janet A Gilbertson, Dorota Rowczenio, Ashutosh Wechalekar, Ana Martinez-Naharro, Helen J Lackmann, Carol J Whelan, David F Hutt, Philip N Hawkins, Marianna Fontana and Julian D Gillmore. 2020. ESC Heart failure; doi 10.1002/ehf2/12989

Permission has been obtained for use this article within my thesis.

Introduction

Cardiac ATTR amyloidosis may be acquired (ATTRwt-CM) or hereditary (ATTRv-CM). The commonest ATTRv-CM is that associated with the V122I (p.V142I) TTR variant (V122I-ATTRv-CM), carried by 3.9% of individuals of African descent.[113] The prevalence of ATTR-CM is not known but high grade cardiac uptake on Tc-DPD scintigraphy was reported in 3.9% of males over 75 years of age in a recent Spanish study.[114] Advances in imaging techniques and the development of validated non-biopsy diagnostic criteria for ATTR-CM have led to an exponential rise in diagnoses of ATTR-CM throughout the world.[2,25,31,44,57,115] Without treatment, the natural history of ATTR-CM is of progression and death within 3-10 years of diagnosis.[31]

Diagnostic delay is common and patients may be diagnosed at any time during the

disease course.[31,116] Recent therapeutic advances, including the TTR stabiliser, tafamidis, and 'gene-silencing' therapies, inotersen and patisiran, show promise in ATTR amyloidosis, although tafamidis is the only therapy to have specifically been shown to alter the natural history at the time of study.[3-5] However, a number of Phase 3 clinical trials of these or even newer agents for ATTR-CM are planned or already in progress.

At the time of diagnosis, prognosis of patients with ATTR-CM can be estimated by stratification into one of three NAC ATTR Stages, according to the NT-proBNP concentration and eGFR.[19,117] Median survival in Stage I, II and III ATTR-CM is approximately 6 years, 4 years and 2 years respectively.[19] However, serial calculation of NAC ATTR Stage in order to determine whether patients progress through the NAC Stages during their disease course and if so, whether an increase in NAC ATTR Stage is of prognostic relevance has not previously been undertaken.

We sought to determine the ability of NAC ATTR Stage to predict survival at various times during the disease course in ATTR-CM rather than simply at the time of diagnosis and to determine the prognostic relevance of an increase from NAC ATTR Stage I to a higher NAC ATTR Stage throughout patient follow up.

Methods

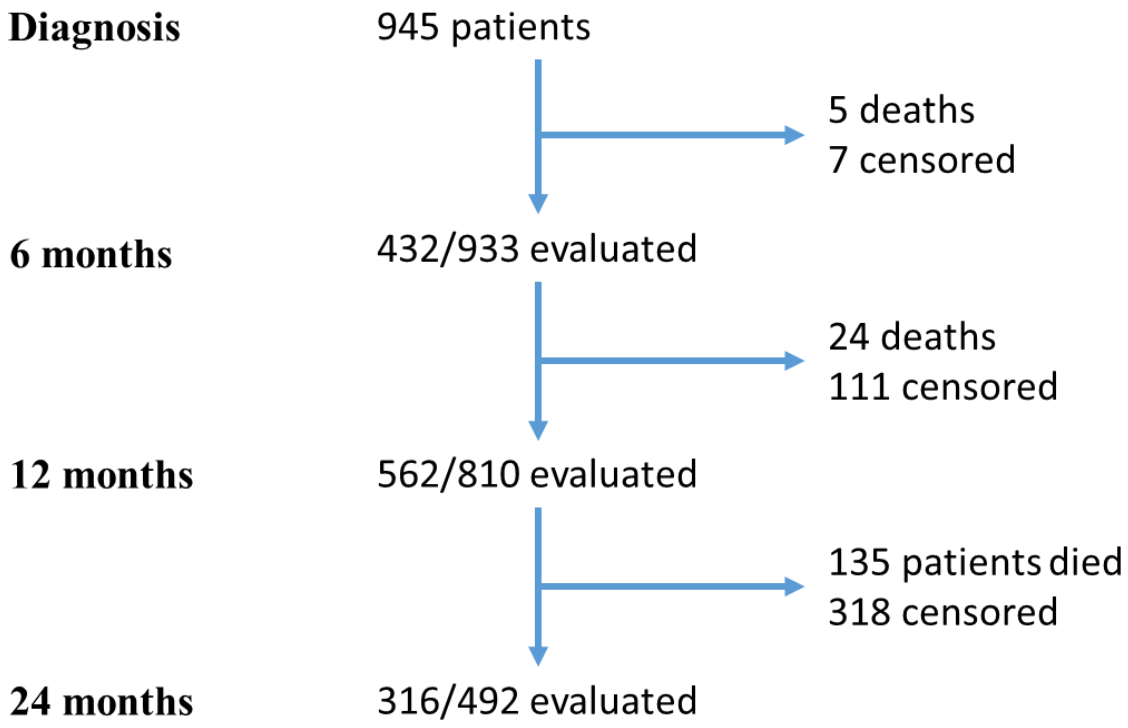
Patients

Patients with symptomatic ATTRwt-CM or V122I-ATTRv-CM, diagnosed between August 2001 and February 2019 on the basis of validated criteria,[2,79] who underwent routine clinical follow up at NAC, were included in this retrospective study. Patients with other amyloidogenic *TTR* mutations were excluded due to their typical 'mixed' phenotype including amyloid neuropathy. Censor date was 18 October 2019;

however, patients receiving any form of disease-modifying therapy were censored at the time of initiation of such treatment to exclude the potential influence on survival of therapeutic intervention; this included diflunisal, tafamadis, patisiran, inotersen and enrolment into interventional clinical trials. Symptomatic heart failure management was according to local protocols.

Nine hundred and forty-five patients were analysed at diagnosis, 432 at 6±1 months from diagnosis, 562 at 12±3 months from diagnosis, and 316 at 24±3 months from diagnosis. The differences in numbers of evaluable patients at each timepoint were due to a combination of the following: appointments occurring outside the specified time windows, patient death, and insufficient follow up time before the censor date. A study consort diagram is shown in Figure 3.1.

Figure 3.1: Consort diagram showing evaluable patients at each follow up timepoint



Disease Staging

The measurement of NT-proBNP and eGFR alongside calculation of NAC ATTR Stage are outlined in Chapter 2. NAC ATTR stage was calculated at baseline and again at each follow up attendance within the 6, 12 and 24-month window [19].

Statistical methods

Date of diagnosis was defined as date of first review at NAC. Mortality date was obtained from central NHS care records. Patients were categorised into NAC ATTR Stage I, II, and III, and further stratified by genotype into ATTRwt-CM and V122I-ATTRv-CM. Kaplan-Meier plots were used to illustrate survival stratified by NAC ATTR Stage; differences in survival were assessed by log rank test. Cox proportional hazard regression analysis was used to estimate hazard ratios for mortality in patient sub-groups.

Patients with attendances at 6±1, 12±3, and 24±3 months were then re-staged based on eGFR and NT-proBNP at the relevant timepoint. Landmark KM analyses provided survival curves from the relevant timepoint stratified by NAC ATTR Stage recalculated at the relevant timepoint. Cox proportional hazard regression analysis was used to estimate hazard ratios for mortality from each attendance stratified by NAC ATTR Stage, and further sub-group analyses were conducted for both genotypes.

Landmark KM analyses in the sub-group of patients with NAC ATTR Stage I at diagnosis, stratified by whether the NAC ATTR Stage was stable or had increased since diagnosis were performed at each timepoint; differences in survival were assessed by log rank test. Cox proportional hazard regression analysis was also used in this patient sub-group to compare mortality from each follow up timepoint

among those in whom NAC ATTR Stage was stable (i.e., still Stage I) and those in whom NAC ATTR Stage had increased since diagnosis.

Data is presented as median (interquartile range [IQR]) or number (percentage) unless otherwise stated. A p value of < 0.05 was deemed significant unless otherwise stated. Summary statistics were obtained using SPSS (IBM Corp, 2017) and all other analyses were performed using Stata (Stata Corp, 2019).

Results

Baseline characteristics

Baseline characteristics of 945 patients (727 ATTRwt-CM and 218 V122I-ATTRv-CM) diagnosed at NAC are shown in Table 3.1. At diagnosis, ATTRwt-CM were more commonly male ($p<0.001$), had less severe NYHA class heart failure ($p<0.001$), better LVEF ($p<0.001$), higher 6MWT distance ($p<0.001$), and fewer Perugini grade 3 Tc-DPD scans ($p<0.001$) compared to patients with V122I-ATTRv-CM (Table 3.1).

Table 3.1: Baseline characteristics in patients with ATTRwt-CM and V122I-ATTRv-CM

	ATTRwt-CM n=727	**V122I-ATTRv-CM** n=218	**p-value**
Age at diagnosis (years)	79 (73-83)	77 (72-81)	0.056
Male gender	683 (94%)	154 (71%)	<0.001
Caucasian Ancestry	678 (94%)	30 (14%)	<0.001
NAC ATTR Stage I	330 (45%)	106 (49%)	0.464
NAC ATTR Stage II	277 (38%)	73 (34%)	
NAC ATTR Stage III	120 (17%)	39 (18%)	
NT-proBNP (ng/L)	3036 (1717-5310)	2636 (1581-5193)	0.254
eGFR (MDRD, mL/min)	58 (47-71)	57 (46-69)	0.721
CKD Stage I	38 (5%)	13 (6%)	
CKD Stage II	305 (42%)	79 (36%)	
CKD Stage IIIa	235 (32%)	76 (35%)	
CKD Stage IIIb	120 (17%)	34 (16%)	
CKD Stage IV	29 (4%)	16 (7%)	
CKD Stage V	0 (0%)	0 (0%)	
NYHA Class (n=596, 189)			<0.001
I	54 (9%)	10 (5%)	
II	416 (69%)	112 (59%)	
III	126 (21%)	66 (35%)	
IV	3 (1%)	3 (2%)	
Systolic blood pressure (mmHg)	123 (113-137)	121 (110-135)	0.480
Diastolic blood pressure (mmHg)	74 (68-80)	74 (66-82)	0.612
IVSd (mm)	17 (16-18)	17 (16-18)	0.300
LVPWd (mm)	16 (15-18)	17 (15-18)	0.896
Left ventricular ejection fraction (%)	49 (41-56)	45 (35-51)	<0.001
6MWT (metres)	363 (274-439)	272 (184-368)	<0.001
Tc-DPD cardiac uptake (n=639, 170)			<0.001
Perugini grade 2	597 (93%)	109 (64%)	
Perugini grade 3	42 (7%)	60 (36%)	
Follow up (months)	26 (15-39)	24 (15-34)	0.195
Deaths	225 (31%)	114 (52%)	<0.001

Results displayed as median (percentage) or median (interquartile range).

Survival by NAC ATTR Stage throughout the disease course

At diagnosis 436/945 (46%) patients were NAC ATTR Stage I, 350 (37%) were Stage II and 159 (17%) were Stage III with a median survival of 58, 41, and 30 months respectively (Stage II vs I, HR 1.95; p<0.001; Stage III vs II, HR 2.25; p<0.001). In ATTRwt-CM, 330 (45%) were stage I, 277 (38%) Stage II, and 120 (17%) Stage III with median survival of 63, 46 and 33 months respectively (Stage II vs I, HR 2.41; p<0.001, Stage III vs II, HR 2.46; p<0.001). In V122I-ATTRv-CM, 106 (49%) were stage I, 73 (34%) stage II, and 39 (18%) Stage III with median survival of 39, 35, and 26 months (Stage II vs I, HR 1.62; p=0.030, Stage III vs II, HR 1.63; p=0.062; Figure 3.2A, Table 3.2 and Table 3.3).

At 6 months of follow up, 186/432 (43%) were Stage I, 147 (34%) were Stage II, and 99 (23%) were Stage III with median survival from this timepoint of 56, 36, and 28 months respectively (Stage II vs I, HR 2.45; p<0.001, Stage III vs II, HR 1.86; p=0.001; Figure 3.2B, Tables 3.2 and Table 3.3).

At 12 months of follow up, 216/562 (38%) were Stage I, 211 (38%) were Stage II, and 135 (24%) were Stage III with median survival from this timepoint of 51, 32, and 23 months respectively (Stage II vs I, HR 2.45; p<0.001, Stage III vs II, HR 1.75; p<0.001; Figure 3.2C, Tables 3.2 and Table 3.3).

At 24 months of follow up, 105/316 (33%) were Stage I, 119 (38%) were Stage II, and 92 (29%) were Stage III with median survival from this timepoint of 43, 28, and 19 months respectively (Stage II vs I, HR 2.67; p<0.001, Stage III vs II, HR 1.64; p=0.013; Figure 3.2D, Tables 3.2 and Table 3.3).

Table 3.2: Cox regression analyses showing risk of mortality from different follow up timepoints in relation to NAC ATTR Disease Stage calculated at the relevant timepoint

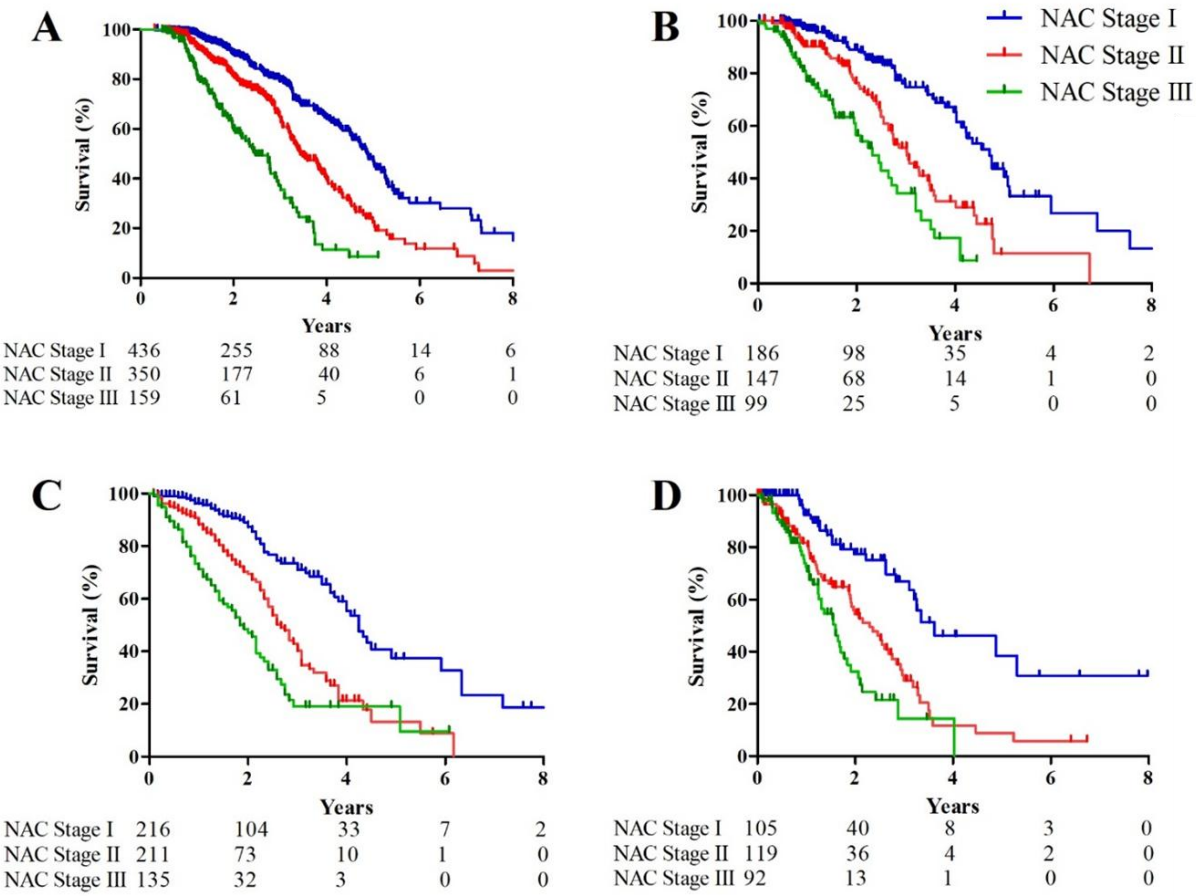
	All patients		**ATTRwt-CM**			**V122I-ATTRv-CM**		
	HR	p-value		HR	p-value		HR	p-value
At diagnosis, n=945			n=727			n=218		
Stage II vs I	1.95 (1.52-2.49)	<0.001	Stage II vs I	2.41 (1.77-3.29)	<0.001	Stage II vs I	1.62 (1.05-2.49)	0.030
Stage III vs I	4.38 (3.27-5.87)	<0.001	Stage III vs I	5.92 (4.09-8.56)	<0.001	Stage III vs I	2.63 (1.59-4.34)	<0.001
Stage III vs II	2.25 (1.70-2.98)	<0.001	Stage III vs II	2.46 (1.76-3.42)	<0.001	Stage III vs II	1.63 (0.97-2.72)	0.062
At 6 month timepoint, n=432			n=336			n=96		
Stage II vs I	2.45 (1.67-3.58)	<0.001	Stage II vs I	2.96 (1.85-4.74)	<0.001	Stage II vs I	1.90 (0.96-3.74)	0.065
Stage III vs I	4.55 (2.98-6.96)	<0.001	Stage III vs I	5.32 (3.11-9.11)	<0.001	Stage III vs I	2.71 (1.34-5.47)	0.006
Stage III vs II	1.86 (1.27-2.72)	0.001	Stage III vs II	1.80 (1.13-2.87)	0.014	Stage III vs II	1.43 (0.71-2.85)	0.313
At 12 month timepoint, n=562			n=432			n=130		
Stage II vs I	2.45 (1.74-3.45)	<0.001	Stage II vs I	2.36 (1.56-3.55)	<0.001	Stage II vs I	2.29 (1.21-4.31)	0.011
Stage III vs I	4.29 (2.99-6.16)	<0.001	Stage III vs I	4.07 (2.64-6.27)	<0.001	Stage III vs I	4.32 (2.19-8.53)	<0.001
Stage III vs II	1.75 (1.28-2.40)	<0.001	Stage III vs II	1.73 (1.17-2.55)	0.006	Stage III vs II	1.89 (1.10-3.24)	0.020
At 24 month timepoint, n=316			n=251			n=65		
Stage II vs I	2.67 (1.68-4.23)	<0.001	Stage II vs I	2.53 (1.51-4.24)	<0.001	Stage II vs I	3.91 (1.29-11.6)	0.016
Stage III vs I	4.36 (2.66-7.16)	<0.001	Stage III vs I	3.73 (2.08-6.68)	<0.001	Stage III vs I	6.48 (2.07-20.3)	0.001
Stage III vs II	1.64 (1.11-2.42)	0.013	Stage III vs II	1.47 (0.91-2.38)	0.112	Stage III vs II	1.66 (0.79-3.48)	0.182

Table 3.3: Median survival in months from each follow up timepoint according to NAC ATTR Stage calculated at the relevant timepoint

		NAC ATTR Stage		
		I	**II**	**III**
Diagnosis	All	58 (54-63)	41 (36-46)	30 (25-35)
	ATTRwt-CM	63 (59-68)	46 (41-51)	33 (27-38)
	V122I-ATTRv-CM	39 (30-49)	35 (26-43)	26 (19-33)
6 months	All	56 (50-63)	36 (32-41)	28 (22-34)
	ATTRwt-CM	61 (56-66)	39 (34-45)	32 (23-42)
	V122I-ATTRv-CM	36 (6-66)	23 (22-23)	25 (13-37)
12 months	All	51 (46-56)	32 (28-36)	23 (19-27)
	ATTRwt-CM	53 (46-60)	35 (31-39)	26 (22-30)
	V122I-ATTRv-CM	44 (30-58)	21 (11-31)	17 (11-23)
24 months	All	43 (23-64)	28 (21-34)	19 (15-23)
	ATTRwt-CM	59 (29-88)	30 (22-37)	21 (17-24)
	V122I-ATTRv-CM	32 (26-37)	23 (6-41)	15 (11-20)

Survival (in months) from diagnosis (baseline), 6, 12, and 24 months of follow up by NAC ATTR Disease Stage recalculated at the relevant timepoint; displayed as median (95% confidence interval).

Figure 3.2: Survival stratified by NAC ATTR Stage at diagnosis and during follow up



Landmark Kaplan Meier analyses showing survival proportions in cardiac ATTR amyloidosis stratified by NAC ATTR Stage calculated at the following follow up timepoints; A) Diagnosis ($p<0.001$), B) 6 month follow up timepoint ($p<0.001$), C) 12 month follow up timepoint ($p<0.001$), D) 24 months follow up timepoint ($p<0.001$). The number at risk are displayed below each figure.

Change in NAC ATTR Stage in patients with NAC ATTR Stage I disease at diagnosis

Among 436 (46%) patients with NAC ATTR Stage I disease at baseline, 204 were evaluated at 6 months, 2 had died, 2 were censored prior to the 6 month timepoint, and 228 were alive but not evaluated within the 6 month timepoint window. Of the 204 evaluable patients, 43 (21%) had an increase in NAC ATTR Stage and the remaining 161 (79%) were still NAC ATTR Stage I at this timepoint. Cox regression analysis showed a highly significant increase in ongoing mortality risk among patients with an increase in NAC ATTR Stage compared to stable NAC ATTR Stage (HR 3.19 [95% CI 1.76-5.77]; p<0.001), with consistent results across both genotypes (Table 3.4). Landmark KM survival analysis stratified by stable or increased NAC ATTR Stage at 6 months is shown in Figure 3.3A.

Among 436 patients with NAC ATTR Stage I disease at baseline, 283 were evaluated at 12 months, 4 had died, 46 were censored prior to the 12 month timepoint, and 103 were alive but not evaluated within the 12 month timepoint window. Of the 283 evaluable patients, 90 (32%) had an increase in NAC ATTR Stage and the remaining 193 (68%) were still NAC ATTR Stage I at this timepoint. Cox regression analyses showed a highly significant increase in ongoing mortality risk among patients with an increase in NAC ATTR Stage compared to stable NAC ATTR Stage (HR 2.58 [95% CI 1.67-3.99]; p<0.001) with consistent results across both genotypes (Table 3.4). Landmark KM survival analysis stratified by stable or increased NAC ATTR Stage at 12 months is shown in Figure 3.3B.

Among 436 patients with NAC ATTR Stage I disease at baseline, 166 were evaluated at 24 months, 34 had died, 148 were censored prior to the 24 month timepoint, and 88 were alive but not evaluated within the 24 month timepoint window.

Of the 166 evaluable patients, 73 (44%) had an increase in NAC ATTR Stage and the remaining 93 (56%) were still NAC ATTR Stage I at this timepoint. Cox regression analyses showed a highly significant increase in ongoing mortality risk among patients with an increase in NAC ATTR Stage compared to stable NAC ATTR Stage (HR 3.22 [95% CI 1.87-5.52]; p<0.001) with consistent results across both genotypes (Table 3.4). Landmark KM survival analysis stratified by stable or increased NAC ATTR Stage at 24 months is shown in Figure 3.3C.

Increase in NAC ATTR Stage or death occurred in a significantly higher proportion of NAC ATTR Stage I patients with V122I-ATTRv-CM than ATTRwt-CM at the 12 (p=0.01) and 24 month (p=0.001) follow up timepoints (Table 3.5). Among 397 ATTRwt-CM patients with NAC ATTR Stage II or III disease at diagnosis, 2 (1%), 10 (3%) and 70 (17%) had died at 6, 12 and 24 months of follow up respectively. Among 112 V122I-ATTRv-CM patients with NAC ATTR Stage II or III disease at diagnosis 1 (1%), 10 (9%), and 31 (27%) had died at 6, 12 and 24 months of follow up respectively.

Table 3.4: Cox regression analyses showing risk of ongoing mortality among patients who were NAC ATTR Stage I at diagnosis according to whether the recalculated NAC ATTR Stage was stable or had increased at the relevant timepoint

	All Patients			ATTRwt-CM			V122I-ATTRv-CM		
	N	HR (95% CI)	p-value	N	HR (95% CI)	p-value	N	HR (95% CI)	p-value
6 month FU timepoint	204			152			52		
Stable NAC ATTR Stage I	161	1		123	1		38	1	
Increased NAC ATTR Stage	43	3.19 (1.76-5.77)	<0.001	29	2.77 (1.16-6.70)	0.024	14	3.28 (1.37-7.87)	0.008
12 month FU timepoint	283			210			73		
Stable NAC ATTR Stage I	193	1		152	1		41	1	
Increased NAC ATTR Stage	90	2.58 (1.67-3.99)	<0.001	58	1.86 (1.01-3.43)	0.048	32	2.52 (1.28-4.95)	0.007
24 month FU timepoint	166			134			32		
Stable NAC ATTR Stage I	93	1		78	1		15	1	
Increased NAC ATTR Stage	73	3.22 (1.87-5.52)	<0.001	56	2.98 (1.58-5.64)	0.001	17	4.38 (1.38-13.95)	0.012

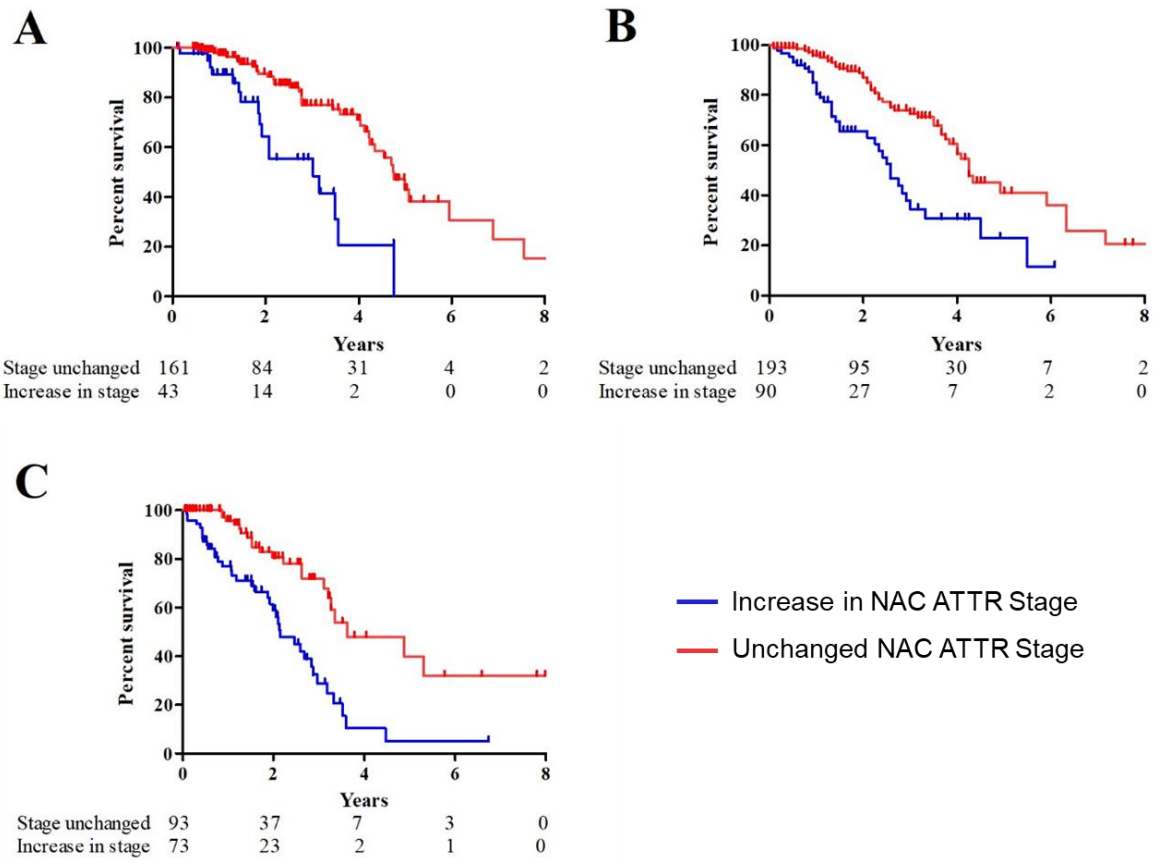
FU – follow up

Table 3.5: Comparison of change in NAC ATTR Stage and mortality between patients with ATTRwt-CM and V122I-ATTRv-CM at different timepoints among those with NAC ATTR Stage I at diagnosis

	N	Died	NAC ATTR Stage		Progressed[≠] or died	p-value*
			Increased	Stable		
6 month timepoint						
ATTRwt-CM	154	2 (1%)	29 (18%)	123 (80%)	31 (20%)	
V122I-ATTRv-CM	52	0 (0%)	14 (27%)	38 (73%)	14 (27%)	0.334
12 month timepoint						
ATTRwt-CM	212	2 (1%)	58 (27%)	152 (72%)	60 (28%)	
V122I-ATTRv-CM	75	2 (3%)	32 (43%)	41 (55%)	34 (45%)	0.010
24 month timepoint						
ATTRwt-CM	146	12 (8%)	56 (38%)	78 (53%)	68 (47%)	
V122I-ATTRv-CM	54	22 (41%)	17 (31%)	15 (28%)	39 (72%)	0.001

[≠] - Progressed' defined as increase in NAC ATTR Stage. *P-values comparing proportion (shown in brackets) of ATTRwt-CM and V122I-ATTRv-CM patients who progressed or died

Figure 3.3: Landmark KM survival analyses in patients with NAC ATTR Stage I at diagnosis stratified by whether the NAC ATTR Stage was stable or had increased at each timepoint



A) At 6 month follow up timepoint patients with stable NAC ATTR Stage I disease had median ongoing survival of 57 months and patients with increased NAC ATTR Stage had median ongoing survival of 36 months ($p<0.001$). B) At 12 month follow up timepoint patients with stable NAC ATTR Stage I disease had median ongoing survival of 51 months and patients with increased NAC ATTR Stage had median ongoing survival of 31 months ($p<0.001$). C) At 24 month follow up timepoint patients with stable NAC ATTR Stage I disease median ongoing survival was 43 months, and patients with increased NAC ATTR Stage had median ongoing survival of 26 months ($p<0.001$). The number at risk are displayed below each figure.

Discussion

This study shows that NAC ATTR Stage, which has been validated as a prognostic tool for ATTR-CM at the time of diagnosis, is applicable throughout the disease course with patients tending to increase their NAC ATTR Stage as the condition progresses.[19] The natural history of ATTR-CM is one of relentless progression and eventual death, although the rate of clinical decline varies between individuals. This study shows that patients tend to increase their NAC ATTR Stage by one point every ~2 years, consistent with the published median survival associated with each diagnostic NAC ATTR Stage which differs by about 2 years per stage, and that the prognostic significance of the NAC ATTR Stage holds up throughout the disease course.[19] The proportion of patients who increased their NAC ATTR Stage during follow up was higher in V122I-ATTRv-CM than ATTRwt-CM and taken together with the higher mortality rate in V122I-ATTRv-CM, provides further evidence of a more aggressive phenotype in the hereditary condition.[31,118]

Despite diagnostic delays being common, there is evidence that the recent development and validation of non-invasive diagnosis of ATTR-CM, coupled with an increase in disease-awareness amongst cardiologists, partly because of therapeutic advances, is leading to earlier diagnosis.[2,4,6,31,57] It seems probable that the proportion of patients who are NAC ATTR Stage I at diagnosis will rise to > 50% within the next decade. Furthermore, NAC ATTR Stage I encompasses a broad range of disease severity from virtually asymptomatic imaging or histological abnormalities to disease associated with significant morbidity. Our demonstration that progression from NAC ATTR Stage I to a higher NAC ATTR Stage during follow up is prognostically important is therefore likely to have substantial clinical relevance. One might postulate that the absence of an increase in NAC ATTR Stage could be

used to demonstrate efficacy of novel therapeutic agents in ATTR-CM, although this hypothesis needs further study.

Limitations of our study include the variation in patient numbers, in part because of evaluations occurring outside the specified timepoint windows; however, it is not anticipated that this will introduce bias since appointment delays in our Centre almost invariably occur due to issues of capacity rather than on clinical grounds. The consistency of the findings across the studied timepoints supports NAC ATTR Stage being applicable at any time during the disease natural history. A further limitation is the relatively small number of patients with V122I-ATTRv-CM compared to ATTRwt-CM, particularly among those evaluated at later timepoints.

In summary, this study demonstrates that NAC ATTR Stage predicts survival in ATTR-CM throughout follow up and that an increase in NAC ATTR Stage from a diagnostic NAC ATTR Stage of I predicts mortality throughout the disease natural history.

Chapter Four: Change in NT-proBNP at one year predicts mortality in wild-type transthyretin cardiac amyloidosis

This chapter is based on the following publication[119]:

Change in N-terminal pro-B-type natriuretic peptide at 1 year predicts mortality in wild-type transthyretin amyloid cardiomyopathy. Steven Law, Aviva Petrie, Liza Chacko, Oliver C Cohen, Sriram Ravichandran, Janet A Gilbertson, Dorota Rowczenio, Ashutosh D Wechalekar, Ana Martinez-Naharro, Helen J Lackmann, Carol J Whelan, David F Hutt, Philip N Hawkins, Marianna Fontana and Julian D Gillmore. 2022. Heart; doi:10.1136/heartjnl-2021-319063

Permission has been obtained for use this article within my thesis.

Introduction

Transthyretin amyloid cardiomyopathy is an increasingly recognised cause of heart failure. Prognosis can be estimated at the time of diagnosis by stratifying patients by NAC ATTR Stage, however there is substantial interpatient variability in rate of disease progression such that there is an urgent need for a widely applicable marker of disease progression in this population.[19,117]

Chapter 3 demonstrated that NAC ATTR Stage was prognostic during follow up and that an increase from NAC ATTR Stage 1 was associated with mortality. However, as a marker of disease progression it has limitations as patients diagnosed with NAC ATTR Stage III cannot progress further, and it does not distinguish between the heterogeneous group of patients diagnosed with NAC ATTR Stage I, who can have an NT-proBNP ranging from normal to 3000ng/L. Chapter 3 also

highlighted the more rapid disease progression in patients with V122I-ATTRv-CM compared to ATTRwt-CM.

This study evaluates the change in a range of disease-related variables between diagnosis and 12 months of follow up to identify the best marker of disease progression and prognosis in ATTR-CM; given the inherent difference in natural history between V122I-ATTRv-CM compared to ATTRwt-CM demonstrated in Chapter 3, I opted to focus on ATTRwt-CM.

Methods

Study Design

A retrospective observational cohort study of 432 patients with symptomatic ATTRwt-CM diagnosed at the UK NAC between June 2006 and October 2018. Patients with ATTRv amyloidosis and those receiving any form of disease-modifying therapy were excluded; no patients underwent heart transplantation. Diagnosis was established at NAC in all patients by meeting the non-biopsy diagnostic criteria for ATTR-CM outlined in chapter 2, or by histology including immunohistochemistry and proteomic analysis of amyloid, undertaken at NAC.[2,79] The diagnostic clinical evaluation at NAC as well as routine clinical follow up performed 12±3 months later, also included a full biochemical profile (renal, liver, bone, cardiac biomarkers), echocardiography, and functional assessment including NYHA class and 6MWT distance.

Statistical methods

Date of diagnosis (baseline) was defined as date of first review at NAC, and patients were censored at death or last clinical contact. Mortality data was obtained from NHS central care records. Censor date was 18th October 2019.

Change in a range of disease-related variables (labelled as Δ variable throughout the manuscript) between baseline and 12 months was calculated and the association between each Δ variable and mortality from the 12 month timepoint was explored by univariable Cox regression analyses and subsequently by a multivariable Cox regression analysis in which the best performing biochemical (NT-proBNP), echocardiographic (IVSd) and functional (NYHA class) variable significant in the univariable analyses were included. The 12 month timepoint was selected to identify an early marker of disease progression which would be reached by the majority of patients diagnosed with ATTR-CM; it also matches the timepoint of the primary endpoint measure being used in part A of the ongoing phase 3 clinical trial ATTRibute-CM. Time-dependent receiver operator characteristic (ROC) curve analyses were performed using both absolute Δ NT-proBNP concentration from baseline and percentage Δ NT-proBNP from baseline to identify the optimal measure of Δ NT-proBNP. The area under the curve was higher for absolute Δ NT-proBNP than percentage Δ NT-proBNP (0.63 versus 0.60); therefore, absolute Δ NT-proBNP was used for all subsequent analyses. The relationship between Δ NT-proBNP and mortality from the 12 month timepoint was further explored by multivariable Cox regression analyses including a range of previously reported prognostic factors assessed at diagnosis.[120] Landmark KM survival curves illustrate survival from the 12 month timepoint stratified by different Δ NT-proBNP values.

Data is presented as median (IQR) or number (percentage) unless otherwise stated. A p value of < 0.05 was deemed significant. Summary statistics were obtained using SPSS v25 (IBM Corp, 2017) and all other analyses were performed using Stata v16 (Stata Corp, 2019).

Results

Patient characteristics

Baseline characteristics of all 432 patients are shown in Table 4.1. Median age at diagnosis of ATTRwt-CM was 77 years and 95% were male. The majority of patients (69%) had NYHA class 2 heart failure at diagnosis and median 6MWT distance was 358 meters. Median time from diagnosis to 12±3 month follow up timepoint was 12 (11-13) months, median follow up from the 12 month timepoint was 19 (10-31) months. At censor, 146 patients had died and 286 were alive.

Table 4.1: Baseline patient characteristics

	Number	ATTRwt-CM n=432
Age at diagnosis (years)	432	77 (73 to 82)
Male gender	432	409 (95%)
Caucasian Ancestry	432	402 (93%)
NAC ATTR Stage I	432	210 (49%)
NAC ATTR Stage II	432	161 (37%)
NAC ATTR Stage III	432	61 (14%)
NT-proBNP (ng/L)	432	2760 (1568 to 4904)
eGFR (MDRD, mL/min)	432	60 (49-74)
CKD Stage ≤I		24 (6%)
CKD Stage II		196 (45%)
CKD Stage IIIa		130 (30%)
CKD Stage IIIb		66 (15%)
CKD Stage IV		16 (4%)
CKD Stage V		0 (0%)
Troponin T (ng/L)	389	58 (40 to 81)
Serum albumin (g/L)	432	44 (42 to 46)
NYHA Heart Failure Class	432	
I		53 (12%)
II		298 (69%)
III		77 (18%)
IV		4 (1%)
Co-morbidities	432	
Hypertension		147 (34%)
Atrial fibrillation		226 (52%)
Diabetes mellitus		54 (13%)
Pacemaker		66 (15%)
Systolic blood pressure (mmHg)	432	122 (111 to 135)
Diastolic blood pressure (mmHg)	432	72 (67 to 79)
Body mass index (kg/m^2)	432	26 (24 to 29)
IVSd (mm)	427	17 (16 to 18)
LVPWd (mm)	427	16 (15 to 18)
LVEF (%)	423	48 (42 to 56)

6MWT distance (metres)	313	358 (230 to 449)
Perugini grade on Tc-DPD scintigraphy	380	
Grade 2		349 (92%)
Grade 3		31 (8%)

Results displayed as number (percentage) for categorical variables and median (IQR) for numerical variables.

Survival impact of change in disease-related variables from baseline to 12 months

Univariable Cox regression analyses identified an association between mortality from the 12 month timepoint and Δ NT-proBNP (p=0.001) and Δ NYHA class (p=0.005) (Table 4.2). Multivariable analysis showed both Δ NT-proBNP (HR 1.04 [95% CI: 1.01-1.07] per 500 ng/L increase; p=0.003) and increasing NYHA class (HR 1.65 [95% CI: 1.11-2.47]; p=0.014) to be predictive of mortality from the 12 month timepoint, independent of change in other disease related variables (Table 4.2).

Multivariable Cox regression analysis incorporating Δ NT-proBNP along with a range of baseline variables which are known to be prognostic in ATTRwt-CM at the time of diagnosis, also showed Δ NT-proBNP (HR 1.07 [95% CI: 1.02-1.13] per 500 ng/L increase; p=0.007; Table 4.3) to be an independent predictor of mortality along with age and NT-proBNP concentration at diagnosis. When diagnostic eGFR and NT-proBNP as individual variables were exchanged for diagnostic NAC ATTR Stage (which is calculated from the same two variables) and this multivariable analysis was repeated, Δ NT-proBNP continued to predict mortality (HR 1.07 [95% CI: 1.01-1.14] per 500 ng/L increase; p=0.017).

Time-dependent ROC curve analyses identified absolute Δ NT-proBNP concentration from baseline to be a better predictor of mortality than percentage

Δ NT-proBNP. Median Δ NT-proBNP was not significantly different between different diagnostic NAC ATTR Stages (p=0.19 by Kruskal-Wallis test) and there was no evidence of an association between Δ NT-proBNP and the follow up interval from diagnosis which ranged from 9 to 15 months (p=0.13). The proportion of patients with Δ NT-proBNP > 500 ng/L, > 1000 ng/L and > 2000 ng/L at 12 months was 45%, 35% and 16% respectively. Landmark KM survival curves stratified for Δ NT-proBNP cutoffs of > or ≤ 500 ng/L, > or ≤ 1000 ng/L and > or ≤ 2000 ng/L are shown in Figure 1. Among the 150/432 (35%) patients who had a Δ NT-proBNP of ≥ 1000 ng/L median survival was 29 (95% CI: 25 to 33) months compared to 46 (95% CI: 38 to 54) months in 282/432 (65%) patients with Δ NT-proBNP < 1000 ng/L (p<0.001). Both Δ NT-proBNP of ≥ 500 ng/L (HR 1.65 [95% CI: 1.18-2.31]; p=0.003) and ≥ 2000 ng/L (HR 2.87 [1.93-4.27]; p<0.001) also predicted ongoing mortality.

Table 4.2: Association between mortality and change (Δ) from baseline to 12 months in disease-related variables by Cox regression analyses

Variable		**Univariable Analysis**			**Multivariable Analysis**		
	Median change (IQR)	HR	95% CI	p-value	HR	95% CI	p-value
Δ NT-proBNP (ng/L)	375 (-258 to 1350)	1.05*	1.02 to 1.07	**0.001**	1.04*	1.01-1.07	**0.003**
Δ eGFR (ml/min)	-5 (-12 to 1)	1.00	0.98 to 1.01	0.732			
Δ Albumin (g/L)	-1 (-3 to 1)	1.01	0.95 to 1.06	0.840			
Δ Troponin T (ng/L)	12 (4 to 25)	1.01	1.00 to 1.02	0.055			
Δ IVSd (mm)	0 (0 to 1)	1.15	0.99 to 1.34	0.075	1.03	0.85-1.25	0.741
Δ LVPWd (mm)	0 (0 to 1)	1.12	0.98 to 1.27	0.099			
Δ LVEF (%)	-1 (-6 to 4)	1.00	0.98 to 1.02	0.886			
Increasing NYHA Class	0 (0 to 1)	1.72	1.18 to 2.52	**0.005**	1.65	1.11-2.47	**0.014**
Δ Systolic BP (mmHg)	-1 (-11 to 9)	1.00	0.99 to 1.01	0.741			
Δ Diastolic BP (mmHg)	1 (-7 to 7)	1.00	0.98 to 1.01	0.556			
Δ NAC ATTR Stage	0 (0 to 0)	1.39	0.94 to 2.06	0.096			
Δ 6MWT (meters)	-11 (-70 to 16)	0.85†	0.70 to 1.02	0.080			

*HR for NT-proBNP is per 500 ng/L increase; †HR for 6 minute walk test (6MWT) distance is per 100 meter increase; remainder of HRs are per unit increase. Increasing NYHA class is displayed as a binary measure.

Table 4.3: Multivariable analysis including Δ NT-proBNP at 12 months and a range of variables and baseline patient characteristics known to affect prognosis.

		HR	95% CI	p-value
Δ NT-proBNP at 12 months*		1.07	1.02-1.13	**0.007**
Diagnostic NT-proBNP (ng/L) *		1.07	1.02-1.13	**0.006**
Diagnostic Troponin T (ng/L)		1.01	1.00-1.02	0.147
Diagnostic eGFR (ml/min/1.73m^2)		1.01	0.99-1.03	0.377
Age at diagnosis		1.08	1.02-1.13	**0.004**
NYHA class at diagnosis	I	1		
	II	0.74	0.33-1.65	0.461
	≥III	0.39	0.14-1.15	0.089
IVSd at diagnosis		0.93	0.81-1.07	0.321
Body mass index (kg/m^2)		0.95	0.88-1.03	0.244
6MWT at diagnosis (m)†		0.91	0.72-1.16	0.457
Atrial Fibrillation		0.90	0.50-1.63	0.738
Hypertension		1.01	0.54-1.89	0.986
Diabetes		2.19	0.99-4.86	0.054
Permanent Pacemaker in situ		0.73	0.31-1.68	0.454
Aortic stenosis§		1.47	0.53-4.11	0.459

*HR for NT-proBNP is per 500 ng/L increase; †HR for 6 minute walk test (6MWT) distance is per 100 meter increase; § ≥ moderate aortic stenosis at diagnosis; remainder of HRs are per unit increase. Δ NT-proBNP was calculated at the 12 month timepoint, all other variables were assessed at diagnosis.

Figure 4.1: Landmark Kaplan-Meier survival curves stratified by Δ NT-proBNP during the first year of follow up.

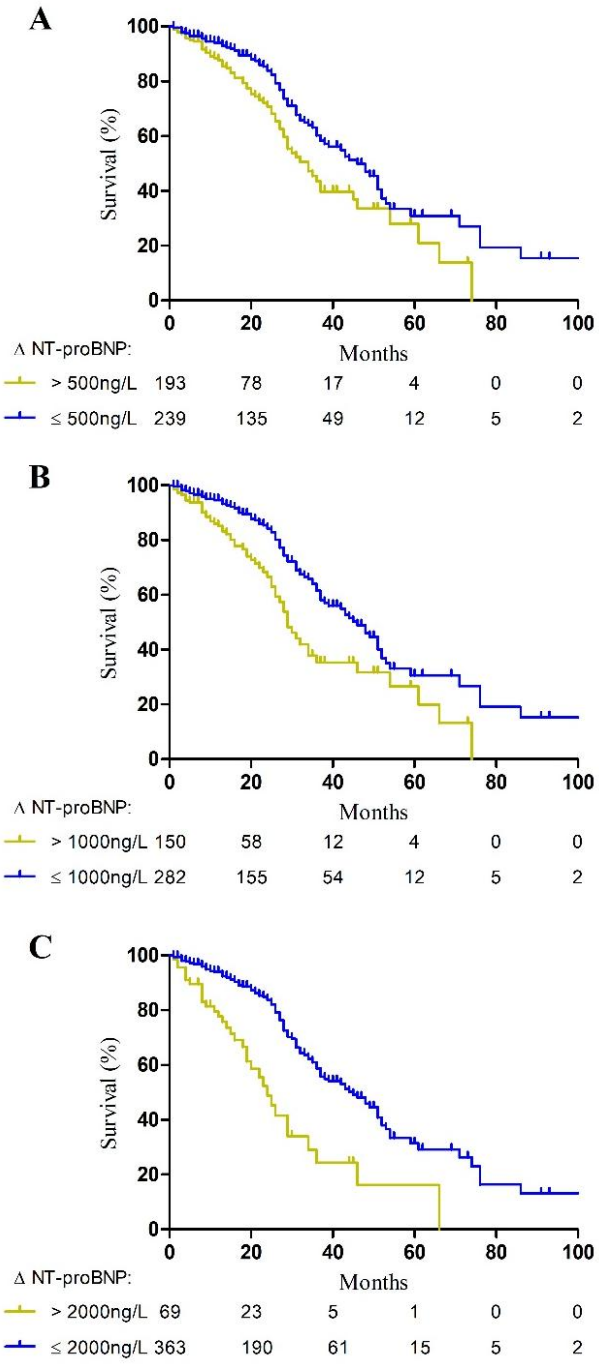


Figure 4.1: Numbers at risk are shown below each curve. A) Patient survival stratified by Δ NT-proBNP > or ≤ 500 ng/L (HR 1.65 [95% CI: 1.18-2.31]; p=0.003). B) Patient survival stratified by Δ NT-proBNP > or ≤ 1000 ng/L (HR 1.92 [95% CI: 1.37-2.70]; p<0.001). C) Patient survival stratified by Δ NT-proBNP > or ≤ 2000ng/L (HR 2.87 [95% CI: 1.93-4.27]; p<0.001).

Discussion

This study establishes Δ NT-proBNP at 12 months from diagnosis as a powerful independent predictor of ongoing mortality in patients with ATTRwt-CM. Importantly, the prognostic relevance of Δ NT-proBNP was independent of a range of biochemical, functional and echocardiographic parameters at diagnosis including NAC ATTR Stage, troponin T, age, NYHA class, 6MWT distance, presence of atrial fibrillation, and IVSd thickness.

Limitations of our study include its retrospective design, exclusion of patients who die within the first year of follow up (approximately 5% of patients in the UK), exclusion of more detailed echocardiographic variables and absence of cardiac magnetic resonance data.[45,46,51,121] However, the authors maintain that a biomarker-based prognostic system has great attraction due to its simplicity, lack of operator variability, and universal availability and applicability. There may also be concerns that NT-proBNP concentration is known to be confounded by other factors in the ATTRwt-CM population; however, where possible, these were accounted for in our multivariable model. Furthermore, in cardiac AL amyloidosis, a far more heterogeneous disease than ATTRwt-CM, diagnostic NT-proBNP predicts mortality, and both disease progression and treatment response are defined by Δ NT-proBNP which is strongly associated with morbidity, quality of life, and mortality despite the same, and indeed additional, confounders.[54,56] Our study does not include patients on disease-modifying therapy since tafamidis was not available in the United Kingdom at the time of study.

A number of disease-modifying therapies have emerged for ATTR amyloidosis including diflunisal,[88] tafamidis,[4] patisiran[3,91] and inotersen[5,102] and several of the relevant clinical trials suggest a role for Δ NT-proBNP in the

assessment of response to these disease-modifying treatments. Tafamidis is associated with a reduction in mortality among patients with ATTR-CM and the ATTR-ACT trial showed a smaller increase in NT-proBNP in the tafamidis group compared to placebo.[4] Post-hoc analysis of the sub-population with ATTRv-CM accompanying neuropathy in the APOLLO study showed a highly significant reduction in NT-proBNP concentration among patients receiving patisiran compared to those on placebo.[91] Of note, the trial design of ATTRibute-CM, a current global phase 3 clinical trial evaluating the novel TTR stabiliser acoramidis in ATTR-CM, includes a Part A for which the primary endpoint is a comparison of Δ 6MWT distance from enrolment to 12 months between the treatment and placebo groups.[6] Although Δ 6MWT distance undoubtedly provides a valuable functional assessment, it is noteworthy that it did not predict survival in our cohort.

This data supports further study of Δ NT-proBNP at 12 months in patients receiving disease-modifying therapy to assess its utility as a marker of treatment response and potential use as a surrogate endpoint in future clinical trials. Multiple disease-modifying therapies may soon be available for ATTRwt-CM, including both gene silencer and TTR stabiliser therapies, and early markers of treatment response will be required to guide therapeutic decisions.

In summary, this study establishes, for the first time, that increasing NT-proBNP concentration in the first year after diagnosis of ATTRwt-CM is a powerful independent predictor of mortality. Further study of Δ NT-proBNP in a ATTRwt-CM cohort receiving disease-modifying therapy is warranted to establish its potential utility as an indicator of treatment response.

Chapter Five: Characteristics and natural history of early-stage cardiac transthyretin amyloidosis

This chapter is based on the following publication[122]:

Characteristics and natural history of early-stage cardiac transthyretin amyloidosis. Steven Law, Melanie Bezard, Aviva Petrie, Liza Chacko, Oliver C Cohen, Sriram Ravichandran, Olabisi Ogunbiyi, Mounira Kharoubi, Sashiananthan Ganeshananthan, Sharmananthan Ganeshananthan, Janet A Gilbertson, Dorota Rowczenio, Ashutosh Wechalekar, Ana Martinez-Naharro, Helen J Lackmann, Carol J Whelan, David F Hutt, Philip N Hawkins, Thibaud Damy, Marianna Fontana and Julian D Gillmore. 2022. European Heart Journal; doi:10.1093/eurheartj/ehac259
Permission has been obtained for use this article within my thesis.

Introduction

Transthyretin amyloid cardiomyopathy is increasingly diagnosed although only a small proportion of individuals who have ATTR amyloid deposits in their hearts, according to prevalence estimates from post-mortem series, are ever diagnosed with cardiac amyloidosis in life.[1,7,8] Since the diagnosis of ATTR-CM is challenging and often missed, the true disease prevalence remains unknown.

The prognosis of patients who are diagnosed with ATTR-CM can be estimated on the basis of NAC ATTR Stage.[19,117] Initial reports indicated that ~40% patients were diagnosed in Stage I conferring a prognosis of > 5 years in the absence of disease-modifying therapy.[19] Improvements in diagnostic imaging, alongside heightened awareness of ATTR-CM among cardiologists following the

development of life-prolonging therapies, mean that > 50% of patients are now diagnosed with NAC Stage I, a trend which is likely to continue.[2,4,26,31,91,102,123,124] NAC ATTR Stage I represents a heterogeneous group from patients with minimal symptoms and a normal NT-proBNP, to those with significant heart failure symptomatology and an NT-proBNP of 3000ng/L. Chapters 3 and 4 demonstrated the clinical significance of progressing beyond NAC ATTR Stage I, and that increasing NT-proBNP during follow up independently predicts mortality in ATTRwt-CM.

There are few data on the natural history of early-stage ATTR-CM. This study aims to characterise the disease course and clinical outcomes among patients diagnosed with early-stage ATTR-CM in the absence of disease-modifying therapy.

Methods

Patients

A retrospective analysis was conducted of all patients with ATTR-CM attending two large amyloidosis Centres, the UK NAC and the Amyloidosis Mondor Network, France between 27th August 2009 and 30th July 2020, who fulfilled all of the following inclusion criteria; wild-type *TTR* gene sequence or *TTR* mutation encoding the known pathogenic p.V142I variant; NAC ATTR Stage I biomarkers at the time of diagnosis; and absence of administration of disease-modifying therapy during clinical follow up.[2,19] ATTR-CM was defined either by validated non-biopsy diagnostic criteria, or by the presence of ATTR amyloid on histology from any biopsy site coupled with Perugini grade ≥1 cardiac uptake on radionuclide scan. NAC ATTR Stage I was defined as an NT-proBNP ≤3000ng/L and eGFR ≥45ml/min/1.73m^2 at diagnosis. In order to focus on patients with predominant ATTR-CM, those with non-

V122I-associated ATTRv amyloidosis were excluded due to the important but variable contributions of peripheral and autonomic neuropathy to both morbidity and mortality in those conditions.[19]

Patients were systemically evaluated at diagnosis and on a 6-12 monthly basis thereafter as clinically indicated. Evaluations consisted of a full clinical history and examination alongside functional, biochemical, electrocardiography and echocardiographic assessment. Hospitalization data was not reliably captured and therefore excluded from analyses. Throughout the study cardiovascular morbidity is defined by presence of atrial fibrillation, cerebrovascular accident, permanent pacemaker, diuretic requirement, or NYHA functional class ≥2.

The measurement of NT-proBNP, high-sensitivity troponin T, and calculation of eGFR are outlined in Chapter 2 alongside the performance of radionuclide scintigraphy, echocardiography, histological assessment, genetic testing and criteria for non-biopsy diagnosis of ATTR-CM.

Statistical methods

Diagnosis was defined as date of first review at a specialist amyloidosis centre, and follow up was defined as time from diagnosis to date of Censor or death. Patients were censored at the earliest of the following timepoints; 30 July 2020, date of commencement of disease-modifying therapy, enrolment into a clinical trial of disease-modifying therapy, or at 100 months of follow up.

The association between patient characteristics at diagnosis and survival was explored by univariable Cox regression analyses, and subsequently by multivariable Cox regression analyses including variables known to be associated with survival in ATTR-CM according to the published literature.[19,125,126] Due to the independent

prognostic power of NT-proBNP at diagnosis coupled with recently published literature demonstrating the independent association between loop diuretic equivalent dose requirement and survival in ATTR-CM,[126] the study population was stratified into two groups defined as: NAC ATTR Stage Ia - NT-proBNP ≤500ng/L or ≤1000ng/L in the presence of atrial fibrillation with a loop diuretic equivalent requirement of <0.75mg/kg, and NAC ATTR Stage Ib - NT-proBNP >500ng/L or >1000ng/L in the presence of atrial fibrillation or a loop diuretic requirement of ≥0.75mg/kg. The NT-proBNP cut-offs were chosen to reflect the NT-proBNP eligibility criteria for recently conducted clinical trials of novel disease-modifying agents in ATTR-CM, some of which require the use of diuretics and acknowledge the effect of atrial fibrillation on NT-proBNP concentration independently of disease severity (ATTR-ACT [>600ng/L], ATTRibute-CM [>300ng/L], APOLLO-B [>300ng/L and >600ng/L in atrial fibrillation], HELIOS-B [>300ng/L and >600ng/L in atrial fibrillation], ION-CS2 [>600ng/L and >1200ng/L in atrial fibrillation], and ITL-2001-CL-001 [>600ng/L and >1000ng/L in atrial fibrillation]).[4,6,127,128] Loop diuretic doses were converted to a total daily furosemide equivalent dose; for example 1mg bumetanide twice daily was converted to 80mg as a total daily furosemide equivalent dose. A value of <0.75mg/kg was selected to identify patients with a relatively low diuretic requirement of ≤40mg daily for patients >53kg and ≤80kg in weight and ≤60mg daily for patients of >80kg typically administered in mild heart failure or for non-heart failure indications. A single cut off value was chosen for simplicity to maximise clinical utility of the staging system. Multivariable Cox regression analyses were subsequently repeated with replacement of NT-proBNP as a continuous variable and loop diuretic dosage by NAC ATTR Stage Ia and Stage Ib as categorical variables. The proportional hazards assumption was checked and satisfied.

Cox regression analyses on the UK cohort alone could not be performed due to there being no deaths in Stage Ia patients from this subgroup; this prevented the creation of a multivariable Cox regression model on this group with subsequent external validation using the Amyloidosis Mondor Network cohort. We therefore divided the whole cohort into an 80% training group and 20% validation group to validate our multivariable Cox regression model containing NAC ATTR Stage Ia and Ib using the method reported by Royston et al 2006.[129] We assessed the Cox proportional hazards regression model on the training data and calculated R^2 for survival models, a measure of the explained variation in such models. The performance of R^2 was evaluated using 1000 bootstrap samples on the validation data. The difference in R^2 values between the training group and validation group was 0.047 (95% CI: -0.25 to 0.34) consistent with no real difference. In addition, a type of model calibration was performed by evaluation of R^2, allowing regression on the predicted index in the validation sample. The model was developed on the training sample and calibrated on the index in the validation sample using 1000 bootstrap samples. The calibration slope was 0.85 suggesting the model is adequately calibrated. The validation analysis was performed exclusively by statistics expert Dr Aviva Petrie.

Patient characteristics at diagnosis stratified by NAC ATTR Stages Ia and Ib were compared by Kruskal Wallis test (numerical variables) and Chi-squared test (categorical variables). Patients were also stratified by Perugini grading of cardiac uptake on bone scintigraphy. Patient characteristics of those with NAC ATTR Stage Ia disease were subsequently stratified by presenting symptom into cardiovascular, defined as symptoms of cardiac failure, symptomatic arrhythmia, or cerebrovascular accident, and non-cardiovascular presentations. Kaplan-Meier

survival analyses were performed to estimate median survival and illustrate survival percentages stratified by NAC ATTR Stage. Further KM survival analyses were performed on patients with NAC ATTR Stage Ia stratified by presenting symptom; survival is reported at 80 months of follow up in these analyses as 100 months of follow up was not reached in the cardiovascular presentation group. Log rank tests were performed to compare patient survival between subgroups.

United Kingdom general population survival data was obtained from the Office of National Statistics. Each study patient was assigned an expected survival equivalent to the mean survival for a person of the same age and gender, from the year of diagnosis. These UK individuals were deemed to have died at their expected survival time if that time was less than 100 months. Any UK individual whose expected survival time was equal to or greater than 100 months was taken as censored at 100 months. A KM survival curve was created for the expected survival times and this was superimposed on the KM survival curves. UK general population control groups were matched to the NAC ATTR Stage Ia group and the NAC ATTR Stage Ib separately to provide group specific matching given the significant difference in patient age between Stage Ia and Stage Ib. Survival comparisons with the matched UK population controls were restricted to the UK patient cohort due to differences in population life expectancy between France and the United Kingdom.

Data is presented as median (IQR) or number (percentage) unless otherwise stated. A p value of < 0.05 was deemed significant.

Results

Patient characteristics

There were 879 patients with NAC Stage I ATTR-CM at diagnosis included in the analyses (623 from UK and 256 from France); 109 (12%) were Stage Ia and 770 (88%) were Stage Ib. In the UK cohort, 74 (12%) were Stage Ia and 549 (88%) were Stage Ib. Baseline characteristics of all patients are shown in Table 5.1 along with a comparison between those with Stage Ia and Ib disease. NAC ATTR Stage Ia patients were more commonly ATTRwt, and had better biochemical, functional, and echocardiographic parameters of disease compared to Stage Ib patients. A greater proportion of NAC ATTR Stage Ib patients had Perugini grade ≥ 2 radionuclide scans compared to patients with Stage Ia disease (99% vs 90%); both Stages were associated with thickening of the ventricular walls (median IVSd 16 mm [IQR 14-17] and 17 mm [IQR 15-18] for NAC ATTR Stage Ia and Ib respectively). Prevalence of atrial fibrillation, a permanent pacemaker and carpal tunnel syndrome appeared to be high across the whole cohort when compared to those reported in age-matched control populations from a variety of published sources.[130-135]

Patient characteristics stratified by grade of cardiac uptake on radionuclide scintigraphy are shown in Table 5.2. Patients with Perugini grade ≥2 cardiac uptake had worse clinical, biochemical, functional and echocardiographic parameters of cardiac disease when compared to those with Perugini grade 1 cardiac uptake.

Table 5.1: Patient and disease-related characteristics at diagnosis in 879 patients with NAC Stage I ATTR-CM

		All patients n=879	**Stage Ia** n=109	**Stage Ib** n=770	**p-value** Ia vs Ib
Age at diagnosis (years)		77 (71-82)	75 (71-80)	77 (71-80)	0.032
Amyloid type	ATTRwt, n (%)	698 (79)	99 (91)	599 (78)	0.002
	ATTRv, n (%)	181 (21)	10 (9)	171 (22)	
Year of diagnosis	2018-2020	420 (48)	56 (51)	364 (47)	0.540
	2015-2017	294 (33)	37 (34)	257 (33)	
	2012-2014	131 (15)	13 (12)	118 (15)	
	2009-2011	34 (4)	3 (3)	31 (4)	
Male gender, n (%)		775 (88)	101 (93)	674 (88)	0.121
Caucasian ancestry, n (%)		684 (78)	89 (82)	595 (77)	0.303
ATTR histology, n (%)		359 (41)	46 (42)	313 (41)	0.758
Non-biopsy criteria met, n (%)		752 (86)	83 (76)	669 (87)	0.003
NT-proBNP (ng/L)		1496 (913-2254)	367 (205-480)	1684 (1130-2342)	<0.001
Troponin T (ng/L; n=861)		45 (31-64)	27 (20-38)	48 (34-66)	<0.001
eGFR (mls/min/1.73m^2)		70 (59-81)	76 (66-88)	69 (58-81)	<0.001
CKD stage, n (%)	CKD I	120 (14)	26 (24)	94 (12)	0.001
	CKD II	530 (60)	64 (59)	466 (61)	
	CKD IIIa	229 (26)	19 (17)	210 (27)	
ALP (u/L; n=866)		81 (63-103)	72 (58-91)	82 (65-105)	<0.001
Gamma GT (u/L; n=856))		58 (31-112)	33 (22-62)	63 (34-117)	<0.001
Serum albumin (g/L; n=740)		44 (42-46)	44 (42-47)	44 (42-46)	0.093
IVSd (mm; n=865)		17 (15-18)	16 (14-17)	17 (15-18)	<0.001
LVPW (mm; n=858)		16 (14-18)	15 (13-16)	16 (14-18)	<0.001
LVEF (%; n=854)		51 (45-58)	58 (54-61)	51 (44-57)	<0.001
DPD/HDMP grade, n (%)	1	22 (3)	11 (10)	11 (1)	<0.001
	≥2	857 (97)	98 (90)	759 (99)	
Systolic BP (mmHg; n=871)		128 (118-141)	131 (122-143)	127 (117-140)	0.022
Diastolic BP (mmHg; n=871)		75 (69-82)	76 (70-84)	75 (69-81)	0.222
6MWT distance (m; n=636)		360 (255-444)	430 (345-506)	354 (240-436)	<0.001

NYHA score, n (%; n=870)	I	126 (15)		40 (38)	86 (11)	<0.001
	II	611 (70)		58 (55)	553 (72)	
	≥III	133 (15)		8 (8)	125 (16)	
Loop diuretic, n (%)		536 (61)		32 (29)	504 (66)	<0.001
Thiazide diuretic, n (%)		52 (6)		9 (8)	43 (6)	0.270
MRA, n (%)		142 (16)		8 (7)	134 (17)	0.008
Digoxin, n (%)		32 (4)		5 (5)	27 (4)	0.577
ACE inhibitor, n (%)		311 (35)		30 (28)	281 (37)	0.067
ARB, n (%)		166 (19)		19 (17)	147 (19)	0.674
Beta blocker, n (%)		304 (35)		18 (17)	286 (37)	<0.001
		All	**Controls**	**Stage Ia**	**Stage Ib**	**p-value**
CTS, n (%)	Unilateral	119 (14)	<8.1%[135]	16 (15)	103 (13)	0.710
	Bilateral	423 (48)	<8.1%[135]	66 (61)	357 (46)	0.006
Spinal stenosis, n (%)		114 (13)		20 (18)	94 (12)	0.074
Joint replacement, n (%)		174 (20)		25 (23)	149 (19)	0.379
Atrial Fibrillation, n (%)		271 (31)	8%[130]	33 (31)	238 (32)	0.846
PPM, n (%)		132 (15)	3%[131]	14 (13)	118 (15)	0.497
ICD, n (%)		47 (5)	-	1 (1)	46 (6)	0.028
Hypertension, n (%)		388 (44)	64%[132]	47 (43)	341 (44)	0.810
Ischaemic heart disease, n (%)		136 (16)	16%[133]	10 (9)	126 (16)	0.052
Stroke or TIA, n (%)		96 (11)	9%[133]	9 (8)	87 (11)	0.341
Diabetes mellitus, n (%)		143 (16)	14%[134]	22 (20)	121 (16)	0.237

Values displayed as median (IQR) unless otherwise stated. P-values represents comparison testing between NAC ATTR Stage Ia and Ib by Kruskal Wallis test for numerical variables and Chi-squared test for categorical variables. BP: blood pressure; MRA: mineralocorticoid antagonist; ARB: angiotensin receptor blocker; CTS: carpal tunnel syndrome; PPM: permanent pacemaker; ICD: implantable cardiac defibrillator; TIA: transient ischaemic attack. Controls report the disease prevalence in matched general population groups from a variety of published sources. Number reported where missing data is present within a given variable.

Table 5.2: Baseline characteristics of patients with NAC Stage I ATTR-CM stratified by grade of cardiac uptake by radionuclide scanning

		Grade 1 n=22	**Grade ≥ 2** n=857	**p value**
Age at diagnosis (years)		79 (72-83)	77 (71-82)	0.402
Amyloid type	ATTRwt, n (%)	19 (86)	679 (79)	0.414
	V122I-ATTRv, n (%)	3 (14)	178 (21)	
Male gender, n (%)		19 (86)	756 (88)	0.791
NAC ATTR Stage	Ia	11 (50)	98 (11)	
	Ib	11 (50)	759 (89)	
NT-proBNP (ng/L)		513 (245-1239)	1522 (939-2267)	<0.001
Troponin T (ng/L)		20 (14-35)	46 (32-64)	<0.001
eGFR (mls/min/1.73m^2)		73 (61-88)	70 (59-81)	0.381
Alkaline Phosphatase (u/L)		76 (55-105)	81 (63-103)	0.544
IVSd (mm)		12 (11-12)	17 (15-18)	<0.001
LVPW (mm)		12 (11-13)	16 (14-18)	<0.001
LV ejection fraction (%)		55 (45-60)	51 (45-58)	0.340
Systolic blood pressure (mmHg)		132 (121-148)	128 (117-141)	0.216
6MWT distance (m)		365 (235-460)	360 (256-443)	0.696
NYHA class, n (%)	I	9 (41)	117 (14)	0.001
	II	12 (54)	599 (71)	
	≥III	1 (5)	132 (16)	
Carpal tunnel syndrome, n (%)	Unilateral	3 (14)	116 (14)	0.989
	Bilateral	12 (55)	411 (48)	0.541
Spinal stenosis, n (%)		3 (14)	111 (13)	0.925
Joint replacement, n (%)		2 (9)	172 (20)	0.202
Loop diuretic, n (%)		8 (36)	528 (62)	0.017
Thiazide diuretic, n (%)		1 (5)	51 (6)	0.782
Mineralocorticoid antagonist, n (%)		1 (5)	141 (17)	0.134
Digoxin, n (%)		1 (5)	31 (4)	0.820
ACE inhibitor, n (%)		3 (14)	308 (36)	0.031
Angiotensin receptor blocker, n (%)		2 (9)	164 (19)	0.234
Beta blocker, n (%)		4 (18)	300 (35)	0.101
Atrial Fibrillation, n (%)		7 (32)	264 (31)	0.963

PPM, n (%)	2 (9)	130 (15)	0.431
ICD, n (%)	1 (5)	46 (5)	0.866
Hypertension, n (%)	7 (32)	381 (45)	0.237
Ischaemic heart disease, n (%)	3 (14)	133 (16)	0.809
Stroke or TIA, n (%)	3 (14)	93 (11)	0.679
Diabetes mellitus, n (%)	5 (23)	138 (16)	0.406

Values displayed as median (IQR) unless otherwise stated. P-values represent comparison testing between patients with a Perugini grade 1 cardiac uptake versus those with Perugini grade ≥2 cardiac uptake at diagnosis.

Patient Survival

At a median follow up of 21 (IQR 8-40) months, 120 (14%) patients had died, and the overall median estimated survival was 87 (95% CI: 63 - not met) months.

Univariable Cox regression analyses investigating the association between mortality and a range of patient and disease-related variables for the whole cohort are shown in Table 5.3. There was a significant association between mortality and *TTR* genotype, NT-proBNP, troponin T, serum albumin, IVSd, NYHA class, diabetes mellitus, loop diuretic dose, mineralocorticoid requirement, LVEF and systolic blood pressure. Multivariable analyses identified higher NT-proBNP (Table 5.4; Harrell's c-statistic 0.89) or NAC ATTR Stage Ib (Table 5.5: Categorical variable vs Stage Ia; Harrell's c-statistic=0.75), ATTRv, and higher troponin T at diagnosis as independent predictors of mortality. NT-proBNP at diagnosis was the most powerful independent predictor of mortality (HR 4.36 [95% CI 1.69-11.30]; p=0.002, Table 5.4)

Median estimated survival by KM analysis among patients with NAC ATTR Stage Ia disease was not met at 100 months, and was 75 months (95% CI: 57-93) among those with Stage Ib disease (p<0.001, Figure 1A). When limited to the UK cohort, the median estimated survival of patients with NAC ATTR Stage Ia disease was not met at 100 months, and there was no evidence of a difference in survival between UK NAC Stage Ia patients and matched UK general population controls (p=0.297; Figure 1B). The median estimated survival of UK patients with NAC ATTR Stage Ib disease was 85 (95% CI: undefined) months which was significantly reduced when compared to matched UK general population controls (p<0.0001; Figure 1C)

Table 5.3: Univariable Cox regression analyses of mortality indicators at diagnosis in NAC Stage I ATTR-CM

		Univariable		
		HR	95% CI	p-value
NAC ATTR Stage	Ia	1		
	Ib	8.813	2.18-35.66	**0.002**
Age (per 10 years)		1.208	0.92-1.58	0.168
Male gender		0.890	0.51-1.55	0.681
V122I-ATTRv		2.728	1.87-3.96	**<0.001**
Meets non-biopsy criteria		1.673	0.92-3.04	0.091
NT-proBNP (per 100 ng/L)		1.053	1.03-1.08	**<0.001**
Troponin T (per 100 ng/L)		3.285	2.46-4.39	<**0.001**
eGFR (mls/min/1.73m^2)		0.996	0.98-1.01	0.502
Serum albumin (g/L)		0.925	0.88-0.98	**0.005**
IVSd (mm)		1.118	1.03-1.21	**0.005**
LVEF (%)		0.970	0.96-0.98	**<0.001**
Systolic BP (mmHg)		0.991	0.98-1.00	**0.012**
Diastolic BP (mmHg)		0.997	0.98-1.01	0.643
NYHA class	I	1.00		
	II	2.535	1.28-5.04	**0.008**
	III	2.630	1.21-5.72	**0.015**
Atrial fibrillation		1.127	0.78-1.63	0.528
Permanent pacemaker		1.455	0.80-2.65	0.219
Hypertension		1.016	0.70-1.47	0.934
Myocardial infarction		1.174	0.43-3.18	0.753
Stroke/TIA		0.931	0.52-1.66	0.809
Diabetes Mellitus		1.697	1.07-2.70	**0.026**
Loop diuretic dose		1.567	1.32-1.87	**<0.001**
Thiazide diuretic		1.135	0.53-2.44	0.745
Mineralocorticoid		1.819	1.21-2.73	**0.004**
Digoxin		1.615	0.84-3.09	0.147
Beta blockade		0.915	0.64-1.32	0.631
Lipid therapy		0.848	0.59-1.22	0.369

TIA: transient ischaemic attack.

Table 5.4: Multivariable Cox regression analyses of mortality indicators at diagnosis in NAC Stage I ATTR-CM

		Multivariable		
		HR	95% CI	p-value
Age (per 10 years)		1.19	0.88-1.59	0.260
Male gender		1.28	0.68-2.39	0.445
V122I-ATTRv amyloid type		2.10	1.33-3.30	**0.001**
Log serum NT-proBNP (ng/L)		4.36	1.69-11.30	**0.002**
Log serum troponin T (ng/L)		4.32	1.56-11.99	**0.005**
Loop diuretic dose (mg/kg)		1.24	0.97-1.58	0.089
NYHA class	I	1		
	II	1.90	0.87-4.16	0.107
	III	1.78	0.75-4.25	0.192

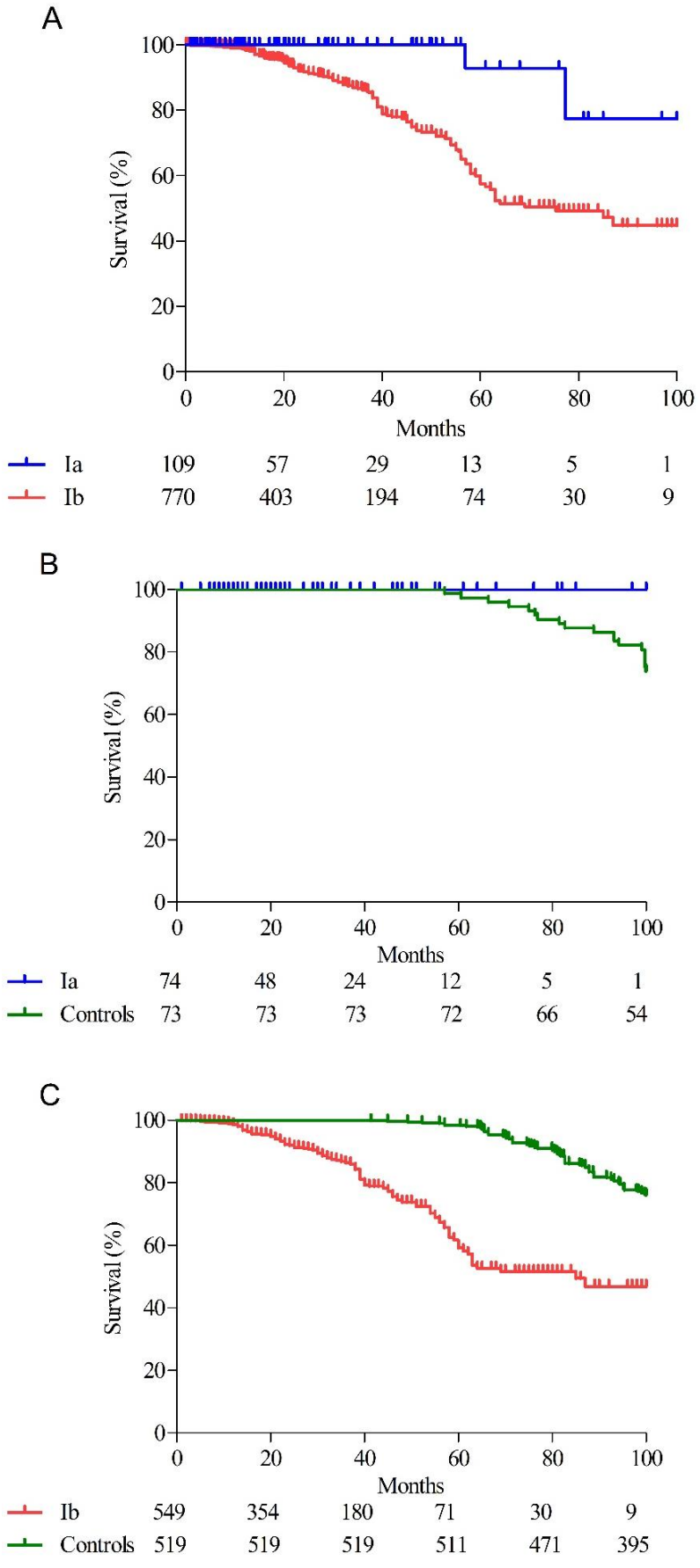
Serum NT-proBNP and serum troponin T have undergone log transformation due to variable skew. Harrell's c-statistic 0.89.

Table 5.5: Multivariable Cox regression analyses of mortality indicators at diagnosis in NAC Stage I ATTR-CM

		Multivariable		
		HR	95% CI	p-value
NAC ATTR Stage	Ia	1		
	Ib	5.06	1.23-20.87	**0.025**
Age (per 10 years)		1.14	0.85-1.52	0.374
Male gender		1.28	0.70-2.37	0.424
V122I-ATTRv amyloid type		2.33	1.54-3.53	**<0.001**
Troponin T (per 100 ng/L)		2.76	1.92-3.97	**<0.001**
NYHA class	I	1		
	II	1.96	0.90-4.29	0.091
	III	1.78	0.75-4.27	0.194

Harrell's C statistic 0.75.

Figure 5.1: Kaplan-Meier survival curves stratified by NAC ATTR Stage



A) Kaplan-Meier survival curves stratified by NAC ATTR Stage for the whole cohort. Median estimated survival among patients with Stage Ia was not met at 100 months and was 75 months (57-93) in patients with Stage Ib (p=0.0002); B) Survival of the UK NAC cohort with Stage Ia compared with matched UK general population controls. Median estimated survival was not met in either group at 100 months; there was no evidence of a difference in survival between the two groups (p=0.297); C) Survival of the UK NAC cohort with Stage Ib compared with matched UK general population controls. Median estimated survival among patients with Stage Ib was 85 months (95% CI: undefined) and was not met at 100 months in a matched UK general population control group; patient survival was reduced in the Stage Ib group compared to UK general population controls (p<0.0001).

Cardiovascular and non-cardiovascular morbidity in Stage Ia ATTR amyloidosis

Among the 109 patients from the whole cohort who were NAC ATTR Stage Ia at diagnosis, followed for a median of 28 months (range: 8-46), regular diuretic use increased from 39% of patients at diagnosis to 56% of patients, prevalence of atrial fibrillation increased from 31% at diagnosis to 40% of patients, NYHA class ≥ 2 heart failure increased from 62% to 82% of patients, stroke or TIA from 8% to 15% of patients, and PPM implants from 13% to 20% of patients. Of the 6 permanent pacemakers implanted after diagnosis of ATTR-CM, 3 were for complete heart block and the remainder for non-specified bradyarrhythmia. Median NT-proBNP increase during follow up was 145 ng/L/year (IQR: 47-440) and median eGFR reduction was 2.8 mls/min/1.73m^2/year (IQR: 0-7). 21 of 80 (26%) patients with follow up biomarkers went from Stage Ia to Stage Ib ATTR-CM and 14 (18%) developed NAC ATTR Stage ≥ II disease during the follow up period.

Among 63 patients with NAC ATTR Stage Ia disease who had a primary cardiovascular presentation, cardiac biomarkers, cardiac imaging and functional markers of cardiac disease were significantly worse than in the 46 patients with a primary non-cardiovascular presentation (Table 5.6). Similarly, a significantly higher proportion of patients with a primary cardiovascular presentation required diuretic therapy, had atrial fibrillation and met the non-biopsy diagnostic criteria for ATTR-CM. The commonest cardiovascular presentations were cardiac failure (60%) and atrial arrhythmias (25%) (Table 5.6).

Table 5.6: Baseline characteristics of 109 patients diagnosed with NAC ATTR Stage Ia disease stratified by clinical presentation

		Cardiovascular Presentation		
		No	Yes	
		n=46	n=63	p value
Age (years)		75 (70-80)	75 (71-80)	0.988
Male gender, n (%)		41 (89)	60 (95)	0.227
Caucasian ancestry, n (%)		35 (76)	54 (86)	0.200
Amyloid type	ATTRwt, n (%)	39 (85)	60 (95)	0.062
	V122I-ATTRv, n (%)	7 (15)	3 (5)	
ATTR histology, n (%)		24 (52)	22 (35)	0.072
Meets non-biopsy criteria, n (%)		30 (65)	53 (84)	0.022
NT-proBNP (ng/L)		263 (155-456)	403 (288-668)	0.001
Troponin T (ng/L)		21 (17-30)	32 (24-48)	<0.001
eGFR (mls/min/1.73m^2)		76 (71-90)	74 (64-88)	0.237
IVSd (mm)		15 (12-17)	16 (15-17)	0.024
DPD/HDMP	1	11 (24)	0 (0)	<0.001
grade, n (%)	≥2	35 (76)	63 (100)	
NYHA score,	I	25 (57)	15 (24)	0.003
n (%)	II	16 (36)	42 (68)	
	≥III	3 (7)	5 (8)	
Atrial fibrillation, n (%)		6 (13)	27 (43)	0.001
Hypertension, n (%)		19 (41)	28 (44)	0.744
Diabetes Mellitus, n (%)		9 (20)	13 (21)	0.891
Pacemaker, n (%)		6 (13)	8 (13)	0.958
Stroke or TIA, n (%)		3 (7)	6 (10)	0.574
Diuretic requirement, n (%)		10 (22)	33 (52)	0.001
Presentation				
Cardiac failure, n (%)			38 (60)	
Atrial fibrillation/flutter, n (%)			16 (25)	
Complete heart block, n (%)			1 (2)	
Stroke, n (%)			3 (5)	
Chest pain, n (%)			3 (5)	

Aortic stenosis, n (%)			1 (2)
Syncope			1 (2)
Asymptomatic ECG abnormality, n (%)		5 (11)	
Asymptomatic cardiac imaging abnormality, n (%)		22 (48)	
Histology	Carpal Tunnel, n (%)	5 (11)	
	Gastrointestinal, n (%)	6 (13)	
	Bladder, n (%)	7 (15)	
	Prostate, n (%)	1 (2)	

Values displayed as median (IQR) unless stated otherwise. P-values represent comparison testing between NAC ATTR Stage Ia patients with a symptomatic cardiovascular clinical presentation versus those with a non-cardiovascular clinical presentation at diagnosis.

Non-cardiovascular clinical presentations included urological symptoms accompanied by histological evidence of bladder or prostatic ATTR amyloid, carpal tunnel syndrome with ATTR amyloid deposits in the flexor retinaculum or tenosynovium, and incidental discovery of ATTR amyloid deposits in the gastrointestinal tract. Biopsy sites in which ATTR amyloid deposits were identified in patients with a non-cardiovascular primary clinical presentation are shown in Table 5.6. Perugini grade ≥2 cardiac uptake was identified by imaging (usually in the context of screening for it) in 76% of patients with a primary non-cardiovascular presentation (Table 5.6). Follow up data was available for 43 of 46 Stage Ia patients with a non-cardiovascular primary clinical presentation and after a median follow up of 19 months (IQR: 8-50), 12 (28%) patients had atrial fibrillation, 8 (19%) had a PPM, 6 (14%) had a history of stroke or TIA, 29 (69%) had NYHA class ≥ 2 heart failure symptoms, and 16 (37%) required diuretic therapy; median NT-proBNP increase was 109 ng/L/year (IQR: 23-276) and 9 of 32 (28%) patients with follow up biomarkers had progressed to NAC ATTR Stage ≥ Ib. Median estimated survival of patients with NAC ATTR Stage Ia was not met at 80 months in both patients with and without a cardiovascular presentation (p=0.837).

Discussion

Increased awareness of ATTR-CM among cardiologists and improved diagnostic techniques are leading to a reduction in diagnostic delays with a consequent increase in the proportion of patients diagnosed with early-stage disease, defined as NAC ATTR Stage I.[2,26,31,123,124] This trend has been highlighted by the findings from Part 1 of the ATTRibute-CM (acoramidis) study in which the 6 minute walk test distance among patients within the placebo arm declined by < 10 metres in the first

year compared to >50 metres over the same time period among patients on placebo within the older ATTR-ACT trial (tafamidis). This study outlines for the first time, the clinical features and natural history of early-stage ATTR-CM in a large cohort of patients with predominant cardiomyopathic *TTR* genotypes followed in two large European Amyloidosis Centres.

The most important independent predictor of mortality in this cohort was NT-proBNP concentration at diagnosis. A diagnostic NT-proBNP concentration of ≤500 ng/L (or ≤1000 ng/L in the context of atrial fibrillation) coupled with a loop diuretic equivalent dose requirement of <0.75mg/kg, defined here as NAC ATTR Stage Ia, was present in only 109 (12%) of patients. Despite Perugini grade ≥2 radionuclide scintigraphy in 90% of such patients, only 58% of Stage Ia patients had a primary cardiovascular clinical presentation. A comparison between patients diagnosed with Stage Ia and Ib disease showed a significantly higher proportion of ATTRwt, lower troponin T, higher eGFR, lower ALP, less thickening of LV walls and better LVEF on echocardiography, and a better functional status in the former group. Median survival was > 100 months from diagnosis in Stage Ia patients, and there was no evidence of a difference in survival between when compared to matched UK general population controls.

The most common non-cardiac sites in which ATTR amyloid deposits were identified were flexor retinaculum, gastrointestinal tract and bladder; flexor retinaculum biopsies were obtained at carpal tunnel surgery and bladder biopsies were usually performed for haematuria, whereas the finding of ATTR amyloid in the gastrointestinal tract was often ‘incidental’ in association with a second pathology in the relevant organ (e.g., gastritis).

Cardiovascular morbidity at the time of diagnosis was substantial, even in patients with NAC ATTR Stage Ia disease; NYHA class ≥ 2 heart failure symptoms, regular diuretic use, atrial fibrillation, permanent pacemaker implants, and stroke/TIAs were present in 62%, 39%, 31%, 13% and 8% of patients respectively. Furthermore, despite a relatively short median duration of follow up in this patient sub-group, the proportion of patients with these cardiovascular morbidities increased following diagnosis including among patients who did not have a primary cardiovascular clinical presentation. These findings suggest a role for disease-modifying therapy at the time of identification of ATTR amyloid in patients with cardiac uptake by radionuclide imaging, even in patients without a primary cardiovascular presentation or overt heart failure symptoms.[4,91,102] It remains to be determined however, whether therapeutic intervention at this early disease stage will reduce subsequent cardiovascular morbidity.

Study limitations include the smaller size of the ATTRv-CM population compared to ATTRwt-CM population although this probably reflects true disease prevalence in the respective countries. Other limitations include the relatively short median duration of follow up, the absence of hospitalization data, the use of internal validation rather than external validation, the absence of data on cause of death, the use of expected survival rather than actual survival for general population analyses, the restriction of general population analyses to the UK cohort, and the potential bias introduced by the fact that patients were followed in large specialist amyloidosis centres rather than across general cardiology as well as other specialty clinics.

In conclusion, the short and mid-term prognosis of patients diagnosed with Stage Ia ATTR-CM, defined here as an eGFR ≥45ml/min, NT-proBNP ≤500ng/L (or ≤1000ng/L in the context of atrial fibrillation) and a loop diuretic equivalent dose

requirement of <0.75mg/kg, appears to be good and overall prognosis in the absence of disease-modifying therapy may be comparable to the age and gender-matched general population. However, cardiovascular morbidity is high in patients diagnosed with Stage Ia ATTR-CM and increases further during patient follow up. Whether early therapeutic intervention in patients with Stage Ia ATTR-CM will reduce cardiovascular morbidity and prolong survival remains to be determined.

Chapter 6: Clinical features and outcomes of hereditary transthyretin amyloidosis associated with the p.Ser97Tyr TTR gene variant

Introduction

Hereditary transthyretin amyloidosis is a progressive multi-system disorder resulting from a pathogenic TTR gene variant producing amyloidogenic TTR protein which deposits as ATTR amyloid typically in the soft tissues, myocardium and nervous system. Inheritance occurs in an autosomal dominant pattern and over 140 TTR mutations are known to date.[136] The clinical phenotype is largely dictated by the specific TTR variant, for example: a valine to isoleucine substitution at position 122 (V122I variant) of the mature protein typically presents with cardiomyopathy; while the valine for methionine substitution at position 30 (Val30Met variant) typically presents with peripheral neuropathy or mixed neuropathy-cardiomyopathy, depending on age at presentation.[13]

Studies containing small numbers of patients with ATTRv-CM carrying the tyrosine substitution of serine at position 77 (p.Ser97Tyr) in exon 3 of the TTR gene suggest this variant may be associated with less than expected cardiac uptake by radionuclide scintigraphy.[137] Validated non-biopsy diagnostic criteria for ATTR-CM frequently allow diagnosis without histology; these criteria include Perugini grade ≥2 cardiac uptake on radionuclide scintigraphy.[2] I report the presentation and clinical course of thirty patients with the p.Ser97Tyr TTR gene variant.

Methods

Patients

We performed a retrospective analysis of all patients confirmed to carry the p.Ser97Tyr TTR gene variant at the UK NAC between January 1988 and September 2021. All patients referred to the NAC with suspected or confirmed ATTR amyloidosis undergo TTR genotyping. All patients confirmed to carry to p.Ser97Tyr TTR gene variant were invited to the NAC for diagnostic assessment; for those unable to attend, clinical details were extracted from correspondence with the referring teams. Patients were reviewed 6 to 12 monthly as clinically indicated. At each review, patients underwent clinical history, examination, electrocardiogram, urinary dipstick, functional testing, blood and urine biochemical analyses and cardiac imaging as indicated.

Statistical analyses

Date of diagnosis is defined as the date of amyloid confirmation by histology or the date of TTR genotyping. Follow up is defined as time from diagnosis to censor or death. For the purposes of natural history survival analyses patients were censored at the first of the following time points: the 17th of November 2021, death, clinical trial enrolment or disease modifying therapy initiation. Kaplan-Meier survival analyses were performed to estimate the median survival in the absence of disease modifying therapy. Data was also collected following initiation of disease modifying therapy or clinical trial enrolment for descriptive purposes.

Results

Patients

Thirty patients with the p.Ser97Tyr TTR variant were identified representing approximately 3% of all patients with hereditary ATTR amyloidosis and <1% of all patients with ATTR-CM amyloidosis seen at the NAC; twenty-seven patients attended the NAC for full evaluation. At the time of genotyping, twenty-five had symptoms consistent with ATTRv amyloidosis and five were asymptomatic relatives of ATTRv patients, two of whom developed evidence of ATTRv-CM during follow up. Patients were from 23 different families. Twenty-four patients were British, three Western/Northern European, two from New Zealand and one Australia.

DNA analysis

Transthyretin genotyping confirmed that all 30 patients were heterozygous for the tyrosine substitution of serine at position 77 in exon 3 of the TTR gene (p.Ser97Tyr); the remainder of the TTR sequence was normal in all cases.

Diagnosis of amyloidosis

Of the 27 patients developing ATTRv amyloidosis, amyloid deposition was confirmed histologically in 13 (48%). The amyloid type was confirmed as ATTR in 11 patients while the amyloid was too scanty to type in the remaining two. Histological sampling was myocardial in three patients, fat in four, bladder in two, gastrointestinal in one, and peripheral nerve in two; extensive ATTR amyloid deposition was confirmed in one patient at postmortem and was present in most organs.

Of the patients without histological evidence of amyloid deposition: 9 presented with peripheral neuropathy consistent with amyloid neuropathy and 3 with heart failure and characteristic cardiac imaging features of cardiac amyloidosis; two were asymptomatic carriers at the time of genotyping but developed evidence of cardiac amyloidosis during follow up (confirmed by both Tc-DPD and CMR imaging).

Presentation

Of the 27 patients who developed ATTRv amyloidosis, the median age at diagnosis was 64 (58-69) years. The presenting symptom was peripheral neuropathy in 13 (48%), heart failure symptoms in 8 (30%), autonomic neuropathy in two (7%), haematuria in one (4%) and gastro-oesophageal reflux in one (4%); two (7%) patients were mutation carriers without amyloidosis at the time of genotyping and developed imaging evidence of cardiac amyloidosis during follow up. The median delay from symptoms onset to diagnosis for the whole cohort was 18 (9-33) months. Patient characteristics at diagnosis are displayed in Table 6.1.

Table 6.1: Patient characteristics at diagnosis of ATTRv amyloidosis

			ATTRv-S77Y n=27
Age (years)			64 (58-69)
Male, n (%)			20 (74%)
Ethnicity, n (%):	White British		21 (78)
	White European		3 (11)
	Non-European		3 (11)
Presentation, n (%):	Peripheral neuropathy		13 (48)
	Autonomic neuropathy		2 (7)
	Heart failure		8 (30)
	Other		4 (15)
Suggestive family history, n (%)			13 (48%)
First symptom to diagnosis (months)			18 (7-36)
Organ involvement at diagnosis, n (%):			
Cardiac			25 (93%)
Peripheral neuropathy			19 (70%)
Autonomic neuropathy			11 (44%)
NYHA, n (%)		I	8 (31)
		II	14 (54)
		III	4 (15)
PND score, n (%):		0	8 (32)
		I	11 (44)
		II	4 (16)
		IIIa	1 (4)
		IIIb	1 (4)
NT-proBNP (ng/L)			1552 (600-3592)
Troponin T (ng/L)			43 (30-87)
Albumin (g/L)			44 (42-46)
eGFR (mL/min/1.73m^2)			81 (67-90)
IVSd (mm)			16 (14-20)
LVEF (%)			49 (40-58)
Global longitudinal strain (%)			-12% (-7.9% to -16%)
Cardiac uptake on Tc-DPD, n (%):		No uptake	1 (5)
		Grade 1	11 (58)
		Grade 2	7 (37)

Supine systolic BP (mmHg)	130 (114-149)
Supine diastolic BP (mmHg)	79 (74-85)
Standing systolic BP (mmHg)	123 (105-145)
Standing diastolic BP (mmHg)	78 (74-85)
History of carpal tunnel syndrome, n (%)	12 (44)
Six minute walk test (m)	385 (321-473)

Cardiac involvement

Despite the fact that only 30% of patients presented with heart failure, either myocardial histology, echocardiography, Tc-DPD scintigraphy or CMR imaging indicated presence of cardiac amyloid in 25/27 (93%) patients with ATTRv amyloidosis; one patient had isolated peripheral neuropathy without evidence of cardiac amyloid throughout a follow up period of 40 months and the other did not attend for cardiac evaluation.

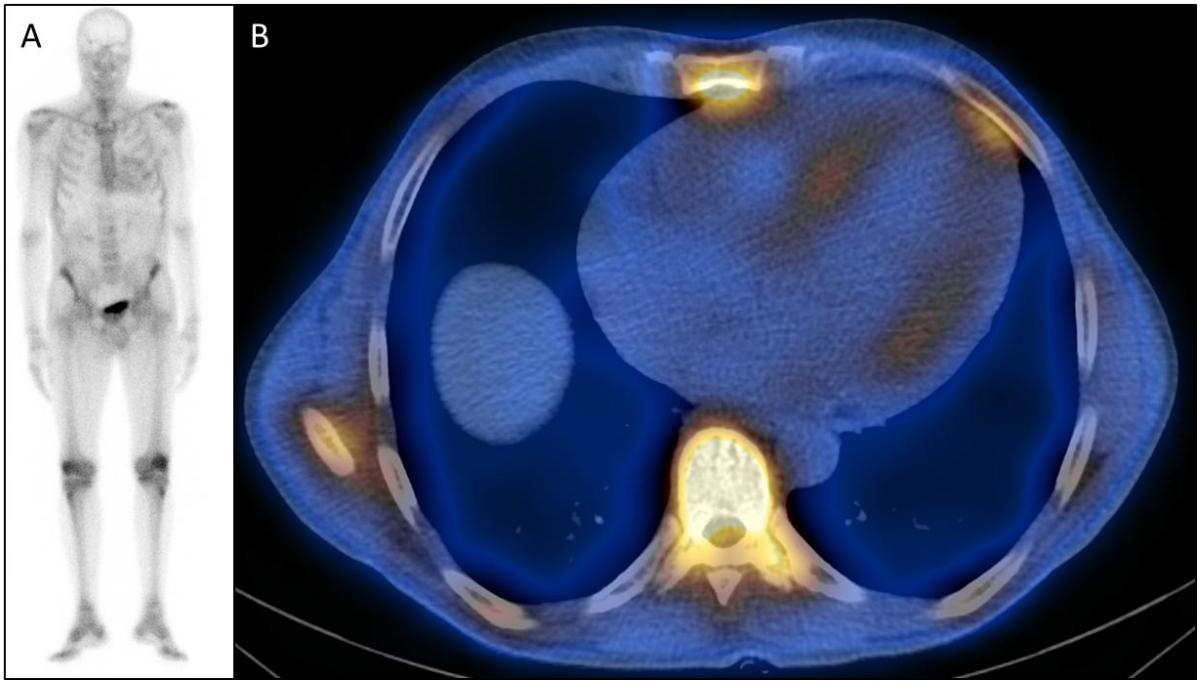
Of the 25 patients with evidence of cardiac amyloid at diagnosis, 20 had an electrocardiogram available for review from the diagnostic evaluation. At the time of diagnosis, 17 were in sinus rhythm, 2 were in atrial fibrillation and one had a permanent pacemaker for complete heart block which was inserted 4 months before diagnosis. Among the 19 patients without a pacemaker at the time of diagnosis, QRS complex voltages were reduced in 4, poor R wave progression was present in 3, and 1st degree heart block was noted in 2 cases. One patient required pacemaker insertion for complete heart block during follow up.

Echocardiography was performed in 22/25 patients and was consistent with cardiac amyloidosis in 20 with a median IVSd of 17mm (15-20). Of the two patients without evidence of cardiac amyloidosis by echocardiography, both had Perugini grade 1 cardiac uptake on Tc-DPD and one underwent CMR imaging which showed

evidence of early cardiac amyloidosis based on an elevated ECV and LGE; the other did not have a CMR.

Nineteen patients underwent Tc-DPD scintigraphy with cardiac uptake absent in 1 (5%), Perugini grade 1 in 11 (58%) and Perugini grade 2 in 7 (37%); no patients had Perugini grade 3 cardiac uptake despite five patients having an IVSd of ≥20mm (Fig 6.1). The patient without cardiac uptake on Tc-DPD scintigraphy had no heart failure symptoms and only minor vascular ATTR deposition identified on myocardial histology. The median IVSd was 16mm (14-18) in patients with Perugini grade 1 cardiac uptake, and 17mm (17-20) in patients with grade 2 cardiac uptake. Full patient characteristics stratified by Perugini grade cardiac uptake are shown in Table 6.2.

Figure 6.1: Tc-DPD scintigraphy with transaxial SPECT-CT imaging



A) Tc-DPD scintigraphy showing Perugini grade 1 cardiac uptake defined as cardiac uptake less intense than bone signal, B) Transaxial SPECT-CT image showing low grade myocardial tracer uptake.

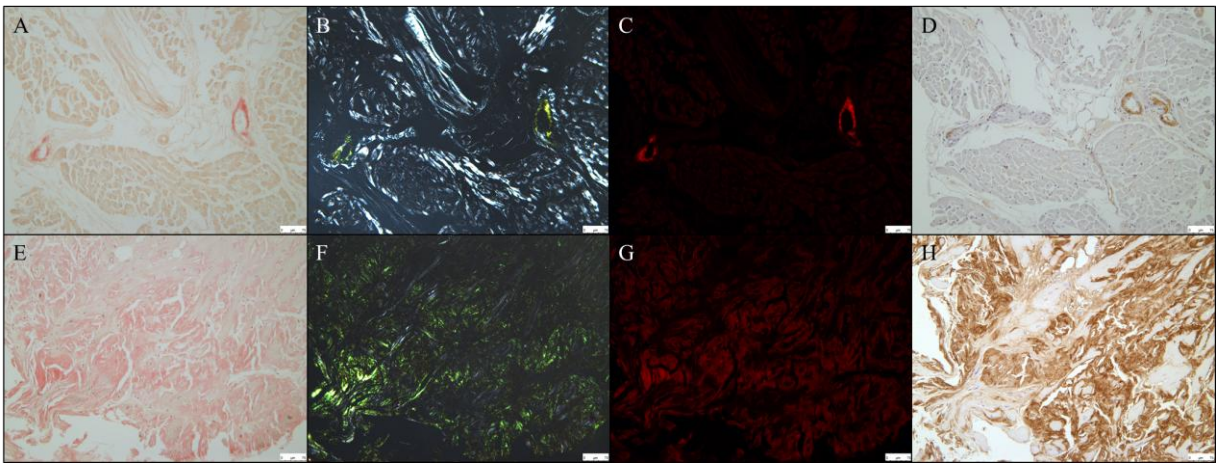
Table 6.2: Patient characteristics stratified by cardiac uptake by radionuclide scintigraphy.

		Perugini Grade on Tc-DPD Scintigraphy		
		No uptake *(n=1)*	Grade 1 *(n=11)*	Grade 2 *(n=7)*
Age (years)		51	68 (63-72)	64 (56-68)
Male gender, n (%)		1	9 (82)	7 (100)
Presentation, n (%):				
Heart Failure		0 (0)	3 (27)	4 (57)
Peripheral neuropathy		1 (100)	4 (36)	2 (29)
Autonomic neuropathy		0 (0)	1 (9)	1 (14)
Other		0 (0)	3 (27)	0 (0)
NT-proBNP (ng/L)		16	846 (220-3592)	1976 (626-5717)
Troponin T (ng/L)		-	32 (30-69)	65 (43-87)
eGFR (mls/min/1.73m^2)		78	80 (75-90)	90 (67-90)
Serum Albumin (g/L)		44	44 (42-46)	43 (42-45)
NYHA	I	1	4 (36)	0 (0)
Class:	II	0	7 (64)	5 (71)
	III	0	0 (0)	2 (29)
Systolic BP (mmHg)		170	133 (115-143)	119 (112-133)
Diastolic BP (mmHg)		80	81 (75-85)	79 (74-85)
IVSd (mm)		11	16 (14-18)	17 (16-20)
LVPW (mm)		12	15 (13-18)	18 (15-19)
LVEF (%)		65	53 (49-55)	39 (33-45)
GLS (%)		-	-12.6 (-11.4 to -14.4)	-6.3 (-5.4 to -7.2)
CMR	T1 (ms)		1054 (1049-1077)	1202 (1200-1205)
(n=0, 5, 2)	T2 (ms)		47.3 (47.0-48.5)	49.3 (47.5-51.0)
	ECV (%)		46 (46-49)	69 (63-76)
	No LGE		0	0 (0)
	Subendocardial LGE		1 (20)	0 (0)
	Transmural LGE		4 (80)	2 (100)

T1: native myocardial T1 signal, T2: native myocardial T2 signal

Three endomyocardial biopsies were performed, all demonstrating ATTR amyloid on immunohistochemistry; two were subsequently confirmed by mass spectrometry. One patient had CMR imaging features of cardiac amyloidosis. Another patient presented with isolated neuropathy and had no symptoms of heart failure, a NT-proBNP of 17ng/L, an echocardiogram not suggestive of cardiac amyloidosis (IVSd 11mm) and no cardiac uptake on Tc-DPD scintigraphy; endomyocardial biopsy was performed during work up for liver transplantation identifying minimal vascular amyloid (Figure 6.2: A-D). The third patient had characteristic amyloid CMR imaging and echocardiography (IVSd of 21mm), Perugini grade 1 cardiac uptake on DPD scintigraphy and an IgM lambda secreting clonal disorder. Cardiac histology confirmed extensive cardiac ATTR amyloid, replacing most of the normal cardiac architecture (Figure 6.2: E to H).

Figure 6.2: Examples of myocardial histology showing ATTR deposition



Myocardial histology from two patients (A to D and E to H): A) Vascular congophilia following congo red staining; B) Apple green birefringence under polarised light; C) Congophilia under fluorescent light; D) Positive immunohistochemistry with transthyretin antibody; E) Myocyte congophilia following congo red staining; F) Apple

green birefringence under polarised light; G) Congophilia under fluorescent light; H) Positive immunohistochemistry with transthyretin antibody.

Neuropathic involvement

At diagnosis, 18 (67%) patients had clinical features of peripheral neuropathy. Eleven (41%) patients had a peripheral nerve disability (PND) score of 1, 4 (15%) were PND 2, 2 (7%) PND 3a, and 1 (4%) PND 4. Symptoms of autonomic neuropathy were present in 11 patients at diagnosis with six patients reporting early satiety, nausea or vomiting, seven altered bowel habits, six weight loss and six postural dizziness. Three patients had a postural systolic blood pressure drop of >20mmHg at diagnosis.

Twenty-two (81%) had a history of carpal tunnel syndrome by the time of diagnosis; two further patients were diagnosed with carpal tunnel syndrome during follow up.

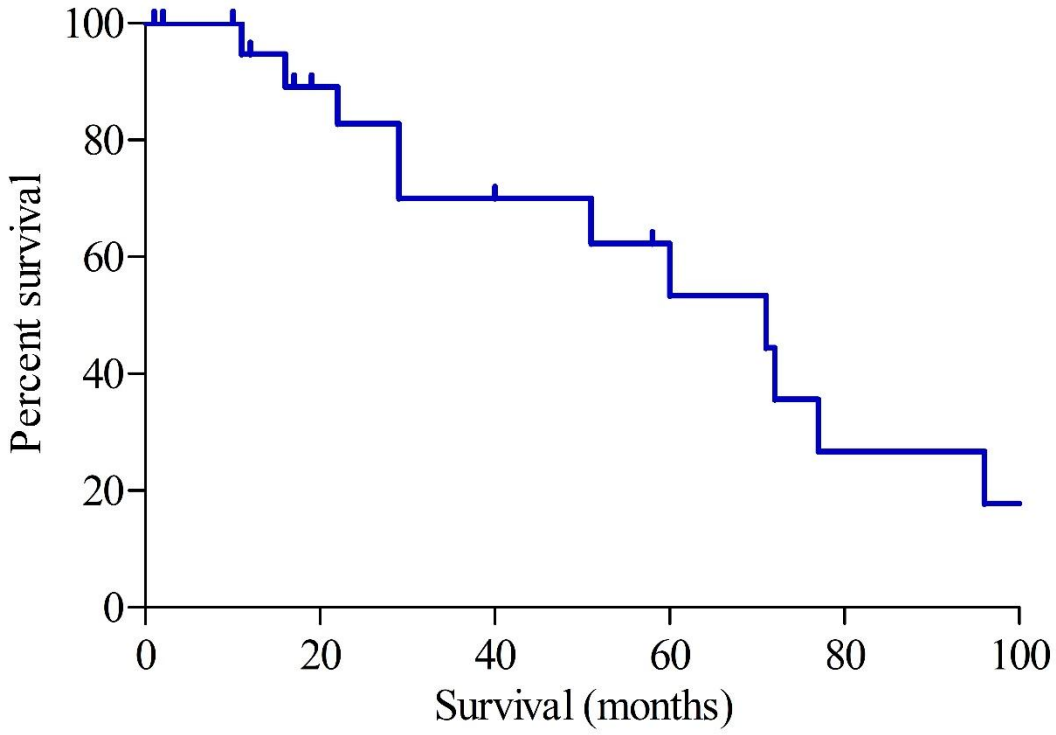
Patient survival and clinical course in the absence of disease-modifying therapy

With censoring at the time of trial enrolment or commencement of disease-modifying therapy, median follow up for the 27 patients was 22 (2-60) months; 12 (44%) patients died and the median expected patient survival was 71 (40-102) months (Fig 6.3). Cause of death was progressive amyloidosis in three patients, sepsis in two, myocardial infarction in one and unknown in six. All patients with established cardiac amyloidosis at diagnosis and subsequent follow up reported worsening heart failure

symptoms; in the 13 patients with follow up NT-proBNP measurements, the median NT-proBNP increase per year was 674 (231-1169) ng/L.

Throughout follow up 8 patients increased their PND score with 5 patients PND 3a, one patient PND 3b and 4 patients PND 4 at censor. Two patients without neuropathy at diagnosis developed PND 1 neuropathy after 12 and 96 months of follow up respectively. The frequency of autonomic symptoms also increased during follow up.

Figure 6.3: Kaplan-Meier survival curve from diagnosis in the absence of disease modifying therapy



Disease modifying therapy

Six patients received diflunisal therapy with three simultaneously receiving patisiran. Two patients received diflunisal monotherapy for 22 and 72 months with both suffering progressive amyloidosis; one patient discontinued diflunisal after a general decline in symptoms attributed to the medication. Of the patients receiving simultaneous diflunisal and patisiran therapy, all three reported improvement of peripheral neuropathy symptoms. Two reported improvements in heart failure symptoms one of whom had stable NT-proBNP values and echocardiographic markers of cardiac amyloidosis whilst the other had a rising NT-proBNP. The third patient had stable heart failure symptoms and markers of cardiac disease. All three were alive at censor having received 22, 36 and 38 months of therapy.

A further six patients received patisiran monotherapy for a median of 17 (11-22) months achieving a median TTR knockdown of 88% (79-93). Follow up review occurred in five of these patients: four patients had peripheral neuropathy at diagnosis and all reported improvement of neuropathy symptoms, four had cardiac amyloidosis at diagnosis which remained symptomatically stable throughout treatment. One patient passed away after 27 months of therapy; they had extensive cardiac amyloidosis on CMR and a left ventricular ejection fraction of 25% at diagnosis; the other four were alive and clinically stable at censor.

Three patients commenced inotersen; one developed progressive neuropathy and cardiomyopathy and was switched to patisiran after 70 months, one developed thrombocytopenia after 12 months and was switched to patisiran, and one patient was clinically stable having been on inotersen for 11 months at censor.

Three patients underwent liver transplantation, one was combined with heart transplantation, and one subsequently required kidney transplantation for non-amyloidosis related kidney disease. Of the patients undergoing isolated liver transplantation, both patients developed progressive cardiac, peripheral and autonomic neuropathy during follow up. The patient receiving combined liver and heart transplantation had stable peripheral and autonomic neuropathy prior to death 20 months later from ischaemic heart disease. All patients undergoing transplantation were treated prior to the availability of TTR stabilisers and RNA silencing therapy.

Transthyretin variant carriers

At genotyping five patients were carriers of the p.Ser97Tyr TTR variant without evidence of ATTRv amyloidosis. The median age at genotyping was 43 years old, and two patients progressed to ATTRv amyloidosis, identified at 55 and 73 years of age. All five patients were evaluated because of a proven family history of the ATTRv amyloidosis. Three patients did not reach the age of onset of symptoms in their affected relative during follow up; none of these patients developed ATTRv amyloidosis. In the patient who did develop ATTR amyloidosis the age of symptom onset in their affected relative was unavailable.

Discussion

We report, to our knowledge, the clinical presentation, natural history and response to treatment of the largest cohort of patients with ATTRv amyloidosis due to the p.Ser97Tyr TTR gene variant. The commonest presentation was with peripheral neuropathy although evidence of cardiac amyloidosis, often asymptomatic, was present in up to 96% at diagnosis. As is common in ATTR amyloidosis, there was a prolonged time from first symptom to diagnosis, with advanced disease and significant morbidity common at diagnosis.[138] In the absence of disease modifying therapy there was predictable progression of symptoms with the majority developing a mixed cardiomyopathy-neuropathic phenotype with notable autonomic symptoms later in the disease course.

Our cohort were mostly of British English ancestry with some exceptions from France, Spain, Australia and New Zealand contrasting the commoner mixed neuropathy-cardiomyopathy ATTRv phenotypes such as T60A which is typically found in patients of Irish ancestry and V30M which is endemic to Portugal, Sweden and Japan. Large healthy genetic control analyses have not reported the p.Ser97Tyr TTR variant. The p.Ser97Tyr TTR variant has been shown to reduce conformational stability of TTR monomers, a key step in the pathophysiology of ATTR amyloidosis.[139-141]

A unique finding in our study population is the finding of Perugini grade 1 cardiac uptake by Tc-DPD scintigraphy despite features of advanced cardiac amyloidosis by other imaging modalities. Eleven patients demonstrated Perugini grade 1 cardiac uptake on Tc-DPD scintigraphy despite their median IVSd by echocardiography being 16mm; five patients underwent CMR imaging all

demonstrating raised ECV, raised native myocardial T1 and abnormal gadolinium kinetics. Transmural LGE, typically a feature of advanced cardiac amyloidosis, was present in four of the five patients.[45] In patients with ATTRwt-CM or V122I-ATTRv-CM, this degree of wall thickness is associated with Perugini grade ≥2 uptake in almost 100% of patients.[112,119] In addition, in a review of 280 ATTR-CM patients with cardiac uptake on Tc-DPD scintigraphy, only 3/17 patients with Perugini grade 1 cardiac uptake had characteristic LGE, all of whom carried the p.Ser97Tyr TTR variant.[137] These findings suggest ATTRv-CM due to the p.Ser97Tyr TTR variant, is associated with a disproportionately low level of cardiac uptake on Tc-DPD scintigraphy to the burden of cardiac amyloidosis, when compared to wild-type and other hereditary forms of ATTR-CM.

The differential diagnosis for low-grade cardiac uptake by DPD scintigraphy and significant cardiac amyloidosis by other imaging modalities includes cardiac AL amyloidosis and apolipoprotein AIV/AI amyloidosis; accurate diagnosis in this context is key to avoid unnecessary chemotherapy and enable access to novel TTR therapies for ATTRv.[142] The mechanism for cardiac uptake in cardiac amyloidosis remains undetermined and the finding of variant dependent degrees of cardiac uptake offers a potential avenue for further investigation.

Response to disease modifying therapy in our small study population was in keeping with that of other ATTRv neuropathy studies.[3,5] Patients on patisiran reported improvements in neuropathic symptoms, although the impact on cardiomyopathy symptoms and disease markers was less clear.[91,92] Disease progression following liver transplantation occurred in 2/3 patients with the third patient passing away 20 months after transplantation.[143,144]

In summary, this study presents a series of 30 patients with p.Ser97Tyr TTR gene variant, 27 of whom had clinical disease typically presenting with a mixed neuropathy and cardiomyopathy phenotype. A unique finding was the low degree of cardiac uptake by Tc-DPD scintigraphy despite substantial cardiac amyloidosis burden. Appreciation of this phenomenon is key to avoiding misdiagnosis and allowing early appropriate treatment.

Chapter 7: Pre-diagnostic healthcare utilization in patients with transthyretin amyloidosis in the United Kingdom

Introduction

Transthyretin amyloidosis is a multisystem life limiting disease resulting from progressive accumulation of amyloid, an extracellular fibrillary material, comprised of misfolded TTR protein.[31,41] Transthyretin amyloidosis may be hereditary due to a pathogenic TTR gene variant, or wild-type, associated with the aging process with normal TTR genotyping.[13] ATTRwt is characterised by cardiomyopathy and is an increasingly recognised cause of heart failure in the elderly.[145] ATTRv is significantly rarer and may present with either an isolated cardiomyopathy, neuropathy or more typically a mixed phenotype.[146] Both ATTRv and ATTRwt are associated with soft tissue disease, typically carpal tunnel syndrome and spinal stenosis, which often precede diagnosis by many years.[122,147] In recent years there has been a dramatic increase in diagnoses of ATTR amyloidosis although the condition is still considered underdiagnosed.[1] Diagnostic delay is often significant with advanced disease at diagnosis common and heralding a poor prognosis.[19,148] Several novel disease modifying therapies, such as the TTR stabiliser tafamidis and ribonucleic acid targeted therapy patisiran, have been licensed for the treatment of ATTR amyloidosis, with many others undergoing phase III clinical trials.[3-5,146,149]

Early diagnosis will be crucial to maximising the benefits of recent therapeutic advances. Several studies have suggested significant healthcare utilization and costs both prior to and following a diagnosis of ATTR amyloidosis although the literature remains sparse with a lack patient specific data.[148,150-152] Our study uses

Hospital Episodes Statistics (HES) data combined with patient specific data to investigate the pre-diagnosis course of patients with ATTR amyloidosis diagnosed at the NAC in the United Kingdom.

Methods

Patients

We performed a retrospective analysis of all patients attending the UK NAC between 1st April 2010 and 31st August 2016 residing in the United Kingdom with a diagnosis of either ATTRwt-CM or ATTRv. ATTRwt-CM was diagnosed in the presence of wild-type TTR genotyping and either validated non-biopsy diagnostic criteria, or the presence of ATTR amyloid on histology from any biopsy site with cardiac imaging evidence of cardiac amyloidosis. ATTRv diagnoses required the identification of a pathogenic TTR gene variant combined with either ATTR amyloid on histology or characteristic clinical features such as peripheral and or autonomic neuropathy; for certain subgroup analyses ATTRv was divided into ATTRv-CM, describing patients with ATTRv and a predominant cardiomyopathy phenotype, and ATTRv-mixed, describing patients with a mixed cardiomyopathy and neuropathy phenotype. Diagnosis date was defined as the date of first assessment at the NAC. Patients were linked to the HES database using their NHS number; hospital attendance data was available from April 2007 onwards while comorbidity data was available from April 2000. The April 2010 patient selection timepoint was chosen to allow a three-year pre-diagnosis period; only linked patients with ≥1 confirmed healthcare utilization episode were included. Data on accident and emergency (A&E) attendances, hospital admissions and outpatient visits were collected in the three years prior to diagnosis; outpatient clinic visits were subsequently divided by clinical

specialty. Diseases suffered within three years of diagnosis were also assessed; including historical and new diagnoses. Data was subsequently collected on cardiac magnetic resonance imaging utility and endomyocardial biopsy due to their role in the diagnosis of ATTR-CM, and stroke and carpal tunnel syndrome due to their known association with ATTR amyloidosis. Patient characteristics at diagnosis in each study group were compared by Kruskal Wallis test (numerical variables) and Chi-squared test (categorical variables). Data is presented as median (IQR) or number (percentage) unless otherwise stated. A p value of < 0.05 was deemed significant. The performance of histological assessment and genetic testing are outlined in Chapter 2. Extraction of HES data via patient NHS number linkage was performed entirely by Rosie McDonald at IQVIA following Confidentiality Advisory Group approval.

Results

Patient characteristics

There were 534 patients included in the analyses: 367 (69%) ATTRwt-CM, 100 (27%) ATTRv-CM and 67 (13%) ATTRv-mixed. Baseline characteristics of all patients are shown in Table 7.1. In the ATTRv-CM group 98% of patients carried the V122I TTR variant, whereas in the ATTRv-mixed group the commonest TTR gene variants were T60A (46%) and V30M (21%). Patients with ATTRv-CM were more likely to be of non-Caucasian ethnicity and had a reduced LVEF, higher NYHA class and reduced 6MWT distance compared to the ATTRwt-CM and ATTRv-mixed groups. The ATTRv-mixed group were significantly younger, had a lower serum NT-proBNP concentration and higher left ventricular ejection fraction than both ATTRwt-CM and ATTRv-CM groups at the time of diagnosis.

Table 7.1: Patient and disease-related characteristics at diagnosis stratified by phenotype

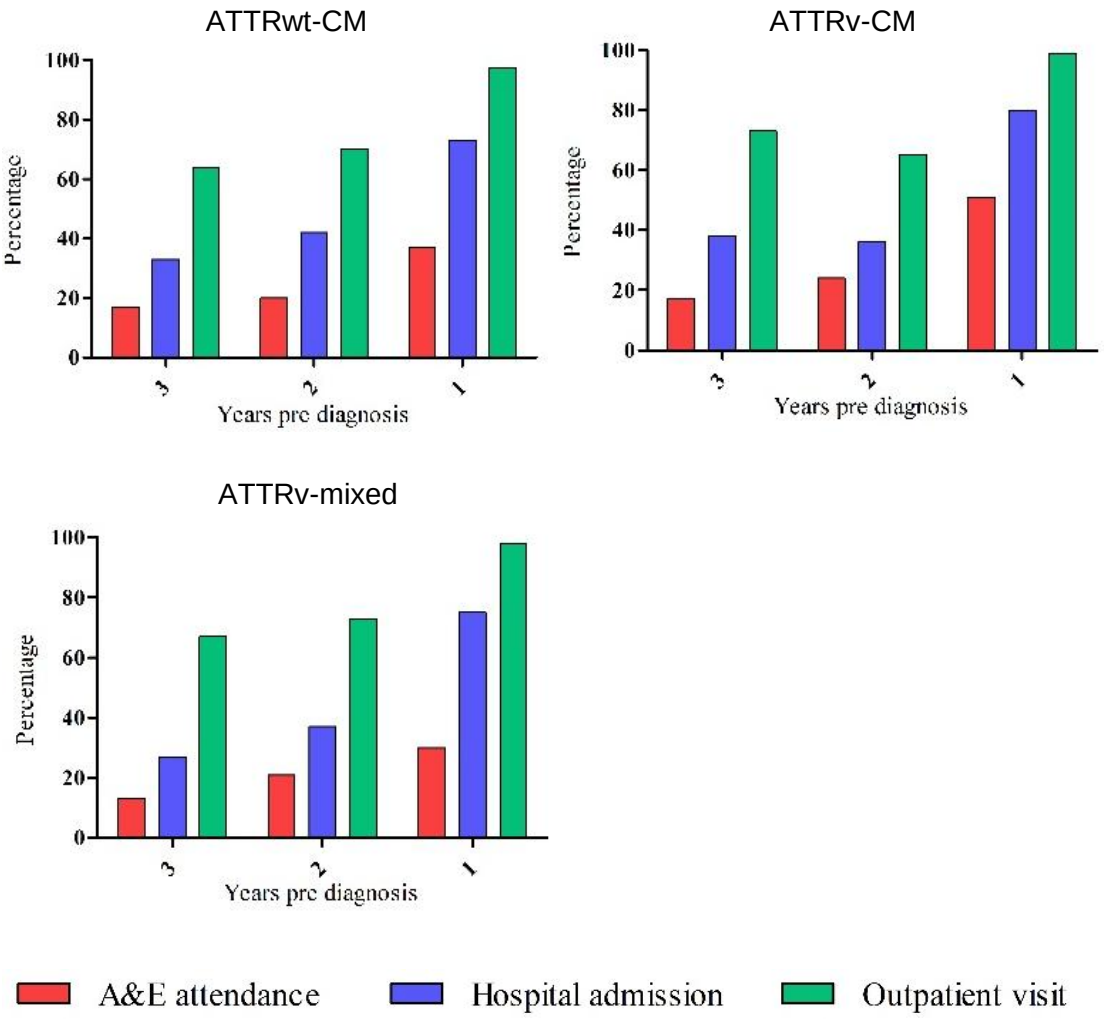
		ATTRwt-CM n=367	**ATTRv-CM** n=100	**ATTRv-mixed** n=67
Age at diagnosis (years)		78 (73-82)	76 (71-80) *	67 (62-73) * ¥
Male gender, n(%)		346 (94)	69 (69) *	50 (75) *
Caucasian ancestry, n(%)		360 (98)	3 (3) *	60 (89) * ¥
Year of diagnosis, n(%)	2009-2011	78 (21)	34 (34)	16 (24)
	2012-2014	197 (54)	48 (48)	34 (51)
	2015-2016	92 (25)	18 (18)	17 (25)
TTR genotype, n(%)	V122I		98 (98) *	0 (0) * ¥
	T60A		0 (0)	31 (46)
	V30M		0 (0)	14 (21)
	Other		2 (2)	22 (33)
NAC ATTR stage, n (%)	1	157 (43)	46 (46)	44 (66) * ¥
	2	153 (42)	36 (36)	17 (25)
	3	57 (16)	18 (18)	6 (9)
eGFR (ml/min/1.73m2)		59 (47-70)	50 (40-59) *	81 (63-90) * ¥
NT-proBNP (ng/L)		3113 (1691-5243)	2901 (1624-5319)	1666 (617-4279) * ¥
Serum albumin (g/L)		44 (42-46)	43 (40-45) *	43 (41-46)
IVS (mm)		17 (15-18)	17 (16-18)	16 (14-19)
LVPW (mm)		16 (15-18)	17 (15-18)	16 (14-19)
LVEF (%)		48 (40-56)	43 (36-51) *	55 (43-60) * ¥
Systolic BP (mmHg)		123 (113-136)	118 (106-135)	123 (108-135)
Diastolic BP (mmHg)		72 (66-80)	73 (66-82)	74 (67-79)
NYHA (class), n(%)	1	53 (14)	9 (9) *	11 (16) ¥
	2	230 (63)	50 (50)	43 (64)
	≥3	84 (22)	41 (41)	13 (19)
6MWT distance (m)		339 (229-406)	236 (82-322) *	367 (230-414) ¥

Values displayed as median (IQR) unless otherwise stated. * Denotes p values <0.05 on comparison testing when compared to the ATTRwt-CM group; ¥ denotes a p value of <0.05 on comparison testing when compared to the ATTRv-CM group.

Healthcare utilization prior to diagnosis

Accident and emergency attendances, hospital admissions and outpatient visits across the three years prior to diagnosis are displayed in Figure 7.1. Overall, A&E attendances increased each year prior to diagnosis with 85 (17%) of patients attending during the 3rd year prior to diagnosis, 101 (21%) attended in the second year prior to diagnosis and 201 (39%) attending A&E within one year of diagnosis; hospital admissions also increased over these three years 172 (33%), 208 (40%) and 386 (74%), as did outpatient visits 343 (66%), 361 (69%), 508 (98%) respectively. On average, ATTRwt-CM patients had 21 healthcare visits within the three years prior to diagnosis increasing annually from 4, to 5, to 12 until diagnosis; 51% attended accident and emergency within three years of diagnosis, at an average of 9.3 months pre diagnosis. ATTRv-CM patients had on average 26 healthcare visits within the three years prior to diagnosis increasing annually from 6, to 6, to 14 and ATTRv-mixed patients had 20 healthcare visits within the three years prior to diagnosis increasing annually from 4, to 5, to 11 until diagnosis. Overall, 55% of patients with ATTRv had an accident and emergency attendance within three years of diagnosis, occurring on average 1.8 months prior to diagnosis.

Figure 7.1: Pre-diagnostic hospital service usage in ATTR amyloidosis

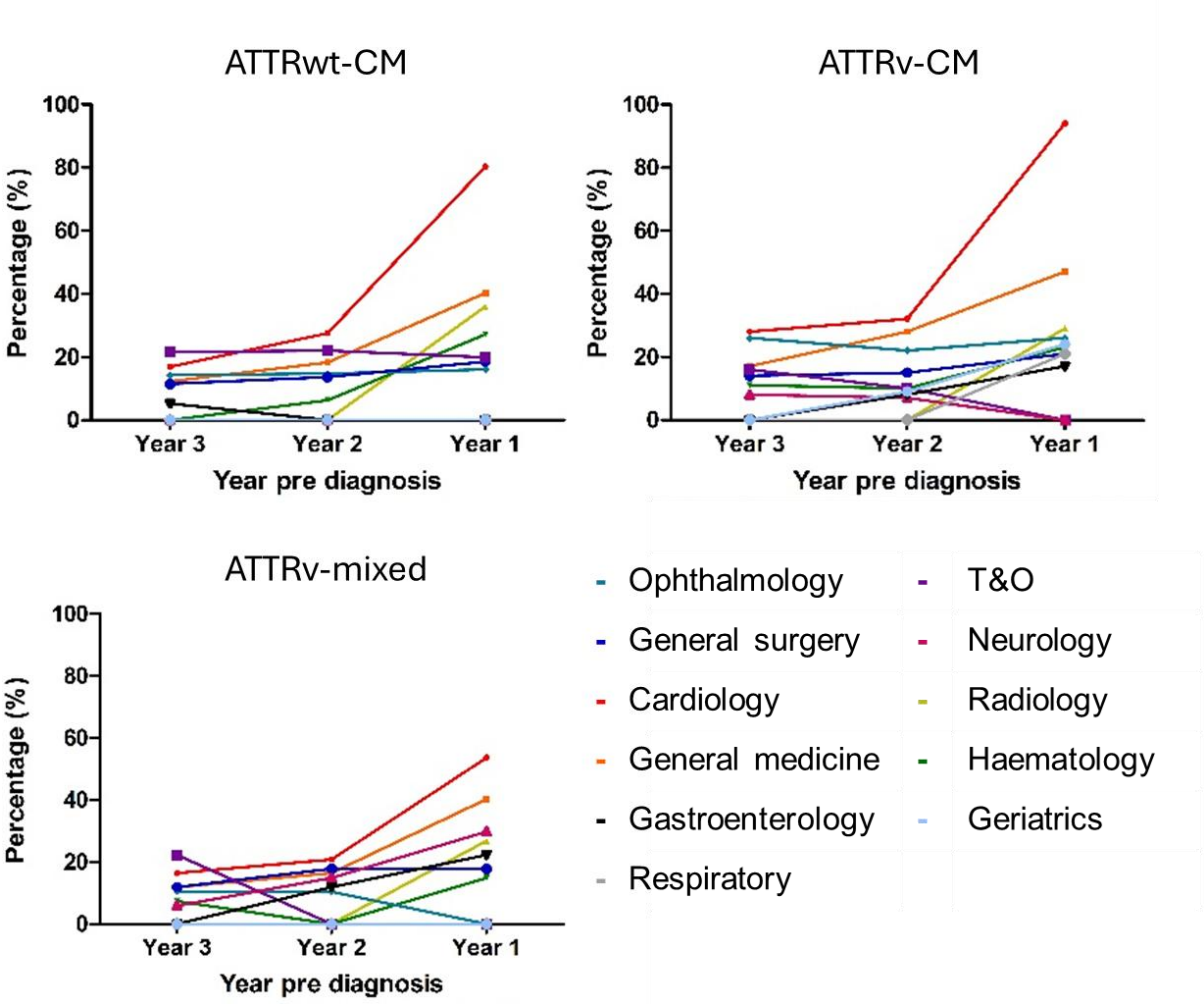


Hospital service usage in the three years prior to diagnosis of ATTR amyloidosis categorized by clinical phenotype.

Outpatient clinic visits increased across the three years before diagnosis. Overall, 515 (96%) of patients had a recorded clinic visit within one year of diagnosis, 373 (70%) in the 2nd year prior to diagnosis and 352 (66%) in the 3rd year pre-diagnosis. Figure 7.2 displays the trend of specialty outpatient visits across the three years prior to diagnosis. Table 7.2 displays the ten most recorded outpatient episodes in the three years prior to diagnosis in each group. Cardiology were more commonly visited by the ATTRwt-CM and ATTRv-CM groups (80% and 94%

respectively in the year prior to diagnosis) compared to ATTRv-mixed group in which 54% visited in the year prior to diagnosis; 17-32% of patients in these two groups also visited cardiology in the second and third year prior to diagnosis. Neurology was visited by 30% of patients in the ATTRv-mixed group within a year of diagnosis; in comparison neurology were rarely visited in the ATTRwt-CM and ATTRv-CM groups. Gastroenterology was also commonly visited by the ATTRv-mixed group with 22% visiting in the year prior diagnosis likely reflecting autonomic neuropathy.

Figure 7.2: Trend in specialty outpatient visits in the three years prior to ATTR amyloidosis diagnosis.



Trend in specialty outpatient visits in the three years prior to ATTR amyloidosis diagnosis stratified by clinical phenotype.

Table 7.2: Ten most common outpatient specialty encounters in the three years pre-diagnosis categorized by clinical phenotype.

Year pre diagnosis	**ATTRwt-CM**			**ATTRv-CM**			**ATTRv-mixed**		
Specialty	1	2	3	1	2	3	1	2	3
Cardiology	295 (80)	101 (28)	62 (17)	94 (4)	32 (32)	28 (28)	36 (54)	14 (21)	11 (16)
General Medicine	148 (40)	67 (18)	45 (12)	47 (47)	28 (28)	17 (17)	27 (40)	11 (16)	8 (12)
Rheumatology	70 (19)	-	-	33 (33)	-	-	16 (24)	-	-
Ophthalmology	59 (16)	54 (14)	52 (14)	26 (26)	22 (22)	26 (26)	-	7 (10)	7 (10)
Haematology	100 (27)	23 (6)	-	23 (23)	10 (10)	11 (11)	10 (15)	-	5 (7)
General Surgery	68 (19)	50 (14)	42 (11)	21 (21)	15 (15)	14 (14)	12 (18)	12 (18)	8 (12)
Trauma and Ortho	73 (20)	81 (22)	79 (22)	-	10 (10)	16 (16)	-	-	15 (22)
Radiology	132 (36)	-	-	29 (29)	-	-	18 (27)	-	-
Nursing episode	59 (16)	28 (8)	20 (5)	-	-	-	-	7 (10)	-
AHP episode	103 (28)	48 (13)	42 (11)	-	-	10 (10)	10 (15)	8 (12)	7 (10)
Dermatology	-	24 (7)	19 (5)	-	-	-	-	-	4 (6)
Urology	-	34 (9)	37 (10)	-	-	9 (9)	-	8 (12)	8 (12)
Gastroenterology	-	-	19 (5)	17 (17)	8 (8)	-	15 (22)	8 (12)	-
Geriatric Medicine	-	-	-	24 (24)	9 (9)	6 (6)	-	-	-
Respiratory	-	-	-	21 (21)	-	-	10 (15)	-	-
Neurology	-	-	-	-	7 (7)	8 (8)	20 (30)	10 (15)	4 (6)
Accident and Emergency	-	-	-	-	9 (9)	-	-	-	-
Ear Nose and Throat	-	-	-	-	-	-	-	7 (10)	-

Reported as number (percentage).

Comorbidity pre-diagnosis

The most common diseases suffered within three years of diagnosis are displayed in Table 7.3. Amongst the ATTRwt-CM cohort: essential primary hypertension occurred in 49% at an average of 7 months pre diagnosis, hypercholesterolaemia in 22% an average of 9 months pre diagnosis, and atherosclerotic heart disease in 13% an average of 10 months pre diagnosis; atrial fibrillation and flutter occurred as a primary diagnosis in 15% an average of 10 months pre to diagnosis and a secondary diagnosis in 36% an average of 5 months pre diagnosis. In the ATTRv group: atrial fibrillation occurred as a secondary diagnosis in 22% of patients an average of 5 months pre diagnosis, left ventricular failure occurred in 11% an average of 4 months pre diagnosis and cardiomegaly occurred in 20% an average of 9 months pre diagnosis.

Carpal tunnel syndrome occurred in 103 (28%) of the ATTRwt-CM group and 37 (22%) of the ATTRv group occurring on average 7 years pre diagnosis. Within the three years prior to diagnosis, 64% and 57% of the ATTRwt-CM and ATTRv-CM patients were placed on an elective trauma and orthopaedic surgical waiting list with 22% and 33% having a procedure booked.

Nine percent of patients with ATTRwt-CM or ATTRv-CM experienced a cerebrovascular accident prior to diagnosis occurring at a median of 33 and 16 months respectively.

Table 7.3: Twenty most common comorbidities recorded within three years of diagnosis

	ATTRwt-CM			ATTRv		
Year pre diagnosis	1	2	3	1	2	3
Comorbidity						
Essential hypertension	147 (40)	55 (15)	44 (12)	72 (43)	23 (14)	30 (18)
Atrial fibrillation / flutter	125 (34)	40 (11)	18 (5)	37 (22)	7 (4)	7 (4)
Hypercholesterolaemia	59 (16)	22 (6)	18 (5)	28 (17)	13 (8)	12 (7)
Atherosclerotic heart disease	55 (15)	18 (5)	15 (4)	18 (11)	8 (5)	2 (1)
Congestive heart failure	66 (18)	7 (2)	7 (2)	37 (22)	8 (5)	2 (1)
Cardiomegaly	51 (14)	7 (2)	4 (1)	27 (16)	12 (7)	5 (3)
Cardiomyopathy in metabolic disease	48 (13)	4 (1)	0 (0)	30 (18)	2 (1)	0 (0)
Type 2 diabetes mellitus without complications	40 (11)	18 (5)	15 (4)	22 (13)	8 (5)	12 (7)
Left ventricular failure	33 (9)	15 (4)	7 (2)	22 (13)	7 (4)	0 (0)
Angina pectoris unspecified	33 (9)	15 (4)	7 (2)	-	-	-
Heart failure unspecified	29 (8)	11 (3)	0 (0)	17 (10)	5 (3)	2 (1)
Chronic ischaemic heart disease unspecified	29 (8)	7 (2)	4 (1)	-	-	-
Other forms of chronic ischaemic heart disease	15 (4)	11 (3)	15 (4)	-	-	-
Asthma unspecified	26 (7)	7 (2)	7 (2)	-	-	-
Hyperplasia of prostate	15 (4)	11 (3)	7 (2)	12 (7)	5 (3)	3 (2)
Diverticular disease	15 (4)	11 (3)	7 (2)	-	-	-
Acute renal failure unspecified	26 (7)	4 (1)	4 (1)	-	-	-
Carpal tunnel syndrome	11 (3)	11 (3)	11 (3)	-	-	-
Pleural effusion not elsewhere classified	26 (7)	4 (1)	4 (1)	17 (10)	3 (2)	0 (0)
Mitral valve insufficiency	22 (6)	4 (1)	4 (1)	8 (5)	2 (1)	2 (1)
Anaemia unspecified	-	-	-	12 (7)	2 (1)	2 (1)

Cataract unspecified	-	-	-	5 (3)	3 (2)	5 (3)
Disorders of both mitral and tricuspid valves	-	-	-	12 (7)	2 (1)	0 (0)
Acute myocardial infarction unspecified	-	-	-	7 (4)	7 (4)	2 (1)
Diaphragmatic hernia without obstruction of gangrene	-	-	-	7 (4)	3 (2)	2 (1)
Obesity unspecified	-	-	-	8 (5)	2 (1)	2 (1)
Unstable angina	-	-	-	7 (4)	5 (3)	2 (1)

The twenty most common comorbidities recorded within the three years prior to diagnosis categorized by TTR genotype; ATTRv included both ATTRv-CM and ATTRv-mixed combined. Data presented as the number (percentage) of patients with recorded co-morbidity within the 1st, 2nd and 3rd year before diagnosis and include both new and longstanding diagnoses.

Investigations and Procedures pre diagnosis

Table 7.4 displays the ten commonest procedures performed in the three years preceding diagnosis. Transthoracic echocardiography was the most frequently occurring procedure prior to diagnosis occurring at 5.8 and 5.5 months prior to diagnosis in the ATTRwt-CM and ATTRv group respectively. Amongst patients diagnosed between April 2007 and March 2012, 23 in the ATTRwt-CM group and 6 in the ATTRv group underwent CMR imaging; this increased to 55 and 16 patients respectively between April 2012 and March 2017. Between April 2007 and March 2012, 11 patients in the ATTRwt-CM group and 9 patients in the ATTRv group underwent endomyocardial biopsy; this reduced to 5 and 5 patients respectively between April 2012 and March 2017.

Table 7.4: Ten most frequently occurring procedures in three years pre diagnosis

	ATTRwt-CM		ATTRv	
	N (%)	Months pre diagnosis	N (%)	Months pre diagnosis
Assessment by uniprofessional team	47 (13)	8.5	-	-
Coronary Arteriography	50 (13)	8.3	33 (20)	9.6
Approach to organ under fluoroscopic control	121 (33)	7.3	55 (33)	8.3
Radiology of one body area	139 (38)	6.9	62 (37)	7.3
Radiology post contrast	44 (12)	6.6	23 (14)	5.5
Transthoracic echocardiography	169 (46)	5.8	67 (40)	5.5
Diagnostic electrocardiography	51 (14)	5.4	-	-
Cardiac magnetic resonance imaging	51 (14)	2.8	-	-
Unspecified radiopharmaceutical imaging	88 (24)	0.4	23 (14)	0.1
Nuclear medicine bone scan	84 (23)	0.3	23 (14)	0.1
Computer tomography of head	-	-	18 (11)	13.4
Endoscopic examination of UGIT and biopsy	-	-	28 (17)	11
Computerised tomography NEC	-	-	23 (14)	5.6

Cardiovascular investigations performed within three years of diagnosis categorized by TTR genotype; ATTRv includes ATTRv-CM and ATTRv-mixed. UGIT: upper gastrointestinal tract.

Discussion

This study illustrates the high level of healthcare resource utilization by patients with ATTR amyloidosis prior to diagnosis. Patients visit a broad range of medical and surgical specialties prior to diagnosis in keeping with the systemic nature of ATTR amyloidosis; these specialties differ between wild-type and hereditary subgroups reflecting their differing clinical phenotypes.[31]

At diagnosis, the ATTRv-CM subgroup, the majority of whom carried the V122I TTR variant, had the most severe cardiac phenotype by imaging, biomarkers and functional assessment. This is consistent with the literature demonstrating V122I-ATTRv-CM presents with more advanced and rapidly progressive disease compared to ATTRwt-CM; it also reflects the relatively low incidence of severe neuropathy commonly found in other forms of ATTRv, for example with the T60A and V30M TTR variants, which more frequently present with peripheral or autonomic neuropathy prior to the onset of severe cardiomyopathy.[31,112] Of note, in the ATTRwt-CM and ATTRv-CM groups 58% and 56% of patients were NAC ATTR Stage ≥2 at diagnosis suggesting significant delays in diagnosis. This is of increasing importance following the development of novel TTR stabilising and RNA targeted therapies shown to reduce disease progression in both cardiomyopathic and neuropathic forms of ATTR amyloidosis, and the increasing evidence suggesting maximal benefit when started early in the disease course.[3-5,91,92,128]

A gradual rise in healthcare utilization across the three years prior to diagnosis is evident across each ATTR subgroup (Figure 7.1). Whilst this is expected in a population over the age of 75, the specialties involved support the

hypothesis that a large proportion of this activity is ATTR amyloidosis related with cardiology visits increasing rapidly in the ATTRwt-CM and ATTRv-CM groups reflecting their typical cardiomyopathic phenotype; whilst cardiology, neurology and gastroenterology all feature prominently in the ATTRv-mixed subgroup given the mixed cardiac and neuropathic phenotype. Each subgroup showed a gradual increase in attendance to general medicine clinics; while the reason for referral is unknown, one might hypothesise that this may reflect ongoing symptoms and investigational abnormalities prior to specialty referral and a confirmed diagnosis. Across each subgroup patients are visiting trauma and orthopaedics and ophthalmology likely reflecting musculoskeletal and retinal involvement by ATTR amyloidosis.[153,154] In particular, an above expected proportion of patients had a history of carpal tunnel syndrome, a known complication of ATTR amyloidosis, with over 50% of patients with ATTR-CM awaiting an orthopaedic procedure in the three years prior to diagnosis.[31]

Across the studied time period, CMR use increased whilst endomyocardial biopsy utility fell. This likely reflects the extensive literature highlighting high sensitivity and specificity of cardiac magnetic resonance imaging in the diagnosis of cardiac amyloidosis alongside the widespread adoption of the non-biopsy ATTR-CM diagnostic criteria enabling a diagnosis of ATTR-CM without the need for histological confirmation in the majority of patients.[2,25,45,57,61,69]

Transthyretin amyloidosis, in particular wild type, is a progressive disease due to gradual distortion of tissue structure by ongoing deposition of amyloid fibrils eventually causing organ dysfunction and symptoms. Our data demonstrates that patients with ATTR amyloidosis are presenting to healthcare at numerous points in the three years prior to diagnosis, to a variety of healthcare staff, medical and

surgical specialties, and through multiple different clinical pathways. Each encounter offers an opportunity for earlier diagnosis. In the absence of approved therapies to actively remove amyloid, early diagnosis remains key to improving patient outcomes, allowing the initiation of TTR stabilisers or RNA targeted therapies to prevent ongoing organ damage and promote amyloid regression. At the time of study, the transthyretin stabiliser tafamidis was not approved for use in the United Kingdom while RNA targeted therapies patisiran and inotersen were approved for use in ATTRv with neuropathy only; both RNA targeted therapies and novel TTR stabilisers are currently being evaluated in the ATTRwt-CM population.[6,127,128] Whether initiation of these therapies would significantly reduce ongoing healthcare utilization and the associated costs remains to be proven.

Chapter 8: Improving outcomes in renal AL amyloidosis, a 20 year specialist centre experience

Introduction

Systemic AL amyloidosis is a rare multi-system disease which involves the kidneys in 70% of cases, and is responsible for up to 1% of end stage renal disease[15,95,111]. Without treatment, disease progression can be rapid with both renal and patient survival often short. Chemotherapy based treatments targeting the abnormal underlying B cell clone have advanced dramatically over the last two decades with increasingly efficacious therapies at diagnosis and relapse prolonging patient survival.[9-11,17] Despite these advances there is often advanced irreversible organ damage at diagnosis and the efficacy of targeted anti-amyloid therapies remains to be established.[81]

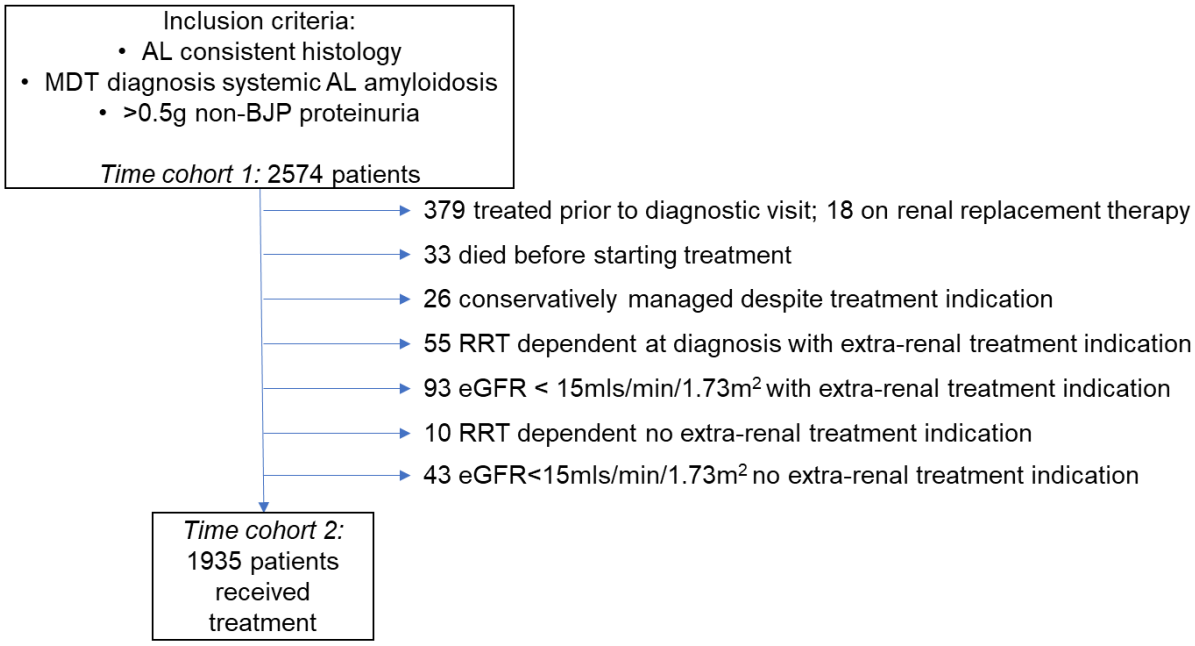
We present the changes in diagnostic characteristics, disease course, patient survival and renal survival in patients treated for renal AL amyloidosis at the UK NAC over the last 20 years.

Methods and Materials

Patients

A retrospective analysis was performed of patients attending the United Kingdom NAC between 01/01/2000 and 09/12/2021 with a diagnosis of renal AL amyloidosis. Renal AL amyloidosis was confirmed in all cases at a specialist multidisciplinary team (MDT) meeting and was defined according to international guidelines for the diagnosis of renal involvement in systemic AL amyloidosis as[60]: histological evidence of AL amyloid deposition from any site with non-Bence Jones proteinuria of >0.5g on 24 hour collection or uPCR >50mg/mmol on spot urine sample; patients meeting these criteria are referred to as Time cohort 1. A further cohort focussing on patients receiving treatment for renal AL amyloidosis, referred to throughout at Time cohort 2, comprised of all patients from Time cohort 1 with the following patients excluded: completion of ≥1 cycle of chemotherapy prior to first attendance at NAC; not treated with chemotherapy; renal replacement therapy (RRT) requirement at diagnosis; eGFR of <15mls/min/m^2 at diagnosis (Figure 8.1). Patients with an eGFR of <15mls/min/m^2 were excluded from the analysis cohort since chemotherapy was typically recommended only for extra-renal organ involvement, given the expected poor renal survival in this subgroup.

Figure 8.1: Consort diagram



MDT: multidisciplinary team, BJP: Bence jones protein, RRT: renal replacement therapy.

Diagnosis date was defined as the date of first appointment at the NAC. Median (IQR) time from histological identification of amyloid to first NAC appointment was 1 (0-2) months. At diagnosis, patients underwent full clinical history, biochemical, imaging and histological assessment including electrocardiogram, proteinuria quantification, echocardiography and SAP scintigraphy. Renal function was evaluated by CKD-EPI eGFR calculation, serum creatinine, 24-hour urinary protein measurement and/or by spot uPCR. Renal and cardiac amyloid stage were defined as outlined in chapter 2.[18,111]; cardiac Mayo stage was only calculated for patients with an IVSd of >12mm as per Gertz et al diagnostic criteria for cardiac involvement.[60]

Available tissue samples were requested from the referring hospital prior to first NAC assessment and fat aspiration was invariably performed at the time of the

first NAC evaluation; all histology was performed in the specialist NAC laboratory as outlined in chapter 2.[79,109]

Diagnostic confirmation and treatment recommendation was via a weekly specialist amyloidosis MDT meeting. Patients were followed 6-12 monthly at NAC as per clinical indication. Data arising from correspondence from local healthcare providers was included where necessary.

Serum protein electrophoresis and serum free light chains were assessed on a 1 to 3 monthly basis as determined by clinical indication with haematologic response to treatment assessed as outlined in chapter 2.[54]

Outcomes

Mode and date of death was obtained from the UK Office of National Statistics (ONS). Patient survival was defined as time from diagnosis (first NAC evaluation) to death or censor, while renal survival was defined as time from diagnosis (first NAC evaluation) to censor, initiation of renal replacement therapy, kidney transplantation, or death primarily due to kidney failure on the basis of UK death certification data.

Statistical analyses

Patient characteristics at diagnosis were compared using the Mann Whitney and chi squared test for numerical and categorical variables respectively. Survival functions were estimated according to the KM method, with groups compared using the log-rank test. Patients were stratified by date of diagnosis; groups were selected to reflect advances in treatment with 2005 representing a shift in first line therapy from melphalan or vincristine doxorubicin-based regimens to thalidomide, 2012 reflecting

the introduction of bortezomib, and 2019 the introduction of daratumumab at first relapse for selected patients. The association between patient characteristics at diagnosis and patient and renal survival were explored by multivariable cox proportion hazard regression analysis using diagnostic patient variables reported to be predictive of these outcomes in the published literature.[18,111] Results are expressed as hazard ratios with calculated 95% CI. A p value of < 0.05 was considered significant.

Patient characteristics are presented as median (IQR) or number (percentage) unless otherwise stated. Data analysis and graph production was performed in SPSS (IBM Corp, 2017).

Results

Patient characteristics

Time cohort 1 included 2574 patients. Time cohort 2 comprised 1935 patients following exclusion of the following patients from the Time cohort 1: completion of ≥1 cycle of chemotherapy prior to first attendance at NAC (n=379); not treated with chemotherapy despite indication (n=59); renal replacement therapy requirement at diagnosis (n=65); eGFR of <15mls/min/m^2 at diagnosis (n=136) (Consort Diagram, Figure 8.1). Of these, 182, 469, 997 and 287 were diagnosed in 2000-04, 2005-11, 2012-18 and 2019 onwards respectively; baseline characteristics stratified by diagnosis date are shown in Table 8.1. There were no significant differences in diagnostic renal amyloid stage between the groups (p=0.833); diagnostic proteinuria (2000-04 vs 2005-11; p=0.299, 2005-11 vs 2012-18; p=0.060; all other p<0.05) and eGFR (2005-11 vs 2012-18; p=0.021, 2005-11 vs 2019 onwards; p=0.004; all others p>0.053) tended to be lower with more recent diagnoses. Age at diagnosis increased

with more recent diagnoses ($p<0.05$ other than 2012-18 vs 2018 onwards group) as did diagnostic NT-proBNP ($p<0.01$ other than 2000-04 vs 2005-11 and 2012-18 vs 2019 onwards). There were no significant differences in dFLC across the time cohorts. First line chemotherapy regimens were markedly different between groups with 84% of patients in the 2000-04 diagnostic group receiving melphalan or vincristine doxorubicin based regimens, 66% of the 2005-11 group receiving a thalidomide-based regimen and >82% of patients diagnosed from 2012 onwards receiving a bortezomib based regimen.

Table 8.1: Patient characteristics at diagnosis of renal AL amyloidosis for Time cohort 2 stratified by date of diagnosis

		Date of diagnosis			
		2000-2004 n=182	2005-2011 n=469	2012-2018 n=997	2019 onwards n=287
Age (years)		61	65	67	68
Male		100 (55)	268 (57)	577 (58)	183 (64)
Renal biopsy		134 (74)	300 (64)	595 (60)	156 (54)
HDM-ASCT		18 (10)	34 (7)	106 (11)	6 (2)
Treatment	Bortezomib	0 (0)	37 (8)	819 (82)	244 (85)
	Thalidomide	11 (6)	307 (66)	79 (8)	1 (0)
	Melphalan	88 (48)	70 (15)	23 (2)	0 (0)
	Up front ASCT	5 (3)	4 (1)	16 (2)	0 (0)
	Lenalidomide	0 (0)	4 (1)	8 (1)	10 (4)
	Bendamustine	0 (0)	0 (0)	27 (3)	15 (5)
	VAD	65 (36)	7 (2)	0 (0)	0 (0)
	Rituximab	1 (1)	12 (3)	12 (1)	2 (1)
	Other	12 (7)	28 (6)	12 (1)	9 (3)
eGFR (mls/min/1.73m^2)		76	75	68	66
CKD Stage	I	52 (29)	152 (32)	289 (29)	69 (24)
	II	66 (36)	149 (32)	297 (30)	95 (33)
	IIIa	25 (14)	62 (13)	144 (14)	35 (12)
	IIIb	21 (12)	52 (11)	132 (13)	51 (18)
	IV	18 (10)	54 (12)	135 (14)	37 (13)
Proteinuria (g/24hr)		5.8	5.7	5.0	4.4
Albumin (g/L)		27	28	29	30
ALP (u/L)		92	90	87	97
NT-proBNP (ng/L)		626	901	1372	1502
TnT (ng/L)		10	24	44	44
dFLC (mg/L)		153	125	140	146
Monoclonal protein serum (g/L)		7.5 (4-10)	7 (4-13)	8 (4-14)	7 (3-10)
Involved light chain	Kappa	33 (18)	73 (16)	159 (16)	55 (19)
	Lambda	147 (81)	390 (83)	835 (84)	232 (81)
	Unknown	2 (1)	6 (1)	3 (0)	0 (0)

Serum IF	IgA	17 (9)	56 (12)	147 (15)	45 (16)
	IgD	0 (0)	3 (1)	11 (1)	0 (0)
	IgG	33 (18)	145 (31)	334 (34)	86 (30)
	IgM	7 (4)	23 (5)	75 (8)	23 (8)
	LC only	5 (3)	63 (13)	214 (22)	76 (27)
	None	124 (66)	179 (38)	216 (22)	57 (20)
Renal	I	54 (30)	155 (33)	343 (34)	94 (33)
Stage	II	98 (54)	232 (49)	480 (48)	147 (51)
	III	30 (16)	82 (17)	173 (17)	45 (16)
Mayo	I	1 (25)	7 (7)	17 (4)	9 (7)
Stage	II	2 (50)	36 (34)	80 (18)	21 (16)
	IIIa	1 (25)	43 (40)	251 (56)	70 (52)
	IIIb	0 (0)	21 (20)	102 (23)	35 (26)
IVSd (mm)		12	12	12	13
LVPW (mm)		12	12	12	13
Systolic BP (mmHg)		135	121	120	118
6MWT (m)				403	399

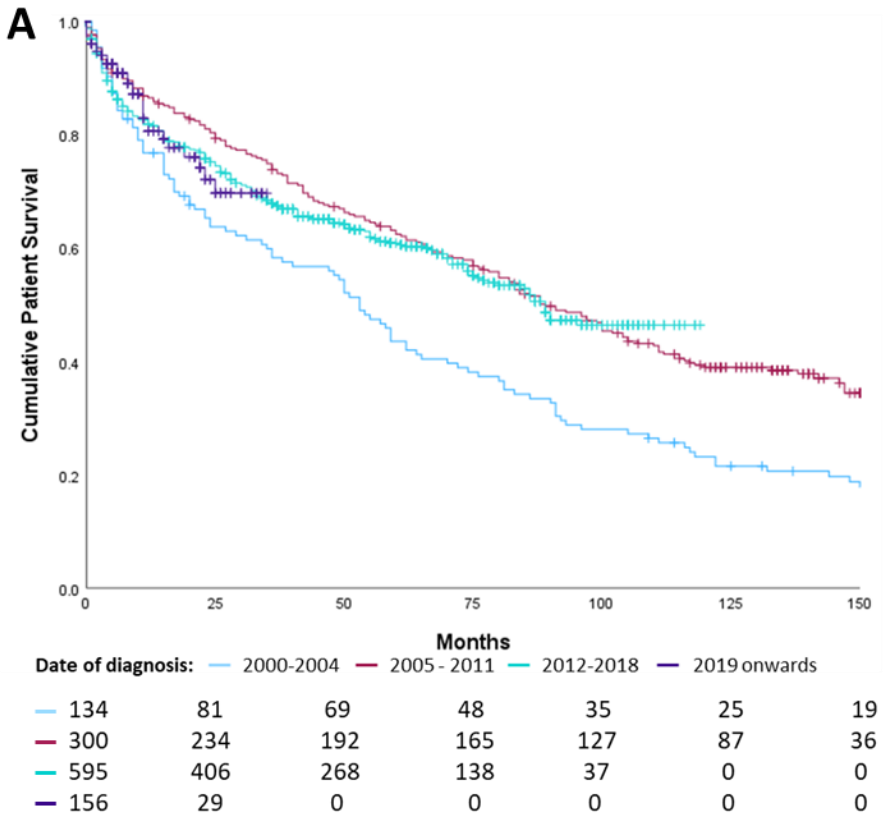
LVPW – left ventricular posterior wall thickness; IF – immunofixation

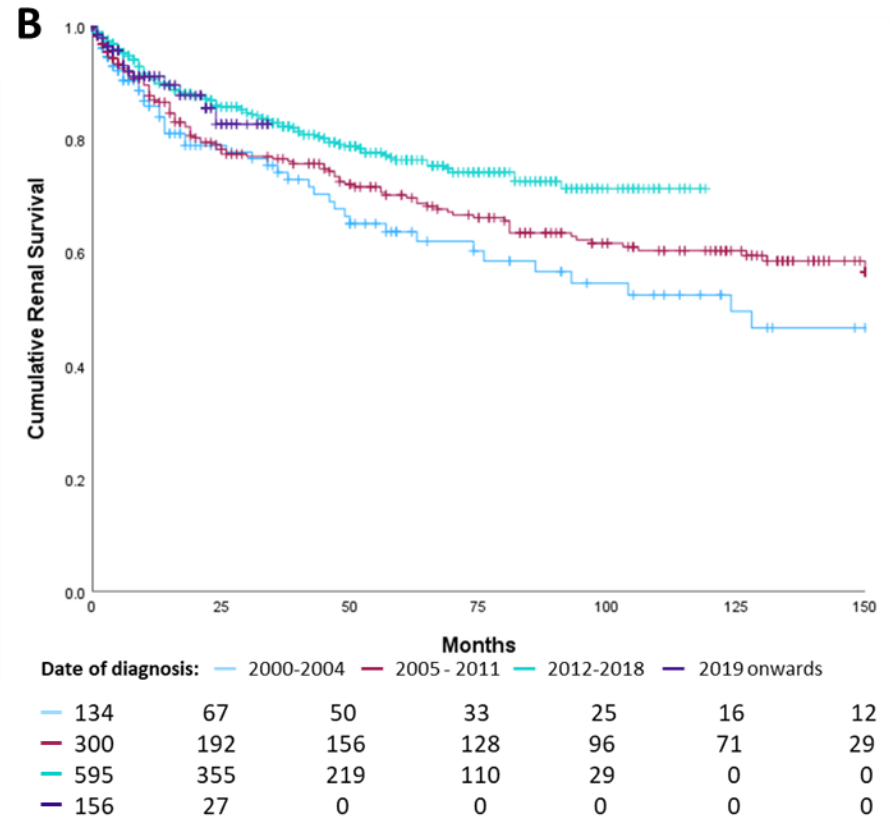
Patient survival

The median follow up of the 1935 patients receiving chemotherapy was 32 (7-74) months during which 1025 (53%) died. The median follow up was 37 (10-96), 66 (17-127), 38 (7-68) and 10 (4-22) months for patients diagnosed between 2000-04, 2005-11, 2012-18 and 2019 onwards respectively. Estimated patient survival in the total study group was 66 (13-168) months; when stratified by diagnostic date group survival was 48 (11-109) months, 73 (20-177), 71 (10-not met) and not met (12-not met) for 2000-2004, 2005-2011, 2012-2018 and 2019 onwards groups respectively (Figure 8.2A). Multivariable cox proportion hazard regression analysis identified increasing age, diagnostic NT-proBNP and dFLC as independent predictors of mortality in the whole study group (Table 8.2); this was consistent throughout the diagnostic year groups other than the 2019 onwards group in which age did not

predict mortality. Proteinuria and eGFR were not independently predictive of mortality in any subgroup. In the 696 patients with an IVSd > 12mm, cardiac Mayo stage strongly predicted patient survival at stages greater than II (Figure 8.3).

Figure 8.2: Patient and death censored renal survival stratified by date of diagnosis



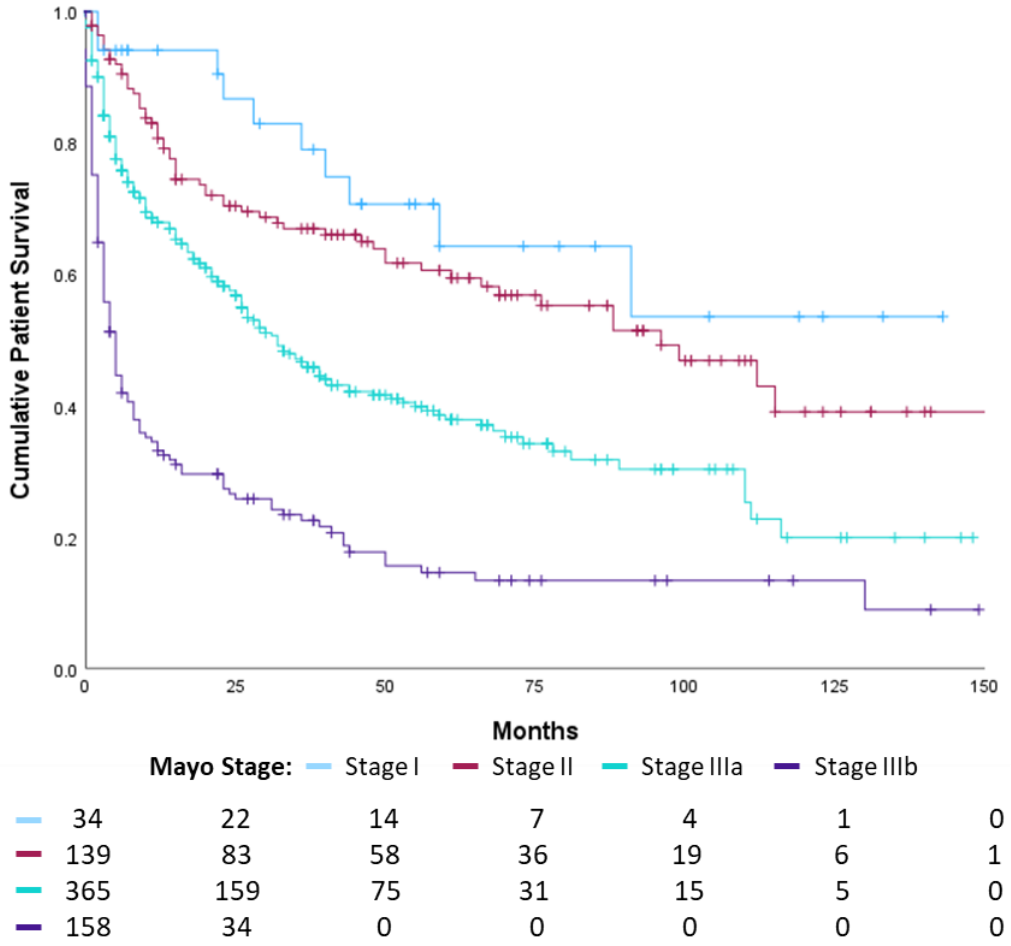


(A) Kaplan-Meier patient survival curves stratified by diagnostic date. Estimated median survival in patients diagnosed in 2000-04 was 48 (11-109) months, 2005-11 73 (20-177) months, 2012-18 71 (10-not met) months, and 2019 onwards was not met (12 months – not met). 2000-04 versus 2005-11; p<0.001, 2005-11 vs 2012-18; p=0.634, 2012-18 vs 2019 onwards; p=0.514. (B) Kaplan-Meier death censored renal survival curves stratified by diagnostic date. Estimated death censored median renal survival in patients diagnosed in 2000-04 was 124 (38-124) months, 2005-11 not met (61-not met) months, 2012-18 and 2019 onward groups median and quartiles not met. 2000-04 versus 2005-11; p=0.009, 2005-11 vs 2012-18; p=0.003, 2012-18 vs 2019 onwards; p=0.950.

Table 8.2: Multivariable cox regression analyses of predictors of patient survival and death censored renal survival stratified by date of diagnosis

			Date of diagnosis							
	All patients		2000-2004		2005-2011		2012-2018		2019 onwards	
	HR	p value	HR	p value	HR	p value	HR	p value	HR	p value
Patient survival										
Age (years)	1.030	<0.001	1.025	0.153	1.036	<0.001	1.031	<0.001	1.020	0.176
eGFR (per 10mls/min/1.73m^2)	0.986	0.310	0.972	0.656	1.023	0.371	0.977	0.210	0.915	0.090
Proteinuria (g/24hrs)	0.990	0.225	0.998	0.957	1.001	0.973	0.994	0.587	0.958	0.262
NT-proBNP (per 100ng/L)	1.006	<0.001	1.002	0.418	1.008	<0.001	1.006	<0.001	1.005	<0.001
dFLC (per 100mg/L)	1.005	<0.001	1.055	0.001	1.037	<0.001	1.004	0.005	1.024	0.002
Renal Survival										
Age (years)	0.965	<0.001	0.955	0.039	0.965	<0.001	0.972	0.003	0.937	0.060
eGFR (per 10mls/min/1.73m^2)	0.741	<0.001	0.818	0.015	0.748	<0.001	0.713	<0.001	0.699	0.010
Proteinuria (g/24hr)	1.059	<0.001	1.143	0.023	1.052	<0.001	1.068	<0.001	1.088	0.140
NT-proBNP (per 100ng/L)	0.999	0.999	0.974	0.104	1.001	0.534	1.000	0.892	1.002	0.757
dFLC (per 100mg/L)	1.000	1.000	1.039	0.119	0.988	0.650	1.000	0.983	0.958	0.722

Figure 8.3: Patient survival stratified by cardiac Mayo stage



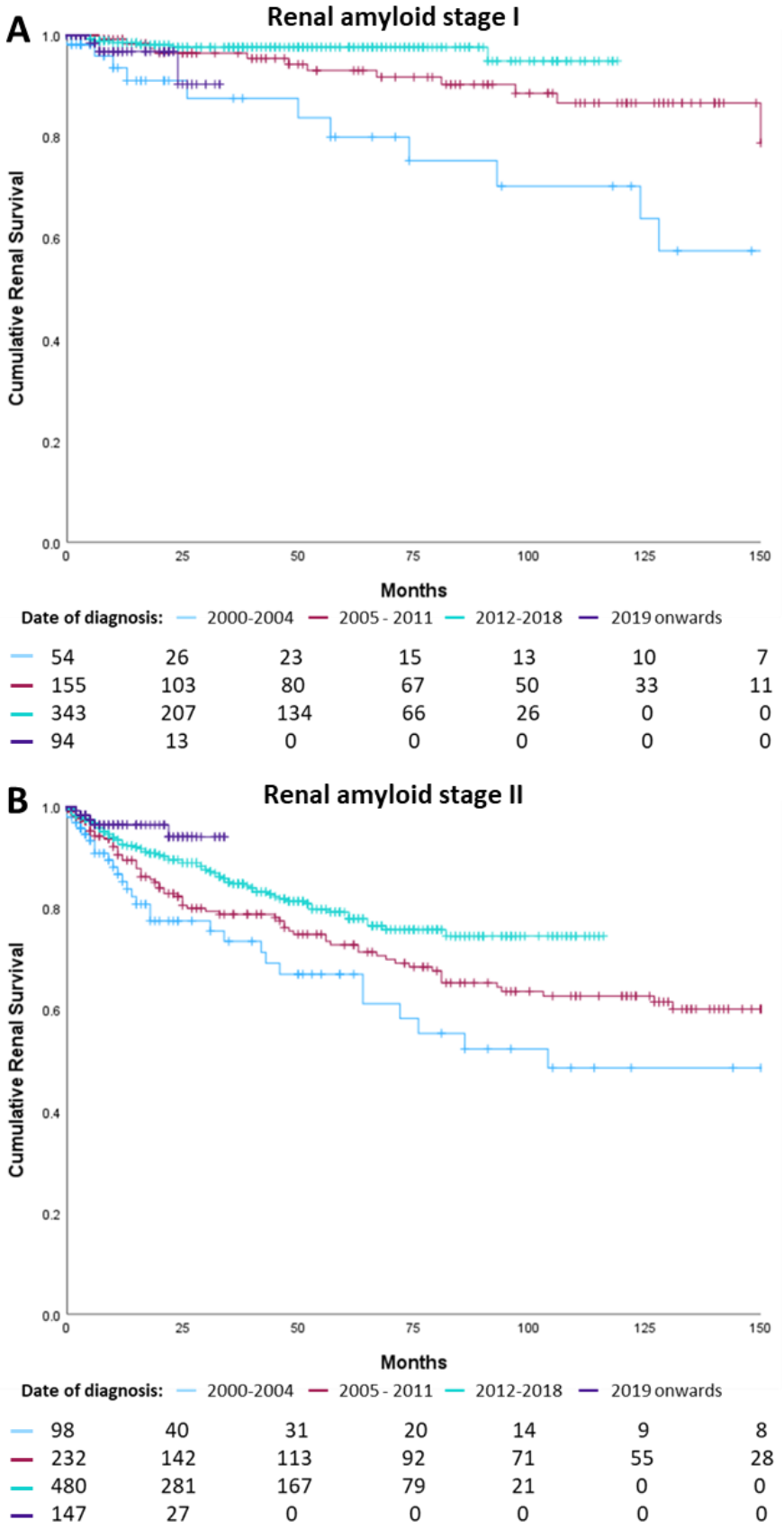
Kaplan-Meier patient survival curves stratified by cardiac Mayo stage at diagnosis in patients with an interventricular septal wall thickness at end diastole of >12mm. Estimated median survival in patients with cardiac Mayo stage I was not met (40 – not met) months, II 96 (15-164) months, IIIa 32 (7-111) months, and IIIb 5 (2-31) months. Stage I vs II; p=0.248, II vs IIIa; p<0.001, and IIIa vs IIIb; p<0.001.

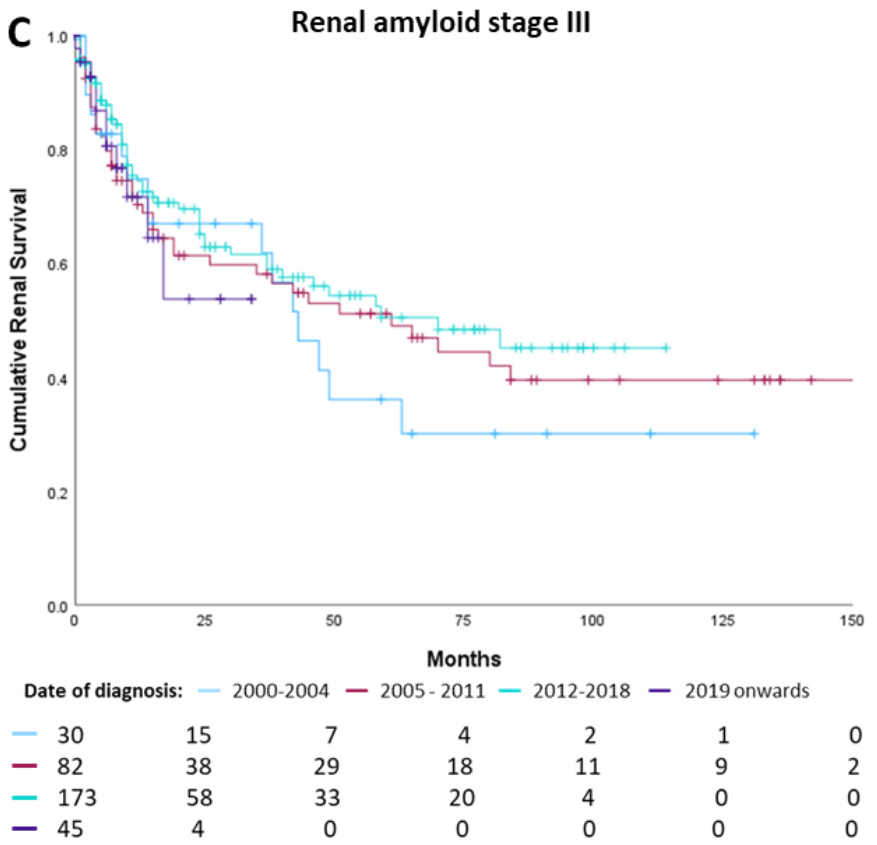
Renal survival

During follow up 325/1935 (17%) patients reached ESRD; overall median death censored renal survival was not met with 60% of living patients not reaching ESRD at 200 months. Death censored renal survival stratified by diagnostic date is shown

in Figure 8.2B. Median death censored renal survival was 124 (38 – not met) months in the 2000-04 group and was not met in other groups; death censored renal survival was significantly longer with more recent diagnoses up to 2018. Death censored renal survival divided by diagnostic amyloid stage and stratified by diagnostic date is shown in Figure 8.4. For patients with renal amyloid stage III at diagnosis there is no difference in death censored renal survival across the diagnostic date groups (Figure 8.3C); patient with renal amyloid stage I and II showed a trend towards prolonged death censored renal survival with more recent diagnoses up to 2018 (Figure 8.4A and 4B). Multivariable cox proportion hazard regression analysis identified reducing diagnostic eGFR, increasing 24-hour proteinuria and increasing age as independent predictors of ESRD in the whole study group (Table 8.2); this was consistent throughout the diagnostic year groups other than the 2019 onwards group in which reducing eGFR was the only predictive variable of ESRD. There were no significant differences in renal survival between the diagnostic date groups in the 500 patients with a diagnostic eGFR of <45mls/min (p=0.271); there was a significant increase in renal survival with more recent diagnoses up to the 2012-18 diagnostic group in the 1435 patients with a diagnostic eGFR of ≥ 45mls/min (p<0.001). NT-proBNP and dFLC were not independently predictive of renal survival in any subgroup.

Figure 8.4: Death censored renal survival divided by amyloid stage stratified by diagnostic date





Kaplan-Meier death censored renal survival curves stratified by diagnostic date for patients with (A) renal amyloid stage I: estimated renal survival for diagnoses in 2000-04 was 155 (93-not met) months, 2005-11 not met (155 months - not met), 2012-18 and 2019 median and quartiles not met. 2000-04 versus 2005-11; p=0.003, 2005-11 vs 2012-18; p=0.066, 2012-18 vs 2019 onwards; p=0.126. (B) Renal amyloid stage II: estimated renal survival for diagnoses in 2000-04 was 104 (34 – not met) months, 2005-11 not met (49 months - not met), 2012-18 not met (82 months – not met) and 2019 onward groups median and quartile not met. 2000-04 versus 2005-11; p=0.148, 2005-11 vs 2012-18; p=0.033, 2012-18 vs 2019 onwards; p=0.178. (C) Renal amyloid stage III: estimated renal survival for diagnoses in 2000-04 was 43 (10 – not met) months, 2005-11 61 (8 - not met) months, 2012-18 70 (12– not met) months and 2019 onward not met (10 months – not med). There were no significant differences in death censored renal survival between diagnostic date groups.

Haematologic treatment and response

Haematology specific follow up data is shown in Table 8.3. Utilization of HDM-ASCT was significantly lower in the 2019 onwards group compared to the other groups ($p<0.002$); there was no difference in HDM-ASCT utilization between the remaining groups ($p=0.121$). The proportion of patients achieving a VGPR or CR at 6 and 12 months rose throughout the study period from 37% and 39% respectively in the 2000-04 diagnostic group to 69% and 71% in the 2019 onwards diagnostic group. Requirement of more than one line of chemotherapy was common throughout.

Table 8.3: Haematologic treatment and response during follow up

		Date of diagnosis			
		2000-2004	2005-2011	2012-2018	2019 onwards
HDM-ASCT		18 (10)	34 (7)	106 (11)	6 (2)
Haematologic response at 6 months	CR	12 (22)	61 (36)	66 (34)	47 (36)
	VGPR	8 (15)	46 (27)	63 (32)	43 (33)
	PR	9 (17)	40 (23)	42 (21)	28 (21)
	NR	25 (46)	24 (14)	26 (13)	14 (11)
Haematologic response at 12 months	CR	7 (21)	42 (29)	52 (34)	39 (35)
	VGPR	6 (18)	33 (23)	56 (37)	41 (36)
	PR	10 (29)	40 (28)	24 (16)	24 (21)
	NR	11 (32)	30 (21)	21 (14)	9 (8)
Total lines of chemotherapy	1	88 (48)	246 (53)	671 (67)	236 (82)
	2	47 (26)	113 (24)	205 (21)	45 (16)
	3	31 (17)	59 (13)	82 (8)	6 (2)
	4	9 (5)	33 (7)	30 (3)	0 (0)
	5	4 (2)	14 (3)	8 (1)	0 (0)
	>5	3 (2)	4 (1)	1 (0)	0 (0)

Survival on renal replacement therapy

Of the patients reaching ESRD 312 commenced dialysis, 222 (71%) commencing haemodialysis and 91 (29%) peritoneal dialysis (Table 8.4); five underwent pre-emptive kidney transplantation; eight received maximal conservative management (MCM; Table 8.4). Median patient survival following commencement of dialysis was 40 (16-88) months; there was no significant difference in survival between those commenced on peritoneal dialysis or haemodialysis (37 [16-114] and 43 [16-88] months respectively; p=0.959) and survival on dialysis did not differ depending on date of diagnosis. Of those commenced on peritoneal dialysis 28 underwent a modality shift to haemodialysis occurring after a median of 5.5 (1.8-28) months. The indications for a switch in modality included: 13 infection and 7 insufficient dialysis. Pre-emptive transplantation occurred in 5 cases with a further 23 patients undergoing transplantation 28 (23-51) months following commencement of dialysis. The median follow up post-transplant was 45 (24-73) months with 23 alive at censor, all with functioning kidney allografts. Five patients died following transplantation occurring at 2, 6, 39, 53 and 73 months. Recurrent amyloidosis of the allograft occurred in one patient requiring chemotherapy; seven patients received treatment for clonal progression or extra-renal amyloidosis progression.

Table 8.4: Management of end stage renal disease

	Date of diagnosis			
	2000-2004 n=56	2005-2011 n=118	2012-2018 n=132	2019 onwards n=19
MCM	3 (5)	3 (3)	2 (2)	0 (0)
Haemodialysis	37 (66)	74 (63)	94 (71)	16 (84)
Peritoneal dialysis	15 (27)	39 (33)	34 (26)	3 (16)
Pre-emptive kidney transplant	1 (2)	2 (2)	2 (2)	0 (0)
Transplanted on dialysis	3 (5)	14 (12)	6 (5)	0 (0)
Survival after commencing dialysis	43 (15-82)	37 (16-87)	54 (16-88)	Not met

MCM – maximal conservative management with renal failure listed as cause of death.

Discussion

This study shows a significant improvement in death censored renal survival over the previous two decades, most pronounced in patients with a renal amyloid stage of ≤ II at diagnosis. This is despite progressively increasing age and NT-proBNP, alongside reducing eGFR, in those more recently diagnosed. Significant renal dysfunction at diagnosis remains common with over half of patients having nephrotic range proteinuria and 31% having an eGFR of <45mls/min. Despite this >75% of patients diagnosed with renal AL amyloidosis from 2012 onwards, and alive at 100 months, are independent of dialysis.

Patient survival has improved across the study period, particularly since the introduction of thalidomide-based treatment regimens in 2005. Diagnostic NT-proBNP and dFLC were strongly associated with mortality in all sub groups consistent with other studies demonstrating the severity of cardiac involvement by amyloidosis and of the underlying clonal disease as crucial determinants of prognosis.[18,56] It is worth noting that both eGFR and proteinuria were not independently predictive of mortality, despite the high mortality associated with ESRD. The established Mayo staging system for cardiac AL amyloidosis remained highly predictive of patient survival in patients with cardiac involvement by amyloidosis despite the reduced sensitivity of NT-proBNP with declining eGFR and increasing proteinuria.[18,155,156]

Across the study period, first line chemotherapy regimens changed from 84% receiving melphalan or vincristine doxorubicin based regimens, to 85% receiving bortezomib based regimens. Numerous studies confirm the efficacy of thalidomide, bortezomib and daratumumab, in achieving rapid and durable haematologic responses[9-12,157], which are associated with improved patient and renal

survival.[157,158] This is reflected in our population with 69% and 71% of patients diagnosed in 2019 onwards achieving a hematologic VGPR / CR at 6 and 12 months respectively compared to 37% and 39% in those diagnosed between 2000-2004. This increased treatment efficacy, alongside comparably reduced adverse event profiles, have likely contributed to the trend of treating older patients with more advanced multisystem amyloidosis.

Despite overall improvements in renal and patient survival, progress has been limited in those with advanced renal dysfunction at diagnosis. At present, treatment of systemic AL amyloidosis is purely chemotherapy or HDM-ASCT based, targeting the rapid reduction of amyloidogenic light chain concentration to prevent progressive amyloid deposition. If a rapid and durable fall in involved light chains is achieved, natural regression of amyloid deposits may gradually occur leading to improvements in organ function; the speed of this varies between individuals and affected organs often taking months to years. Regression of cardiac and liver amyloidosis with subsequent improvement in cardiac function has been shown, however regression of renal amyloid deposits remains less clear, partially due to the lack of imaging modalities to assess treatment response.[65,159-162] Case reports and small series performing repeat renal biopsies show mixed results including: reductions in the amount of amyloid deposition following treatment, no significant change in amyloid deposition despite improvements in proteinuria, and improvement of amyloid load but replacement with glomerular sclerosis.[163-166]

There is significant interest in therapies promoting phagocytic clearance of amyloid deposits alongside standard clone directed therapy. The administration of CPHPC followed by IgG anti-serum amyloid P component antibodies demonstrated reduction in renal amyloid load by radiolabelled SAP scintigraphy in one patient

without change in renal function or proteinuria at 42 days.[81] The phase III randomised controlled VITAL study of the monoclonal antibody NEOD001 suggested a survival benefit in patients with advanced cardiac AL amyloidosis; another monoclonal antibody CAEL-101 is also undergoing phase III trials in advanced cardiac amyloidosis.[167,168] Whether these therapies will enhance regression of renal amyloid deposits, and whether regression will result in meaningful improvements in renal function remain to be determined.

The median survival on dialysis of 40 months is comparable to other causes of ESRD in this age group, although the number of patients going on to receive a kidney transplant was relatively low at 8%.[169,170] Numerous studies have demonstrated good patient and renal outcomes following kidney transplantation for selected patients with AL amyloidosis.[98,171] Given the ongoing improved native renal survival, the increasing duration of haematologic responses achieved with therapies such as daratumumab, and the increasing number of options at haematologic relapse, it is likely more patients may benefit from renal transplantation given the known poor prognosis of remaining on dialysis.[12,172]

Limitations of this study include its retrospective nature, reduced follow up periods for more patients with more recent diagnoses, limited access to cause of death likely resulting in reduced detection of patients passing away primarily from end stage renal disease, and the potential bias introduced by patients being followed up in a large specialist amyloidosis centre rather than across general nephrology and haematology clinics.

In summary, death censored renal survival has significantly improved over the last 20 years, particularly in those with less severe disease at diagnosis. This likely reflects improved chemotherapy options at diagnosis and relapse leading to more

rapid and durable haematologic responses. Whether the addition of therapies promoting amyloid regression will improve renal outcomes further remains to be determined.

Chapter 9: Renal transplant outcomes in Amyloidosis

This chapter is based on the following publication[171]:

Renal Transplant Outcomes in Amyloidosis. Steven Law, Oliver Cohen, Helen J Lachmann, Tamer Rezk, Janet A Gilbertson, Dorota Rowczenio, Ashutosh D Wechalekar, Philip N Hawkins, Reza Motallebzadeh and Julian D Gillmore. 2021. Nephrology Dialysis Transplantation; doi:10.1093/ndt/gfaa293

Permission has been obtained for use this article within my thesis.

Introduction

Amyloidosis is responsible for up to 1% of ESRD and is classified according to the fibril precursor protein, typically identified by immunohistochemistry or mass spectrometry in amyloidotic tissue samples, such as renal histology.[21,95]

The commonest cause of amyloid nephropathy is systemic AL amyloidosis in which renal involvement occurs in up to 70% of patients, typically causing proteinuria and renal dysfunction.[20,173-175] Diagnostic proteinuria of >5g/24 hours and an eGFR of <50ml/min/1.73 m^2, predict progression to dialysis in 60-85% at 3 years.[111] Prompt chemotherapy directed at the underlying clonal disorder improves renal outcomes and patient survival.[54,176] Although patient survival has improved in recent years, up to one third of patients continue to die within one year of diagnosis, with the severity of cardiac involvement being the primary predictor of early mortality.[56,60,177]

Systemic AA amyloidosis, resulting from the chronic overproduction of the acute phase reactant SAA protein, is the second most common form of amyloid

nephropathy.[178] The incidence of AA amyloidosis is reducing, and both patient and renal survival is improving, likely reflecting the increasingly widespread use of biologic therapies revolutionising the treatment of chronic inflammatory conditions.[15,179,180] Kidney involvement is present at diagnosis of AA amyloidosis in 97% of patients; cardiac AA amyloidosis is rare.[22,181] Progression to dialysis is associated with the failure to effectively suppress SAA concentration.[22,182]

More effective biologics in AA amyloidosis, and chemotherapeutic agents in AL amyloidosis, have allowed improved control of the underlying precursor protein in AA and AL amyloidosis. This has resulted in increasing numbers of patients with stable, well managed underlying disease, but with ESRD who may benefit from transplantation. Patient survival on renal replacement therapy in systemic amyloidosis has traditionally been poor, with one large study indicating a median survival from commencement of dialysis of only 2.1 years versus 4.5 in other causes of ESRD.[95,174,183] Historically, outcomes following renal transplantation have also been poor although recent studies suggest some improvement.[95-98]

The primary aim of this study is to determine the risk of graft loss and patient survival for patients with ESRD secondary to AA and AL amyloidosis, and to compare outcomes to a matched cohort of recipients where the primary renal disease does not recur post-transplantation (autosomal dominant polycystic kidney disease [ADPKD]) and those who are at a relatively higher risk of mortality despite transplantation (diabetic nephropathy [DN]).[184-186] The secondary aim is to identify recipient and donor factors predictive of patient and allograft survival, to support physicians in identifying patients with systemic amyloidosis who may benefit from transplantation.

Methods and Materials

Study design

Data for this retrospective observational study was obtained from the UK NAC, the only specialist amyloidosis referral centre in the UK. Donor and recipient transplant details were derived from the UK Transplant Registry of the Organ Donation and Transplant Directorate of NHSBT, which records mandatory data for patients on the waiting list, and details of episodes of transplantation performed by all 23 UK adult kidney transplant centres. Histology reports were obtained from local hospitals.

Study Participants

All patients with ESRD due to AL or AA amyloidosis followed up at the NAC who underwent their first renal transplant between 1st January 1989 and 30th April 2018 were included. Date of amyloidosis diagnosis was defined as date of biopsy confirming amyloid or date of first review at NAC if unavailable. Diagnosis was confirmed by histology with immunohistochemistry and/or mass spectrometry in 97 patients; the remaining two patients were diagnosed with AA amyloidosis on the basis of unequivocal renal amyloid on SAP scintigraphy in association with a chronically elevated SAA concentration in the absence of a plasma cell dyscrasia or mutation in any of the known hereditary amyloidosis genes. Patients attended the NAC 6 to 24 monthly with biochemical evaluation of renal, cardiac and liver function performed at each visit; echocardiography and SAP scintigraphy were performed when indicated. Patients were followed up until the 12th of May 2020. Some of the patients reported in this study were included in a previous publication.[97]

Assessment and monitoring of circulating fibril precursor protein concentration

In patients with systemic AL amyloidosis, haematological response was assessed at each clinic attendance and one to four monthly in the interim. In patients with AA amyloidosis, SAA concentration was measured at each clinic attendance and one to four monthly in the interim.

Assessment of renal allograft and cardiac function

Renal allograft function was evaluated at each visit by CKD-EPI eGFR calculation, serum creatinine and 24-hour urinary protein measurement.[105] Detailed echocardiographic assessment was performed at diagnosis and throughout follow up as indicated. NYHA class and serum NT-proBNP concentration were assessed at each attendance.

Transplant details

Human leukocyte antigen (HLA) mismatch level was defined according to UK allocation policy for donor.[187] Calculated reaction frequency (cRF) recipient sensitization was defined as HLA antibody reaction frequency, which is calculated by comparison of unacceptable HLA specificities with HLA types of donors of identical ABO blood group in a pool of 10,000 donors on the UK transplant database. Delayed graft function (DGF) defined as need for dialysis within 7 days post-transplant.

Outcomes

Primary outcome measures were all-cause patient death after transplantation, and death censored allograft survival. Patient survival was defined as time from transplantation until death. Death censored allograft survival was taken as time from transplantation to the earliest of graft nephrectomy, re-transplantation or return to dialysis with censoring for death with a functioning graft or at last follow-up evaluation. Recurrent amyloid was defined by allograft histology confirming amyloid, or abnormal uptake of ^{123}I-labelled SAP in the allograft.

Statistical Analysis

Control groups were generated from NHSBT registry data for all UK patients with biopsy proven DN, or ADPKD who received their first renal allograft between 1st January 1995 and 30th April 2018. Propensity score matching by logistic regression, using a nearest neighbour approach was used to produce 4:1 DN and ADPKD controls to both AL and AA amyloidosis patient groups.[188] Separate matched groups were created for the AL and AA patient groups due to significant differences in patient characteristics. Patients were matched on factors known to affect transplant outcomes as independent variables. Based on data availability, recipient age, donor status (live vs deceased), immunological mismatch level, transplant year, and pre-emptive transplantation were used to match 84 amyloidosis patients; recipient age, donor status and transplantation year were used for 14 amyloidosis patients, whilst one patient was not matched. Matching was assessed using independent sample testing and Spearman's analysis, to compare baseline characteristics. Recipient age was higher in DN and CRF higher in ADPKD compared with the AA amyloidosis group, and recipient age was lower in ADPKD than AL amyloidosis; a higher

proportion of DN patients were male in both groups. Otherwise, groups were well matched for transplant year, donor status, donor age, HLA mismatch level, pre-emptive transplantation, cold ischaemic time and delayed graft function (Tables 9.1 and 9.2).

Table 9.1: Donor and recipient characteristics of renal transplant patients with primary diagnosis of AA amyloidosis compared to matched diabetic nephropathy and adult polycystic kidney disease patients.

		AA Amyloidosis n=48	**Diabetic Nephropathy** n=188	**ADPKD** n=188
Recipient age		44 (33-54)	56 (47-62)*	49 (42-59)
Male		27 (56%)	138 (73%)*	107 (57%)
Transplant year		1989-2017	1995-2018	1995-2018
Donor status	Live	18 (38)	88 (47)	88 (47)
	Cadaveric	29 (62)	100 (53)	100 (53)
Donor age		41 (30-57)	51 (40-61)	51 (43-59)
HLA mismatch	1	5 (11)	11 (6)	27 (14)
	2	9 (20)	43 (23)	42 (22)
	3	24 (53)	89 (47)	82 (44)
	4	7 (16)	45 (24)	37 (20)
Pre-emptive	Yes	5 (12)	22 (13)	27 (16)
	No	37 (88)	146 (87)	140 (84)
%cRF		0 (0-0)	0 (0-0)	0 (0-21)*
Cold ischaemic time (mins)		710 (180-960)	469 (180-955)	380 (140-848)
Delayed graft function	Yes	3 (8)	26 (19)	23 (16)
	No	33 (92)	113 (81)	118 (84)

* $p<0.05$ when compared to AA amyloidosis group on pairwise testing.

Table 9.2: Donor and recipient characteristics of renal transplant patients with primary diagnosis of AL amyloidosis compared to matched diabetic nephropathy and adult polycystic kidney disease groups.

		AL Amyloidosis n=51	**Diabetic Nephropathy** n=204	**ADPKD** n=204
Recipient age		61 (57-65)	58 (50-64)	55 (49-62)*
Male		28 (55%)	158 (78%)*	108 (53%)
Transplant year		1999-2018	1998-2018	1999-2018
Donor status	Yes	21 (41)	82 (40)	91 (45)
	No	30 (59)	122 (60)	113 (55)
Donor age		56 (40-64)	51 (38-60)	52 (44-60)
HLA mismatch	1	4 (9)	21 (10)	19 (9)
	2	11 (25)	37 (18)	40 (20)
	3	22 (50)	98 (48)	94 (46)
	4	7 (16)	48 (24)	51 (25)
Pre-emptive	Yes	3 (6)	17 (8)	23 (11)
	No	47 (94)	187 (92)	181 (89)
%cRF		0 (0-0)	0 (0-0)	0 (0-28)
Cold ischaemic time (mins)		757 (187-1142)	664 (250-980)	581 (197-954)
Delayed graft function	Yes	10 (29)	43 (25)	41 (24)
	No	25 (71)	128 (75)	128 (76)

* $p<0.05$ when compared to AL amyloidosis group on pairwise testing.

Survival functions were estimated according to the KM method, with groups compared using the log-rank test. Cox proportion hazard regression analysis was used to estimate hazard ratios for death and graft loss for patient and donor variables. Unrelated variables significant at the 10% level were included in multivariable analyses; where variables were known to be strongly correlated, the most predictive variable on univariate analysis was used in the multivariable

analysis. Results are expressed as hazard ratios with calculated 95% CI. A p value of < 0.05 was considered significant.

Patient characteristics are presented as median (IQR) or number (percentage) unless otherwise stated. All data analysis was performed in SPSS (IBM Corp, 2017), with graphs generated in GraphPad Prism Version 5.03.

Results

Baseline characteristics of participants

Ninety-nine patients, 48 with AA and 51 with AL amyloidosis, underwent renal transplantation (Table 9.3). This cohort comprised 7% and 17% of all ESRD patients due to AL and AA amyloidosis attending the NAC during the study period respectively.

Table 9.3: Patient characteristics at diagnosis of AL and AA amyloidosis

		AL Amyloidosis n=51	**AA amyloidosis** n=48
Age, years (Median, IQR)		55 (50-59)	38 (27-46)
Male gender, n (%)		28 (55%)	27 (56%)
Caucasian Ancestry, n (%)		48 (94%)	40 (83%)
Year of amyloid diagnosis		1987 - 2015	1988 - 2013
eGFR at diagnosis, n (%)	<15 ml/min	16 (31%)	18 (38%)
	15-30 ml/min	12 (24%)	7 (15%)
	30-60 ml/min	7 (14%)	7 (15%)
	>60 ml/min	16 (31%)	16 (33%)
24hr Urine Protein (g) (Median, IQR)		9.2 (5.8-11.9)	5.35 (2.5-7.1)
Time to eGFR<15mls/min (yr) (Median, IQR)		1.0 (0 -2.9)	1.1 (0-6.4)
Organ amyloid*, n (%)	Liver	26 (51%)	13 (29%)
	Spleen	44 (86%)	45 (100%)
	Heart	11 (22%)	0 (0%)
Amyloid load at Diagnosis, n (%)	Small	20 (39%)	11 (23%)
	Moderate	16 (31%)	27 (56%)
	Large	15 (29%)	10 (21%)
NT-proBNP (ng/L) (Median, IQR)		1158 (389-6596)	
IVSd (mm) (Median, IQR)		11 (10-12)	
LVEF (%) (Median, IQR)		60 (57-63)	
NYHA Class, n (%)	1	22 (54%)	
	2	19 (46%)	
Underlying disease, n (%)			
Inflammatory arthritis			20 (42)
Hereditary periodic fever syndrome			7 (15)
Chronic Infection			7 (15)
Inflammatory bowel disease			5 (10)
Castleman's disease			1 (2)
Unknown			8 (17)

*Organ amyloid determined by SAP scintigraphy for liver and spleen, and echocardiography for heart.

Treatment of underlying condition

In the AL amyloidosis group first line treatment was with a thalidomide regimen in 20 (39%) patients, a bortezomib regimen in 5 (10%), a vincristine regimen in 14 (27%), and with melphalan-dexamethasone followed by HDM-ASCT in 5 (10%). At the time of renal transplantation, no patients were on maintenance chemotherapy, 27 (53%) had received two or more prior lines of therapy, with 12 (24%) having undergone HDM-ASCT. In the AA amyloidosis group, 12 (25%) received chlorambucil prior to transplantation, 5 (10%) anakinra, 2 (4%) tocilizumab, 3 (6%) infliximab, 5 (10%) etanercept, 5 (10%) another biologic, and 6 (13%) colchicine, whilst 10 (21%) received supportive therapy only.

Patient survival

Median follow up post-transplant among 99 transplant recipients with a primary diagnosis of AA or AL amyloidosis was 6.1 years and at the time of censor, 39 (39%) patients had died (Table 9.4). One-, 5-, and 10-year unadjusted patient survival from renal transplantation respectively was 96%, 84% and 66% in AA amyloidosis and 96%, 79% and 39% in AL amyloidosis.

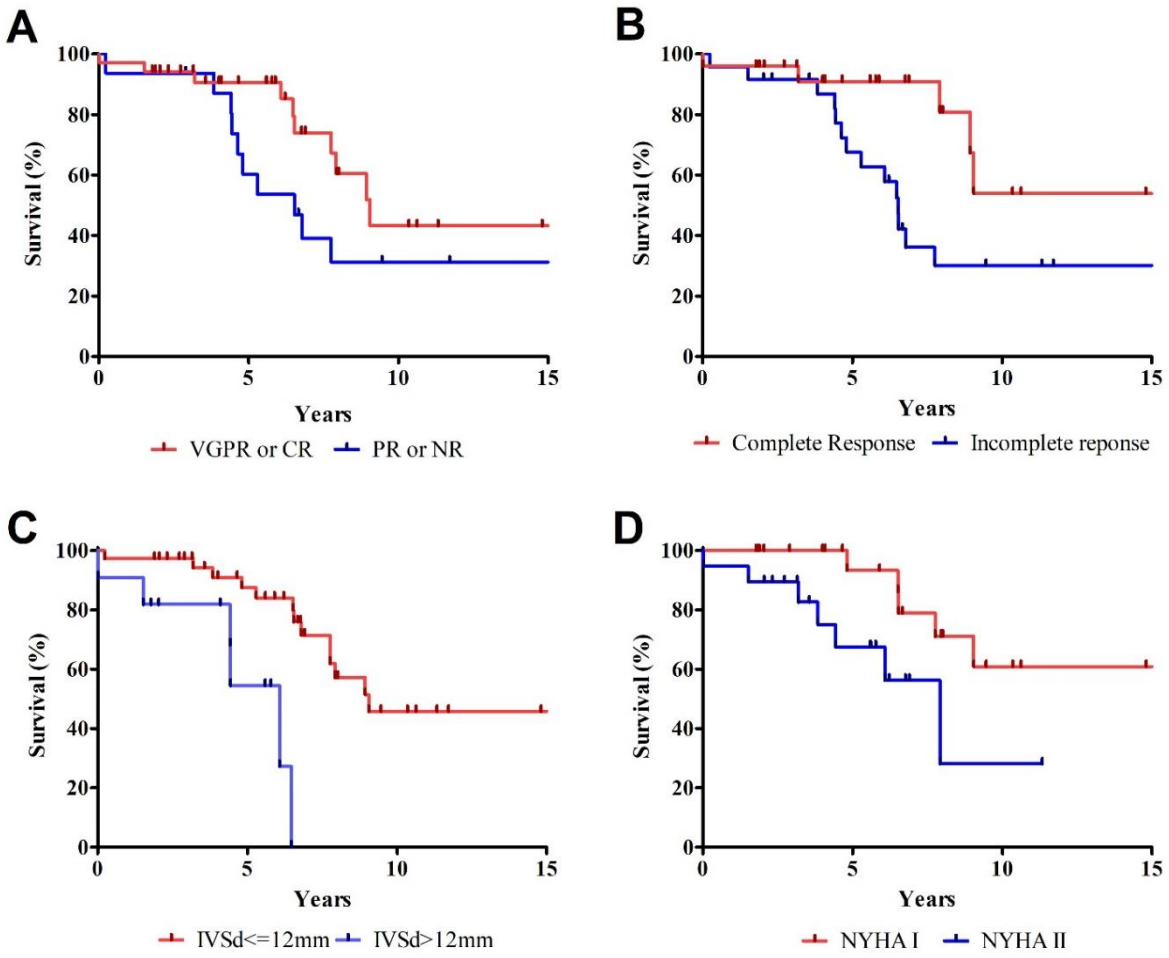
Table 9.4: Transplant details and outcomes in AL and AA amyloidosis

	AL amyloidosis n=51	**AA amyloidosis** n=48
Age at Transplantation (yr)	61 (57-65)	44 (34-53)
Follow-up post-transplant (yr) (Median, IQR)	5.9 (3.6-8.0)	6.7 (4.7-12.5)
Immunosuppression regimen*		
CNI / MMF / Steroids	18 (35%)	25 (52%)
CNI / MMF	10 (20%)	6 (13%)
CNI / Steroids	5 (10%)	7 (15%)
CNI / Azathioprine / Steroids	4 (8%)	1 (2%)
MMF / Steroids	0 (0%)	1 (2%)
CNI only	3 (6%)	1 (2%)
Other	4 (8%)	0 (0%)
Unknown	7 (14%)	7 (15%)
Recurrent amyloid (n)	7 (14%)	9 (20%)
Time to Recurrence (yr) (Median, IQR)	4.5 (3.6-6.3)	4.0 (2.3-9.1)
Death (n)	20 (39%)	19 (40%)
Cause of graft loss (n)		
Death with functioning graft	18	13
Recurrent amyloid	1	4
Primary non function	1	
Post-operative complications	1	1
Renal artery thrombosis		1
Acute rejection		1
Chronic allograft nephropathy		1
Multifactorial		1

*Immunosuppression on first visit to the NAC after renal transplantation. If visit occurred over two years following transplantation immunosuppression was recorded as unknown.

In the AL amyloidosis group, two patients died within three months of transplantation, one due to post-operative complications and the other from cardiac failure. Pre-transplantation IVSd, LVPW thickness, NYHA class, and the presence of a serum paraprotein, predicted mortality on univariate analysis, whilst being in a haematologic CR predicted survival (Table 9.5; Figure 1A, 1C, 1D); haematologic VGPR or CR vs PR or NR did not predict survival in univariate analysis (Figure 1B). Pre-transplant IVSd > 12 mm and being in a haematologic CR were independent predictors of mortality and survival respectively (Table 9.5). When haematologic CR was replaced in the multivariable analysis by haematologic VGPR or CR, this also predicted survival (HR 0.07 [0.01-0.64]; p=0.018). Of note, serum paraprotein, LVPW and NYHA, all of which were significant in univariable analyses, were not included in the multivariable analysis due to their strong association with those that were included.

Figure 9.1: Patient survival post-transplant in AL amyloidosis



Patient survival post-transplant in AL amyloidosis stratified by: A) Pre-transplant haematologic response divided into VGPR or CR versus PR or NR (p=0.12), B) CR vs VGPR, PR, or NR (p=0.03), C) Pre-transplant IVSd wall thickness (p=0.002), D) Pre-transplant NYHA heart failure class (p<0.05).

Table 9.5: Cox regression analyses of predictors of patient survival and death censored graft survival following renal transplantation in AL amyloidosis

	Patient Survival				**Graft Survival**	
	Univariable		Multivariable		Univariable	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
IVSd >12mm*	6.8 (2.0-23)	**0.002**	26.6 (1.5-485)	**0.03**	0.04 (n/a)	0.6
IVSd*	1.4 (1.1-1.8)	**0.006**			0.9 (0.5-1.7)	0.8
LVPW*	1.4 (1.1-1.8)	**0.01**			1.2 (0.7-2.1)	0.6
NYHA*	3.4 (1.0-11.1)	**0.05**			0.7 (0.1-7.8)	0.8
Amyloid echocardiogram	2.5 (0.9-7.0)	0.1			0.04 (n/a)	0.6
LVEF*	1.0 (0.9-1.0)	0.4			1.0 (0.8-1.2)	0.7
NT-proBNP*	1.0 (1.0-1.0)	0.9			1.0 (1.0-1.0)	0.7
CR*	0.3 (0.1-0.9)	**0.03**	0.1 (0.0-0.7)	**0.02**	0.02 (0.0-158)	0.4
CR or VGPR*	0.5 (0.2-1.3)	0.2			0.9 (0.1-9.9)	0.9
>1 treatment line*	0.8 (0.3-2.0)	0.7			57 (0.0-6.4x10^5)	0.4
Serum PP*	2.9 (1.0-7.9)	**0.04**			0.03 (0.00-9069)	0.6
ASCT	0.6 (0.2-1.7)	0.3			1.4 (0.1-15)	0.8
Amyloid recurrence	0.6 (0.2-2.2)	0.5			2.4 (0.2-27)	0.5
Haematological relapse**	1.0 (0.4-2.7)	1.0			0.03 (0.0-976)	0.5
Age at diagnosis	1.1 (1.0-1.1)	0.2			1.1 (0.9-1.3)	0.6
Recipient age	1.0 (1.0-1.1)	0.3	0.9 (0.8-1.0)	0.2	1.1 (0.9-1.3)	0.4
Live donor	0.7 (0.3-1.9)	0.5	2.8 (0.01-601)	0.7	0.0 (0.0-283)	0.4
Donor age	1.0 (1.0-1.0)	0.9	1.0 (1.0-1.1)	0.5	1.1 (1.0-1.2)	0.2
HLA group	1.2 (0.7-2.1)	0.5	1.7 (0.5-5.7)	0.4	3.0 (0.5-17)	0.2
cRF	1.0 (1.0-1.0)	0.3	1.0 (0.9-1.0)	0.09	1.0 (1.0-1.1)	0.5
CIT (mins)	1.0 (1.0-1.0)	0.3	1.0 (1.0-1.0)	0.4	1.0 (1.0-1.0)	0.2
DGF	1.3 (0.5-3.8)	0.6	0.7 (0.1-5.6)	0.8	366 (n/a)	0.4
Pre-emptive	2.2 (0.3-17.5)	0.5	0.0 (0.0-n/a)	1.0	0.05 (n/a)	0.8

CIT – cold ischaemic time. *Evaluated pre-renal transplantation. **Haematological relapse after transplantation requiring treatment. When 'CR' is replaced by 'CR or VGPR' in the multivariable it predicts survival (HR 0.07 [0.01-0.64]; p=0.018).

In the AL amyloidosis cohort, patient survival following transplants performed after 2007, when bortezomib was approved for the treatment of multiple myeloma in the UK, was not longer than transplants performed before 2007 (HR pre 2007 vs post 2007: 1.63 [0.67-3.95]; p=0.284); the same was found in DN (HR 1.38 [0.73-2.61]; p=0.323) and ADPKD (HR 1.94 [0.79-4.81];p=0.151).

In the AA amyloidosis group, there was no significant association between mortality and cause of chronic inflammation, amyloid load by SAP scintigraphy pre transplant, recurrence of amyloid, median SAA in year pre-transplant (10 [5-27] mg/L) or median SAA post-transplant (9 [6-19] mg/L; Table 9.6).

Table 9.6: Univariable Cox regression analyses of predictors of patient survival and death censored graft survival following renal transplantation in AA amyloidosis.

	Patient Survival		**Death Censored Graft Survival**	
	HR	p-value	HR	p-value
Cause of Inflammation				
Inflammatory arthritis	1		1	
Recurrent infection	1.81 (0.47-6.97)	0.4	1.00 (0.12-8.27)	1.0
Unknown	1.51 (0.38-5.96)	0.6	1.00 (0.13-7.97)	1.0
Inflammatory bowel disease	0.58 (0.071-4.65)	0.6	1.00 (0.12-8.26)	1.0
Periodic fever syndrome	0.70 (0.19-2.60)	0.6	1.00 (0.15-6.52)	1.0
Large amyloid load	0.57 (0.073-4.47)	0.6	1.02 (0.12-8.53)	1.0
SAA year pre-transplant (mg/L)	0.99 (0.97-1.01)	0.2	1.01 (1.00-1.02)	0.07
Recurrence	1.10 (0.38-3.22)	0.9	2.08 (0.55-7.89)	0.3
SAA post-transplant (mg/L)	1.01 (0.99-1.04)	0.3	0.99 (0.95-1.04)	0.8

SAA > 10 mg/L pre-transplant represents median SAA concentration in year prior to renal transplantation; SAA post-transplant represents median concentration from renal transplantation to censor.

Renal allograft survival

Overall, there were 31 deaths with a functioning renal allograft and 12 (3 AL, 9 AA) further renal allograft losses among patients with amyloidosis (Table 9.4). Seven patients (13.7%) had recurrence of AL amyloid in the renal allograft occurring a median of 4.5 years post-transplantation, although recurrent amyloid was the primary cause of renal allograft failure in only one such patient. Pre-transplantation haematologic responses in those seven patients were as follows; three had CR, two VGPR, and two PR. The three patients who were in haematologic CR pre-transplantation all had a haematologic relapse post-transplant; 2 received further chemotherapy; the other patient did not receive chemotherapy in view of a very low

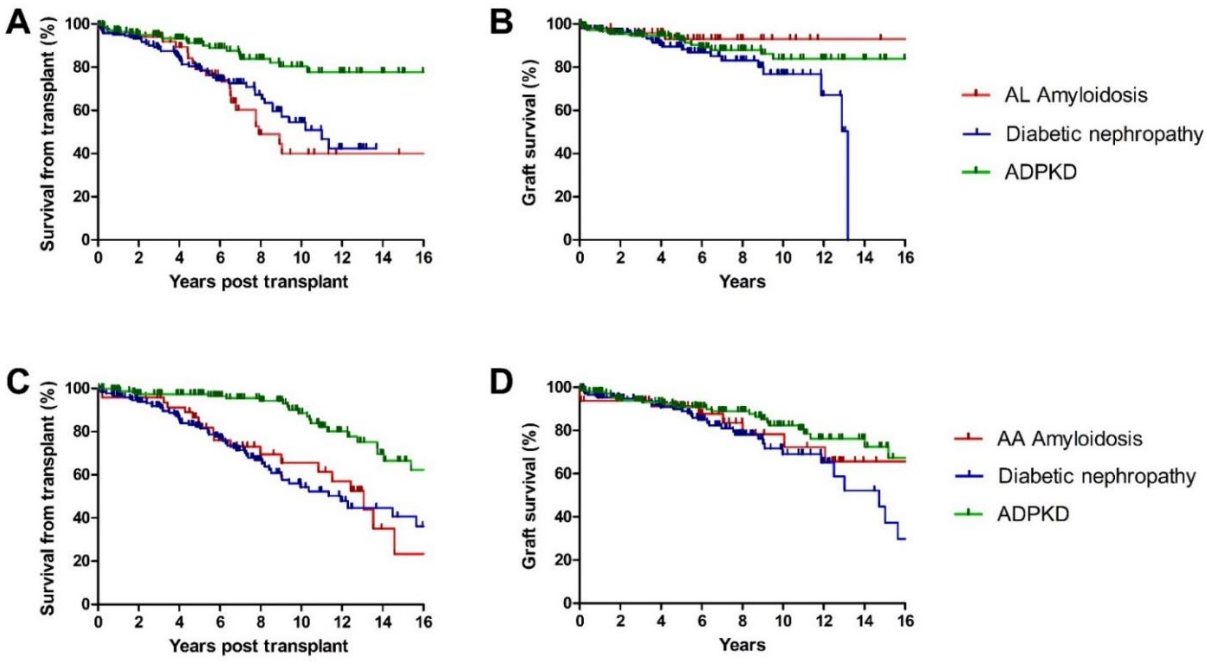
level haematologic relapse, a stable eGFR with proteinuria <0.6g, and a poor performance status. Following transplant, a total of 11 patients required further chemotherapy for haematologic relapse, and one patient underwent autologous stem cell transplantation; 4 (33%) of these had amyloid in their allograft. Three of these patients commenced maintenance chemotherapy following transplantation, with regimens including daratumumab, lenalidomide and ixazomib.

In the AA amyloidosis cohort, nine patients (19%) had recurrent amyloid in their allograft occurring at a median of 4.0 years post-transplant. Recurrent amyloid was the primary cause of graft failure in 4 (44%) of these patients, occurring a median of 4.3 years after recurrence. Median (IQR) SAA levels post-transplant were 26 (11-36) mg/L and 9 (6-15) mg/L in those with and without recurrence of amyloid in the renal allograft respectively (Mann Whitney U test, p=0.007); the median SAA from transplant to graft loss in the 4 patients who lost their allograft with recurrence was 21 (9-39) mg/L, whilst their median SAA in the year prior to transplantation was 38 (11-152) mg/L. Of note, recurrence in the renal allograft occurred in 4 (57%) hereditary periodic fever syndrome patients and caused graft loss in 3/4 such cases, a median of 8 years post-transplantation. They also had the highest median SAA post-transplant 20mg/L, compared to 9.5mg/L in inflammatory arthritis patients, 6.5mg/L recurrent infection, 10mg/L cause unknown and 9mg/L in inflammatory bowel disease. Following transplantation 6 (13%) patients received treatment with anakinra, 4 (8%) with tocilizumab, 1 (2%) infliximab, 4 (8%) etanercept, 1 (2%) adalimumab, 3 (6%) colchicine, and 1 (2%) chlorambucil, whereas 26 (54%) received no anti-inflammatory therapy other than their standard transplant immunosuppression.

Comparison to matched ADPKD and DN groups

Patient survival following renal transplantation was poorer in AL and AA amyloidosis than in matched ADPKD patients ($p<0.001$), but comparable with matched DN patients (Table 9.7, Figure 2A and 2C). Death censored allograft survival was comparable between all groups ($p>0.05$, Table 9.7, Figure 2B and 2D).

Figure 9.2: Patient and death censored allograft survival following transplantation.



Kaplan-Meier estimates of patient and death censored allograft survival following transplantation for AL (A and B) and AA (C and D) amyloidosis compared to matched diabetic nephropathy and ADPKD patients.

Table 9.7: Patient and death-censored renal allograft survival from renal transplantation in AL amyloidosis compared to matched patients with DN and ADPKD.

Patient survival post transplantation

	Years (95% CI)	HR (95% CI)	p-value
AL	7.9 (5.5-10.3)	1	
DN[1]	11.0 (8.7-13.3)	0.85 (0.50-1.44)	0.5
ADPKD[1]	Unable	0.32 (0.17-0.59)	**<0.001**
AA	13.1 (10.5-45.7)	1	
DN[2]	11.4 (8.5-14.2)	1.14 (0.67-1.92)	0.6
ADPKD[2]	Unable	0.32 (0.18-0.60)	**<0.001**

Renal allograft survival

	Years (95% CI)	HR (95% CI)	p-value
AL	Unable	1	
DN[1]	12.9 (12.3-13.5)	3.1 (0.9-10.3)	0.07
ADPKD[1]	Unable	1.8 (0.5-6.1)	0.4
AA	Unable	1	
DN[2]	14.7 (11.3-18.1)	1.5 (0.7-3.0)	0.3
ADPKD[2]	Unable	0.7 (0.4-1.6)	0.4

AL: AL amyloidosis; AA: AA amyloidosis. [1]Diabetic nephropathy and ADPKD groups matched to AL amyloidosis group (Table 9.2). [2]Diabetic nephropathy and ADPKD groups matched to AA amyloidosis group (Table 9.1). Unable – unable to estimate due to low number of events

Discussion

This study demonstrates that selected patients with ESRD from systemic AA and AL amyloidosis achieve post transplantation patient survival comparable to DN but inferior to ADPKD; death censored allograft survival was comparable to DN and ADPKD. Recurrence of amyloid in the renal allograft did not necessarily preclude lengthy graft survival. Outcomes following renal transplantation in systemic amyloidosis have undoubtedly improved, likely reflecting increasingly effective suppression of the amyloid fibril precursor protein with chemotherapy in AL amyloidosis and biologic therapies in AA amyloidosis.[10,95,97,98,180]

Among patients with AL amyloidosis, pre-transplantation IVSd was the strongest predictor of mortality, and pre-transplant haematologic VGPR or CR was the strongest predictor of survival. These findings are consistent with published literature in AL amyloidosis demonstrating the prognostic importance of cardiac involvement and haematological response to chemotherapy.[18,189,190] Cardiac amyloidosis is defined by international amyloidosis consensus criteria by an IVSd > 12 mm, and it is noteworthy that despite its lack of specificity in the context of CKD, where an IVSd > 12 mm without cardiac amyloidosis is common, it predicted patient survival.[60,191,192] Achieving a haematologic VGPR or CR pre-transplantation was associated with prolonged patient survival post transplantation in the AL amyloidosis cohort, corroborating the recently published findings of the Boston Amyloidosis Centre, in which patients who had achieved a haematologic CR or VGPR fared better with renal transplantation than those in PR/NR.[98] It is interesting to hypothesize that with a larger cohort, there may be further stratification of renal transplant outcomes according to each category of haematologic response, analogous to outcomes in AL amyloidosis generally.[54]

In this cohort of patients with AL amyloidosis, amyloid recurrence in the renal allograft was a contributor to only one graft loss, despite eleven patients receiving post-transplant chemotherapy. It is interesting that amyloid recurrence did not predict graft loss in this patient cohort, likely reflecting the gradual nature of amyloid accumulation among patients who remain in a degree of haematologic response albeit not in haematologic CR. Recurrence of amyloid in the renal allograft was more common in AA amyloidosis than in AL amyloidosis and associated with persistent elevation of SAA concentration, most evident among patients with hereditary periodic fever syndromes underlying their AA amyloidosis. Once again, recurrence of amyloid in the renal allograft did not predict graft loss in our cohort which may reflect a 'threshold effect' in which reasonable, albeit incomplete, suppression of inflammation as evidenced by moderately elevated SAA concentration (median (IQR) 26 mg/L (11-36)) leads to very gradual AA amyloid accumulation, without a rapid decline in allograft function. A larger study population with longer follow up may highlight more subtle implications of amyloid allograft recurrence. Overall, the time course and impact on graft survival of disease recurrence in amyloidosis appear to differ substantially from those reported in other causes of ESRD, although this warrants further study.[184,193]

We did not find a significant difference in post-transplantation survival between patients with AL amyloidosis transplanted before and after 2007, when bortezomib was approved in the UK. This is in contrast to Angel-Korman et al who found improved survival in patients transplanted after 2007.[98] This may reflect smaller patient numbers in our pre-2007 cohort (14 versus 24), or a broadening of patient selection criteria with a higher median age, IVSd, NT-proBNP, and higher

proportion with echocardiographic evidence of cardiac amyloidosis among patients transplanted from 2007 onwards.

It is important to acknowledge that this amyloidosis cohort represents a carefully selected group of patients who were deemed by a multi-disciplinary team to benefit from renal transplantation, comprising only 7% and 17% of dialysis-dependent AL and AA amyloidosis patients attending our national centre respectively. This highlights the fact that the majority of patients with systemic AA and AL amyloidosis continue to be considered unsuitable for kidney transplantation, usually due to severe extra renal organ involvement, functional disability or advancing age. Transplantation was more likely in AA compared to AL amyloidosis, likely reflecting the younger age and less frequent cardiac involvement.[22]

Limitations of our study include the inherent difficulties associated with retrospective cohort matching. There were significant differences in gender and recipient age, whilst recipient ethnicity and dialysis vintage data were unavailable; all of which are known to affect transplant outcomes. The transplantation date time frame used to select control and amyloidosis patients were different due to limitations in NHSBT data availability and a desire to maximise amyloidosis patient numbers for powered analyses; despite this comparison analysis showed no significant differences in transplantation year between patient groups. A median follow up of 6.1 years in the amyloidosis cohorts also limits our ability to comment on longer term renal transplant outcomes.

In summary, this study demonstrates that selected individuals requiring RRT due to AA and AL amyloidosis achieve comparable outcomes following renal transplantation to DN. This study confirms previous evidence that IVSd thickness and achieving a deep haematologic response pre-transplant predict mortality and

survival respectively in AL amyloidosis. It also reaffirms that suppression of SAA concentration in AA amyloidosis reduces the risk of amyloid recurrence in the renal allograft, although our data indicates that gradual amyloid accumulation post-transplant in association with incomplete suppression of the respective fibril precursor protein does not preclude excellent graft and patient survival.

General Conclusions

The studies reported in this thesis contribute to an improved understanding of two areas of systemic amyloidosis:

1. The natural history of ATTR-CM, including early in the disease course
2. Patient outcomes in renal AL amyloidosis and patient selection for kidney transplantation in renal amyloidosis

At the onset of the study, interest in ATTR-CM was increasing dramatically. Diagnoses were rising exponentially driven by advances in diagnostics, such as CMR imaging, bone scintigraphy and the widespread adoption of the non-biopsy criteria, alongside significant interest in therapeutics following the ATTR-ACT study confirming improved survival with tafamidis, and a range of novel RNA based therapies entering clinical trials.[1-6,61,124,127] There were no validated markers of disease progression and questions regarding the spectrum of 'disease' which ranges from >25% of >80 year old male hearts having ATTR deposition at autopsy despite the majority not being symptomatic in life, to being present in >10% of patients with heart failure and preserved ejection fraction. This posed questions in evaluating new therapies, with trials using non-validated markers of disease progression such as 6MWT, and having arbitrary NT-proBNP cut-offs for trial inclusion to include patients considered to have a certain degree of ATTR-CM felt more likely to benefit from treatment. Indeed, ATTRibute-CM did not meet its primary end point of 6MWT at 12-month interim analysis in 2022 despite returning a positive outcome in 2024.

The data reported in Chapter 3 highlight the utility of NAC ATTR stage throughout the disease course in ATTR-CM and the importance of increasing from NAC ATTR stage I to II which strongly predicted mortality. This is informative to

physicians and supports patients understanding of the severity of their disease. As a marker of disease progression there are limitations as patients with NAC ATTR Stage III cannot progress further, and it does not differentiate between the increasing number of patients diagnosed with NAC ATTR Stage I, which encompasses patients with a normal NT-proBNP up to one of 3000ng/L. Chapter 4 built on these findings with a more extensive analysis of potential markers of disease progression including functional assessments, biomarkers and imaging findings. This highlighted increasing NT-proBNP as the strongest independent predictor of mortality at 12 months.

Throughout Chapter 3 and 4, it became clear that NAC ATTR stage I was a heterogeneous group warranting further study and that NT-proBNP was the strongest predictor of disease severity and progression. Clinical trials were utilizing NT-proBNP based eligibility criteria including adjustments for atrial fibrillation and in some, a minimum diuretic dose requirement. Chapter 5 focussed on a group of patients considered to have early stage ATTR-CM (NT-proBNP ≤500ng/L or ≤1000ng/L in the presence of atrial fibrillation and a loop diuretic requirement equivalent to <0.75mg/kg of furosemide) who would be considered ineligible for the majority of ATTR-CM clinical trials. This study demonstrates that while these patients do have a good prognosis, with no demonstrable reduction in survival compared to matched controls, they encountered progressive cardiovascular morbidity suggesting a potential benefit from disease modifying therapy.

Chapter 6 highlights a group of patients with the p.Ser97Tyr TTR gene variant who present atypically with low level cardiac uptake on radionuclide scintigraphy. Awareness of this group is important to avoid misdiagnosis as cardiac AL amyloidosis and subsequent incorrect treatment. It also provides a potential avenue

for study into understanding why radionuclide cardiac uptake is a feature in ATTR-CM.

Chapter 7 presents the high level of healthcare resource utilization by patients with ATTR-CM prior to diagnosis, and the range of different specialties involved in their care. This highlights opportunities for early diagnosis and suggests a significant opportunity to reduce healthcare utilization with earlier diagnosis and treatment.

Chapters 8 and 9 focussed on renal amyloidosis. Chapter 8 demonstrated a significant improvement in patient and renal survival across the last two decades. This trend is likely to continue with increased follow up of more recently diagnosed patients in whom daratumumab is now being widely used, and with further novel agents in clinical trials. It is noticeable however improvements in renal survival are significantly more noticeable in patients without advanced disease at diagnosis. Whether direct anti-amyloid therapies will improve renal survival in patients with advanced disease at diagnosis remains an area for future study. Chapter 9 highlights that kidney transplant survival for end stage renal failure secondary to either renal AA or AL amyloidosis, in selected individuals, is comparable to patients with diabetic nephropathy. This likely relates to improved chemotherapeutic options at diagnosis and relapse leading to deeper, more durable haematologic responses in AL amyloidosis, and biologic therapies allowing improved control of underlying inflammation in AA amyloidosis. It is likely more patients with renal amyloidosis will benefit from kidney transplantation going forward.

References

1. Ravichandran S, Lachmann HJ, Wechalekar AD. Epidemiologic and Survival Trends in Amyloidosis, 1987–2019. New England Journal of Medicine 2020;382:1567-1568. doi: 10.1056/NEJMc1917321
2. Gillmore Julian D, Maurer Mathew S, Falk Rodney H, et al. Nonbiopsy Diagnosis of Cardiac Transthyretin Amyloidosis. Circulation 2016;133:2404-2412. doi: 10.1161/CIRCULATIONAHA.116.021612
3. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. New England Journal of Medicine 2018;379:11-21. doi: 10.1056/NEJMoa1716153
4. Maurer MS, Schwartz JH, Gundapaneni B, et al. Tafamidis Treatment for Patients with Transthyretin Amyloid Cardiomyopathy. 2018;379:1007-1016. doi: 10.1056/NEJMoa1805689
5. Benson MD, Waddington-Cruz M, Berk JL, et al. Inotersen Treatment for Patients with Hereditary Transthyretin Amyloidosis. New England Journal of Medicine 2018;379:22-31. doi: 10.1056/NEJMoa1716793
6. Gillmore JD, Garcia-Pavia P, Grogan M, et al. Abstract 14214: ATTRibute-CM: A Randomized, Double-Blind, Placebo-Controlled, Multi-Center, Global Phase 3 Study of AG10 in Patients With Transthyretin Amyloid Cardiomyopathy (ATTR-CM). Circulation 2019;140:A14214-A14214. doi:10.1161/circ.140.suppl_1.14214
7. Tanskanen M, Peuralinna T, Polvikoski T, et al. Senile systemic amyloidosis affects 25% of the very aged and associates with genetic variation in alpha2-macroglobulin and tau: a population-based autopsy study. Ann Med 2008;40:232-239. doi: 10.1080/07853890701842988
8. Mirzoyev SA, Edwards WD, Mohammed SF, et al. Abstract 17926: Cardiac Amyloid Deposition is Common in Elderly Patients with Heart Failure and Preserved Ejection Fraction. Circulation 2010;122:A17926-A17926. doi: doi:10.1161/circ.122.suppl_21.A17926

9. Seldin DC, Choufani EB, Dember LM, et al. Tolerability and Efficacy of Thalidomide for the Treatment of Patients with Light Chain–Associated (AL) Amyloidosis. Clinical Lymphoma 2003;3:241-246. doi: https://doi.org/10.3816/CLM.2003.n.005
10. Kastritis E, Anagnostopoulos A, Roussou M, et al. Treatment of light chain (AL) amyloidosis with the combination of bortezomib and dexamethasone. Haematologica 2007;92:1351-1358. doi: 10.3324/haematol.11325
11. Palladini G, Kastritis E, Maurer MS, et al. Daratumumab plus CyBorD for patients with newly diagnosed AL amyloidosis: safety run-in results of ANDROMEDA. Blood 2020;136:71-80. doi: 10.1182/blood.2019004460
12. Kaufman GP, Schrier SL, Lafayette RA, et al. Daratumumab yields rapid and deep hematologic responses in patients with heavily pretreated AL amyloidosis. Blood 2017;130:900-902. doi: 10.1182/blood-2017-01-763599
13. Law S, Fontana M, Gillmore JD. Advances in Diagnosis and Treatment of Cardiac and Renal Amyloidosis. Cardiology Clinics 2021;39:389-402. doi: https://doi.org/10.1016/j.ccl.2021.04.010
14. Law S, Gillmore JD. When to Suspect and How to Approach a Diagnosis of Amyloidosis. Am J Med 2022;135 Suppl 1:S2-s8. doi: 10.1016/j.amjmed.2022.01.004
15. Wechalekar AD, Gillmore JD, Hawkins PN. Systemic amyloidosis. The Lancet 2016;387:2641-2654. doi: https://doi.org/10.1016/S0140-6736(15)01274-X
16. Lachmann HJ, Hawkins PN. Systemic amyloidosis. Curr Opin Pharmacol 2006;6:214-220. doi: 10.1016/j.coph.2005.10.005
17. Manwani R, Cohen O, Sharpley F, et al. A prospective observational study of 915 patients with systemic AL amyloidosis treated with upfront bortezomib. Blood 2019;134:2271-2280. doi: 10.1182/blood.2019000834
18. Wechalekar AD, Schonland SO, Kastritis E, et al. A European collaborative study of treatment outcomes in 346 patients with cardiac stage III AL amyloidosis. Blood 2013;121:3420-3427. doi: 10.1182/blood-2012-12-473066

19. Gillmore JD, Damy T, Fontana M, et al. A new staging system for cardiac transthyretin amyloidosis. European Heart Journal 2017;39:2799-2806. doi: 10.1093/eurheartj/ehx589
20. Merlini G, Seldin DC, Gertz MA. Amyloidosis: Pathogenesis and New Therapeutic Options. Journal of Clinical Oncology 2011;29:1924-1933. doi: 10.1200/jco.2010.32.2271
21. Sipe JD, Benson MD, Buxbaum JN, et al. Nomenclature 2014: Amyloid fibril proteins and clinical classification of the amyloidosis. Amyloid 2014;21:221-224. doi: 10.3109/13506129.2014.964858
22. Lachmann HJ, Goodman HJB, Gilbertson JA, et al. Natural History and Outcome in Systemic AA Amyloidosis. New England Journal of Medicine 2007;356:2361-2371. doi: 10.1056/NEJMoa070265
23. Pinney JH, Smith CJ, Taube JB, et al. Systemic amyloidosis in England: an epidemiological study. British journal of haematology 2013;161:525-532. doi: 10.1111/bjh.12286
24. Quock TP, Yan T, Chang E, Guthrie S, Broder MS. Epidemiology of AL amyloidosis: a real-world study using US claims data. Blood advances 2018;2:1046-1053. doi: 10.1182/bloodadvances.2018016402
25. Maceira Alicia M, Joshi J, Prasad Sanjay K, et al. Cardiovascular Magnetic Resonance in Cardiac Amyloidosis. Circulation 2005;111:186-193. doi: 10.1161/01.CIR.0000152819.97857.9D
26. Perugini E, Guidalotti PL, Salvi F, et al. Noninvasive Etiologic Diagnosis of Cardiac Amyloidosis Using 99mTc-3,3-Diphosphono-1,2-Propanodicarboxylic Acid Scintigraphy. Journal of the American College of Cardiology 2005;46:1076-1084. doi: https://doi.org/10.1016/j.jacc.2005.05.073
27. Sattianayagam PT, Gibbs SDJ, Rowczenio D, et al. Hereditary lysozyme amyloidosis – phenotypic heterogeneity and the role of solid organ transplantation. Journal of

Internal Medicine 2012;272:36-44. doi: https://doi.org/10.1111/j.1365-2796.2011.02470.x

28. Vaxman I, Gertz M. Recent Advances in the Diagnosis, Risk Stratification, and Management of Systemic Light-Chain Amyloidosis. Acta Haematologica 2019;141:93-106. doi: 10.1159/000495455

29. Rezk T, Lachmann HJ, Fontana M, et al. Cardiorenal AL amyloidosis: risk stratification and outcomes based upon cardiac and renal biomarkers. British Journal of Haematology 2019;186:460-470. doi: 10.1111/bjh.15955

30. Colon W, Kelly JW. Partial denaturation of transthyretin is sufficient for amyloid fibril formation in vitro. Biochemistry 1992;31:8654-8660. doi: 10.1021/bi00151a036

31. Lane T. Natural History, Quality of Life, and Outcome in Cardiac Transthyretin Amyloidosis. Circulation 2019;140:16-26. doi: https://doi.org/10.1161/CIRCULATIONAHA.118.038169

32. Adams D, Ando Y, Beirão JM, et al. Expert consensus recommendations to improve diagnosis of ATTR amyloidosis with polyneuropathy. Journal of Neurology 2020. doi: 10.1007/s00415-019-09688-0

33. Hewitt K, McGuckin M, Giblin G, Murphy L, Joyce E. The Clinical Spectrum of T60a Variant Hereditary Transthyretin Amyloidosis In Ireland. Journal of Cardiac Failure 2020;26:S33. doi: https://doi.org/10.1016/j.cardfail.2020.09.101

34. Rapezzi C, Quarta CC, Riva L, et al. Transthyretin-related amyloidoses and the heart: a clinical overview. Nature Reviews Cardiology 2010;7:398-408. doi: 10.1038/nrcardio.2010.67

35. Ladefoged B, Dybro A, Povlsen JA, et al. Diagnostic delay in wild type transthyretin cardiac amyloidosis - A clinical challenge. Int J Cardiol 2020;304:138-143. doi: 10.1016/j.ijcard.2019.12.063

36. Hester L, Gifkins D, Bellew K, et al. Diagnostic delay and characterization of the clinical prodrome in AL amyloidosis related cardiomyopathy among 1,173 patients

diagnosed between 2001-2018. JACC Heart Failure and Cardiomyopathies 2020;75:900. doi: 10.1016/S0735-1097(20)31527-8

37. Bishop E, Brown EE, Fajardo J, et al. Seven factors predict a delayed diagnosis of cardiac amyloidosis. Amyloid 2018;25:174-179. doi: 10.1080/13506129.2018.1498782

38. Sanchis K, Cariou E, Colombat M, et al. Atrial fibrillation and subtype of atrial fibrillation in cardiac amyloidosis: clinical and echocardiographic features, impact on mortality. Amyloid 2019;26:128-138. doi: 10.1080/13506129.2019.1620724

39. Giancaterino S, Urey MA, Darden D, Hsu JC. Management of Arrhythmias in Cardiac Amyloidosis. JACC Clinical Electrophysiology 2020;6:351-361. doi: doi:10.1016/j.jacep.2020.01.004

40. Shin SC, Robinson-Papp J. Amyloid neuropathies. The Mount Sinai journal of medicine, New York 2012;79:733-748. doi: 10.1002/msj.21352

41. Pinney JH, Whelan CJ, Petrie A, et al. Senile Systemic Amyloidosis: Clinical Features at Presentation and Outcome. Journal of the American Heart Association 2013;2:e000098. doi: doi:10.1161/JAHA.113.000098

42. Martinez-Naharro A, Hawkins PN, Fontana M. Cardiac amyloidosis. Clin Med (Lond) 2018;18:s30-s35. doi: 10.7861/clinmedicine.18-2-s30

43. Rapezzi C, Quarta CC, Guidalotti PL, et al. Usefulness and limitations of 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy in the aetiological diagnosis of amyloidotic cardiomyopathy. European Journal of Nuclear Medicine and Molecular Imaging 2011;38:470-478. doi: 10.1007/s00259-010-1642-7

44. Fontana M, Banypersad SM, Treibel TA, et al. Native T1 Mapping in Transthyretin Amyloidosis. JACC: Cardiovascular Imaging 2014;7:157-165. doi: https://doi.org/10.1016/j.jcmg.2013.10.008

45. Fontana M, Pica S, Reant P, et al. Prognostic Value of Late Gadolinium Enhancement Cardiovascular Magnetic Resonance in Cardiac Amyloidosis. Circulation 2015;132:1570-1579. doi: 10.1161/circulationaha.115.016567

46. Martinez-Naharro A, Kotecha T, Norrington K, et al. Native T1 and Extracellular Volume in Transthyretin Amyloidosis. JACC Cardiovasc Imaging 2019;12:810-819. doi: 10.1016/j.jcmg.2018.02.006

47. Kapoor M, Rossor AM, Jaunmuktane Z, Lunn MPT, Reilly MM. Diagnosis of amyloid neuropathy. Practical Neurology 2019;19:250-258. doi: 10.1136/practneurol-2018-002098

48. Hawkins PN, Lavender JP, Pepys MB. Evaluation of Systemic Amyloidosis by Scintigraphy with 123I-Labeled Serum Amyloid P Component. New England Journal of Medicine 1990;323:508-513. doi: 10.1056/nejm199008233230803

49. Dungu JN, Valencia O, Pinney JH, et al. CMR-based differentiation of AL and ATTR cardiac amyloidosis. JACC Cardiovasc Imaging 2014;7:133-142. doi: 10.1016/j.jcmg.2013.08.015

50. Boldrini M, Salinaro F, Musca F, et al. An ECG/ECHO comparison between AL and ATTR cardiac amyloidosis at diagnosis. European Heart Journal 2013;34. doi: 10.1093/eurheartj/eht309.P2991

51. Chacko L, Martone R, Bandera F, et al. Echocardiographic phenotype and prognosis in transthyretin cardiac amyloidosis. Eur Heart J 2020;41:1439-1447. doi: 10.1093/eurheartj/ehz905

52. Cheng Z, Zhu K, Tian Z, et al. The findings of electrocardiography in patients with cardiac amyloidosis. Ann Noninvasive Electrocardiol 2013;18:157-162. doi: 10.1111/anec.12018

53. Palladini G, Campana C, Klersy C, et al. Serum N-Terminal Pro–Brain Natriuretic Peptide Is a Sensitive Marker of Myocardial Dysfunction in AL Amyloidosis. Circulation 2003;107:2440-2445. doi: doi:10.1161/01.CIR.0000068314.02595.B2

54. Palladini G, Dispenzieri A, Gertz MA, et al. New Criteria for Response to Treatment in Immunoglobulin Light Chain Amyloidosis Based on Free Light Chain Measurement and Cardiac Biomarkers: Impact on Survival Outcomes. Journal of Clinical Oncology 2012;30:4541-4549. doi: 10.1200/jco.2011.37.7614

55. Palladini G, Dispenzieri A, Gertz MAA, et al. Validation of the Criteria of Response to Treatment In AL Amyloidosis. Blood 2010;116:1364-1364. doi: 10.1182/blood.V116.21.1364.1364

56. Kumar S, Dispenzieri A, Lacy MQ, et al. Revised Prognostic Staging System for Light Chain Amyloidosis Incorporating Cardiac Biomarkers and Serum Free Light Chain Measurements. Journal of Clinical Oncology 2012;30:989-995. doi: 10.1200/jco.2011.38.5724

57. Maurer MS, Bokhari S, Damy T, et al. Expert Consensus Recommendations for the Suspicion and Diagnosis of Transthyretin Cardiac Amyloidosis. Circulation Heart Failure 2019;12:e006075. doi: 10.1161/CIRCHEARTFAILURE.119.006075

58. Koyama J, Ray-Sequin PA, Falk RH. Longitudinal Myocardial Function Assessed by Tissue Velocity, Strain, and Strain Rate Tissue Doppler Echocardiography in Patients With AL (Primary) Cardiac Amyloidosis. Circulation 2003;107:2446-2452. doi: 10.1161/01.CIR.0000068313.67758.4F

59. Pagourelias ED, Mirea O, Duchenne J, et al. Echo Parameters for Differential Diagnosis in Cardiac Amyloidosis: A Head-to-Head Comparison of Deformation and Nondeformation Parameters. Circ Cardiovasc Imaging 2017;10:e005588. doi: 10.1161/circimaging.116.005588

60. Gertz MA, Comenzo R, Falk RH, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. Am J Hematol 2005;79:319-328. doi: 10.1002/ajh.20381

61. Fontana M, Chung R, Hawkins PN, Moon JC. Cardiovascular magnetic resonance for amyloidosis. Heart Failure Reviews 2015;20:133-144. doi: 10.1007/s10741-014-9470-7

62. Karamitsos TD, Piechnik SK, Banypersad SM, et al. Noncontrast T1 Mapping for the Diagnosis of Cardiac Amyloidosis. JACC: Cardiovascular Imaging 2013;6:488-497. doi: https://doi.org/10.1016/j.jcmg.2012.11.013

63. Baggiano A, Boldrini M, Martinez-Naharro A, et al. Noncontrast Magnetic Resonance for the Diagnosis of Cardiac Amyloidosis. JACC Cardiovasc Imaging 2020;13:69-80. doi: 10.1016/j.jcmg.2019.03.026

64. Banypersad SM, Fontana M, Maestrini V, et al. T1 mapping and survival in systemic light-chain amyloidosis. European heart journal 2015;36:244-251. doi: 10.1093/eurheartj/ehu444

65. Martinez-Naharro A, Abdel-Gadir A, Treibel TA, et al. CMR-Verified Regression of Cardiac AL Amyloid After Chemotherapy. JACC Cardiovasc Imaging 2018;11:152-154. doi: 10.1016/j.jcmg.2017.02.012

66. Fontana M, Martinez-Naharro A, Chacko L, et al. Reduction in CMR Derived Extracellular Volume With Patisiran Indicates Cardiac Amyloid Regression. JACC: Cardiovascular Imaging 2021;14:189-199. doi: 10.1016/j.jcmg.2020.07.043

67. Woolen SA, Shankar PR, Gagnier JJ, et al. Risk of Nephrogenic Systemic Fibrosis in Patients With Stage 4 or 5 Chronic Kidney Disease Receiving a Group II Gadolinium-Based Contrast Agent: A Systematic Review and Meta-analysis. JAMA Internal Medicine 2020;180:223-230. doi: 10.1001/jamainternmed.2019.5284

68. Ali A, Turner DA, Rosenbush SW, Fordham EW. Bone Scintigram in Cardiac Amyloidosis: A Case Report. Clinical Nuclear Medicine 1981;6. doi: 10.1097/00003072-198103000-00003

69. Hutt D, McPhillips H, McKnight S, et al. DPD Scintigraphy for diagnosis of amyloidosis in 1191 patients– a single centre experience. Orphanet Journal of Rare Diseases 2015;10:O16-O16. doi: 10.1186/1750-1172-10-S1-O16

70. Visram A, Al Saleh AS, Parmar H, et al. Correlation between urine ACR and 24-h proteinuria in a real-world cohort of systemic AL amyloidosis patients. Blood Cancer J 2020;10:124. doi: 10.1038/s41408-020-00391-2

71. Mendelson L, Sanchorawala V, Connors L, et al. Correlation Between 24-Hour Urine Protein and Random Urine Protein-Creatinine Ratio in Amyloid Light-Chain

Amyloidosis. Kidney medicine, doi: 10.1016/j.xkme.2022.100427. Published online ahead of print.

72. Gillmore JD, Lachmann HJ, Rowczenio D, et al. Diagnosis, pathogenesis, treatment, and prognosis of hereditary fibrinogen A alpha-chain amyloidosis. Journal of the American Society of Nephrology : JASN 2009;20:444-451. doi: 10.1681/ASN.2008060614

73. Rezk T, Gilbertson JA, Rowczenio D, et al. Diagnosis, pathogenesis and outcome in leucocyte chemotactic factor 2 (ALECT2) amyloidosis. Nephrology Dialysis Transplantation 2016;33:241-247. doi: 10.1093/ndt/gfw375

74. Soares SM, Fervenza FC, Lager DJ, et al. Bleeding complications after transcutaneous kidney biopsy in patients with systemic amyloidosis: single-center experience in 101 patients. Am J Kidney Dis 2008;52:1079-1083. doi: 10.1053/j.ajkd.2008.05.022

75. Palladini G, Russo P, Bosoni T, et al. Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. Clin Chem 2009;55:499-504. doi: 10.1373/clinchem.2008.117143

76. Hutchison CA, Harding S, Hewins P, et al. Quantitative Assessment of Serum and Urinary Polyclonal Free Light Chains in Patients with Chronic Kidney Disease. Clin J Am Soc Nephrology 2008;3:1684-1690. doi: 10.2215/CJN.02290508

77. Cohen OC, Sharpley F, Gilbertson JA, et al. The value of screening biopsies in light-chain (AL) and transthyretin (ATTR) amyloidosis. European Journal of Haematology 2020;105:352-356. doi: 10.1111/ejh.13458

78. Gilbertson JA, Hunt T, Hawkins PN. Amyloid Typing: Experience from a Large Referral Centre. In: Picken Md PFMM, Dogan MDPDA, Herrera MDGA, eds. Amyloid and Related Disorders: Surgical Pathology and Clinical Correlations. Totowa, NJ: Humana Press; 2012, p231-238.

79. Rezk T, Gilbertson JA, Mangione PP, et al. The complementary role of histology and proteomics for diagnosis and typing of systemic amyloidosis. The journal of pathology. Clinical research 2019;5:145-153. doi: 10.1002/cjp2.126

80. Vrana JA, Gamez JD, Madden BJ, et al. Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. Blood 2009;114:4957-4959. doi: 10.1182/blood-2009-07-230722

81. Richards DB, Cookson LM, Berges AC, et al. Therapeutic Clearance of Amyloid by Antibodies to Serum Amyloid P Component. New England Journal of Medicine 2015;373:1106-1114. doi: 10.1056/NEJMoa1504942

82. Wechalekar AD, Gillmore JD, Bird J, et al. Guidelines on the management of AL amyloidosis. British Journal of Haematology 2015;168:186-206. doi: https://doi.org/10.1111/bjh.13155

83. Manwani R, Hegenbart U, Mahmood S, et al. Deferred autologous stem cell transplantation in systemic AL amyloidosis. Blood Cancer J 2018;8:101. doi: 10.1038/s41408-018-0137-9

84. Comenzo RL, Reece D, Palladini G, et al. Consensus guidelines for the conduct and reporting of clinical trials in systemic light-chain amyloidosis. Leukemia 2012;26:2317-2325. doi: 10.1038/leu.2012.100

85. Kastritis E, Wechalekar AD, Dimopoulos MA, et al. Bortezomib with or without dexamethasone in primary systemic (light chain) amyloidosis. J Clin Oncol 2010;28:1031-1037. doi: 10.1200/jco.2009.23.8220

86. Kristen AV, Dengler TJ, Hegenbart U, et al. Prophylactic implantation of cardioverter-defibrillator in patients with severe cardiac amyloidosis and high risk for sudden cardiac death. Heart Rhythm 2008;5:235-240. doi: 10.1016/j.hrthm.2007.10.016

87. Coelho T, Maia LF, da Silva AM, et al. Long-term effects of tafamidis for the treatment of transthyretin familial amyloid polyneuropathy. Journal of Neurology 2013;260:2802-2814. doi: 10.1007/s00415-013-7051-7

88. Berk JL, Suhr OB, Obici L, et al. Repurposing Diflunisal for Familial Amyloid Polyneuropathy: A Randomized Clinical Trial. JAMA 2013;310:2658-2667. doi: 10.1001/jama.2013.283815

89. Lohrmann G, Pipilas A, Mussinelli R, et al. Stabilization of Cardiac Function With Diflunisal in Transthyretin (ATTR) Cardiac Amyloidosis. J Card Fail 2020;26:753-759. doi: 10.1016/j.cardfail.2019.11.024

90. Judge DP, Heitner SB, Falk RH, et al. Transthyretin Stabilization by AG10 in Symptomatic Transthyretin Amyloid Cardiomyopathy. JACC 2019;74:285-295. doi: 10.1016/j.jacc.2019.03.012

91. Solomon SD, Adams D, Kristen A, et al. Effects of Patisiran, an RNA Interference Therapeutic, on Cardiac Parameters in Patients With Hereditary Transthyretin-Mediated Amyloidosis. Circulation 2019;139:431-443. doi:10.1161/CIRCULATIONAHA.118.035831

92. Dasgupta NR, Benson M. Improved survival of patients with transthyretin amyloid cardiomyopathy with inotersen (TTR specific antisense oligonucleotide). JACC 2019;73:811. doi: 10.1016/S0735-1097(19)31418-4

93. Yamashita T, Ando Y, Okamoto S, et al. Long-term survival after liver transplantation in patients with familial amyloid polyneuropathy. Neurology 2012;78:637-643. doi: 10.1212/WNL.0b013e318248df18

94. Carvalho A, Rocha A, Lobato L. Liver transplantation in transthyretin amyloidosis: Issues and challenges. Liver Transplantation 2015;21:282-292. doi: 10.1002/lt.24058

95. Tang W, McDonald SP, Hawley CM, et al. End-stage renal failure due to amyloidosis: outcomes in 490 ANZDATA registry cases. Nephrology Dialysis Transplantation 2012;28:455-461. doi: 10.1093/ndt/gfs492

96. Sawinski D, Lim MA, Cohen JB, et al. Patient and Kidney Allograft Survival in Recipients With End-Stage Renal Disease From Amyloidosis. Transplantation 2018;102:300-309. doi: 10.1097/tp.0000000000001930

97. Pinney JH, Lachmann HJ, Sattianayagam PT, et al. Renal Transplantation in Systemic Amyloidosis—Importance of Amyloid Fibril Type and Precursor Protein Abundance. American Journal of Transplantation 2013;13:433-441. doi: 10.1111/j.1600-6143.2012.04326.x

98. Angel-Korman A, Stern L, Sarosiek S, et al. Long-term outcome of kidney transplantation in AL amyloidosis. Kidney International 2019;95:405-411. doi: 10.1016/j.kint.2018.09.021

99. Milani P, Basset M, Nuvolone M, et al. Indicators of profound hematologic response in AL amyloidosis: complete response remains the goal of therapy. Blood Cancer Journal 2020;10:90. doi: 10.1038/s41408-020-00355-6

100. Muchtar E, Dispenzieri A, Leung N, et al. Depth of organ response in AL amyloidosis is associated with improved survival: new proposed organ response criteria. Amyloid 2019;26:101-102. doi: 10.1080/13506129.2019.1582486

101. Leung N, Glavey SV, Kumar S, et al. A detailed evaluation of the current renal response criteria in AL amyloidosis: is it time for a revision? Haematologica 2013;98:988-992. doi: 10.3324/haematol.2012.079210

102. Dasgupta NR, Benson MD. Treatment of ATTR cardiomyopathy with a TTR specific antisense oligonucleotide, inotersen. Amyloid 2019;26:20-21. doi: 10.1080/13506129.2019.1582490

103. Association AH. Classes and Stages of Heart Failure. https://www.heart.org/en/health-topics/heart-failure/what-is-heart-failure/classes-of-heart-failure (Feb 2021)

104. ATS Statement: Guidelines for the Six-Minute Walk Test. American Journal of Respiratory and Critical Care Medicine 2002;166:111-117. doi: 10.1164/ajrccm.166.1.at1102

105. Matsushita K, Selvin E, Bash LD, Astor BC, Coresh J. Risk Implications of the New CKD Epidemiology Collaboration (CKD-EPI) Equation Compared With the MDRD Study Equation for Estimated GFR: The Atherosclerosis Risk in Communities (ARIC)

Study. American Journal of Kidney Diseases 2010;55:648-659. doi: https://doi.org/10.1053/j.ajkd.2009.12.016

106. Galat A, Van der Gucht A, Guellich A, et al. Early Phase 99Tc-HMDP Scintigraphy for the Diagnosis and Typing of Cardiac Amyloidosis. JACC: Cardiovascular Imaging 2017;10:601-603. doi: https://doi.org/10.1016/j.jcmg.2016.05.007

107. Gillmore JD, Tennent GA, Hutchinson WL, et al. Sustained pharmacological depletion of serum amyloid P component in patients with systemic amyloidosis. British Journal of Haematology 2010;148:760-767. doi: 10.1111/j.1365-2141.2009.08036.x

108. Hawkins PN. Serum amyloid P component scintigraphy for diagnosis and monitoring amyloidosis. Current Opinion in Nephrology and Hypertension 2002;11:649-655. doi: 10.1097/00041552-200211000-00013

109. Puchtler H, Sweat F, Levine M. On the binding of congo red by amyloid. Journal of Histochemistry & Cytochemistry 1962;10:355-364. doi: 10.1177/10.3.355

110. Rowczenio DM, Noor I, Gillmore JD, et al. Online Registry for Mutations in Hereditary Amyloidosis Including Nomenclature Recommendations. Human Mutation 2014;35:E2403-E2412. doi: 10.1002/humu.22619

111. Palladini G, Hegenbart U, Milani P, et al. A staging system for renal outcome and early markers of renal response to chemotherapy in AL amyloidosis. Blood 2014;124:2325-2332. doi: 10.1182/blood-2014-04-570010 %J Blood

112. Law S, Petrie A, Chacko L, et al. Disease progression in cardiac transthyretin amyloidosis is indicated by serial calculation of National Amyloidosis Centre transthyretin amyloidosis stage. ESC Heart Fail 2020;7:3942-3949. doi: https://doi.org/10.1002/ehf2.12989

113. Jacobson DR, Pastore RD, Yaghoubian R, et al. Variant-Sequence Transthyretin (Isoleucine 122) in Late-Onset Cardiac Amyloidosis in Black Americans. New England Journal of Medicine 1997;336:466-473. doi: 10.1056/nejm199702133360703

114. Mohamed-Salem L, Santos-Mateo JJ, Sanchez-Serna J, et al. Prevalence of wild type ATTR assessed as myocardial uptake in bone scan in the elderly population. International Journal of Cardiology 2018;270:192-196. doi: https://doi.org/10.1016/j.ijcard.2018.06.006

115. Rapezzi C, Quarta CC, Guidalotti PL, et al. Role of 99mTc-DPD Scintigraphy in Diagnosis and Prognosis of Hereditary Transthyretin-Related Cardiac Amyloidosis. JACC: Cardiovascular Imaging 2011;4:659-670. doi: https://doi.org/10.1016/j.jcmg.2011.03.016

116. Lousada I, Maurer MS, Warner MT, et al. Amyloidosis Research Consortium Cardiac Amyloidosis Survey: Results from Patients with AL and ATTR Amyloidosis and Their Caregivers. Journal of Cardiac Failure 2019;25:S69. doi: 10.1016/j.cardfail.2019.07.197

117. Cappelli F, Martone R, Gabriele M, et al. Biomarkers and Prediction of Prognosis in Transthyretin-Related Cardiac Amyloidosis: Direct Comparison of Two Staging Systems. Canadian Journal of Cardiology 2020;36:424-431. doi: https://doi.org/10.1016/j.cjca.2019.12.020

118. Dungu Jason N, Papadopoulou Sofia A, Wykes K, et al. Afro-Caribbean Heart Failure in the United Kingdom. Circulation: Heart Failure 2016;9:e003352. doi: 10.1161/CIRCHEARTFAILURE.116.003352

119. Law S, Petrie A, Chacko L, et al. Change in N-terminal pro-B-type natriuretic peptide at 1 year predicts mortality in wild-type transthyretin amyloid cardiomyopathy. Heart 2021:heartjnl-2021-319063. doi: 10.1136/heartjnl-2021-319063 %J Heart

120. Hutt DF, Fontana M, Burniston M, et al. Prognostic utility of the Perugini grading of 99mTc-DPD scintigraphy in transthyretin (ATTR) amyloidosis and its relationship with skeletal muscle and soft tissue amyloid. European Heart Journal - Cardiovascular Imaging 2017;18:1344-1350. doi: 10.1093/ehjci/jew325

121. Rubin J, Steidley DE, Carlsson M, Ong M-L, Maurer MS. Myocardial Contraction Fraction by M-Mode Echocardiography Is Superior to Ejection Fraction in Predicting

Mortality in Transthyretin Amyloidosis. Journal of Cardiac Failure 2018;24:504-511. doi: https://doi.org/10.1016/j.cardfail.2018.07.001

122. Law S, Bezard M, Petrie A, et al. Characteristics and natural history of early-stage cardiac transthyretin amyloidosis. European Heart Journal 2022;43:2622-2632. doi: 10.1093/eurheartj/ehac259

123. Maceira AM, Joshi J, Prasad SK, et al. Cardiovascular Magnetic Resonance in Cardiac Amyloidosis. Circulation 2005;111:186-193. doi:10.1161/01.CIR.0000152819.97857.9D

124. Rapezzi C, Guidalotti P, Salvi F, Riva L, Perugini E. Usefulness of 99mTc-DPD scintigraphy in cardiac amyloidosis. J Am Coll Cardiol 2008;51:1509-1510; author reply 1510. doi: 10.1016/j.jacc.2007.12.038

125. Grogan M, Scott CG, Kyle RA, et al. Natural History of Wild-Type Transthyretin Cardiac Amyloidosis and Risk Stratification Using a Novel Staging System. Journal of the American College of Cardiology 2016;68:1014-1020. doi: https://doi.org/10.1016/j.jacc.2016.06.033

126. Cheng RK, Levy WC, Vasbinder A, et al. Diuretic Dose and NYHA Functional Class Are Independent Predictors of Mortality in Patients With Transthyretin Cardiac Amyloidosis. JACC CardioOncol 2020;2:414-424. doi: 10.1016/j.jaccao.2020.06.007

127. ClinicalTrials.gov. APOLLO-B: A Study to Evaluate Patisiran in Participants With Transthyretin Amyloidosis With Cardiomyopathy (ATTR Amyloidosis With Cardiomyopathy). https://www.clinicaltrials.gov/ct2/show/NCT03997383 (02 Jan 2022)

128. ClinicalTrials.gov. HELIOS-B: A Study to Evaluate Vutrisiran in Patients With Transthyretin Amyloidosis With Cardiomyopathy. https://clinicaltrials.gov/ct2/show/NCT04153149 (02 Jan 2022)

129. Royston P. Explained Variation for Survival Models. The Stata Journal 2006;6:83-96. doi: 10.1177/1536867x0600600105

130. Davis RC, Hobbs FDR, Kenkre JE, et al. Prevalence of atrial fibrillation in the general population and in high-risk groups: the ECHOES study. EP Europace 2012;14:1553-1559. doi: 10.1093/europace/eus087 %J EP Europace

131. Bradshaw PJ, Stobie P, Knuiman MW, Briffa TG, Hobbs MS. Trends in the incidence and prevalence of cardiac pacemaker insertions in an ageing population. Open Heart 2014;1:e000177. doi: 10.1136/openhrt-2014-000177

132. Zhou B, Danaei G, Stevens GA, et al. Long-term and recent trends in hypertension awareness, treatment, and control in 12 high-income countries: an analysis of 123 nationally representative surveys. The Lancet 2019;394:639-651. doi: https://doi.org/10.1016/S0140-6736(19)31145-6

133. S Scholes JM. Health Survey for England 2017 Cardiovascular diseases. http://healthsurvey.hscic.gov.uk/media/78646/HSE17-CVD-rep.pdf (02 Jan 2022)

134. UK D. Diabetes in the UK 2010: Key statistics on diabetes. https://www.diabetes.org.uk/resources-s3/2017-11/diabetes_in_the_uk_2010.pdf (02 Jan 2022)

135. Atroshi I, Gummesson C, Johnsson R, et al. Prevalence of Carpal Tunnel Syndrome in a General Population. JAMA 1999;282:153-158. doi: 10.1001/jama.282.2.153 %J JAMA

136. Rowczenio DM, Noor I, Gillmore JD, et al. Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. Hum Mutat 2014;35:E2403-2412. doi: 10.1002/humu.22619

137. Martinez-Naharro A, Treibel TA, Abdel-Gadir A, et al. Magnetic Resonance in Transthyretin Cardiac Amyloidosis. Journal of the American College of Cardiology 2017;70:466-477. doi: https://doi.org/10.1016/j.jacc.2017.05.053

138. Hawkins PN, Ando Y, Dispenzeri A, et al. Evolving landscape in the management of transthyretin amyloidosis. Annals of Medicine 2015;47:625-638. doi: 10.3109/07853890.2015.1068949

139. Altland K, Benson MD, Costello CE, et al. Genetic microheterogeneity of human transthyretin detected by IEF. Electrophoresis 2007;28:2053-2064. doi: https://doi.org/10.1002/elps.200600840

140. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285-291. doi: 10.1038/nature19057

141. Ruberg FL, Grogan M, Hanna M, Kelly JW, Maurer MS. Transthyretin Amyloid Cardiomyopathy: JACC State-of-the-Art Review. Journal of the American College of Cardiology 2019;73:2872-2891. doi: 10.1016/j.jacc.2019.04.003

142. Quarta CC, Zheng J, Hutt D, et al. 99mTc-DPD scintigraphy in immunoglobulin light chain (AL) cardiac amyloidosis. European Heart Journal - Cardiovascular Imaging 2021;22:1304-1311. doi: 10.1093/ehjci/jeab095 %J European Heart Journal - Cardiovascular Imaging

143. Fermin DR, Cohle SD, Twydell PT, Dickinson MG. Early Recurrence of Myocardial Transthyretin Amyloid Deposition Three Years Post Heart Transplantation for Hereditary V40I Amyloidosis. Journal of Cardiac Failure 2019;25:S170. doi: https://doi.org/10.1016/j.cardfail.2019.07.484

144. Sattianayagam PT, Hahn AF, Whelan CJ, et al. Cardiac phenotype and clinical outcome of familial amyloid polyneuropathy associated with transthyretin alanine 60 variant. Eur Heart J 2012;33:1120-1127. doi: 10.1093/eurheartj/ehr383

145. AbouEzzeddine OF, Davies DR, Scott CG, et al. Prevalence of Transthyretin Amyloid Cardiomyopathy in Heart Failure With Preserved Ejection Fraction. JAMA Cardiol 2021;6:1267-1274. doi: 10.1001/jamacardio.2021.3070

146. Griffin JM, Rosenthal JL, Grodin JL, et al. ATTR Amyloidosis: Current and Emerging Management Strategies. JACC: CardioOncology 2021;3:488-505. doi:10.1016/j.jaccao.2021.06.006

147. Garcia-Pavia P, Rapezzi C, Adler Y, et al. Diagnosis and treatment of cardiac amyloidosis: a position statement of the ESC Working Group on Myocardial and

Pericardial Diseases. European Heart Journal 2021;42:1554-1568. doi: 10.1093/eurheartj/ehab072

148. Rozenbaum MH, Large S, Bhambri R, et al. Impact of Delayed Diagnosis and Misdiagnosis for Patients with Transthyretin Amyloid Cardiomyopathy (ATTR-CM): A Targeted Literature Review. Cardiol Ther 2021;10:141-159. doi: 10.1007/s40119-021-00219-5

149. Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis. New England Journal of Medicine 2021;385:493-502. doi: 10.1056/NEJMoa2107454

150. Guthrie SD RS, Tieu RS, Munday JS, Tarbox MH, Broder MS, Pollock MR. Healthcare Utilization and Cost Among Patients with Hereditary Transthyretin (HATTR) Amyloidosis. Value in Health 2018;21. doi: https://doi.org/10.1016/j.jval.2018.04.1753

151. Asher C, Guilder A, Finocchiaro G, Carr-White G, Rodríguez-Guadarrama Y. Healthcare resource use associated with the diagnosis of transthyretin amyloidosis cardiomyopathy. Health Sci Rep 2022;5:e466. doi: 10.1002/hsr2.466

152. Lauppe R, Liseth Hansen J, Fornwall A, et al. Healthcare resource use of patients with transthyretin amyloid cardiomyopathy. ESC Heart Fail 2022;9:1636-1642. doi: 10.1002/ehf2.13913

153. Beirão JM, Malheiro J, Lemos C, et al. Ophthalmological manifestations in hereditary transthyretin (ATTR V30M) carriers: a review of 513 cases. Amyloid 2015;22:117-122. doi: 10.3109/13506129.2015.1015678

154. Wininger AE, Phelps BM, Le JT, et al. Musculoskeletal pathology as an early warning sign of systemic amyloidosis: a systematic review of amyloid deposition and orthopedic surgery. BMC Musculoskeletal Disorders 2021;22:51. doi: 10.1186/s12891-020-03912-z

155. Srisawasdi P, Vanavanan S, Charoenpanichkit C, Kroll MH. The Effect of Renal Dysfunction on BNP, NT-proBNP, and Their Ratio. American Journal of Clinical Pathology 2010;133:14-23. doi: 10.1309/ajcp60htpgigfcnk

156. Zhou L, Cai X, Li M, Han X, Ji L. Plasma NT-proBNP is independently associated with albuminuria in type 2 diabetes. J Diabetes Complications 2016;30:669-674. doi: 10.1016/j.jdiacomp.2016.01.017

157. Ravichandran S, Cohen OC, Law S, et al. Impact of early response on outcomes in AL amyloidosis following treatment with frontline Bortezomib. Blood Cancer J 2021;11:118. doi: 10.1038/s41408-021-00510-7

158. Rezk T, Lachmann HJ, Fontana M, et al. Prolonged renal survival in light chain amyloidosis: speed and magnitude of light chain reduction is the crucial factor. Kidney International 2017;92:1476-1483. doi: https://doi.org/10.1016/j.kint.2017.05.004

159. Ioannou A, Patel RK, Martinez-Naharro A, et al. Tracking Multiorgan Treatment Response in Systemic AL-Amyloidosis With Cardiac Magnetic Resonance Derived Extracellular Volume Mapping. JACC: Cardiovascular Imaging 2023;16:1038-1052. doi: https://doi.org/10.1016/j.jcmg.2023.02.019

160. Lovat LB, Persey MR, Madhoo S, Pepys MB, Hawkins PN. The liver in systemic amyloidosis: insights from 123I serum amyloid P component scintigraphy in 484 patients. Gut 1998;42:727-734. doi: 10.1136/gut.42.5.727

161. Martinez-Naharro A, Patel R, Kotecha T, et al. Cardiovascular magnetic resonance in light-chain amyloidosis to guide treatment. European Heart Journal 2022;43:4722-4735. doi: 10.1093/eurheartj/ehac363

162. Chacko L, Martinez-Naharro A, Kotecha T, et al. Regression of cardiac amyloid deposits with novel therapeutics: reaching new frontiers in cardiac ATTR amyloidosis. European Heart Journal 2020;41. doi: 10.1093/ehjci/ehaa946.2117

163. Toriu N, Sawa N, Hiramatsu R, et al. Regression of renal amyloid deposits by VAD therapy plus autologous stem cell transplantation in a patient with primary AL amyloidosis. CEN Case Rep 2020;9:6-10. doi: 10.1007/s13730-019-00416-1

164. Rowland TA, Gillmore J, Bhandari S. Regression of chronic kidney disease in a patient with AL amyloidosis: A case report Clin Nephrol 2019;91:317-320. doi: 10.5414/cn109676

165. Zhang P, Chen X, Zou Y, Wang W, Feng Y. Value of repeat renal biopsy in the evaluation of AL amyloidosis patients lacking renal response despite of complete hematologic remission: a case report and literature review. BMC Nephrology 2022;23:127. doi: 10.1186/s12882-022-02752-4

166. Zeier M, Perz J, Linke RP, et al. No regression of renal AL amyloid in monoclonal gammopathy after successful autologous blood stem cell transplantation and significant clinical improvement. Nephrology Dialysis Transplantation 2003;18:2644-2647. doi: 10.1093/ndt/gfg436

167. Gertz MA, Cohen AD, Comenzo RL, et al. Results of the Phase 3 VITAL Study of NEOD001 (Birtamimab) Plus Standard of Care in Patients with Light Chain (AL) Amyloidosis Suggest Survival Benefit for Mayo Stage IV Patients. Blood 2019;134:3166-3166. doi: 10.1182/blood-2019-124482

168. Valent J, Liedtke M, Zonder JA, et al. 1-Year Results from a Phase 2 Study to Determine Safety and Tolerability of Treating Patients with Light-Chain (AL) Amyloidosis with Cael-101, an Anti-Amyloid Monoclonal Antibody, Combined with Anti-Plasma Cell Dyscrasia. Blood 2022;140:10127-10128. doi: 10.1182/blood-2022-162597

169. Rishi Pruthi RS, Terry Feest. UK Renal Registry 16th Annual Report: Chapter 8 Survival and Cause of Death of UK Adult Patients on Renal Replacement Therapy in 2012: National and Centre-specific Analyses. https://ukkidney.org/sites/renal.org/files/Chap%2008.pdf

170. Vernooij RWM, Law W, Peters SAE, et al. The probability of receiving a kidney transplantation in end-stage kidney disease patients who are treated with haemodiafiltration or haemodialysis: a pooled individual participant data from four randomised controlled trials. BMC Nephrology 2021;22:70. doi: 10.1186/s12882-021-02265-6

171. Law S, Cohen O, Lachmann HJ, et al. Renal transplant outcomes in amyloidosis. Nephrology Dialysis Transplantation 2021;36:355-365. doi: 10.1093/ndt/gfaa293

172. Strohmaier S, Wallisch C, Kammer M, et al. Survival Benefit of First Single-Organ Deceased Donor Kidney Transplantation Compared With Long-term Dialysis Across Ages in Transplant-Eligible Patients With Kidney Failure. JAMA Network Open 2022;5:e2234971-e2234971. doi: 10.1001/jamanetworkopen.2022.34971

173. Dember LM. Amyloidosis-Associated Kidney Disease. Journal of the American Society of Nephrology 2006;17:3458-3471. doi: 10.1681/asn.2006050460

174. Gertz MA, Kyle RA, O'Fallon WM. Dialysis Support of Patients With Primary Systemic Amyloidosis: A Study of 211 Patients. Archives of Internal Medicine 1992;152:2245-2250. doi: 10.1001/archinte.1992.00400230061010

175. Said SM, Sethi S, Valeri AM, et al. Renal Amyloidosis: Origin and Clinicopathologic Correlations of 474 Recent Cases. Clinical Journal of the American Society of Nephrology 2013;8:1515-1523. doi: 10.2215/CJN.10491012

176. Pinney JH, Lachmann HJ, Bansi L, et al. Outcome in Renal AL Amyloidosis After Chemotherapy. Journal of Clinical Oncology 2011;29:674-681. doi: 10.1200/jco.2010.30.5235

177. Weiss BM, Lund SH, Bjorkholm M, et al. Improved Survival in AL Amyloidosis: A Population-Based Study on 1,430 Patients Diagnosed in Sweden 1995-2013. Blood 2016;128:4448-4448. doi: 10.1182/blood.V128.22.4448.4448

178. Real de Asúa D, Costa R, Galván JM, et al. Systemic AA amyloidosis: epidemiology, diagnosis, and management. Clinical epidemiology 2014;6:369-377. doi: 10.2147/CLEP.S39981

179. Lane T, Pinney JH, Gilbertson JA, et al. Changing epidemiology of AA amyloidosis: clinical observations over 25 years at a single national referral centre. Amyloid 2017;24:162-166. doi: 10.1080/13506129.2017.1342235

180. Immonen K, Finne P, Grönhagen-Riska C, et al. A marked decline in the incidence of renal replacement therapy for amyloidosis associated with inflammatory rheumatic diseases – data from nationwide registries in Finland. Amyloid 2011;18:25-28. doi: 10.3109/13506129.2010.549252

181. Kuroda T, Tanabe N, Kobayashi D, et al. Significant association between renal function and area of amyloid deposition in kidney biopsy specimens in reactive amyloidosis associated with rheumatoid arthritis. Rheumatology International 2012;32:3155-3162. doi: 10.1007/s00296-011-2148-8

182. Ahbap E, Kara E, Sahutoglu T, et al. Outcome of 121 patients with renal amyloid a amyloidosis. Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences 2014;19:644-649.

183. Gertz MA, Leung N, Lacy MQ, et al. Clinical outcome of immunoglobulin light chain amyloidosis affecting the kidney. Nephrology Dialysis Transplantation 2009;24:3132-3137. doi: 10.1093/ndt/gfp201

184. Pruthi R, McClure M, Casula A, et al. Long-term graft outcomes and patient survival are lower posttransplant in patients with a primary renal diagnosis of glomerulonephritis. Kidney International 2016;89:918-926. doi: 10.1016/j.kint.2015.11.022

185. Kiberd B, Panek R. Cardiovascular Outcomes in the Outpatient Kidney Transplant Clinic: The Framingham Risk Score Revisited. Clinical Journal of the American Society of Nephrology 2008;3:822-828. doi: 10.2215/CJN.00030108

186. Kasiske BL, Guijarro C, Massy ZA, Wiederkehr MR, Ma JZ. Cardiovascular disease after renal transplantation. Journal of the American Society of Nephrology 1996;7:158-165. doi: 10.1681/ASN.V71158

187. NHSBT KAG. Kidney Transplantation: Deceased Donor Organ Allocation. In. POL186/10 ed; 2019.

188. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. Multivariate behavioral research 2011;46:399-424. doi: 10.1080/00273171.2011.568786

189. Kumar S, Dispenzieri A, Lacy MQ, et al. Revised prognostic staging system for light chain amyloidosis incorporating cardiac biomarkers and serum free light chain measurements. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2012;30:989-995. doi: 10.1200/JCO.2011.38.5724

190. Wong SW, Toskic D, Warner M, et al. Impact of Cardiac Stage and Hematologic Response on AL Amyloidosis Patients with Renal Involvement. Blood 2016;128:2136-2136. doi: 10.1182/blood.V128.22.2136.2136 %J Blood

191. Foley RN, Curtis BM, Randell EW, Parfrey PS. Left ventricular hypertrophy in new hemodialysis patients without symptomatic cardiac disease. Clinical journal of the American Society of Nephrology : CJASN 2010;5:805-813. doi: 10.2215/CJN.07761109

192. Nubé MJ, Hoekstra T, Doganer V, et al. Left ventricular geometric patterns in end-stage kidney disease: Determinants and course over time. Haemodialysis International 2018;22:359-368. doi: 10.1111/hdi.12644

193. Ponticelli C, Glassock RJ. Posttransplant Recurrence of Primary Glomerulonephritis. Clinical Journal of the American Society of Nephrology 2010;5:2363-2372. doi: 10.2215/CJN.06720810