UNIVERSITY COLLEGE LONDON

Cardiac Amyloidosis: Clinical Characteristics, Prognostic Factors and Treatment

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Chapter 0

Declarations, publications arising from this work, acknowledgements, abstract and impact statement

0.1 Declaration

I, Ketna Shrikant Patel confirm that the work presented in this thesis is my own. I have collected and analysed the data presented in this thesis in my role as a Clinical Fellow at the National Amyloidosis Centre, UK. I have declared where information has been derived from other sources. Several diagnostic methods were carried out by other individuals in the department: Histological and immunohistochemical analyses were performed by Janet Gilbertson and Karen Boniface. Gene sequencing was performed by Dorota Rowczenio and Hadija Trojer. Echocardiography was performed by Babita Pawarova and Oliver Manalo. Cardiac magnetic resonance imaging was performed by Dr. Marianna Fontana. ^{123}I SAP and ^{99m}Tc DPD scintigraphy was performed by David Hutt, Stephanie McKnight, and Dorothea Gopal. ^{99m}Tc DPD scintigraphy was reported by Dr. Ann-Marie Quigley and Dr. Thomas Wagner. Measurement for biochemical and haematological data were performed by the Royal Free Hospital laboratory services.

0.2 Publications arising from this work

Publications:

Patel KS, Hawkins PN. Cardiac amyloidosis: where are we today? J Intern Med. 2015 Aug;278(2):126-44.

(Case Report): Patel KS, Hawkins PN, Whelan CJ, Gillmore JD. Life-saving implantable cardioverter defibrillator therapy in cardiac AL amyloidosis. BMJ Case Rep. 2014 Dec 22;2014.

Abstracts:

Patel KS, Fontana M, Sachchithanantham S, Sayed R, Wechalekar AD, Lachmann HJ, et al. Abstract 16030: Heterogeneity of Electrocardiographic Findings in Cardiac Transthyretin (ATTR) Amyloidosis and Impact on Survival. Circulation. 2014;130(suppl2):A16030-A.

Patel KS, Pinney JH, Sachchithanantham S, Mahmood S, Wechalekar AD, Lachmann HJ, et al. Cardiac transthyretin (ATTR) amyloidosis - clinical and echocardiographic findings from the largest single cohort worldwide. European Heart Journal. 2013;34(suppl1).

0.3 Acknowledgements

I am grateful to the many wonderful patients, friends and relatives who attend the NAC. The advances that have been made in our understanding of this condition could not have been possible without your strength and curiosity; it truly was a privilege to have been able to contribute to your care.

Thank you to the many members of staff at the NAC whom I have had the pleasure of working with and the wonderful friends I have made along the way.

A special thanks to my parents for the endless support you have always given me - I would not be where I am today without you; to my children for bringing endless light and laughter in to my life - you have been such a blessing; and finally to my quietly amazing husband, without whose wisdom, endurance and IT skills this work would not have been possible.

0.4 Abstract

Cardiac amyloidosis occurs when misfolded protein fibrils are deposited in the extracellular space of the myocardium. The most common causes for cardiac amyloidosis are light chain (AL) amyloidosis and transthyretin (ATTR) amyloidosis. ATTR amyloidosis can occur with ageing due to the accumulation of wild-type transthyretin or can due to an inherited genetic mutation. One of the more common inherited forms in the United Kingdom (UK) is associated with the V122I mutation.

I have described the clinical features, electrocardiogram and multimodality imaging findings in the most common forms of ATTR cardiac amyloidosis diagnosed in the UK. I found significant differences between ATTR amyloidosis types. In particular, those with V122I associated ATTR cardiac amyloidosis present at a younger age than those with wild-type disease but have a more advanced cardiac phenotype at diagnosis and a worse survival. I identified predictors of survival in transthyretin cardiac amyloidosis which were predominantly those associated with a more severe cardiac phenotype and found that the most common form of death in these patients is from heart failure.

I examined the use of DPD in ATTR and AL cardiac amyloidosis and showed that higher DPD grades are associated with more severe cardiac disease in ATTR amyloidosis. However, DPD grade does not predict survival in ATTR amyloidosis and I demonstrated that this is also the case in AL amyloidosis.

I conducted a retrospective study to examine the effect of the non-steroidal anti-inflammatory drug, diflunisal, in ATTR amyloidosis. I found that even in highly selected patients, diflunisal was poorly tolerated and I did not find any evidence for benefit. I described the UK experience of a phase 2 trial of a silencing RNA treatment, revusiran. I described challenges to recruitment

and patient characteristics for those I recruited.

0.5 Impact statement

Cardiac amyloidosis cannot be considered a single entity owing to the extensive heterogeneity in pathophysiology, clinical phenotype and prognosis. This is well established when considering the differences between amyloid light chain (AL) and transthyretin (ATTR) cardiac amyloidoses, however, the importance of differences between subgroups of patients with ATTR cardiac amyloidosis is often underappreciated. ATTR cardiac amyloidosis can be caused by accumulation of wild-type transthyretin (TTR) in older patients or by accumulation of mutant forms of TTR due to a mutation in the TTR gene, with more than 100 mutations identified. These different aetiologies may have important implications when considering all aspects of ATTR cardiac amyloidosis from clinical phenotype and natural history to response to treatment in clinical trials.

I have demonstrated that significant differences exist between types of ATTR cardiac amyloidosis, particularly between wild-type transthyretin (ATTR-wt) cardiac amyloidosis and amyloidosis associated with the V122I transthyretin variant (ATTR-V122I). There are differences in findings at diagnosis in the electrocardiogram, echocardiogram, cardiac magnetic resonance imaging and cardiac biomarkers. These differences suggest that patients with ATTR-V122I cardiac amyloidosis present with a more severe cardiac phenotype than those with ATTR-wt cardiac amyloidosis. This is further corroborated by my finding that survival in those with the ATTR-V122I is significantly worse than those with wild-type ATTR cardiac amyloidosis. I found that Perugini DPD grade correlated with disease severity in ATTR cardiac amyloidosis but there was also a signal to suggest that differences in DPD grade may reflect different ATTR types.

This constellation of differences between ATTR-wt and ATTR-V122I cardiac amyloidoses may

point to diverse underlying pathophysiology between the types of ATTR cardiac amyloidosis, and would suggest that it is important to separate types of ATTR amyloidosis when describing observational data and should be a key consideration when designing clinical trials.

An additional impact of my data relates to treatment. Diflunisal has been used in familial amyloid polyneuropathy but little data is available regarding its use in ATTR cardiac amyloidosis. In a retrospective trial I found that the drug was poorly tolerated and there were no signals for benefit. While these data are flawed by their retrospective nature and a randomised trial is required to address this question, it seems unlikely that diflunisal will have a significant impact on the disease trajectory of cardiac ATTR amyloidosis.

The field of cardiac amyloidosis research and drug development is currently a particularly active one. This work has contributed to a better understanding of this sometimes overlooked debilitating disease.

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Abbreviations

^{123}I SAP scintigraphy ^{123}I Serum Amyloid P component scintigraphy.

^{99m}Tc DPD scintigraphy ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy.

2D 2-dimensional.

6MWT six minute walk test.

ACEi Angiotensin Converting Enzyme inhibitor.

AE adverse event.

AF atrial fibrillation.

AL amyloid light chain.

ALT alanine transaminase.

ANOVA analysis of variance.

ApoA1 amyloidosis Apoprotein A-1-Related Amyloidosis.

ARB Angiotensin II Receptor Blocker.

AST aspartate transaminase.

ATTR transthyretin amyloid.

ATTR-m mutant transthyretin amyloid.

ATTR-T60A amyloidosis associated with the T60A transthyretin variant.

ATTR-V122I amyloidosis associated with the V122I transthyretin variant.

ATTR-V30M Amyloidosis associated with the V30M transthyretin variant.

ATTR-wt amyloidosis due to wild-type transthyretin.

BMI body mass index.

BP blood pressure.

CMR Cardiac Magnetic Resonance.

CPHPC (R) -1-[6-[(R)-2- Carboxy-pyrrolidin-1yl]-6-oxo-hexanoyl] pyrrolidine-2 carboxylic acid.

CRT cardiac resynchronisation therapy.

CT computed tomography.

DNA deoxyribonucleic acid.

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

DPD grade Perugini grading of ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy.

ECG Electrocardiogram.

ECOG Eastern Cooperative Oncology Group.

ECV extra-cellular volume.

eGFR estimated glomerular filtration rate.

EMB endomyocardial biopsy.

EU European Union.

FAC familial amyloid cardiomyopathy.

FAP familial amyloid polyneuropathy.

FLC free light chain.

GI gastrointestinal.

GLS global longitudinal strain.

HMDP hydroxymethylene diphosphonate.

ICD Implantable Cardioverter Defibrillator.

IVRT isovolumic relaxation time.

IVSd Interventricular septal thickness in diastole.

JVP jugular venous pressure.

LA Left atrium.

LBBB left bundle branch block.

LFT liver function test.

LGE late gadolinuium enhancement.

LV left ventricle.

LVEF left ventricular ejection fraction.

LVH left ventricular hypertrophy.

LVPWd left ventricular posterior wall thickness in diastole.

LVSD left ventricular systolic dysfunction.

MACE major adverse cardiovascular events.

MAPSE mitral annular plane systolic excursion.

MI myocardial infarction.

mRNA messenger ribonucleic acid.

MV Dec Time mitral valve deceleration time.

NAC National Amyloidosis Centre.

NHS National Health Service.

NSAID non-steroidal anti-inflammatory drug.

NSICD non-specific intraventricular conduction delay.

NT-proBNP N-terminal-pro B-type Natiuretic Peptide.

NYHA New York Heart Association.

PBS phosphate-buffered saline.

PCR polymerase chain reaction.

PYP pyrophosphate.

QTcB QT correction with Bazett formula.

RBBB right bundle branch block.

RNA Ribonucleic acid.

SAA serum amyloid A.

SAE serious adverse event.

SAP serum amyloid P component.

SC subcutaneous.

SCD sudden cardiac death.

ShMOLLI shortened modified look-locker inversion recovery sequence.

siRNA small interfering ribonucleic acid.

SPECT single photon emission computed tomography.

STE speckle-tracking echocardiography.

TAPSE tricuspid annular plane systolic excursion.

TDI tissue Doppler imaging.

THAOS Transthyretin Amyloid Outcome Survey.

TTR transthyretin.

TUDCA tauroursodeoxycholic acid.

UK United Kingdom.

ULN upper limit of normal.

US United States.

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Chapter 1

Introduction

The systemic amyloidoses are a rare group of diseases characterised by the progressive extracellular accumulation of insoluble fibrillar proteins [1–3]. This process results in the disruption of architecture in affected tissues and subsequent organ dysfunction. The amyloidoses are classified according to the responsible amyloid fibril protein. There are more than 30 proteins identified that can form amyloid in man [4] with amyloid light chain (AL) amyloidosis being the most common form diagnosed in developed countries [5] . Reactive systemic (AA) amyloidosis is the most common form of amyloidosis worldwide but rarely affects the heart in a clinically meaningful way [6].

The two predominant types of cardiac amyloidosis are AL amyloidosis and transthyretin amyloid (ATTR) amyloidosis [7]. AL amyloidosis is caused by the accumulation of amyloid fibrils composed of monoclonal immunoglobulin light chains. These light chains are typically produced in patients with myeloma or other plasma cell dyscrasias [8]. ATTR amyloidosis is subdivided into hereditary and acquired types. Hereditary ATTR amyloidosis is due to misfolding of a mutated form of transthyretin (TTR) and can be termed mutant transthyretin amyloid (ATTR-m) while the acquired form is due to misaggregation of wild-type TTR and is termed amyloidosis due to wild-type transthyretin (ATTR-wt) [9]. ATTR-wt has previously

been termed senile cardiac amyloidosis.

Much of the literature describing cardiac amyloidosis refers to AL amyloidosis while ATTR amyloidosis is less well described. There has been a significant change in referrals to the National Amyloidosis Centre (NAC), UK over the last two decades with the rate of ATTR-wt amyloidosis, as a proportion of all new referrals, increasing from 0.2% for the period 1988-99, to 6.4% for the period 2009-12 [10]. The absolute number of new referrals with ATTR amyloidosis is illustrated in Figure 1.1. The NAC has seen a greater than 10-fold increase in the number of patients diagnosed with cardiac ATTR-wt amyloidosis over the last decade.

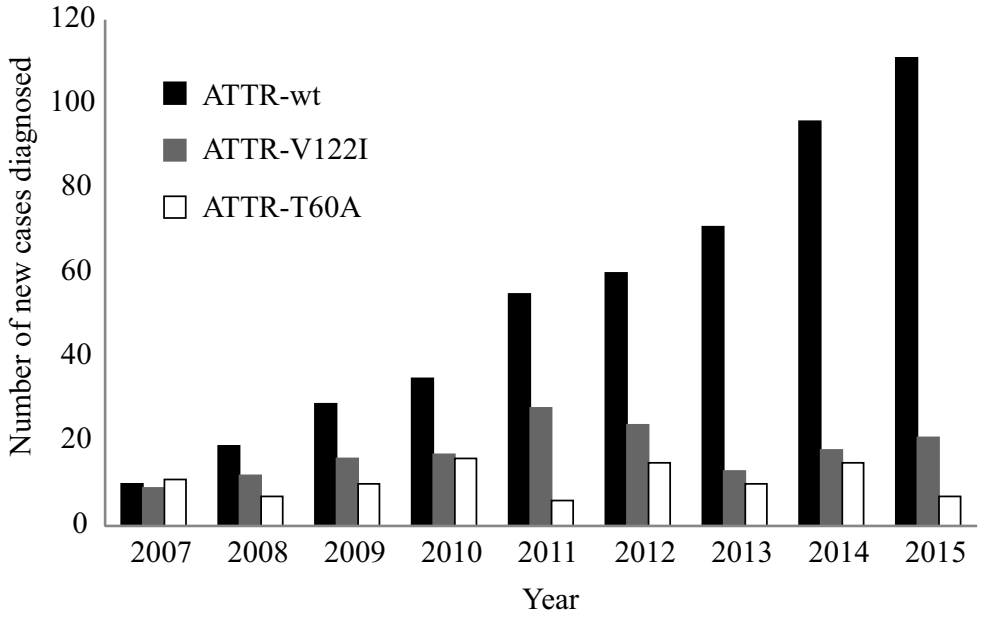


Figure 1.1: New cases of cardiac transthyretin amyloidosis diagnosed at the National Amyloidosis Centre, UK from 2007 to 2015. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis.

The treatment of cardiac amyloidosis is determined by the underlying amyloid type. The published data on therapies for cardiac amyloidosis predominantly focus on AL amyloidosis but there are emerging therapeutics designed to target ATTR amyloidosis [7].

The subject matter for this thesis is cardiac amyloidosis and as such I will predominantly discuss AL and ATTR amyloidoses. In particular, I will focus on ATTR amyloidosis since there is a sparsity of published data in this condition and it may become more important in coming years

due to an increase in new diagnoses and the potential future impact of emerging therapeutics.

1.1 Pathophysiology

Amyloidosis is classified according to the respective amyloid fibril precursor protein. More than 30 different amyloid forming proteins have been identified [9]. The process of amyloidogenesis involves substantial unfolding of the native protein structure and aberrant misfolding into an alternative highly ordered aggregated form with a predominant β-sheet fold [11]. Under the light microscope all types of amyloid appear in tissue sections as homogeneous amorphous eosinophilic material that stains with Congo red dye to produce characteristic apple-green birefringence when viewed under cross-polarised filters [12]. Under electron microscopy amyloid appears as a meshwork of randomly dispersed non-branching fibrils of 7-10 nm diameter and indeterminate length [13]. Immunohistochemical staining using a panel of anti-fibril protein antibodies enables determination of amyloid type in most cases, but does have pitfalls [14]. Accumulation of amyloid in the extracellular space progressively disrupts the structure and function of affected tissues, ultimately leading to organ failure.

Some types of amyloid have a greater predilection for the heart than others. The two most commonly encountered forms of cardiac amyloidosis are the AL and ATTR types [3]. AL amyloidosis may involve almost any organ in the body and cardiac involvement occurs in 50-70% of patients [15–17]. Inherited ATTR amyloidosis may involve the heart, autonomic and peripheral nerves. Wild-type ATTR amyloidosis has an almost exclusive cardiac phenotype although deposition of amyloid may be found at other sites.

A simplified schematic representing the basic pathophysiology of transthyretin and AL cardiac amyloidoses is displayed in Figure 1.2.

TTR is a homo-tetrameric plasma protein encoded by a gene on chromosome 18. Each

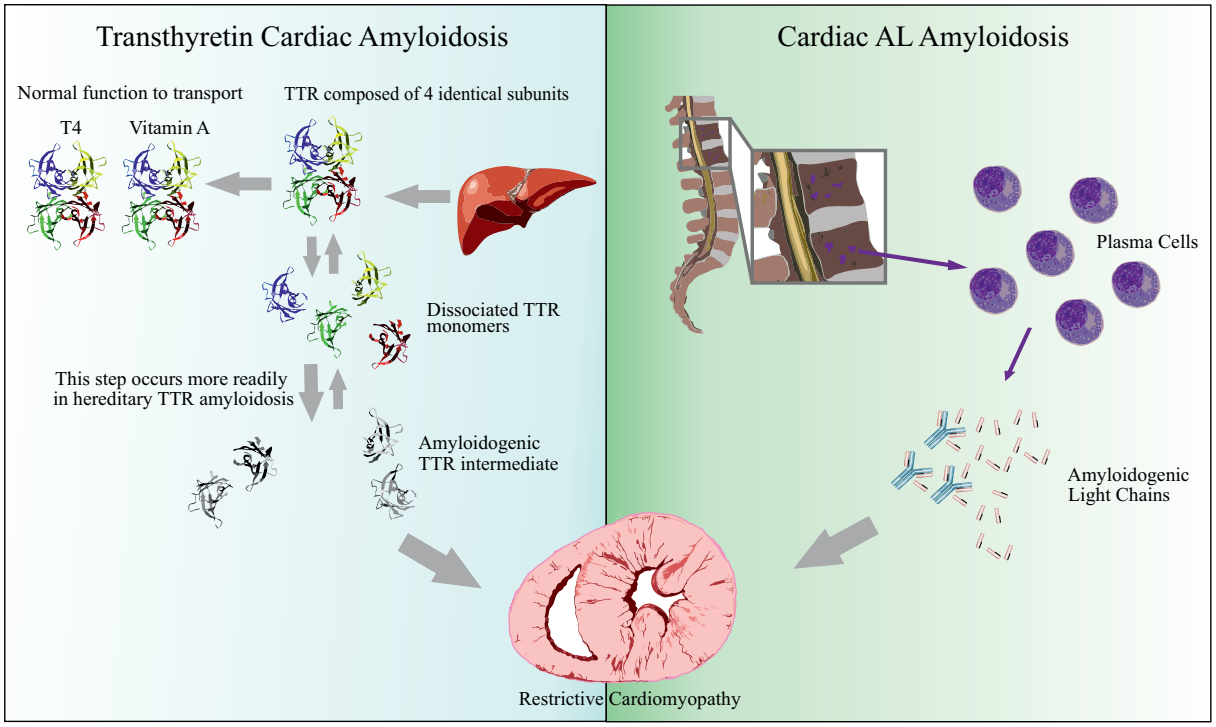


Figure 1.2: Pathophysiology of transthyretin and light chain cardiac amyloidosis. Detailed description in main text. TTR, transthyretin. Reproduced with permission.

monomer is comprised of 127 amino acids. TTR is produced almost exclusively by the liver but also in minor amounts within the choroid plexus and retina. The physiological function of serum TTR is transportation of retinol-binding protein and thyroxine, although thyroxine-binding globulin has the chief role for the latter [18]. Normal (wild-type) TTR is an inherently amyloidogenic protein, but genetic variants have been identified that enhance this property. TTR can evidently undergo a major transformational change to form amyloid fibrils, which is thought to involve dissociation into TTR monomers. Progressive accumulation of ATTR amyloid deposits in the nerves and heart is responsible for causing neuropathic and cardiac amyloidosis respectively. The fibrils in ATTR-wt amyloid are composed of normal wild-type TTR [19], whereas those in FAC and FAP are derived from a combination of both wild-type and variant TTR.

Variant transthyretin amyloidosis is usually associated with a single amino acid substitution caused by a point mutation in the TTR gene. Amyloidogenic TTR variants are thought to be less

stable than their wild-type counterpart, underlying their increased propensity to form amyloid fibrils. To date at least 120 point mutations of the TTR gene have been identified, most of which are associated with amyloidosis [20]. However, just a few of these variants are responsible for the majority of cases for example in the case of Val30Met, where the mutation results in the amino acid methionine being substituted for valine at position 30 of the TTR molecule. Other common variants include Thr60Ala, Ser77Tyr and Val122Ile. Inheritance is autosomal dominant with variable penetrance. Other genetic or environmental factors influencing disease expression are not known, with the exception of the TTR Thr199Met variant, which is more stable than wild-type and reduces the likelihood that a co-existing amyloidogenic TTR variant may cause disease [21], and in the Val30Met TTR variant (see below).

Cardiac AL amyloidosis is associated with a B cell dyscrasia and is the most commonly diagnosed form of cardiac amyloidosis. AL amyloidosis fibrils are composed of fragments of either kappa or lambda monoclonal immunoglobulin light chains. In some patients overt multiple myeloma may co-exist and is associated with a poorer prognosis [22]. One or many vital organ systems may be involved, commonly the kidneys, liver, peripheral and autonomic nervous systems and soft tissues. The heart is frequently the predominant organ affected by amyloid deposition, and is the sole organ involved in a smaller proportion of patients [17]. Cardiac involvement is a key determinant in the prognosis in AL amyloidosis [23].

1.2 Epidemiology

The incidence of systemic AL amyloidosis has been estimated to be 9/million/year based on a study of the population of Olmstead County, Minesota, USA [24], and similar findings were obtained in a more recent study in the UK [5]. In contrast, the prevalence of cardiac ATTR amyloidosis is unknown and likely to vary according to the population

examined. The small number of patients referred to the NAC, approximately 100 per year, contrasts sharply with published autopsy series that have described cardiac ATTR amyloid deposits in up to 25% of individuals over 80 years of age [25]. In a 2015 study of 120 patients with heart failure and preserved ejection fraction aged over 60 years, screening with ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy (^{99m}Tc DPD scintigraphy) detected ATTR amyloidosis as a cause of heart failure in more than 13% of patients [26]. ATTR amyloidosis may go undiagnosed because differentiation of cardiac amyloid from left ventricular hypertrophy is often not possible by standard echocardiography. The diagnostic gold standard remains endomyocardial biopsy (EMB) but this is an invasive test with a risk of complications in what is a relatively aged and frail population. More recently, Cardiac Magnetic Resonance (CMR) imaging and scintigraphy with ^{99m}Tc phosphate derivatives have been shown to be sensitive, very informative, and in selected cohorts highly specific methods for imaging amyloid deposits in the heart [27–33]. These techniques have the potential for identifying amyloid at an early stage in its natural history, however cardiac uptake with ^{99m}Tc phosphate derivatives can be found in other conditions, such as dilated cardiomyopathy and sarcoidosis [34], and following acute myocardial infarction [35].

In selected cohorts within specialist centres, the commonest types of cardiac amyloidosis are the AL and TTR types with greatest proportion being the AL type [10, 23, 36]. However recent data from a heart failure clinic in a tertiary cardiac centre found that ATTR amyloidosis was a more common cause of heart failure than AL amyloidosis [37].

1.3 Clinical Features

The clinical features observed in patients with amyloidosis depends on the type of amyloid and this is summarised in Table 1.1.

	AL Amyloidosis	ATTR-wt	ATTR-V122I	ATTR-T60A	ATTR-V30M
Precursor/ amyloidogenic protein	Monoclonal immunoglobulin light chain	Wild-type transthyretin	Variant transthyretin	Variant transthyretin	Variant transthyretin
Age at presentation (yrs)	60-70	70-80	≥60	≥60	30-40 or 50-60
Common ethnicity	Any	Caucasian	African/ Caribbean	Caucasian (Irish)	Any (Portuguese, Swedish, Japanese)
Frequency of cardiac involvement	40-50%	Almost all	Almost all	Detectable in at least 90%	Uncommon
Other systemic involvement	Kidney, liver, soft tissue, nerves, spleen	Carpal tunnel, (bladder, spine)	Carpal tunnel	Nerves	Nerves
Treatment	ASCT or chemotherapy. Consider cardiac transplantation followed by ASCT	Supportive	Supportive Cardiac transplantation in young patients	Supportive	Liver transplantation (+cardiac transplantation) in select cases
Prognosis/ median survival from diagnosis	Generally poor but variable.	3-5 years	2-3 years	2.5-5.5years	Good with liver transplantation but variable

Table 1.1: Characteristics of the most common cardiac amyloidoses. AL amyloidosis, Light chain amyloidosis; ASCT, Autologous peripheral blood stem cell rescue/transplantation; ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-V30M, Amyloidosis associated with the V30M transthyretin gene mutation; ATTR-wt, Wild-type transthyretin amyloidosis.

1.3.1 Cardiac amyloid light chain amyloidosis

The clinical presentation in cardiac amyloid light chain amyloidosis reflects the multisystem nature of the disease with a variety of non-specific findings that depend on the nature and severity of organ involvement. Examination findings may reflect soft tissue and small vessel changes as a result of amyloid deposition and include periorbital purpura, macroglossia, submandibular gland enlargement and nail dystrophy [23]. Some patients have hoarseness of voice due to amyloid deposition on vocal cords. Fatigue and weight loss are common. Patients with co-existing myeloma may have bone disease and a history of infections. Hepato/splenomegaly may occur, and hepatic or splenic infiltration may result in palpable organomegaly. Renal dysfunction is common, typically manifesting as nephrotic syndrome often with very heavy proteinuria, dependent peripheral oedema, hypoalbuminaemia, and varying degrees of impaired glomerular filtration. Complications of nephrotic syndrome such as deep vein thrombosis and infection may ensue. Early cardiac amyloidosis may be difficult to detect from the history or examination, but a history of rapidly diminishing exercise tolerance is a strong clue. The classical features of right-sided congestive heart failure may not be evident until cardiac disease is very advanced. Elevated jugular venous pressure (JVP), third heart sound, hepatomegaly and peripheral oedema may be very subtle or absent in patients who have already commenced diuretics before the diagnosis has been made.

Minor peripheral neuropathy is relatively common. Patients may describe symptoms of parasthesiae or dysasthesiae typically in a 'glove and stocking' distribution reflecting the symmetrically ascending pattern of axonal neuropathy. Carpal tunnel syndrome and previous decompression surgery may significantly pre-date the diagnosis. Autonomic neuropathy may manifest as orthostatic hypotension, alternating diarrhoea and constipation, early satiety due to delayed gastric emptying, and erectile failure. Monoclonal whole immunoglobulin or free light

chains are identifiable in the serum and/or urine in at least 95% of patients with AL amyloidosis using the most sensitive assays, though routine electrophoresis is negative in about half of cases. The absence of an identifiable and measurable monoclonal protein is problematic for diagnosis and tracking response to treatment. The proportion of plasma cells identified on bone marrow trephine is usually in the order of 5-10%. In contrast to myeloma there is a predominance of lambda light chain idiotype in patients with AL amyloidosis [38].

1.3.2 Cardiac transthyretin amyloidosis

Cardiac amyloidosis is the predominant manifestation in ATTR-wt and familial amyloid cardiomyopathy (FAC) (hereditary ATTR causing amyloid cardiomyopathy), whilst the peripheral and autonomic nerves are predominantly affected in familial amyloid polyneuropathy (FAP) (hereditary ATTR causing neuropathy). Biopsies of other tissues often contain ATTR amyloid deposits that appear to have no clinical significance. This is the basis of diagnosis by screening rectal tissue and fat sampling.

The clinical presentation of cardiac transthyretin amyloidosis may be indistinguishable from cardiac AL amyloidosis, and the features of neuropathy may also be similar. There are differences in characteristics between wild-type and most hereditary forms of ATTR amyloidosis.

Wild-type transthyretin amyloidosis (also known as senile cardiac amyloidosis or senile systemic amyloidosis)

This is almost exclusively a disease affecting older individuals with most patients being more than 70 years of age at the time of diagnosis. There is a tenfold male predominance. Most patients have substantial left ventricular wall thickening at diagnosis, and early diagnosis

remains an elusive goal which could affect outcomes. Only small cohorts of patients with ATTR-wt amyloidosis have been characterised systematically [39–41], and the natural history remains unclear. However, various new investigational tools are now available, with expectations that this will translate into improved diagnosis, potentially without the need for invasive biopsies and may facilitate improved monitoring.

The heart appears to be the only vital organ to be affected clinically in most patients, but carpal tunnel syndrome in association with ATTR-wt amyloid deposits in the flexor retinaculum is common and may precede the development of cardiac symptoms by 10-15 years. More recently however, it is increasingly recognised that involvement of other tissues may also occur and cause symptoms. As an example, histological analysis of specimens taken at surgery for spinal canal stenosis in a small case series revealed a high frequency of ATTR amyloid deposits [42]. Furthermore, the NAC has reported the finding of ATTR amyloid deposition in the bladders of 13 patients presenting with painless haematuria [43]. In this latter example, TTR gene sequencing confirmed the absence of a mutation.

As in cardiac AL amyloidosis, features of right-sided heart failure are often present at diagnosis. Hepatomegaly may be palpable but is caused by hepatic venous congestion rather than liver amyloid infiltration, contrasting with AL amyloidosis in which hepatic infiltration by amyloid may be the cause.

The presence of an incidental plasma cell dyscrasia in up to one quarter of ATTR-wt amyloidosis patients [39] complicates the diagnostic pathway, necessitating cardiac biopsy to obtain a definitive diagnosis in some patients. However, the presence of ATTR amyloid in extra-cardiac tissue in conjunction with characteristic findings on ^{99m}Tc DPD scintigraphy may be sufficient to diagnose cardiac ATTR amyloidosis, avoiding the need for cardiac biopsy in patients who are often elderly and frail and may be unkeen to undergo invasive diagnostic

procedures.

Variant (hereditary) transthyretin amyloidosis

The most prevalent amyloidogenic TTR variants in the UK population are V122I and T60A types about which more detail is provided below. The V30M type is the most common ATTR type worldwide and is also discussed below.

ATTR V122I amyloidosis

Approximately 3 to 4% of African Americans carry the TTR V122I variant allele [44], which increases susceptibility to developing amyloidosis after the age of 60-65 years. The chief clinical manifestation is restrictive cardiomyopathy that is indistinguishable from ATTR-wt amyloidosis except for the clear racial difference. It has a late-onset and marked male preponderance [45]. Neuropathy is infrequent, but may be present in some cases. One small prospective US study of 11 patients suggests a more severe phenotype with a worse prognosis than ATTR-wt amyloidosis [41]. The diagnosis may be overlooked in a patient of African descent with heart failure symptoms, as increased LV wall thickness may be attributed to hypertension, or race. A high index of suspicion is necessary to prompt further relevant investigations such as TTR gene sequencing and bone scintigraphy with ^{99m}Tc labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) or pyrophosphate (PYP), fat aspirate or endomyocardial biopsy to secure the diagnosis.

ATTR T60A amyloidosis

Another variant, termed amyloidosis associated with the T60A transthyretin variant (ATTR-T60A), is the commonest cause of FAP in the United Kingdom (UK) and United States (US). Clinical features comprise any permutation of peripheral or autonomic neuropathy and cardiac involvement, although the latter is present in virtually all cases [46]. Most patients are aged 50 years or more at presentation. The genetic variant was first described in 1986

in a kindred from the Appalachian region of the US [47]. Seven cases of hereditary amyloid polyneuropathy from seven different families originating from a small area of the north-west coast of County Donegal in Ireland were reported in 1987 [48], and this TTR variant is now thought to be prevalent throughout Ireland [49].

ATTR-V30M amyloidosis

Amyloidosis associated with the V30M transthyretin variant (ATTR-V30M) is thought to be the commonest cause of hereditary ATTR amyloidosis in the world, with large foci in Portugal, Japan and Sweden. The condition was first described by Andrade C. in 1952 in Portugal where 74 cases were assessed [50]. ATTR-V30M usually causes a predominant sensorimotor peripheral neuropathy and autonomic neuropathy, also known as type 1 FAP. Interestingly the same mutation can causes a variety of phenotypes and is associated with a different age of onset and different initial symptoms depending on a variety of factors including the geographical area, even within the same country, and whether inheritance is from the mother or father [51–53]. Although cardiac amyloidosis may occur in older patients with this TTR mutation, the vast majority of patients do not have cardiac involvement, which has favourable implications for treatment by liver transplantation [54, 55]. A summary of the clinical differences between AL cardiac amyloidosis and the transthyretin cardiac amyloidoses is displayed in Table 1.2.

1.4 Prognosis

1.4.1 AL cardiac amyloidosis

The Mayo Staging system proposed in 2004 for AL amyloidosis [56] remains widely in clinical use. Stage 1, with cardiac biomarkers below threshold values (TnT $<0.035\mu$g/L and N-terminal-pro B-type Natiuretic Peptide (NT-proBNP) $<$332ng/L) had the lowest risk of

Amyloid type	Symptoms	Signs
ATTR amyloidosis and AL amyloidosis	Hoarse voice	Peripheral neuropathy; 'glove and stocking,' carpal tunnel syndrome or scar from previous release
	Numbness hand/feet	Elevated JVP, 3 rd heart sound, pleural effusions
	Breathlessness, reduced exercise tolerance, fatigue	Peripheral oedema, ascites
	Lower limb swelling, abdominal swelling	Orthostatic hypotension
	Light-headedness on standing or bending forwards	Weight loss
	Alternating diarrhoea/constipation; early satiety	
	Erectile failure/dysfunction	
AL amyloidosis	Easy bruising/bleeding	Peri-orbital purpura, purpura at multiple sites
	Dry mouth, altered taste, difficulty with swallowing/speech	Macroglossia, submandibular gland enlargement
	Brittle nails	Nail dystrophy
	Abdominal discomfort	Hepatomegaly/splenomegaly

Table 1.2: Clinical features of transthyretin and light chain cardiac amyloidoses. AL amyloidosis, Light chain amyloidosis; ATTR amyloidosis, Transthyretin amyloidosis; JVP, jugular venous pressure.

death with a median survival of 26.4 months; stage 2, with either one of the biomarkers above threshold values had a median survival of 10.5 months; and Stage 3, with both biomarkers above the threshold values had a median survival of 3.5 months.

In 2012 the Mayo Clinic group published an updated staging system [57] which incorporated amyloidogenic free light chain (FLC) levels and amended the cut-off values for cardiac biomarkers in an attempt to better discriminate between groups. More recently, a European collaborative piece of work analysed outcomes in 346 patients with stage 3 disease [58] based on the original 2004 staging system and reported better median survival for patients diagnosed between 2001 and 2010 of 7.1 months. Multivariate analysis of this cohort identified serum NT-proBNP $>$8500ng/L and low systolic blood pressure as the only independent factors impacting death.

1.4.2 Transthyretin cardiac amyloidosis

Previous published data in small studies have reported that patients with ATTR cardiac amyloidosis fair far better than those with AL amyloidosis [36, 59] but did not comprise significant numbers of patients with amyloidosis associated with the V122I transthyretin variant (ATTR-V122I) in the hereditary cohorts. There are limited data comparing the clinical presentation and outcomes in patients with cardiac ATTR-wt versus ATTR-V122I amyloidosis. The findings from two small studies suggest that despite a younger age at diagnosis, patients with cardiac ATTR-V122I amyloidosis present with a more severe cardiac phenotype and have a higher mortality rate compared to patients with ATTR-wt amyloidosis [41, 60]. It is unclear whether inequalities in access to medical care may contribute to this difference in the US. More recently, however, these findings have been disputed by the publication of the much

larger Transthyretin Amyloid Outcome Survey (THAOS) [61]. In this multinational cohort, there was no difference in survival between patients with cardiac ATTR-wt amyloidosis and those with cardiac ATTR-V122I amyloidosis, despite the ATTR-V122I group presenting with a more severe cardiac phenotype. Concerns have been raised about the reliability of the findings from THAOS [62]. Thus there is uncertainty as to whether patients with cardiac ATTR-V122I amyloidosis have a worse prognosis compared to those with ATTR-wt amyloidosis.

THAOS should be recognised for its ambition; it is a multinational, longitudinal, observational survey enrolling patients with all ATTR types. Patients are enrolled from Europe, US, South America and Japan. A US subgroup of this population comprising 280 patients with either ATTR-wt or ATTR-V122I amyloidosis was recently described [61]. In comparison, the largest prior study included only 65 patients. Based on the size of the population alone, one might expect the findings from THAOS to be the most accurate, however, there are a number of concerns about the reliability of the conclusions from THAOS. Firstly, the two year follow-up period is relatively short and the median survival was not reached by the end of the study. This may be a particularly critical flaw as separation of the Kaplan-Meier survival curves does not begin until two years of follow-up in other data [62], so a survival difference at two years would not be expected. There are also concerns about the amount of missing data in THAOS, for example left ventricular ejection fraction was only available in 44% of the population, natriuretic peptide in 27% and date of diagnosis in 71%. Missing data for date of diagnosis and the consequent reliance on date of enrolment to THAOS could particularly affect estimates of survival from diagnosis.

No large systematic study of prognostic markers in patients with cardiac ATTR amyloidosis had been reported at the outset of this thesis. Recently, two proposed staging systems for individuals with cardiac ATTR amyloidosis have been published [33, 63], using cardiac biomarkers and/or

renal function as assessed by estimated glomerular filtration rate (eGFR). Neither are in widespread clinical use and both have limitations as discussed in more detail in Chapter 4.

1.5 Investigations of cardiac amyloidosis

1.5.1 Overview

A flow chart illustrating the typical work-up for a patient with possible cardiac amyloidosis is presented in Figure 1.3.

1.5.2 Electrocardiogram

There are limited data describing the Electrocardiogram (ECG) in patients with cardiac amyloidosis, particularly in those with ATTR amyloidosis. This is in part due to the small populations studied, but also there has been focus on the development of more advanced imaging modalities.

The combination of increased left ventricular wall thickness and low voltage QRS complexes should rightly raise suspicion of an infiltrative cardiomyopathy. However, the classical description that cardiac amyloidosis is associated with low voltage QRS complexes is not universally correct. Although low voltage QRS complexes are common in cardiac AL amyloidosis, variable definitions of 'low voltage' have been used in published studies, making the true prevalence of this finding uncertain [64]. Low voltage QRS complexes are, however, less frequent in cardiac transthyretin amyloidosis [36]. A recent study of 64 patients with ATTR-V122I showed that 44% of these patients did not have low voltage QRS complexes [45], indeed the ECG in 26% of cases demonstrated left ventricular hypertrophy by standard criteria. As such, the absence of low voltage QRS complexes should not divert the clinician from

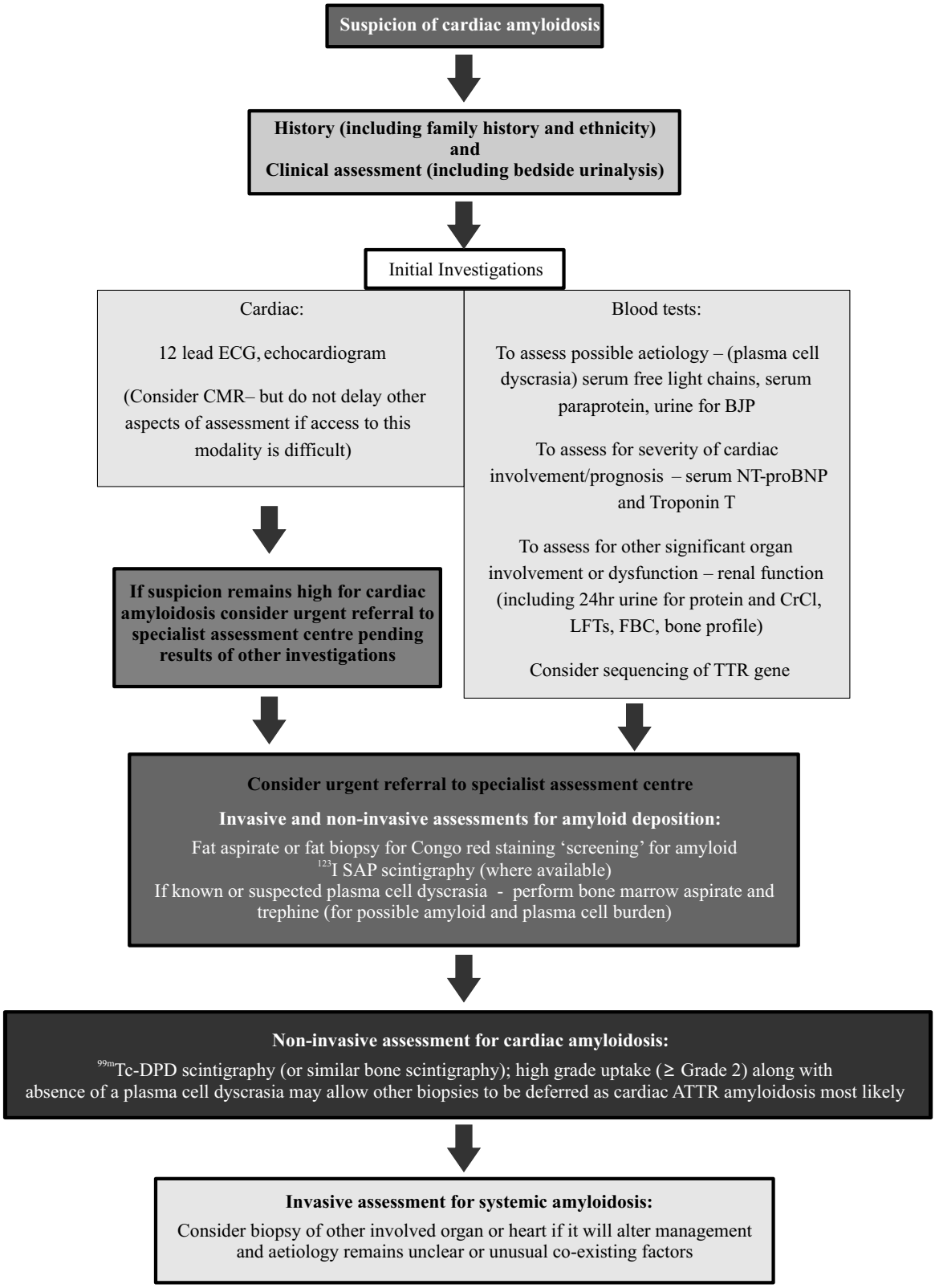


Figure 1.3: Schematic of a diagnostic pathway for patients with possible cardiac amyloidosis. ^{123}I SAP scintigraphy, ^{123}I Serum Amyloid P component scintigraphy; ^{99m}Tc DPD scintigraphy, ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy; ATTR amyloidosis, Transthyretin amyloidosis; BJP, Bence Jones Protein; CMR, cardiac magnetic resonance; CrCl, creatinine clearance; ECG, Electrocardiogram; FBC, full blood count; LFT, liver function test. Reproduced with permission.

considering and further investigating a patient in whom cardiac amyloidosis, particularly of the transthyretin type, is a possibility.

A pseudoinfarct pattern and poor R wave progression is another common ECG finding, and atrial fibrillation (AF) is particularly common in ATTR-wt [39, 41].

Conduction system disease is a commonly cited finding in cardiac amyloidosis but data have only been published in small studies. First degree AV block was reported in 23 out of 41 patients with ATTR-V122I in sinus rhythm [45] and 13 of 25 patients in cardiac AL [65]. Bundle branch block is also common, occuring in 20% of patients with cardiac AL amyloidosis [65] and 17% patients with hereditary ATTR [36]. Although these are small studies there appears to be a significantly higher prevalence of bundle branch block in patients with ATTR-wt amyloidosis occuring in 53% [36].

AF is common in ATTR cardiac amyloidosis (present in 33% of 18 patients with ATTR-wt and 9% out of 11 patients with V122I [41] and in 17% of AL amyloidosis [66]).

One study of 264 individuals with cardiac AL amyloidosis identified fragmentation of the QRS as a prognostic factor [67]. There have been no large studies examining the prognostic value of the ECG in ATTR cardiac amyloidosis.

1.5.3 Cardiac biomarkers

Cardiac biomarkers may raise the clinical suspicion of cardiac amyloidosis in a patient with a known plasma cell dyscrasia and should prompt further investigation with ECG and echocardiography. The combination of elevated serum NT-proBNP and troponin is associated with a poor prognosis in AL amyloidosis as discussed above.

1.5.4 Imaging

The goals of cardiac imaging in amyloidosis are to aid diagnosis and where possible provide prognostic information. The greatest body of experience has been with standard echocardiography, although strain and strain-rate imaging, CMR imaging and radionuclide scintigraphy have lately emerged as highly informative clinical tools.

Transthoracic echocardiography

Transthoracic echocardiography is an essential investigation for cardiac assessment in amyloidosis. Cardiac involvement in systemic AL amyloidosis is defined according to consensus opinion by either an endomyocardial biopsy demonstrating amyloidosis or when echocardiographic evidence of cardiac amyloidosis (mean left ventricle (LV) wall thickness $>$12mm with no other cardiac cause) is found in a patient with amyloid confirmed on a non-cardiac biopsy [68].

Standard echocardiography with M-mode, 2-dimensional (2D), pulsed wave, continuous wave and tissue Doppler, may reveal all or some of the characteristic abnormalities of cardiac amyloidosis. These include: concentric LV wall thickening with normal or small LV cavity and normal or mildly impaired systolic function; moderate to severe diastolic dysfunction or restrictive filling pattern; bi-atrial dilatation, pleural and pericardial effusions; and diffuse valvular and inter-atrial septal thickening. However, many of these features may be absent, especially in early disease where the differential from hypertensive disease is challenging, and a diagnosis of cardiac amyloidosis may be delayed or missed. Standard echocardiography is affected by inter and intra-observer variability which can limit its use for the detection of subtle cardiac changes and identifying disease progression or regression following treatment.

Myocardial strain imaging is emerging as a useful additional echocardiographic technique

in cardiac amyloidosis. Strain and strain-rate imaging derived from tissue Doppler have been reported to be more sensitive than standard tissue Doppler for the detection of systolic impairment [69]. 2D speckle-tracking echocardiography (STE) uses automatic frame by frame tracking of natural acoustic markers in the myocardium (speckles) which move together with the tissue. An example is displayed in Figure 1.4.

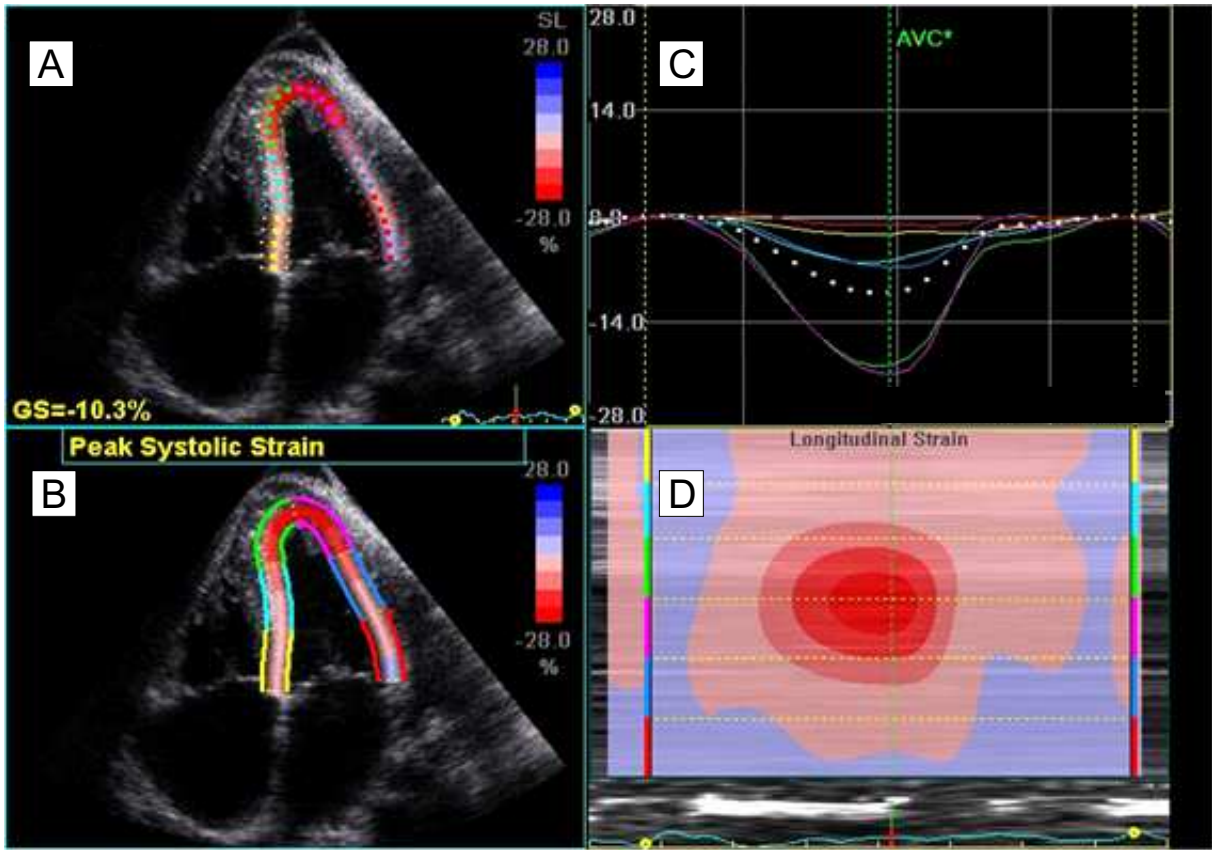


Figure 1.4: Representation of 2D-strain using speckle-tracking echocardiography of the left ventricle in the apical 4-chamber view in a patient with cardiac amyloidosis. The upper left panel (A) depicts each of the myocardial segments and shows the calculated value for the global myocardial strain (in this case -10.3%; cf. for normal individuals approximately -20%). The lower left panel (B) shows the peak systolic strain for each myocardial segment. The upper right panel (C) shows the strain curves sampled in each of the analysed myocardial segments. The lower right panel (D) depicts longitudinal strain processed according to a colour map for each segment. It clearly shows a longitudinal base to apex strain gradient (reduced apical strain compared to basal strain), giving rise to the typical 'bulls eye' appearance. Reproduced with permission.

It is a more reproducible technique than Doppler-derived strain imaging, and has been reported to be an independent predictor of survival [70]. Terminology in myocardial strain imaging includes strain (deformation) which is the percentage change in an objects dimension in

comparison to the object's original dimension and strain-rate which is the speed at which deformation occurs.

A relative apical sparing pattern of longitudinal strain has been shown to be sensitive and specific for the diagnosis of cardiac amyloidosis in a study of 55 patients with moderately increased LV wall thickness [71].

Strain imaging has significant advantages in terms of reproducibility but there remain significant inter-vendor differences in global longitudinal strain (GLS) measurements [72] which limits comparison between studies.

Radionuclide imaging in amyloidosis

^{123}I Serum Amyloid P component scintigraphy (^{123}I SAP scintigraphy)

All amyloid deposits contain serum amyloid P component (SAP), a normal plasma protein that binds specifically to all types of amyloid fibril. Radiolabelled SAP scintigraphy was developed at the NAC, UK and has been used routinely for more than 20 years [73] with an excellent safety record. It can provide diagnostic images for the majority of patients with systemic amyloidosis and is a non-invasive and quantitative method for monitoring response of amyloid deposits to treatment. Renal impairment is not a contraindication to SAP scintigraphy. While the technique can provide useful information about amyloid burden affecting the liver, spleen, kidneys, adrenal glands and bones, it cannot image amyloid burden in the moving heart, gastrointestinal tract, skin or nerves.

^{99m}Tc DPD scintigraphy and 99mTc-PYP scintigraphy

There has lately been a resurgence of interest in the off-label use of the bone scintigraphy tracers ^{99m}Tc DPD and 99mTc-PYP for the diagnosis of cardiac ATTR amyloidosis (an example is illustrated in Figure 1.5). Although the basis for localisation of these agents to cardiac amyloid remains unknown, the technique appears to be very sensitive for imaging cardiac

ATTR amyloid deposits and are even able to identify pre-symptomatic disease. ^{99m}Tc DPD scintigraphy is favoured in Europe but not available in the US, where broadly similar results appear to have been obtained using 99mTc-PYP. CT-SPECT (fused X-ray CT with single photo emission CT) imaging can be performed simultaneously to enable more accurate localisation of tracer and, potentially, quantification of cardiac amyloid. There are data to suggest that a higher Perugini grading of ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy (DPD grade) is associated with a greater burden of cardiac amyloidosis [74–76] but this has not been systematically analysed in a large population utilising multiple measures of cardiac involvement.

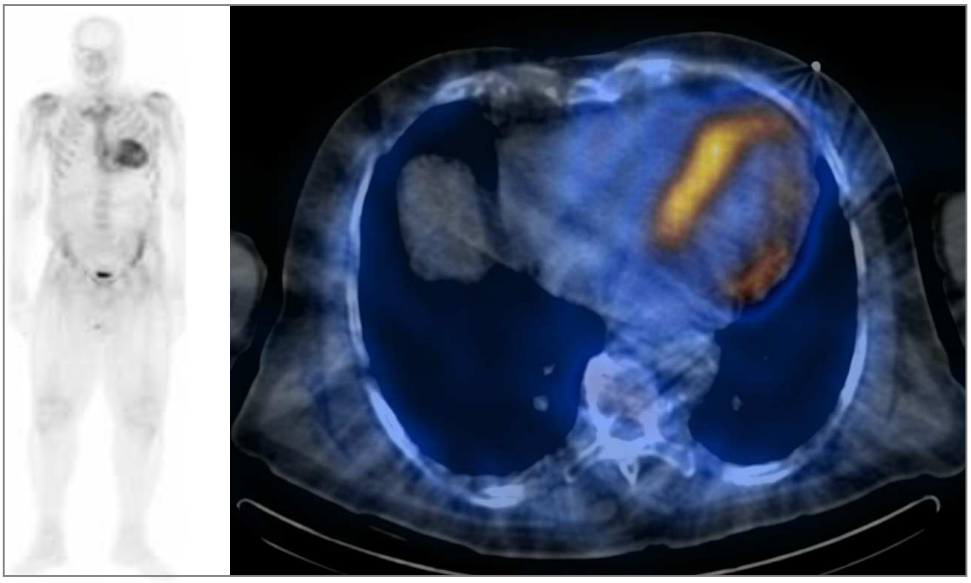


Figure 1.5: ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy (left) in a patient with cardiac wild-type transthyretin amyloidosis and a cross-sectional Single-photon emission computed tomography (SPECT-CT) image at the cardiac level (right). In this case cardiac uptake is of equal intensity to bone uptake (grade 2 scan). A cross-sectional SPECT-CT image at the cardiac level (right) confirms tracer localisation to the heart (predominantly in septum in this case where it is seen as a bright yellow colour). Reproduced with permission.

While ^{99m}Tc DPD scintigraphy appears to show cardiac uptake in all patients with clinically significant cardiac ATTR amyloidosis, it is not specific for this amyloid type. Cardiac localisation also occurs in a significant proportion of patients with AL amyloidosis [75] and cardiac Apoprotein A-1-Related Amyloidosis (ApoA1 amyloidosis) [76, 77].

Cardiovascular magnetic resonance

CMR has recently been found to provide utility in the evaluation of suspected cardiac amyloidosis. Although advanced cardiac amyloidosis with classical imaging features may be easy to detect by standard echocardiography techniques, CMR provides more accurate measurements of LV volume, mass and wall thickness than echocardiography which may be of particular benefit in sequential assessment. Furthermore, CMR has the ability to perform myocardial tissue characterisation which has many potential advantages over other imaging techniques (an example of CMR imaging is displayed in Figure 1.6). CMR utilises the magnetic properties of hydrogen nuclei protons within a magnetic field. After an intravenous bolus of chelated gadolinium contrast (Gd-DTPA; gadolinium diethylenetriamine penta-acetic acid) cardiac amyloidosis reveals a unique appearance of global subendocardial late gadolinuium enhancement (LGE) [78] which, when classical, is virtually pathognomonic of cardiac amyloidosis and may be found even before increased LV wall thickness is evident, thus potentially serving as an early marker of disease. Typically in amyloidosis, there is global subendocardial LGE in a non-coronary distribution with a dark blood pool although various other characteristic findings have been identified including localised, diffuse transmural or patchy LGE [79].

CMR enables quantification of amyloid deposits which are confined to the interstitial space. In the LGE technique, this extracellular agent passes through the vascular wall and into the extracellular space but the molecules are too large to pass into intact myocardial cells. Amyloidosis is the exemplar of an interstitial disease, and the massive expansion of the extracellular compartment by amyloid can be quantified by the technique of T1 mapping through the measurement of the native (non-contrast) myocardial T1, post-contrast T1 and

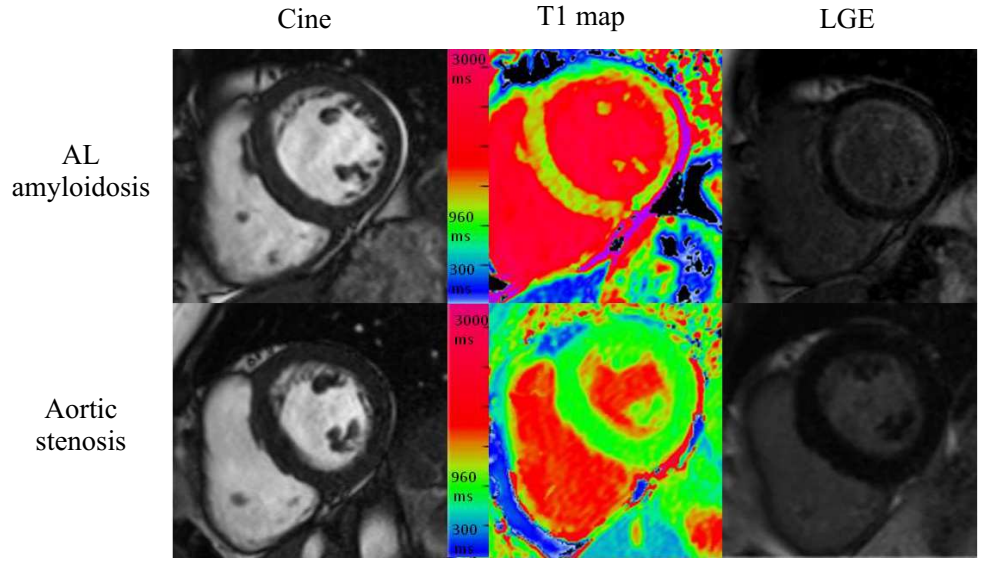


Figure 1.6: Example cross-sectional images from cardiac cardiac magnetic resonance scans in a patient with cardiac AL amyloidosis (top) and a patient with aortic stenosis (bottom). End-diastolic frames from cine (left), non-contrast T1 maps (middle), and late gadolinium enhancement LGE images (right). Note in the upper panes: no significant increase in left ventricular (LV) wall thickness (left); red pixels amongst the green LV wall (middle) in keeping with high T1 values; and diffuse patchy subendocardial late gadolinium enhancement LGE (right). T1 is the longitudinal relaxation time of a tissue (ie. the time taken for recovery of longitudinal magnetization); a property of tissue predominantly determined by the water content of the tissue see CMR section. Note in the lower panes: the increased LV wall thickness (left), green LV wall without red pixels (middle) in keeping with normal T1 values and no LGE (right). Reproduced with permission.

extra-cellular volume (ECV). T1 is the longitudinal relaxation time of a tissue (i.e. the time taken for recovery of longitudinal magnetization). Relaxation times are properties of tissue which are predominantly determined by water content. In the T1 mapping technique each pixel on a CMR image can be colour-coded to reflect the T1 value and aid interpretation. Increases in native T1 occurs in various pathological states including diffuse fibrosis and localised scar, but T1 is markedly elevated in the presence of cardiac amyloid and has been proven to be useful in differentiating the commonest cardiac amyloidosis types (AL and ATTR) from other causes of increased LV wall thickness. This may find clinical utility particularly in patients with severe renal failure, when the use of gadolinium contrast is contraindicated. The measurement of native myocardial T1, shows promise for the detection and tracking of cardiac AL amyloidosis and ATTR amyloidosis [29, 80] thus offering a much desired and useful measure in current and

future clinical trials of treatments for cardiac amyloidosis.

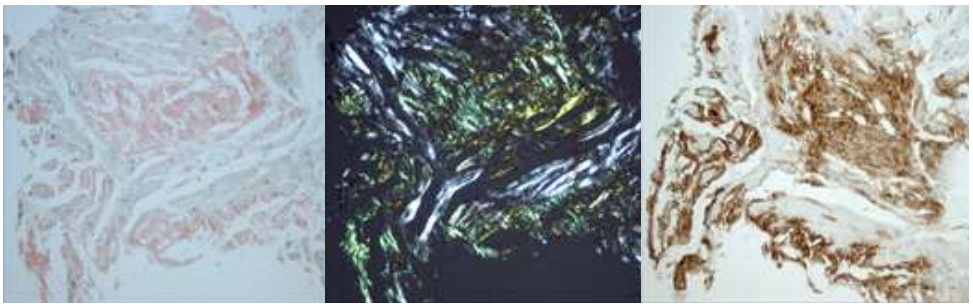
Following the administration of gadolinium-chelated contrast, post-contrast T1 may also be assessed and shows lower values in the presence of expanded interstitial space (as in amyloid). However, interpretation must be cautious in view of the potential for misleading alterations in these values due to body fat percentage, variation in time taken from contrast administration to T1 measurement, and renal function. Measurement of ECV (the proportion of tissue that is interstitial space) after contrast administration has been shown to be hugely increased in AL amyloidosis compared with normal individuals and patients with other causes of increased LV wall thickness such as aortic stenosis, and hypertrophic cardiomyopathy and is thought to reflect interstitial expansion due to amyloid deposition [81]. ECV correlates with CMR measures of LV mass, wall thickness and cardiac function, as well as functional assessment by six minute walk test (6MWT) distance in AL amyloidosis [82], and highlights its potential for the serial quantification of cardiac amyloid burden and therefore also to serve as a useful non-invasive surrogate in clinical trials assessing novel treatments.

Histological validation of these techniques are limited. This is largely due to ethical considerations as cardiac biopsy is not necessary for the clinical management of the patients in the majority of cases. This approach to ECV measurement has some further limitations, the most significant of which is that it is more time consuming than standard CMR, however technical developments are underway to simplify the approach to ECV measurement [83]. Other limitations of CMR include that it is contraindicated in the presence of non-MRI conditional pacemakers and Implantable Cardioverter Defibrillator (ICD)s and is relatively contraindicated in the presence of significant renal failure due to the rare possibility of causing nephrogenic systemic sclerosis. Furthermore, it is less clinically useful than echocardiography for certain functional assessments; namely diastolic function and myocardial strain.

1.5.5 Histology, immunohistochemistry and proteomics

A definitive diagnosis of amyloidosis is obtained through biopsy of an involved organ in a patient with a compatible clinical phenotype. This may require endomyocardial biopsy in patients with suspected cardiac amyloidosis. However, a multidisciplinary approach, potentially including echocardiography, ^{99m}Tc DPD scintigraphy, CMR along with a non-cardiac biopsy containing amyloid, may be sufficient [33]. A fat aspirate is a simple non-invasive way to obtain tissue for histological studies, which can be easily performed in minutes under local anaesthesia in the clinic room, but sensitivity is limited to about 25-70% depending on the amyloid type [84].

Confirmation of amyloid deposition in a tissue section through Congo red histology should be followed-up by immunohistochemical typing using a panel of monospecific antisera to serum amyloid A (SAA), transthyretin and kappa and lambda light chain proteins. Histology images of an endomyocardial biopsy taken from a patient with cardiac ATTR amyloidosis are shown in Figure 1.7. Characterisation of amyloid fibril type is a crucial step in guiding further clinical management and treatment.



Panel A Panel B Panel C

Figure 1.7: Histology images from an endomyocardial biopsy taken from a patient with cardiac transthyretin amyloidosis. The presence of amyloid deposits is demonstrated throughout and is seen as a pink amorphous material when stained with Congo red (panel A) and displaying apple-green birefringence when viewed under high intensity cross polarised light (panel B). Immunohistochemical staining using monospecific antibodies reactive with transthyretin (TTR) show the amyloid stains with antibodies to TTR (seen in panel C stained dark brown), thereby confirming amyloidosis of the TTR type. Reproduced with permission.

Immunohistochemical staining of amyloid deposits has numerous pitfalls and may fail to identify fibril type, even in experienced hands. Proteomic identification of amyloid type through mass spectrometry, using amyloidotic material cut out from tissue sections by laser dissection, is being used increasingly in specialist amyloidosis practice with a substantial improvement in diagnostic yield. The technique has been reported to be useful in assessing samples of subcutaneous fat which may increase its clinical utility [85]. At the NAC, immunohistochemistry and mass spectrometry are regarded as complementary and often mutually informative investigations.

1.5.6 Genetic sequencing

Hereditary types of amyloidosis may present with clinical features that cannot be distinguished from acquired types, and the anticipated dominant family history is often absent due to reduced penetrance [86]. Genetic sequencing using deoxyribonucleic acid (DNA) extracted from blood is therefore often required during the initial assessment of a new patient, and is indicated in all patients with suspected or proven ATTR amyloidosis to establish what form of ATTR amyloidosis the patient has.

1.6 Treatment of cardiac amyloidosis

1.6.1 Heart failure management

Medical management

Management of cardiac amyloidosis can be challenging. Patient education and multi-disciplinary input from cardiologists, heart failure nurse specialists and General Practitioners is essential. Daily weighing is useful to guide pre-emptive adjustment of fluid

intake and diuretic therapy. Sodium and fluid restriction is paramount to managing symptoms of heart failure. The combination of different diuretic types (loop diuretics and thiazides) can be helpful in resistant cases, and the addition of an aldosterone antagonist can help to maintain potassium homeostasis. Hypotension and orthostatic hypotension contribute to difficulties in management [87].

There have been no prospective studies to evaluate the prognostic benefit of Angiotensin Converting Enzyme inhibitor (ACEi)s, Angiotensin II Receptor Blocker (ARB)s and beta blockers in cardiac amyloidosis. The use of such agents, particularly in larger doses, is often limited by hypotension and low cardiac output. Digoxin should in general be avoided due to its potential for cardiotoxicity as a result of possible accumulation in amyloidotic tissues despite normal serum levels [88]. Calcium channel antagonists are also relatively contraindicated [89, 90]. Warfarin or an alternative anti-coagulant for AF is recommended unless a major contraindication exists since intra-cardiac thrombus formation is prevalent in cardiac amyloidosis [91].

Orthostatic hypotension may be treated with the alpha1-adrenoceptor agonist, midodrine, and compression stockings. Fludrocortisone is relatively ineffective and may exacerbate fluid retention. Frequency of diarrhoea can be improved with loperamide and opioids. Subcutaneous octreotide prior to eating is effective in some patients [92]. Pro-kinetic agents such as metoclopramide can be useful for gastroparesis. Painful peripheral neuropathy can be eased with gabapentin and pregabalin, though some patients may require opiate analgesia.

Cardiac devices - pacemakers and implantable cardioverter defibrillators

Post-mortem histological studies have demonstrated fibrotic changes in various parts of the heart [93] and progressive conduction system involvement commonly necessitates pacemaker implantation. In our UK clinical practice pacemaker implantation is guided by the current

general guidelines; no specific recommendations exist for pacemaker, ICD or cardiac resynchronisation therapy (CRT) implantation in patients with cardiac amyloidosis in European guidelines. The most recent US guidelines on device usage suggest consideration on a case by case basis for ICD implantation for primary and secondary prevention of sudden cardiac death (SCD) [94]. This guidance is in light of reported cases where benefit has been demonstrated, although data are very limited [95].

Cardiac transplantation

Cardiac transplantation has been performed in a relatively small number of patients with either AL or ATTR amyloidosis. When undertaken for life-threatening heart failure in cardiac AL amyloidosis, it must be followed by chemotherapy to suppress the associated plasma cell dyscrasia to prevent recurrent cardiac amyloidosis and disease progression in other organ systems; outcomes in highly selected cases is comparable with patients receiving a heart transplant for non-amyloid heart disease [96]. Given the typically advanced age at diagnosis among patients with cardiac ATTR amyloidosis cardiac transplant is rarely feasible, but outcomes in highly selected patients have been excellent in our experience and recurrence has not occurred [97].

1.6.2 Disease modifying treatments for cardiac amyloid light chain amyloidosis

Cardiac AL amyloidosis has a poor prognosis but it may respond to chemotherapy that rapidly suppresses the underlying population of clonal plasma cells thereby reducing production of amyloidogenic light chains and inhibiting further amyloid deposition. Selection and intensity of chemotherapy regimen should be tailored individually, taking account of patient's age,

co-morbidities and performance status, presence and degree of extra-cardiac organ involvement, and treatment-related toxicities. The effects of chemotherapy need to be monitored closely in terms of haematological response and tolerability, and the treatment adjusted or changed accordingly.

Chemotherapy for systemic amyloid light chain amyloidosis

Chemotherapy for AL amyloidosis has evolved greatly over recent years, tracking progress and development of new agents in the treatment of myeloma. In our current practice, most patients with cardiac AL amyloidosis who are deemed suitable for chemotherapy receive a cyclic combination of the alkylating agent cyclophosphamide, the proteasome inhibitor bortezomib (Velcade) and dexamethasone (CVD). If tolerated, this treatment is associated with high haematological response rates and can achieve prolonged progression-free survival in a proportion of patients with Mayo Stage 3 disease who would otherwise have amongst the poorest prognosis [98, 99]. In 43 patients at the NAC the estimated 2-year survival was 94.4% for Mayo Stage 3 disease and 97.7% overall [98]. Other combination regimens may be more appropriate in patients with significant peripheral or autonomic neuropathy, given the potential neurotoxicity of bortezomib, and in patients with underlying lymphoplasmacytic lymphoma in whom other combinations may be more effective. Although often a useful treatment, bortezomib for patients with cardiac AL amyloidosis can cause worsening of heart failure symptoms with a measurable fall in ejection fraction, worsening peripheral oedema, orthostatic hypotension and hypotension. Dexamathasone is also frequently associated with fluid retention and may require dose reduction or omission in some circumstances. In our practice, patients with the very worst prognostic markers, are typically treated with lower dose regimes initially and are up-titrated according to tolerability.

ASCT for systemic amyloid light chain amyloidosis

High dose chemotherapy comprising intravenous melphalan at 140-200mg/m^2 supported by autologous peripheral blood stem cell rescue/transplantation (ASCT) is widely used in younger patients with multiple myeloma, in whom there is usually no significant impairment of vital organ function. This rationale prompted its early trials in AL amyloidosis. ASCT was first described as a potential treatment strategy for AL amyloidosis in 1993 when it was given to a 53 year old Italian patient. [100]. This first patient succumbed but more promising outcomes were reported in 1998 in a series of 25 patients treated at the Boston University Medical Center [101], and the approach has been refined progressively since. Clonal response rates and durability of response following ASCT surpass those achieved with cyclophosphamide, thalidomide and dexamethasone (CTD), lenalidamide based regimens [102], and oral melphalan and dexamethasone [103]. However, ASCT in AL amyloidosis remains associated with substantial treatment related morbidity and mortality in patients with cardiac involvement, and is unsuitable for most of these patients.

Immunotherapy

Therapeutic antibodies that recognise an epitope expressed in human light chain amyloid fibrils have been shown to trigger clearance of experimentally induced amyloidomas in mice [104]. A chimeric form of this monoclonal antibody currently designated 11-1F4 has similar activity [105], and entered Phase I clinical trials in patients with AL amyloidosis (ClinicalTrials.gov Identifier: NCT02245867) in 2014 with promising results presented at the 16th International Symposium on Amyloidosis (March 2018). Another monoclonal antibody designated NEOD001 also entered Phase I clinical testing in AL amyloidosis in 2013 (ClinicalTrials.gov Identifier: NCT01707264) and has reported promising results [106].

An alternative immunotherapeutic approach, designed to have application in all types of amyloidosis, comprises the combination of a small molecule that depletes circulating SAP co-administered with a monoclonal antibody that targets SAP that remains associated with the amyloid deposits. The novel compound (R) -1-[6-[(R)-2-Carboxy-pyrrolidin-1yl]-6-oxo-hexanoyl] pyrrolidine-2 carboxylic acid (CPHPC) cross-links pairs of SAP molecules in the plasma, triggering their rapid removal by the liver [107]. Whilst sustained depletion of circulating SAP is well tolerated and may itself be therapeutic with prolonged administration [108], this treatment does not deplete SAP from amyloid deposits in the very short term. This novel pharmacological phenomenon enables residual amyloid-associated SAP to be targeted by anti-SAP antibodies [109], and the validity of this strategy has been confirmed in experimental murine amyloidosis. The combination of CPHPC and a fully human monoclonal anti-SAP antibody has favourable outcomes with reduction in hepatic, kidney and lympth node amyloid burden in a phase 1 trial [110].

1.6.3 Disease modifying treatments for cardiac ATTR amyloidosis

A summary of the potential therapeutic strategies in cardiac transthyretin cardiac amyloidosis is displayed in Figure 1.8 with detailed explanation in the following sections.

RNA silencing - small interfering RNA and anti-sense oligonucleotide therapies

Ribonucleic acid (RNA) silencing, or RNA interference, is a promising strategy being developed to inhibit hepatic synthesis of both wild-type and variant TTR. RNA silencing therapeutics potently silence specific messenger ribonucleic acid (mRNA) sequences, thereby having potential to prevent disease-causing proteins from being made. This new technology is particularly able to target liver derived proteins. Different approaches to RNA silencing can

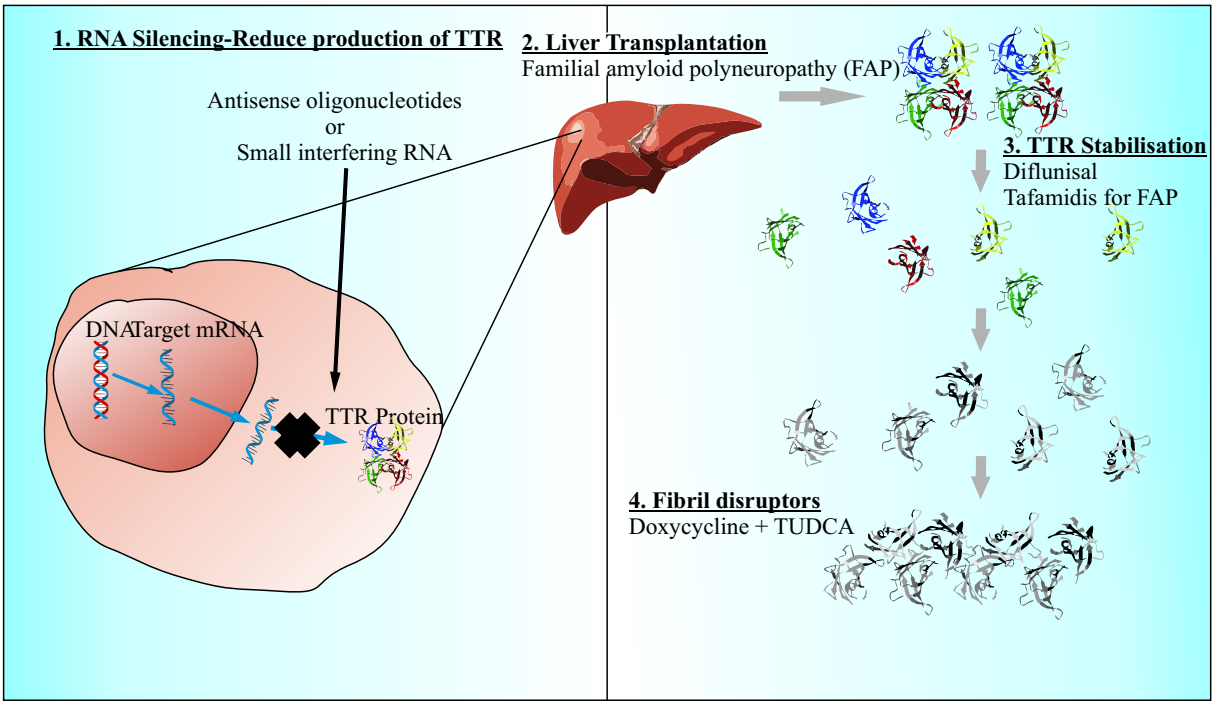


Figure 1.8: Schematic illustrating potential therapeutic strategies in transthyretin cardiac amyloidosis. See main text for detailed explanation. DNA, deoxyribonucleic acid; FAP, Familial amyloid polyneuropathy; mRNA, messenger ribonucleic acid; RNA, ribonucleic acid; TUDCA, Tauroursodeoxycholic acid; TTR, transthyretin. Reproduced with permission.

be employed but relevant to the development of treatments for TTR amyloidosis are the use of small interfering ribonucleic acid (siRNA)s and anti-sense oligonucleotides.

SiRNAs bind to a specific complementary mRNA sequence resulting in cleavage of the mRNA thus causing a reduction of the targeted protein. Systemic administration of unmodified and unformulated siRNA is an ineffective strategy as insufficient tissue distribution is attained due to rapid degradation and elimination [111]. Patisiran (Alnylam Pharmaceuticals) is an investigational product administered as an intravenous infusion and comprises an siRNA encapsulated in a lipid nanoparticle which has been shown to effectively reduce serum TTR levels in healthy volunteers (NCT01814839) and patients with ATTR amyloidosis [112]. In a Phase 2 open-label extension study in 27 patients with hereditary ATTR amyloidosis with polyneuropathy, Patisiran administered every 3 weeks was generally well tolerated, and disease stabilization was observed over 24 months treatment. Liver specificity of RNAi therapeutics

also appears to be achievable by conjugation with an N-acetylgactosamine (GalNAc) ligand which can be taken up into hepatocytes by the asialoglycoprotein receptor [113]. Revusiran (ALN-TTRSC-002) is a second generation investigational product of this type and has the advantage over Patisiran that it can be administered subcutaneously. Revusiran is an siRNA consisting of 21 base pair double-stranded oligonucleotides in a staggered duplex with a covalently attached triantennary GalNAc ligand which targets the 3'UTR of TTR mRNA and is thus homologous in wild-type TTR and all known TTR mutations, and is formulated in water for injection. When administered subcutaneously to healthy volunteers in a phase 1 multiple ascending dose study up to 10 mg/kg, it was generally well-tolerated and resulted in mean reduction in plasma TTR concentration of approximately 90% at doses of $\geq$5mg/kg per week [114]. The development of revusiran for cardiac TTR amyloidosis will be discussed further in Chapter 7.

Anti-sense oligonucleotide therapy is an alternative approach that also inhibits production of TTR by some 80% or more. This approach has been investigated in a variety of diseases such as cytomegalovirus retinitis and homozygous familial hypercholesterolaemia. A short synthetic single chain of nucleotides bind to RNA preventing translation and synthesis of the target protein. Inotersen is a second generation agent administered subcutaneously on a weekly basis for 64 weeks that binds to TTR mRNA and results in its degradation. Results published in 2018 of a phase 3 randomised, double blind trial showed improvement in quality of life scores and modified the course of neuropathy in patients with FAP. However, there were safety concerns regarding thrombocytopenia and glomerulonephritis and there were more deaths in the treatment arm [115].

Liver transplantation

Orthotopic liver transplantation for FAP was first performed in 1990 in Sweden [116] to remove the source of variant TTR in the plasma. Over 2000 liver transplants have been performed since for this indication, and outcomes have been better among patients with the ATTR-V30M variant, in whom cardiac involvement is much less common than in patients with other TTR mutations. Stabilisation or even some improvement in neuropathy can occur when the procedure is performed at an early stage [117]. However, liver transplantation appears to precipitate rapid progression of cardiac ATTR amyloidosis in patients with any significant cardiac involvement prior to the procedure. This has been shown to be due to continued deposition of wild-type ATTR amyloid [46, 118, 119]. This has largely ruled out liver transplantation among British FAP patients, most of whom have the TTR T60A variant with cardiac involvement. In these patients, combined cardiac and liver transplantation remains a mainly theoretical option for reasons of feasibility.

Explanted livers from FAP patients may be re-used to transplant patients with end-stage liver disease in a procedure known as domino liver transplantation, but some recipients have developed iatrogenic FAP 5-10 years later.

TTR tetramer stabilisation

Dissociation of the TTR tetramer into its subunits has been hypothesised to be a crucial step in ATTR amyloid fibril formation, prompting interest in and development of agents that bind to and thermodynamically stabilise the circulating protein tetramer in order to maintain its normal soluble conformation. The properties of drugs that are bound in the plasma by TTR, diflunisal and tafamadis, have recently been investigated in patients with ATTR amyloidosis.

Diflunisal

In vivo studies have found that diflunisal, a non-steroidal anti-inflammatory drug (NSAID) stabilises the TTR tetramer which may prevent mis-folding monomers from forming amyloid deposits in the heart [120]. However, diflunisal appears to be variably tolerated due to adverse gastro-intestinal effects, fluid retention which exacerbates heart failure symptoms and impairment of renal function. In a recent placebo-controlled clinical study, diflunisal 250mg taken orally twice daily in patients with FAP was associated with reduced progression of neuropathy [121]. There was however, substantial attrition of participants during the trial reportedly due to disease progression, raising questions about the effectiveness of the treatment and adverse effect profile. In one small single-arm open-label study in 2012 of 12 patients with cardiac ATTR amyloidosis diflunisal was reported to be reasonably well-tolerated accepting some renal decline at 1 year follow-up [122].

Tafamidis meglumine

Tafamidis meglumine (Vyndaqel, Pfizer) is licensed in the European Union (EU) and Japan for use in patients with early polyneuropathy associated with hereditary transthyretin amyloidosis. A randomised placebo-controlled trial was conducted in 125 patients with early peripheral neuropathy associated with the ATTR-V30M mutation. Progression of neuropathy was slightly slower among patients receiving tafamidis (20mg orally once daily) compared to placebo [123]. Recently, Vyndaqel has been approved by the FDA for use in ATTR cardiomyopathy after the publication of the ATTR-ACT trial, a double-blind placebo-controlled trial which showed a reduction in the combined primary end-point of all-cause mortality and cardiovascular-related hospitalisations, and secondary outcomes of lower rate of decline in both 6MWT and KCCQ-OS score compared with placebo [124]. The National Health Service (NHS) in the UK does not currently support funding tafamidis meglumine for the treatment of FAP.

Interrupting fibrillogenesis

Doxycycline and Tauroursodeoxycholic Acid (TUDCA)

The combination of doxycycline and tauroursodeoxycholic acid (TUDCA), a bile acid, has been investigated in an ATTR-V30M transgenic mouse model of FAP with a suggestion that it may interfere with ATTR amyloid formation [125]. It has been proposed that doxycycline acts as a fibril disrupter and TUDCA as an anti-apoptotic and anti-oxidant agent. An open-label phase 2 study of 20 patients, including 17 with hereditary ATTR amyloidosis, suggests this combination therapy is safe and tolerated [126]. An 18-month open label phase 1/2 study in inherited and acquired cardiac ATTR amyloidosis has yet to report its findings (ClinicalTrials.gov identifier: NCT01855360).

1.7 Aims

- To compare the clinical features and outcomes between patients with the most common forms of cardiac ATTR amyloidosis in the UK; cardiac ATTR-wt, ATTR-V122I and ATTR-T60A amyloidosis.

- To identify prognostic markers in ATTR-wt and ATTR-V122I cardiac amyloidosis.

- To investigate ^{99m}Tc DPD scintigraphy as a marker of amyloid burden in cardiac transthyretin amyloidosis and prognosis in cardiac AL amyloidosis.

- To evaluate the safety and tolerability of diflunisal for cardiac transthyretin amyloidosis in a retrospective study.

- To describe experience in a prospective multi-centre phase 2 clinical trial of revusiran in patients with cardiac ATTR amyloidosis, with particular focus on the effect of enrolment

criteria on the generalisability of the findings from such a trial.

1.8 Structure of thesis

Chapter 3 and Chapter 4 describe and compare the clinical characteristics and investigation findings for individuals with different forms of cardiac ATTR amyloidosis. Published data in this uncommon disease have only been reported in modestly sized cohorts, often combining patients with a variety of hereditary types of ATTR amyloidosis to form heterogeneous groups, potentially limiting clinical usefulness and disease understanding. Furthermore, data on prognosis from the largest study, THAOS, conflicts significantly with smaller studies. However, there are major limitations to the THAOS study that adds further uncertainty to the expected prognosis in these patients. Chapter 3 focuses on the ECG characteristics of the most common forms of cardiac ATTR amyloidosis in the UK; cardiac ATTR-wt, ATTR-V122I and ATTR-T60A amyloidosis. Chapter 4 focuses on multi-modality imaging, describing prognosis and identifying prognostic markers in cardiac ATTR-wt and ATTR-V122I amyloidosis. Patients with ATTR-T60A were ultimately excluded from Chapter 4 due to significant missing imaging data as described in the chapter. The data presented in these two chapters describe the largest comparable populations of cardiac ATTR-wt and ATTR-V122I amyloidosis to date.

In Chapter 5 I investigated the visual grading score of ^{99m}Tc DPD scintigraphy in cardiac ATTR-wt, ATTR-V122I and AL amyloidosis. I examined associations between DPD grade and clinical, biochemical and echocardiographic findings. I went on to evaluate the value of visual scoring of ^{99m}Tc DPD scintigraphy as a measure of disease severity and prognosis.

In Chapter 6 I evaluated the real world use of diflunisal for cardiac transthyretin amyloidosis at the NAC, UK, and undertook a retrospective case-control study to investigate the safety and efficacy of diflunisal for those individuals receiving treatment for 1 year.

Chapter 7 summarises my work as a sub-investigator for, and the results of, a phase 2 clinical trial of a novel treatment for cardiac transthyretin amyloidosis. Additionally I investigated the applicability of the results to the NAC cardiac ATTR amyloidosis cohort, and evaluated the effect of patient selection criteria on recruitment to the trial.

Chapter 2

General Methods

2.1 Patient population

The patients described in this thesis have been evaluated at the NAC, UK. A Microsoft Access database at the NAC is maintained with details of all patients who have amyloidosis. All patients provided explicit informed consent for their data to be used for research purposes. Survival status was ascertained by hospital attendance and telephone communication. Death certificate data for patients who died in England and Wales were provided by the Office for National Statistics.

2.2 Functional evaluation

New York Heart Association (NYHA) Classification was used to classify functional status. This method provides a standardised and widely accepted method of characterising patients with heart failure according to their symptom severity (Table 2.1) and was performed by the assessing clinician.

Functional evaluation of patients with cardiac AL amyloidosis was also evaluated using the Eastern Cooperative Oncology Group (ECOG) performance status (Table 2.2). Historically,

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

Table 2.1: New York Heart Association Classification. NYHA, New York Heart Association.

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

Table 2.2: Eastern Co-operative Group (ECOG) performance status

this tool has been used to determine suitability for chemotherapy and to guide dosing.

In addition, the Karnofsky performance status scale was used to assess eligibility for enrolment in a phase 2 study of revusiran (Table 2.3).

2.3 Six-minute walk test

The 6MWT was used as a marker of functional capacity and measures the distance an individual can walk over six minutes on a hard, flat surface. The individual walked back and forth along a straight, marked, 30m walkway supervised by a nurse trained in overseeing the test who also recorded the walk distance on completion of the test. The instructions provided to the individual were standardized. Patients were informed of the time and encouraged each minute.

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary;active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

Table 2.3: Karnofsky performance status scale

2.4 Electrocardiogram

A standard 12-lead ECG (MAC 1200 ST digital 12-lead electrocardiograph system, GE Medical Systems, Milwaukee) was recorded at each clinic attendance. The calibration was 0.1mV/mm and paper speed was 25mm/s. Heart rate, PR interval, QRS duration, QT correction with Bazett formula (QTcB), and cardiac axis were determined by built-in software and confirmed manually. Manual measurements to the nearest 1mm were performed to evaluate for low QRS voltage and left ventricular hypertrophy. Low QRS voltage was defined using the standard criteria: a) all QRS amplitudes in the limb leads (I, II, III, aVL, and aVF) $<$5mm; or as described by Carroll et al. [127] (b) sum of S wave in V1 + R wave in V5 or V6 $<$15mm. Left ventricular hypertrophy was characterised using both the (a) Sokolow-Lyon criteria (sum of S wave in V1 + R wave in V5 or V6 $>$35mm [128]) and (b) Cornell criteria (sum of R wave in aVL + S wave in V3 $>$ 28mm in men and 20mm in women) [129].

2.5 Transthoracic echocardiography

Standard transthoracic echocardiography using the parasternal long and short axes, apical 4, 3, and 2 chamber, sub-costal and suprasternal views was performed with Vivid E9 Version 112 with an M5S active matrix single-crystal phased array transducer, frequency range 1.5-4.5mHz, GE Healthcare. Scans were performed with the patient in the supine left lateral decubitus position. Images were analysed offline using standard methods in accordance with recommendations from the British Society of Echocardiography and the American Society of Echocardiography . Left ventricular systolic function was categorised based on left ventricular ejection fraction (LVEF) calculated by Simpson's biplne in to the following categories: normal LV function (LVEF$\geq$55%), mild left ventricular systolic dysfunction (LVSD) (LVEF 45-54%), moderate LVSD (LVEF 36-44%) and severe LVSD (LVEF$\leq$35%). Strain analysis was performed using the speckle tracking method; the GLS is reported. Data were not gathered in cases where non-diagnostic images precluded accurate assessment.

2.6 Cardiac magnetic resonance imaging

Subjects underwent standard CMR imaging on a 1.5-T clinical scanner (Avanto, Siemens Healthcare, Erlangen, Germany). A standard volume and LGE study was performed. Native T1 measurement was performed with the use of the shortened modified look-locker inversion recovery sequence (ShMOLLI) [130] with regions of interest drawn in the 4-chamber view at the level of the basal and mid inferoseptum (2 segments, large region of interest) [131]. Native T1, a composite signal from both the interstitium and cells, is sensitive to the presence of amyloid, fibrosis and oedema. After a bolus of gadoterate meglumine (0.1 mmol/kg, gadolinium-DOTA, Dotarem, Guerbet S.A. France) and standard LGE imaging (standard fast

low-angle shot inversion recovery or balanced steady state free precession sequence with MAG-IR and PSIR reconstruction). At this stage, the patient was removed from the scanner. The ECV measurement approach used equilibrium CMR with a primed infusion: At 15 minutes after bolus, an infusion at a rate of 0.0011 mmol/kg/min contrast (equivalent to 0.1 mmol/kg over 90 minutes) was given. Between 45 and 80 minutes after bolus, the patient was returned to the scanner with the infusion continuing, and the T1 measurement was repeated using the same parameters of the pre-contrast ShMOLLI sequence. ECV represents the fraction of myocardium that is amyloid and is calculated with the equation: myocardial ECV = (1-haematocrit) x (ΔR1myocardium/ΔR1blood) where R1 = 1/T1.

2.7 ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy

Subjects were scanned using two GE Medical Systems (Fairfield, Connecticut) hybrid single photon emission computed tomography (SPECT) and computed tomography (CT) gamma cameras (Infinia Hawkeye 4 and Discovery 670) following administration of 700 MBq of intravenously-injected 99mTc DPD. The 3 hour (delayed) whole body planar images were acquired, followed by SPECT of the heart with a low-dose, non-contrast CT scan. Gated/non-gated cardiac SPECT reconstruction and SPECT-CT image fusion was performed on the GE Xeleris workstation. Visual scoring was reported as described by Perugini et al. [132] and is summarised in Table 2.4.

^{99m}Tc DPD visual grade	Description
0	Absent cardiac uptake and normal bone uptake
1	Mild cardiac uptake, inferior to bone uptake
2	Moderate cardiac uptake associated with attenuated bone uptake
3	Strong cardiac uptake with mild/absent bone uptake

Table 2.4: Perugini scoring for ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid ^{99m}Tc scintigraphy.

2.8 ^{123}I SAP scintigraphy

SAP scintigraphy was performed in all subjects at initial assessment. Each subject received approximately 200μg of SAP with 190MBq of ^{123}I as an intravenous injection. Thyroid uptake was blocked by the administration of 60mg of potassium iodide as an oral suspension immediately prior to the study. Five further doses of potassium iodide were dispensed for self-administration over the subsequent three days. Anterior and posterior imaging was performed at either 6 or 24 hours after injection using an IGE-Starcam gamma-camera (IGE Medical Systems, Slough, UK).

2.9 Diagnosis of cardiac involvement

Patients were diagnosed with cardiac amyloidosis if they had the combination of histological confirmation of amyloid (cardiac or non-cardiac biopsy) and increased wall thickness (Interventricular septal thickness in diastole (IVSd) and left ventricular posterior wall thickness in diastole (LVPWd) greater than 1.2cm in the absence of any other causes of increased wall thickness) [68].

Alternatively, patients were diagnosed with cardiac amyloidosis without biopsy evidence

if other criteria were satisfied; in 2016 following a meeting of an international panel of experts, non-invasive diagnostic criteria for cardiac ATTR amyloidosis were proposed [33] in which in patients with heart failure and an echocardiogram or CMR suggestive of amyloidosis, the combination of grade 2 or 3 cardiac uptake by radionuclide (99mTc-DPD/ 99mTc-PYP/99mTc-HMDP) scintigraphy and the absence of a detectable monoclonal protein despite serum immunofixation, urine immunofixation and serum FLC (Freelite) assay can reliably establish the diagnosis of cardiac ATTR amyloidosis.

Patients were excluded if echocardiography showed severe aortic stenosis, regional wall motion abnormalities, or LV wall thinning consistent with a previous large transmural myocardial infarction.

2.10 Mayo staging system for amyloid light chain amyloidosis

The Mayo Staging system proposed in 2004 for AL amyloidosis [56] is used in clinical practice for prognostic purposes. Mayo stage 1, with cardiac biomarkers below threshold values (cTnT $<0.035\mu$g/L and NT-proBNP $<$332ng/L) is associated with the lowest risk of death. Mayo stage 2, with either one of the biomarkers above threshold values confers an intermediate prognosis; and Mayo stage 3, with both biomarkers above the threshold values confers the poorest prognosis. There is currently no widely used validated staging system for patients with transthyretin amyloidosis.

2.11 Histology and immunohistochemistry

Histological assessment for amyloid deposition was undertaken on all histological samples at the NAC. Formalin fixed de-paraffinised tissue sections 6-8μm thick were rehydrated, and counterstained with haematoxylin under running tap water. Sections were then stained using the alkaline-alcoholic Congo-red method as described by Puchtler et al. in 1962. A series of ascending ethanol concentrations to xylene were used to dehydrate the sections which were then mounted in distyrene plasticiser xylene (DPX) mounting medium. Stained slides were then viewed in bright field and under cross polarised light. Positive controls were obtained from a known Congo-red positive block validated by laser micro dissection and mass-spectrometry based proteomic analysis which was always processed in parallel. Samples deemed to be positive for amyloid deposition demonstrated the characteristic apple-green birefringence and were confirmed after review by at least one other individual experienced in this assessment.

The amyloid type was characterised by immunohistochemical staining. Formalin fixed de-paraffinised 2μm sections of tissue were rinsed with water. Endogenous peroxidise activity was quenched by incubation in aqueous (0.3%) hydrogen peroxide (H_2O_2) for 30 minutes. Samples were then rinsed in phosphate-buffered saline (PBS) containing 0.05% Tween (Calbiochem). Prior to the application of antisera, non-specific tissue binding was abolished by incubation for a further 30 minutes in normal non-immune serum from the species providing the secondary antibody (Vector Part of the ImmPRESS Kit). Sections were then incubated overnight with primary antisera at 4°C. They were then rinsed with PBS containing 0.05% Tween (Calbiochem) and labelled with secondary antibodies. Sections were washed in PBS and bound enzyme-antibody bound complexes were then visualised using a metal-enhanced 3,3'-diaminobenzidine (DAB) (Fisher Scientific solution).

Gene (exon)	Forward primer sequence	Reverse primer sequence
Transthyretin (2)	5'-TTTCGCTCCAGATTTCTAATAC -3'	5'-CAGATGATGTGAGCCTCTCTC -3'
Transthyretin (3)	5'-GGTGGGGGTGTATTACTTTGC -3'	5'-TAGGACATTTCTGTGGTACAC -3'
Transthyretin (4)	5'-GGTGGTCAGTCATGTGTGTC-3'	5'-TGGAAGGGACAATAAGGGAAT -3'

Table 2.5: Primers used in the polymerase chain reaction process for genotyping Transthyretin Amyloidosis

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

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

2.12 Transthyretin gene sequencing

Transthyretin genotyping was performed in all patients with suspected transthyretin amyloidosis. A whole blood sample was taken in an ethylenediaminetetraacetic vial and was frozen and stored for gene sequencing. Genomic DNA was isolated. polymerase chain reaction (PCR) was used to amplify the coding regions for the transthyretin gene (exons 2, 3 and 4). The primers used as part of the PCR process are outlined in Table 2.5.

2.13 Ethical approval

All patients whose data have been used in this thesis have given informed written consent at the National Amyloidosis Centre. The consent form was approved by the Royal Free Hospital Ethics Committee (REC Ref 06/Q0501/42). The dosage and administration of radioactive isotopes were approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health.

2.14 Statistical analyses

Statistical analyses were performed with SPSS 22 (IBM). A detailed description of the statistical methods will be presented in the relevant chapters.

Chapter 3

Heterogeneity of Electrocardiographic Findings in Biopsy Proven Cardiac Transthyretin Amyloidosis and Electrocardiographic Markers of Prognosis

3.1 Introduction

ATTR amyloidosis can be classified as being either ATTR-wt or secondary to an underlying genetic abnormality in which case it is described interchangeably as variant, hereditary or mutant ATTR (ATTR-m). The clinical characteristics of patients with ATTR-m amyloidosis depend predominantly on the underlying genetic abnormality, but can also be affected by other factors including the area of their ethnic origin and whether the genetic abnormality is inherited from the mother or father [51–53]. The limited published data examining a heterogeneous group of patients with cardiac transthyretin amyloidosis, consider this group as a single entity when describing ECG findings, or at best make a simple distinction between wild-type and variant (mutant) and have not directly compared the different forms of ATTR-m

amyloidosis [36, 40]. As the clinical phenotype of those with ATTR amyloidosis is hugely dependant on the underlying genotype, heterogeneity in the ECG findings amongst patients with the different types of ATTR is highly plausible and may have been overlooked. Studies have demonstrated that certain ECG characteristics may portend a poor prognosis in cardiac AL amyloidosis [64, 133]. Such data and analyses are lacking in patients with cardiac ATTR amyloidosis with only one case series describing the ECG findings at diagnosis in cardiac ATTR-V122I amyloidosis in detail [45] and there has been no systematic comparison of ECG findings between the types of ATTR. An ECG marker of prognosis in cardiac ATTR amyloidosis, as has been identified in systolic heart failure [134], may be of significant clinical utility given the paucity of data to guide prognosis in this condition.

3.2 Aims

- To describe and compare baseline ECG findings in the most common forms of cardiac ATTR amyloidosis in the UK; cardiac ATTR-wt, ATTR-V122I and ATTR-T60A amyloidosis.
- To determine if abnormalities of the ECG at baseline (diagnosis) predict survival.
- To determine if there is progression of ECG abnormalities during follow-up.

3.3 Methods

3.3.1 Patient population

Medical records for patients assessed at the NAC, UK, between May 2007 and June 2013 were reviewed retrospectively. All patients with histologically proven cardiac ATTR amyloidosis

who had TTR gene sequencing confirming either wild-type, V122I or T60A gene mutations were included in the study. All patients were followed from the date of their initial assessment until death, 1st January 2014 or last patient contact for any patients lost to follow-up.

3.3.2 Data collection

A standard 12-lead ECG was recorded at diagnosis (baseline) and at follow-up visits. Follow-up ECGs were included if they were performed at defined intervals from baseline: 1 year (+/- 3months), 2 years (+/- 3months), 3 years (+/- 3months) and 4 years (+/-3months). Baseline and follow-up ECGs were analysed as described in the general methods, Chapter 2. Patients with left bundle branch block or ventricular pacing were excluded from the analysis of left ventricular hypertrophy. Patients with ventricular pacing were also excluded from the analysis of QRS duration. A QRS duration of 120ms or more was considered to be a broad QRS. Following initial analysis, to determine if any baseline ECG abnormalities were independent predictors of mortality, selected additional data from transthoracic echocardiography and cardiac biomarker assessment were retrospectively collected as appropriate for patients in the wild-type group.

3.3.3 Statistics

Continuous variables are expressed as median and interquartile ranges, and categorical variables as number of patients (percentage). Comparisons between the three cardiac ATTR amyloidosis groups were made using the Kruskal Wallis test for continuous variables, and Chi square test for categorical variables except where expected frequencies were small, where the alternative Fisher's exact test was used. All reported P values are two-sided. A P value of less than 0.05 was considered to be statistically significant. For co-variates that were statistically significant across the three populations, pairwise comparisons were performed with the Mann-Whitney

U test, Chi square or Fisher's exact tests as appropriate. Cox regression analysis was used to identify co-variates that predict survival. All data analyses were performed using IBM SPSS Statistics 22 software.

3.4 Results

3.4.1 Patient characteristics

One hundred and fifty-eight patients satisfied the inclusion criteria: 75 with ATTR-wt, 49 with ATTR-V122I and 34 with ATTR-T60A.

The baseline characteristics of the patients are displayed in Table 3.1 and those co-variates that reached a statistically significant difference across the three groups are represented as pairwise comparisons in Figure 3.1 (categorical variables) and Figure 3.2 (continuous variables).

There were significant differences in age, proportion of males and ethnic origin between the three groups. All groups showed a male predominance but this was greatest in the ATTR-wt and ATTR-V122I groups. All patients in the ATTR-T60A group were Caucasian, as were the majority of patients in the ATTR-wt group which contrasts with the 88% of patients in the ATTR-V122I group who were of African origin. Echocardiographic assessment demonstrated similar degrees of LV wall thickening (median 1.7cm), however, LVEF as assessed by Simpson's Biplane was significantly lower in the ATTR-V122I group compared with ATTR-T60A.

	ATTR-wt n=75	ATTR-V122I n=49	ATTR-T60A n=34	*P*
Patient Characteristics:				
Age at diagnosis (years)	78 (73, 81)	75 (69, 78)	70 (66, 71)	<0.001
Male	70 (93)	39 (80)	21 (62)	<0.001
African descent	4 (5)	43 (88)	0	<0.001
ECG Characteristics:				
Heart rate (bpm)	77 (66, 89)	79 (68, 87)	78 (71, 85)	0.87
Sinus rhythm	35 (47)	36 (73)	26 (76)	0.001
Atrial arrhythmia: **Atrial fibrillation** **Atrial flutter** **Atrial tachycardia**	39 (52) 37 (95) 2 (5) 0	12 (24) 9 (75) 2 (17) 1 (8)	8 (24) 6 (75) 0 2 (25)	0.001
A-Paced	1 (1)	1 (2)	0	
A or V-Paced	8 (11)	4 (8)	5 (15)	0.64
Cardiac axis:** **Normal** **LAD** **RAD**	 25 (37) ‡ 35 (52) 7 (10)	 14 (31) 24 (55) 5 (11)	 3 (10) 18 (62) 8 (28)	 0.03 0.66 0.09
First degree AV block*	17 (55)	16 (47)	18 (72)	0.77
PR interval (ms) *	208 (184, 246)	196 (174, 224)	214 (194, 248)	0.30
QRSd, ms **	108 (92, 134)	100 (90, 112)	118 (110, 138)	0.002
Broad QRS (≥ 120ms):** **LBBB** **RBBB** **NSICD**	25 (37) 11 (44) 11 (44) 3 (12)	9 (20) 3 (33) 2 (22) 4 (44)	14 (48) 9 (64) 3 (21) 2 (14)	0.03
QTcB (ms) **	458 (438, 488)	449 (433, 462)	481 (462, 495)	0.001
Low QRS voltage **: **Limb leads** **Low voltage (S in V1+ R in V5 or V6 <15mm)**	 18 (27) 34 (51)	 11 (24) 26 (58)	 3 (10) 15 (62)	 0.20 0.75
Q waves*:** **Anterior** **Inferior** **Lateral** **Anterior and inferior** **Anterior and lateral** **Anterior, inferior and lateral**	24 (43) 7 (29) 14 (58) 0 2 (8) 0 1 (4)	13 (31) 3 (23) 7 (54) 0 2 (15) 1 (8) 0	12 (38) 4 (33) 3 (25) 1 (8) 4 (33) 0 0	0.48
LVH*:** **Cornell voltage criteria** **Sokolow-Lyon Index**	 3 (5) 2 (4)	 8 (19) 0	 6 (30) 0	 0.01 0.66
Echocardiography Characteristics:				
IVSd (cm)	1.7 (1.5, 1.9)	1.7 (1.6, 1.8)	1.7 (1.5, 1.9)	0.89
LVPWd (cm)	1.7 (1.5, 1.8)	1.6 (1.5, 1.8)	1.7 (1.5, 1.8)	0.74
LVEF (%)	48 (39, 55)	42 (32, 49)	50 (45, 56)	0.005

Table 3.1: Description and comparison of baseline ECG and echocardiographic findings in each type of ATTR cardiac amyloidosis. Categorical data are presented as n (%) and continuous variables as median (interquartile range). * of those in sinus rhythm, ** of those not paced, *** of those not paced or LBBB. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; ECG, Electrocardiogram; IVSd, Interventricular septal thickness in diastole; LAD, left axis deviation; LBBB, left bundle branch block; LVEF, Left Ventricular Ejection Fraction; NSICD, non-specific intraventricular conduction delay; QRSd, QRS duration; QTcB, QT correction with Bazett formula; RAD, right axis deviation; RBBB, right bundle branch block; LVH, Left Ventricular Hypertrophy; LVPWd, Left ventricular posterior wall thickness in diastole.

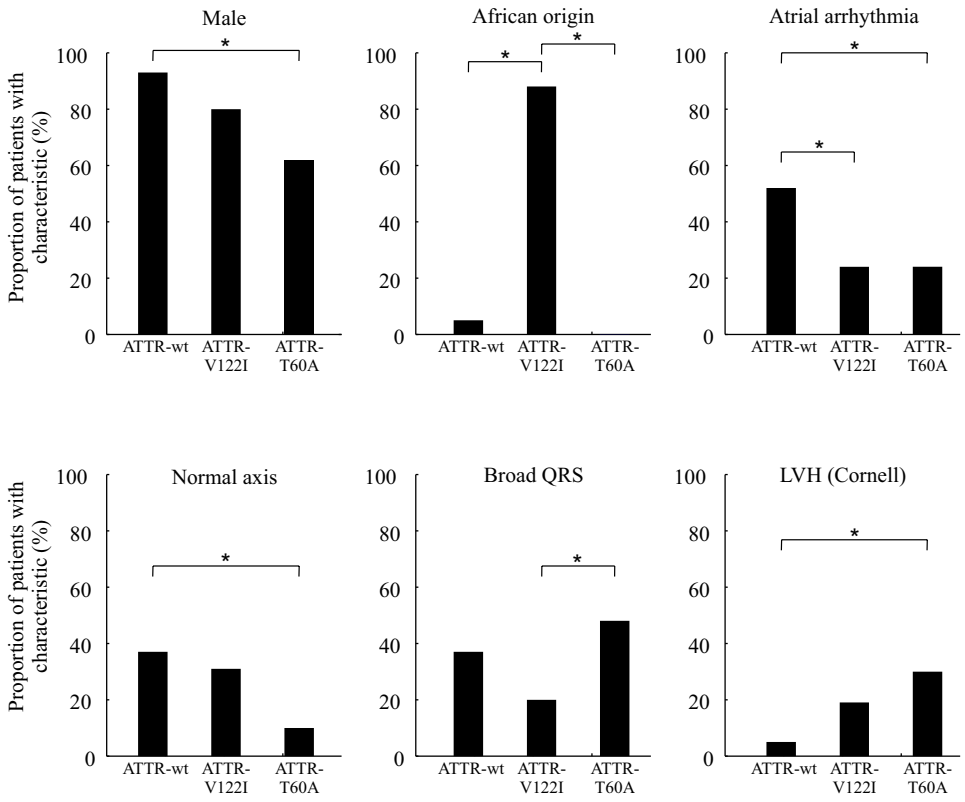


Figure 3.1: Pairwise comparisons of baseline categorical co-variates found to be significantly different between types of cardiac transthyretin amyloidosis. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; LVH, Left Ventricular Hypertrophy.

3.4.2 Baseline electrocardiogram characteristics

Heart rhythm and cardiac axis

Atrial arrhythmias (predominantly AF) were relatively common in all three groups of ATTR but there were significant differences in the prevalence between the groups (P=0.0014) with the ATTR-wt group having significantly more atrial arrhythmias than both ATTR-V122I and ATTR-T60A (52% in ATTR-wt vs 24% in both ATTR-V122I and ATTR-T60A). There was no statistically significant difference between the groups in the proportion of patients with pacemakers at diagnosis (P=0.64). Cardiac axis was deviated to the left in the majority of patients in all three groups, but was normal in more patients with ATTR-wt than those with ATTR-T60A (37% vs 10% $P<0.05$) despite similar degrees of LV wall thickness by

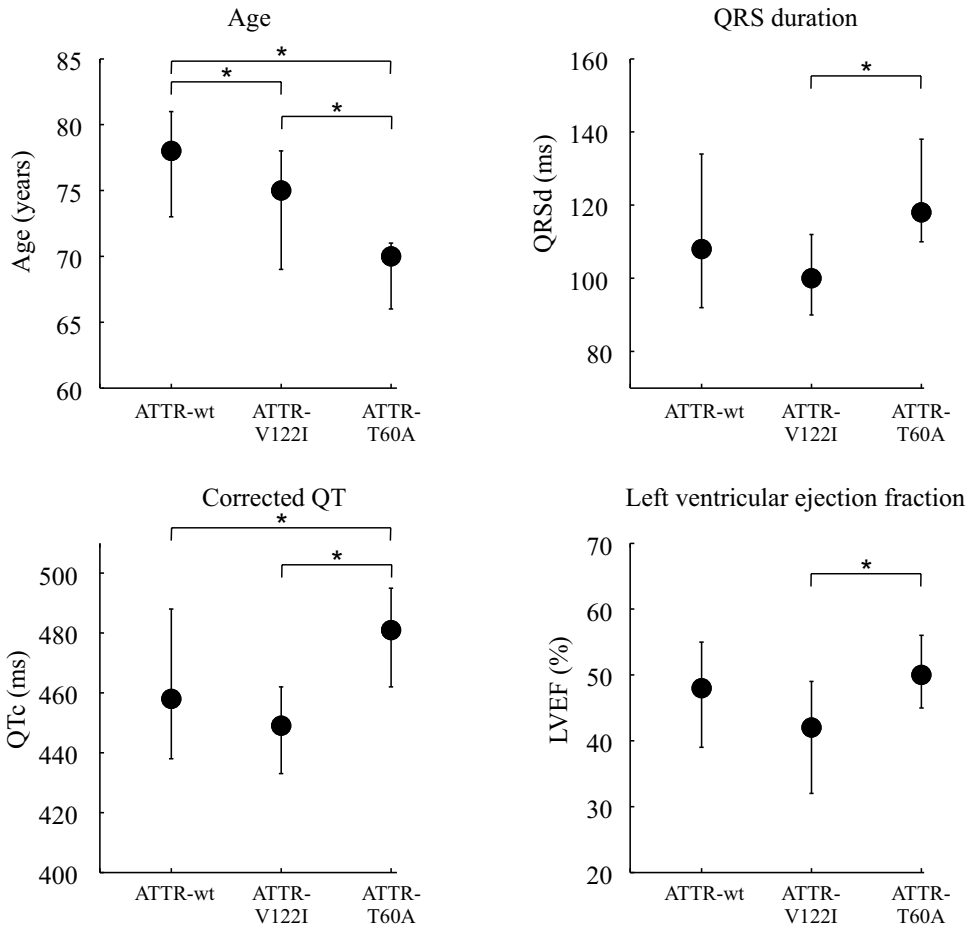


Figure 3.2: Pairwise comparisons of baseline co-variates (continuous) found to be significantly different between types of cardiac ATTR amyloidosis. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; LVEF, left ventricular ejection fraction; QRSd, QRS duration; QTc, corrected QT interval.

echocardiography in all three groups.

Atrioventricular and intraventricular conduction delay

First degree AV block was present in a large proportion of patients in all three groups (55% in ATTR-wt, 47% in ATTR-V122I and 72% in ATTR-T60A). There was no statistical difference in PR interval or the prevalence of first degree AV block at diagnosis between groups. There was a significant difference in QRS duration between the three groups. Broad QRS was present in 37% of ATTR-wt patients, 20% of ATTR-V122I patients and 48% of ATTR-T60A patients, with a statistical difference between the ATTR-V122I and ATTR-T60A groups. Statistical analyses

were not performed to compare proportions of left bundle branch block (LBBB), right bundle branch block (RBBB) and non-specific intraventricular conduction delay (NSICD) due to the small numbers in each group.

QRS amplitude, left ventricular hypertrophy and pathological Q waves

The prevalence of low voltage QRS complexes depended on the method used. The most common method employed is to interrogate the QRS amplitude in the limb leads. Utilising this method, less than one third of patients in each group exhibited low voltage QRS complexes and there was no significant difference in the prevalence between groups. When assessed by the method of summation of the S wave in V1 and the R wave in either V5 or V6 [127], low voltage was much more commonly found (more than half of patients in each group) but the lack of statistical difference in this respect between groups remained. The prevalence of left ventricular hypertrophy (LVH) by ECG criteria was also dependent on the methodology employed. The Cornell voltage criteria identified LVH in 30% of patients with ATTR-T60A which was significantly greater than the 5% of patients in the ATTR-wt group but using the Sokolow-Lyon Index identified very few patients with LVH. Pathological Q waves were common (43% of ATTR-wt, 31% of ATTR-V122I, and 30% of ATTR-T60A); these were most commonly seen in the anterior and inferior leads and did not correspond to echocardiographic appearances of transmural infarcts in any of the patients studied (i.e. these were true pseudo-infarct waves).

3.4.3 Prognostic features of baseline electrocardiogram

The mean follow-up for the ATTR-wt, ATTR-V122I, and ATTR-T60A groups was 22.7+/-14.6, 23.8+/-12.8, and 31.3+/-20.4 months respectively. There were 63 deaths in total: 29 deaths in the ATTR-wt group (12% of ATTR-wt group); 24 deaths in the ATTR-V122I group (49% of

	Univariate analysis		
	Hazard ratio	**95% CI**	***P* value**
TTR aetiology			0.072
ATTR-wt vs ATTR-T60A	1.69	0.82-3.51	0.157
ATTR-V122I vs ATTR-T60A	2.34	1.12-4.88	0.023
Age	1.06	1.01-1010	0.008
Gender (female vs male)	0.64	0.31-1.29	0.212
QRS duration (broad vs narrow)	1.95	1.15-3.32	0.013
Atrial arrhythmia	1.30	0.79-2.13	0.303
1st degree HB	1.00	0.50-2.01	0.986
Low voltage QRS in limb leads	1.60	0.92-2.77	0.095
LV impairment			0.029
Moderate impairment vs normal	1.36	0.67-2.77	0.40
Severe impairment vs normal	2.83	1.24-6.48	0.014
IVSd	2.03	0.80-5.15	0.138

Table 3.2: Univariate analysis for survival in ATTR cardiac amyloidosis. Categorical data are presented as n (%) and continuous variables as median (interquartile range). ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; CI, confidence interval; HB, heart block; IVSd, Interventricular septal thickness in diastole; LV, left ventricular.

ATTR-V122I group); 10 deaths in the ATTR-T60A group (29% of ATTR-T60A group). The median survival in the ATTR-wt group was 3.8years (95% CI 2.2-5.3) and in the ATTR-V122I group the median survival was 2.6 years (95% CI 2.2-3.1); the median survival was not reached in the ATTR-T60A group (log rank P=0.65)

Univariate Cox regression analysis suggests that predictors of survival in cardiac ATTR amyloidosis are TTR type (specifically ATTR-V122I vs. ATTR-T60A), age, broad QRS and severity of LV systolic impairment (Table 3.2).

When combined in a multivariate Cox regression model the only significant predictor of survival was broad QRS (Table 3.3).

There was a trend for TTR type to be a significant predictor of survival with a trend for ATTR-V122I having a higher mortality than ATTR-T60A (Figure 3.3) (HR 2.4 [0.98-5.88] P=0.056).

Since there were significant baseline differences between the three types of ATTR, the effect of

	Multivariate analysis		
	Hazard ratio	**95% CI**	***P*-value**
TTR aetiology			0.157
ATTR-wt vs ATTR-T60A	1.79	0.742-4.33	0.195
ATTR-V122I vs ATTR-T60A	2.40	0.98-5.88	0.056
Age (years)	1.03	0.98-1.09	0.245
QRS duration (broad vs narrow)	2.28	1.17-4.42	0.015
Low voltage QRS in limb leads	1.32	0.68-2.57	0.412
LV impairment			0.199
Moderate impairment vs normal	0.87	0.40-1.90	0.726
Severe impairment vs normal	1.76	0.66-4.71	0.258

Table 3.3: Multivariate analysis of survival in ATTR cardiac amyloidosis. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; CI, confidence interval; LV, left ventricular; TTR, transthyretin

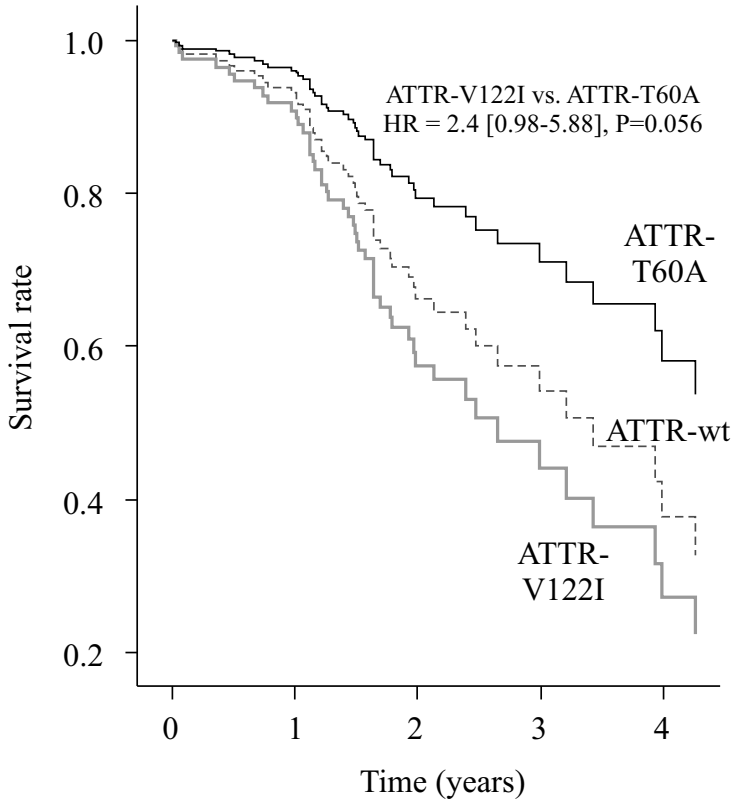


Figure 3.3: Cox regression analysis survival plots for different types of transthyretin cardiac amyloidosis adjusted for age, presence of broad QRS, presence of low voltage QRS and degree of left ventricular impairment. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis.

	HR for broad QRS	95% CI	P value
ATTR-wt	4.79	2.08-11.05	<0.001
ATTR-V122I	1.33	0.43-4.09	0.624
ATTR-T60A	1.75	0.42-7.35	0.445

Table 3.4: Cox regression analysis assessing the effect of a broad QRS on survival in different types of ATTR cardiac amyloidosis. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; CI, confidence interval; HR, hazard ratio.

a broad QRS on survival was assessed separately for the three groups (Table 3.4).

Only in the ATTR-wt groups was survival significantly dependent on the presence of a broad QRS (HR 4.79 [2.08-11.05] P<0.001). Given the apparent utility of the ECG in predicting survival in ATTR-wt this group was assessed in further detail.

Predictors of survival in the ATTR-wt group

Patients in the ATTR-wt group with a broad QRS ($\geq$120ms) had a median survival of 1.6 years (95% CI 1.3-1.9 years) while patients with a normal QRS duration did not reach their median survival by six years follow-up (Figure 3.4) (log rank P<0.0001).

Univariate Cox regression survival analyses are displayed in Table 3.5 for the ATTR-wt group. A multivariate Cox regression model was developed using variables which on univariate analysis were significant at a level of P<0.01 (i.e. broad QRS at baseline, age at diagnosis, supine systolic blood pressure (BP), Ln NT-proBNP and Ln TropT). This more strict level of statistical significance was chosen post-hoc as the number of events in this sub-population was relatively low and as such the number of covariates represented in the multivariate model would be too large if a less strict P value was used. In this multivariate model, broad QRS (P=0.024, HR 2.9; 95% CI 1.2-7.5) and Ln NT-proBNP (P=0.026, HR 2.9; 95% CI 1.1-7.4) predicted mortality (Table 3.6).

Cox regression analysis survival plots in ATTR-wt comparing those with a broad and narrow QRS, adjusted for age, supine systolic BP, Ln NT-proBNP and Ln TropT are displayed in Figure

	Hazard ratio	*P* value	(95% CI) for Hazard ratio
Broad QRS	4.72	<0.001	(2.06-10.81)
Age at diagnosis (years)	1.10	0.007	(1.03-1.18)
Duration of symptoms prior to diagnosis	1.00	0.820	(0.999-1.00)
Systolic BP (mmHg)	0.96	0.007	(0.93-0.99)
IVSd (cm)	1.47	0.550	(0.42-5.20)
LVEF by Simpson's biplane method (%)	0.97	0.052	(0.93-1.00)
Global longitudinal strain	1.06	0.290	(0.95-1.18)
Ln NT-proBNP	3.49	<0.001	(1.98-6.16)
Ln TropT	3.40	<0.001	(1.77-6.52)
eGFR (ml/min)	0.98	0.016	(0.968-0.996)
Hypertension	0.98	0.961	(0.44-2.17)
T2DM	0.61	0.506	(0.15-2.98)
IHD	0.70	0.443	(0.29-1.73)
Stroke	0.71	0.635	(0.17-2.98)

Table 3.5: Univariate survival analysis in ATTR-wt amyloidosis. CI, confidence interval; eGFR, estimated glomerular filtration rate; IHD, ischaemic heart disease; IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; T2DM, type 2 diabetes mellitus.

Characteristics at diagnosis	Hazard ratio	*P* value	(95% CI for Hazard ratio
Broad QRS (≥120ms)	2.943	0.024	(1.156-7.491)
Age (years)	1.074	0.084	(0.990-1.164)
Systolic BP (mmHg)	1.000	0.984	(0.971-1.029)
Ln NT-proBNP	2.905	0.026	(1.138-7.419)
Ln TropT	1.383	0.541	(0.489-3.916)

Table 3.6: Multivariate analysis of survival in ATTR-wt amyloidosis. ATTR-wt, Wild-type transthyretin amyloidosis; BP, blood pressure; CI, confidence interval; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; Trop T, troponin T.

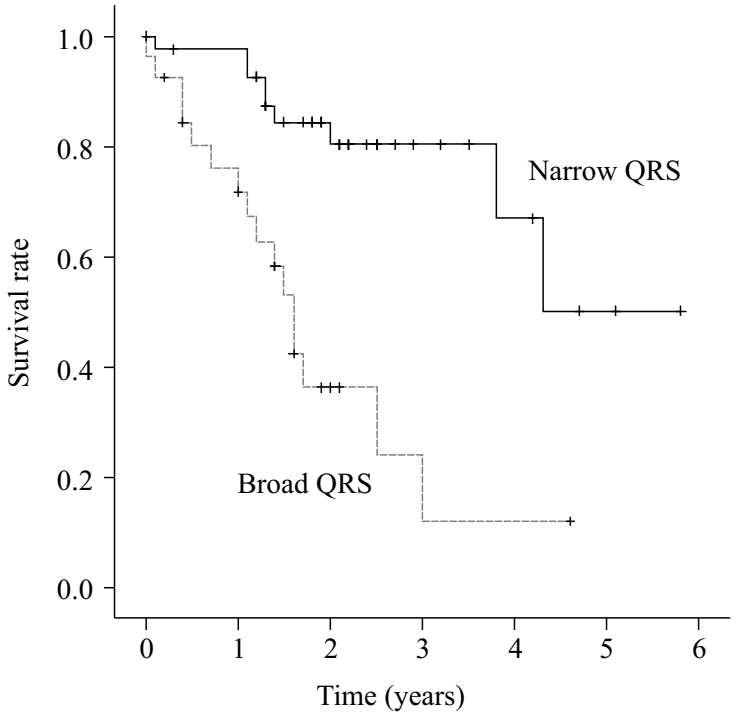


Figure 3.4: Unadjusted Kaplan Meier plot of survival in patients with ATTR-wt cardiac amyloidosis comparing those with a broad QRS to those with a narrow QRS.

3.5.

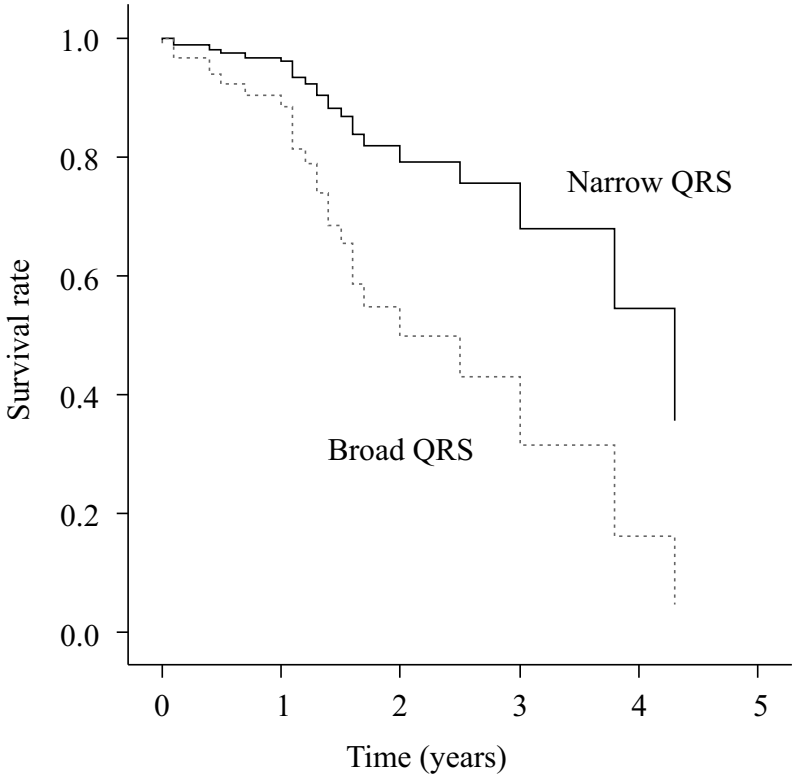


Figure 3.5: Cox regression analysis survival plots in ATTR-wt amyloidosis comparing those with a broad and narrow QRS, adjusted for age, systolic BP, Ln NT-proBNP and Ln TropT

Patient characteristic	Narrow QRS (QRSd < 120ms) n*=41	Broad QRS (QRSd ≥ 120ms) n*=27	*P*
Age (years)	77 (73, 80)	79 (76, 82)	0.1314
Male	37 (90)	27 (100)	0.1516
Caucasian	38 (93)	25 (93)	1.0000
LVEF (%)	50 (42, 56)	48 (39, 55)	0.3754
Global longitudinal strain (%)	-11.1 (-8.6, -15.0)	-10.2 (-7.9, -12.8)	0.491
IVSd (cm)	1.7 (1.5, 1.7)	1.8 (1.6, 1.9)	0.0552
NT-pro BNP (ng/L)	2483 (1975, 4822)	4670 (2511, 8861)	0.027
TropT (μg/L)	0.047 (0.042, 0.064)	0.056 (0.038, 0.128)	0.569
Hypertension	13 (32)	9 (33)	1.0000
Diabetes Mellitus	4 (10)	2 (7)	0.6944
Ischaemic heart disease	12 (29)	6 (22)	0.5843
Stroke	4 (10)	3 (11)	1.0000

Table 3.7: Comparison of baseline patient characteristics and co-morbidity data in patients with ATTR-wt amyloidosis. Continuous variables are presented as median (interquartile range) and categorical variables are presented as n (%). *Excludes patients with paced electrocardiogram. ATTR-wt, Wild-type transthyretin amyloidosis; IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; TropT, Troponin T.

In a sensitivity analysis, patients in the ATTR-wt group who had a narrow QRS were compared to those with a broad QRS. There were no significant differences in other characteristics which could be independently associated with survival other than NT-proBNP (Table 3.7).

3.4.4 Electrocardiogram changes during follow-up

There was attrition in the number of ECGs that could be analysed during follow-up due to patient deaths, patients lost to follow-up and failure to satisfy inclusion criteria with regards to time interval between sequential ECGs. At 1 year, there were 44 ATTR-wt, 40 ATTR-V122I and 28 ATTR-T60A evaluable ECGs. At 2 years, this was 22, 18 and 14 respectively. In total 364 ECGs were examined. Of the 99 patients who did not have an atrial arrhythmia at baseline, 24 (24%) developed an atrial arrhythmia during follow-up [ATTR-wt: 5/36 (14%); ATTR-V122I: 9/37 (24%); ATTR-T60A: 10/26 (38%), P=0.087], and of these, this occurred in 15 patients by the end of the first year of follow-up [ATTR-wt: 4/36 (11%); ATTR-V122I: 6/37 (16%); ATTR-T60A: 5/26 (19%), P=0.62)]. In those patients with normal PR interval at baseline,

	ATTR-wt	ATTR-V122I	ATTR- T60A	*P*
Number of ECGs evaluable at one year follow-up	44	40	28	
New atrial arrhythmias at one year follow-up	5/36 (14%)	6/37 (16%)	5/26 (19%)	0.62
New first degree heart block at one year follow-up	3/10 (30%)	5/16 (31%)	2/7 (29%)	0.94
New broad QRS at one year follow-up	7/42 (17%)	4/36 (11%)	8/15 (53%)	0.003

Table 3.8: New Electrocardiogram abnormalities at one year. Data are presented as number of new cases per number of patients who did not have said abnormality at baseline (%). Groups are compared using Fisher's Exact test. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; ECG, Electrocardiogram.

3/10 (30%) of the ATTR-wt, 5/16 (31%) of ATTR-V122I and 2/7 (29%) of ATTR-T60A developed first degree AV block after one year of follow-up (P=0.94). In those with a narrow QRS at baseline, progression to broad QRS at one year was significantly different between the three groups: 7/42 (17%) of the ATTR-wt, 4/36 (11%) of ATTR-V122I and 8/15 (53%) of ATTR-T60A group (P=0.003. Pairwise comparisons; $P<0.05$ for ATTR-wt vs. ATTR-T60A, and ATTR-V122I vs. ATTR-T60A). The changes that occurred at one year follow-up are summarised in Table 3.8.

Twelve patients required pacemaker implantation during the follow-up period (all within 2 years of diagnosis/baseline ECG); 4 (6%) ATTR-wt, 5 (11%) ATTR-V122I and 3 (10%) ATTR-T60A (P=0.52). All patients who required pacemakers during follow-up and who had been in sinus rhythm, had evidence of first degree AV block at baseline. However, of patients with first degree AV block, only 1/17 (6%) of the ATTR-wt group, 3/16 (19%) of the ATTR-V122I group, and 3/18 (17%) of the ATTR-T60A group required pacemaker implantation during follow-up (P=0.57).

3.5 Limitations

This study is limited by its retrospective nature. Patients were not reviewed at uniform time intervals and the timescale of ECG changes were therefore variable. Although this

study characterised the ECG findings in a large group of patients with cardiac transthyretin amyloidosis subgroup analyses are likely to have been limited by small sample size and may have therefore lacked the power to detect significant differences amongst these populations (ie. Type II error).

3.6 Discussion

The main findings from data presented in this chapter are:

1. Atrial arrhythmias are common at diagnosis in ATTR cardiac amyloidosis. Furthermore, there are significant differences in prevalence of atrial arrhythmias between the different types of ATTR cardiac amyloidosis.

2. Conduction disease is common at diagnosis in ATTR cardiac amyloidosis. First degree heart block is present in up to 72% of patients. The presence of a broad QRS was common in all groups and there were significant differences in prevalence depending on type of ATTR amyloidosis.

3. The presence of a broad QRS is feature predictive of a higher risk of death in a multivariate survival model.

4. Progression of ECG abnormalities over the first 12 months from diagnosis were common. New atrial arrhythmias developed over the first year and the QRS become broad in a significant proportion of patients; the prevalence depending on the type of ATTR cardiac amyloidosis.

This was a comprehensive study investigating baseline and follow-up ECGs in a large group of patients with the three commonest types of cardiac ATTR amyloidosis. The marked prevalence of atrial arrhythmias, in particular AF, in the ATTR-wt group at diagnosis, is greater than would be anticipated in this age group [135] or amongst patients with hypertension [136]

and raises the possibility that earlier diagnosis of ATTR-wt may be possible though active screening particularly of older Caucasian patients with a diagnosis of atrial arrhythmia. A valuable screening study could be designed to determine the prevalence of ATTR-wt in patients with AF using CMR imaging or DPD scintigraphy. Early diagnosis may permit intervention at a stage before irreversible organ dysfunction has occurred. Interestingly, the prevalence of atrial arrhythmia at baseline in the ATTR-V122I group was lower than the ATTR-wt group despite similarities in age. The cause of this remains unclear but may reflect differences of the underlying pathological process; perhaps different rates or sites within the heart of amyloid deposition.

The differences in age at diagnosis, sex and race were expected based on published data. Although the focus of this study was not on echocardiographic findings, there were similar degrees of LV wall thickness at diagnosis in all three groups but significant differences in systolic function as measured by LVEF between the ATTR-V122I group and ATTR-T60A group, again possibly reflecting pathological differences due to the individual transthyretin variants. Although this may be attributable to differences in age or co-morbidities, it raises the possibility of a differential effect on the heart which could depend on the specific structure of the amyloidogenic transthyretin and/or the site and distribution of amyloid within the heart. This could include differences in the effect between TTR types on the cardiac conduction system, and whether the disease process primarily results in myocardial cell death with myocardial oedema, or perhaps alternatively, myocardial cell hypertrophy without myocardial oedema.

This study demonstrates that there are differences in ECG characteristics not only between cardiac ATTR-wt amyloidosis and cardiac ATTR-m amyloidosis patients but also between the cardiac ATTR-m amyloidosis variants, which historically have not been considered as

separate entities in published series. Despite the small numbers of patients, there was a difference in QRS duration between the ATTR-T60A and ATTR-V122I groups. This is an interesting finding, particularly given similar degrees of LV wall thickening in all three groups. It remains unclear why this should be the case and may be helped by further investigation with pathological correlation.

The classical teaching suggests that most patients with cardiac amyloidosis have low voltage QRS complexes. This stems from the ECG description by Carroll et al. in 14 patients with cardiac AL amyloidosis and a QRS duration $\leq$120ms. A significant proportion of patients with cardiac amyloidosis of transthyretin type have bundle-branch block or pre-existing pacemaker implantation which makes assessment of QRS voltage difficult. The definition of low QRS voltage is variable in published studies which further complicates matters, however the most widely accepted description of QRS voltage $<$5mm in all limb leads, was found much less commonly than reported in patients with cardiac AL amyloidosis, and was least common in the ATTR-T60A group where only 10% of patient had this ECG finding. The finding of QS morphology in lead V1 was common in all three groups (despite a normal QRSd). In this situation, no amplitude was recorded for S wave in V1 as the definition of an S wave (downward deflection after an R wave) was not satisfied. This is likely to have contributed to the high prevalence of apparent low voltage by the technique of 'summation of S wave in V1 and R wave in leads V5 or V6' in contrast to that by the method of assessing the QRS voltage in the all limb leads. Clinician awareness of the low prevalence of low voltage complexes (when assessed by the most commonly used method in daily clinical practice) in these subtypes of cardiac transthyretin amyloidosis is important as this may be contributing to a delay in the diagnosis of this condition. Furthermore, voltage criteria for left ventricular hypertrophy, when assessed by the Cornell method, was present in 30% of the ATTR-T60A group and 19% of

the ATTR-V122I group, and particularly when LBBB was present (the most common pattern of broad QRS complexes in the ATTR-wt and ATTR-T60A groups), QRS voltages are also usually large and may further contribute to a lower clinical suspicion of cardiac amyloidosis and delay in diagnosis. I did not attempt to assess patients with LBBB for voltage criteria for LVH as there are no widely accepted criteria for this.

These data suggest that a broad QRS may help risk stratify patients with cardiac transthyretin amyloidosis. In a multivariate analysis of the whole cohort (ATTR-wt, ATTR-V122I and ATTR-T60A amyloidoses combined), a broad QRS was the only predictor of survival. Due to concerns that this analysis was over represented by the larger wild-type group, and because there were significant differences between the three groups of ATTR amyloidosis, I assessed whether a broad QRS was predictive of survival in each of the types of ATTR amyloidosis separately. These analyses showed that a broad QRS was predictive of survival in ATTR-wt but not in ATTR-V122I or ATTR-T60A amyloidoses. This may be due to inherent differences between the types of ATTR amyloidoses or may be as a result of the smaller populations of the ATTR-V122I and and ATTR-T60A leading to underpowered analyses. The survival difference in the ATTR-wt group could not be accounted for by differences in patient characteristics or comorbidities which might affect outcomes or be independently associated with a wide QRS complex. The presence of a broad QRS in these patients may reflect more severe disease as supported by the finding of a higher serum NT-proBNP.

Interestingly, in multivariate analysis (and although not reaching statistical significance) there was a trend for better survival in the ATTR-T60A group compared with the ATTR-V122I group despite adjusting for various relevant factors including age, degree of left ventricular systolic impairment and broad QRS. This is particularly intriguing as the ATTR-T60A group had the greatest proportion of patients of any group with a broad QRS, in contrast to the ATTR-V122I

group which had the smallest proportion of patients with a broad QRS. Despite apparently having more advanced conduction disease, the ATTR-T60A group had a higher LVEF and trend to better survival compared to the ATTR-V122I group. This finding may suggest that survival in these two ATTR-m amyloidosis groups is determined by a different underlying pathological process as a result of their different genotypes.

Follow-up ECG data in this study highlight the importance of monitoring to detect the development of atrial arrhythmias and/or the progression of conduction system disease (50% of the ATTR-T60A group with a normal QRS duration at baseline had progressed to broad QRS). Of those requiring pacemakers during follow-up, the majority of patients in the ATTR-wt and ATTR-V122I groups were in sinus rhythm, with 1st degree AV block and a normal QRS duration at diagnosis. This poses a particular management challenge as predicting the need for pacemaker implantation in this situation is difficult. This finding may be different compared to the ATTR-T60A group where all three patients were in sinus rhythm with 1st degree AV block and two of the three patients had a broad QRS complex at diagnosis with LBBB morphology.

These data not only suggest that there are significant ECG abnormalities present at diagnosis but that there are significant differences between the different types of ATTR cardiac amyloidosis. This has important implications and suggests that the different types of ATTR cardiac amyloidosis should not be grouped together when it comes to describing natural history and designing clinical trials. However, it must be borne in mind that while this is the largest direct comparison of ECG findings in the different types of ATTR amyloidosis the numbers described are still relatively small.

Chapter 4

Prognostic Markers in Cardiac Transthyretin Amyloidosis: A comparative study of wild-type and V122I-associated cardiac transthyretin amyloidosis using multi-modality imaging

4.1 Introduction

Wild-type transthyretin is innately amyloidogenic but mutations in the TTR gene can augment this potential. Approximately one hundred transthyretin gene mutations enhance amyloidogenesis with varying degrees of cardiac involvement, but the most common mutation to present with a significant cardiac phenotype is the Val122Ile (commonly known as V122I) TTR variant in individuals of African descent. Approximately 3-4% of African Americans are thought to be heterozygote for the V122I allele [137]. In Caucasians, cardiac ATTR amyloidosis is most commonly caused by ATTR-wt. ATTR-wt amyloidosis is a late-onset (typical age 70 years and over), non-hereditary disease with an overwhelmingly male preponderance, and a predominant and often isolated cardiac phenotype typically causing heart failure symptoms and

congestive heart failure signs.

Cardiac amyloidosis is widely considered the archetypal restrictive cardiomyopathy with preserved systolic function, although at presentation a restrictive filling pattern is not universally found [36]. This absence of a restrictive filling pattern may contribute to a delayed diagnosis. Although cardiac ATTR amyloidosis remains probably much under-diagnosed, the increasing availability of CMR imaging in the UK appears to be pivotal in the surge of referrals for specialist assessment. CMR has a high specificity for cardiac amyloidosis with characteristic difficulty in nulling of the myocardium and subendocardial LGE [78]. International experience of ^{99m}Tc DPD scintigraphy is also proving that this non-invasive technique is inexpensive and highly sensitive for diagnosing cardiac transthyretin amyloidosis [132]. The specificity of these tests, however, is not sufficient to be used in isolation but the combination of CMR, echocardiography and DPD scintigraphy may lead to an increase in the diagnosis of ATTR amyloidosis in the future.

Historically, therapeutic options have been limited with no evidence-based treatments to offer patients with cardiac ATTR amyloidosis and this may have contributed to the diagnosis being considered one of academic interest. Clinical characteristics and outcomes for patients with transthyretin amyloidosis have been reported [39–41,45,46] but a comprehensive understanding of disease progression and prognostic markers remains lacking. With novel treatments for cardiac ATTR amyloidosis on the horizon and the likely increase in the number of cases diagnosed in the future, a more detailed understanding of the condition and its progression is paramount to aid clinical management and assess the potential impact of disease-modifying treatments.

4.2 Aims

- To describe and compare the characteristics at diagnosis of patients with cardiac ATTR-wt and ATTR-V122I amyloidosis using multi-modality imaging.

- To describe and compare the outcomes of patients with cardiac ATTR-wt and ATTR-V122I amyloidosis.

- To identify clinically useful prognostic markers in cardiac ATTR-wt and ATTR-V122I amyloidosis.

4.3 Methods

4.3.1 Study design

I conducted a retrospective observational cohort study of patients with cardiac ATTR-wt amyloidosis and cardiac ATTR-V122I amyloidosis between March 2005 and December 2012 at the NAC, UK.

4.3.2 Patient population

Consecutive patients with a new diagnosis of cardiac ATTR-wt or ATTR-V122I amyloidosis were included. Patients were identified by a local database search. All patients had echocardiographically defined amyloid cardiomyopathy. Where possible, histological confirmation of amyloid was obtained. Histological confirmation of amyloid deposition was deemed to have been gained if at least one tissue sample showed: a) Congo red staining positivity with apple-green birefringence under cross-polarized light, and

immuno-histochemical analysis confirmed amyloid of the TTR-type; or b) if Congo red staining positivity with apple-green birefringence under cross-polarized light in which confirmatory immuno-histochemical staining was not possible, and there was no evidence of plasma cell dyscrasia based on a combination of clinical history, examination, blood and urine analyses. Patients with secondary amyloidosis were excluded. Reflecting the change in clinical practice at our tertiary referral centre [33], patients with transthoracic echocardiography findings consistent with cardiac amyloidosis, a supportive history and examination, were included even without histological diagnosis if they had undergone ^{99m}Tc DPD scintigraphy yielding a positive result with intense cardiac uptake (consistent with Perugini grade 2 or 3) [132], or demonstrated characteristic CMR imaging findings, providing there was no evidence of plasma cell dyscrasia. TTR gene sequencing was performed in all patients. Patients with hereditary ATTR amyloidosis caused by TTR mutations other than V122I were excluded.

The initial intention of this thesis had been to describe the three most common forms of cardiac ATTR amyloidois; cardiac ATTR-wt, ATTR-V122I and ATTR-T60A amyloidosis. Data for patients with cardiac ATTR-T60A amyloidosis were available for Chapter 3 describing the ECG findings, however, these patients were excluded from this imaging chapter due to lack of data. The majority of patients with cardiac ATTR-T60A amyloidosis were assessed at the NAC at a time when fully comprehensive echocardiographic data were not systematically recorded and most patients did not undergo ^{99m}Tc DPD scintigraphy or CMR imaging.

4.3.3 Data collection

Demographic and other relevant clinical, serological, 12 lead ECG and transthoracic echocardiogram data (as described in the general methods, Chapter 2) were collected in all patients at diagnosis. Patients were re-assessed at 6 monthly intervals and follow-up data

were collected. At our institution, the troponin assay changed to a higher sensitivity type, in December 2012, so only results prior to this point were analysed. Electrocardiogram data analysis was limited to the presence or absence of AF and broad QRS reflecting the key findings from Chapter 3. Data from ^{99m}Tc DPD scintigraphy and CMR imaging are reported for scans performed at our institution as per the techniques described in the general methods, Chapter 2. These scans were clinically indicated scans performed as part of the patient's diagnostic work-up and not all patients underwent these scans. For patients who died during the follow-up period, cause of death was ascertained from death certificates held in the national registry.

4.3.4 Statistics

Continuous parametric data are expressed as mean +/- standard deviation. Continuous non-parametric data are expressed as median (interquartile range). Categorical variables are expressed as absolute values (percentages). Comparisons of characteristics at diagnosis between the two groups of ATTR amyloidosis were made using independent samples T test for parametric data, Mann Whitney U test for non-parametric data, and Pearson Chi-square or Fisher's exact test for proportions. A Bonferroni-type correction was used to account for multiple testing: a P value of less than 0.01 was considered to be significant for comparisons between the groups at baseline. Changes in characteristics over time were assessed with repeated measures analysis of variance (ANOVA); only patients who were regularly followed for 1 year were included in this analysis and serum levels of NT-proBNP were log transformed for this analysis. A P value of less than 0.01 was considered to be significant in the repeated measures ANOVA in view of multiple testing.

Survival curves were constructed according to the Kaplan-Meier method and compared by the log rank test. Predictors of survival were identified in univariate, then multivariate analyses

using the Cox proportional hazards model. A P value of less than 0.05 was considered significant in the Cox proportional hazards models. Separate multivariate models for the two cardiac ATTR groups were constructed. Interactions between variables were sought in multivariate analysis, and only variables that were not collinearly associated were included in the final multivariate models. In all survival analyses, follow-up data were censored on 1st January 2014, and for patients who declined follow-up the censor date was taken as the date of last contact.

4.4 Results

The study population comprised 293 consecutive patients with cardiac transthyretin amyloidosis; 206 patients with ATTR-wt amyloidosis and 87 with ATTR-V122I amyloidosis. Confirmatory histology was available in 136 (66%) of the ATTR-wt amyloidosis group and in 54 (62%) of the ATTR-V122I group. Of these, 57% and 56% respectively were attained from endomyocardial biopsy.

4.4.1 Patient demographics and characteristics at diagnosis

Patient demographics

Patient demographics are displayed in Table 4.1. The ATTR-V122I group were younger than the ATTR-wt group (75.3+/-5.8 years vs. 77.3+/-6.2 years, P=0.008). The majority of patients studied were male. In the ATTR-V122I group 21% (n=18) were female compared to 7% (n=15) in the ATTR-wt group (P=0.002). In the ATTR-wt group, 94% were Caucasian compared with 7% of the ATTR-V122I group. In the ATTR-V122I group 87% were black and 5% were of mixed ethnic origin. The vast majority of Caucasian patients in the wild-type group were of

English origin. The majority of patients in the ATTR-V122I group described themselves to be of West-Indian origin and a smaller proportion of African origin.

Patient characteristics

Patient characteristics at diagnosis are displayed in Table 4.2. Patients in both groups had symptoms for more than 12 months prior to diagnosis. There was a significant difference in the mode of presentation before diagnosis; with the majority of patients in the ATTR-V122I group diagnosed following an emergency admission to hospital while patients with ATTR-wt were predominantly diagnosed during outpatient investigations ($P<0.001$). A history of hypertension was more common in the ATTR-V122I group (53% vs. 31%; $P<0.001$), and ischaemic heart disease more common in the ATTR-wt group (24% vs. 5%; $P<0.001$). At diagnosis, systolic blood pressure was lower and more patients were receiving diuretic therapy in the ATTR-V122I group. Serum troponin T was greater in the ATTR-V122I group, however NT-proBNP was similar in the groups, as were the proportions of patients in the different NYHA classes.

Possible markers of nutritional status (body mass index (BMI) and serum albumin) were similar in the two groups, although interestingly, there was a significant difference in haemoglobin concentration which may reflect the differing racial make-up of the two groups. Renal function assessment by race-corrected eGFR was similar in both groups.

AF was common to both groups but more prevalent in the ATTR-wt group at diagnosis [126 (61%) vs. 29 (33%); $P<0.001$] with increasing prevalence in both groups during follow-up [133 (65%) vs. 42 (48%); $P=0.013$). A broad QRS (as in Chapter 3) had a greater prevalence in the ATTR-wt group ($P=0.003$). By the study conclusion, pacemaker implantation had been performed in 42 (20%) of the ATTR-wt group and 12 (14%) of the ATTR-V122I group in comparison with 25 (12%) and 7 (8%) respectively at diagnosis.

Patient demographics	ATTR-wt n=206	ATTR-V122I n=87	*P*
Age (years)	77.3±6.2	75.3±5.8	0.008
Gender (% male)	191 (93)	69 (79)	0.002
Ethnic origin			<0.001
Caucasian:	194 (94)	6 (7)	
England	174	3	
Scotland	1	1	
Ireland	8		
Wales	1		
Hungary	1		
Italy	2		
Austria/Czech Rep.	1		
Sweden	1		
Switzerland	1		
Australia	1		
Greece/Cyprus	1		
Spain	2	1	
Germany		1	
Black:	10(5)	76(87)	
African			
Nigeria	2	12	
Gambia	1		
Ghana		5	
Sierra Leone		1	
Guyana		1	
Mauritius		1	
Unknown		1	
West-Indian			
Jamaica	5	23	
Trinidad	1		
Monseratt	1	1	
Barbados		8	
St Lucia		2	
St Kitts		2	
Unknown		19	
Mixed:	0	4(5)	
Jamaica/England		2	
Jamaica/Scotland		1	
India/Guyana		1	
Asian:	2(1)	1(1)	
India	1		
Pakistan	1	1	

Table 4.1: Patient demographics at diagnosis. Data are presented as mean+/-SD for parametric variables, or as number (% of group). A P value < 0.01 was considered statistically significant. ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis.

Patient characteristics	ATTR-wt n=206	ATTR-V122I n=87	*P*
Disease presentation			
Duration of symptoms before diagnosis (months)	12.6 (0.4, 157.8)	14.9 (1.2, 123.0)	0.123
Emergency presentation **Outpatient presentation**	69 (33%) 137 (67%)	52 (60%) 35 (40%)	<0.001
Co-morbidities			
Hypertension	64 (31)	46 (53)	<0.001
Diabetes mellitus	19 (9)	13 (15)	0.142
Cerebrovascular disease	24 (12)	7 (8)	0.359
Ischaemic heart disease	49 (24)	4 (5)	<0.001
Cardiac failure medications			
ACEi/ARB	133 (65)	65 (75)	0.102
Diuretic/Aldosterone antagonist	160 (78)	80 (92)	0.003
Beta-blocker	92 (45)	51 (59)	0.031
Other anti-arrhythmics			
Digoxin	21 (10)	7 (8)	
Amiodarone	14 (7)	0	
Systolic BP (mmHg)	122±20	115±17	0.002
Diastolic BP (mmHg)	73±11	72±12	0.311
BMI	26.9±3.9	25.9±4.2	0.090
NYHA Class **I** **II** **III** **IV**	 23 (11) 142 (69) 36 (17) 5 (2)	 4 (5) 63 (72) 18 (21) 2 (2)	0.284
Hb (g/L)	136±17	127±15	<0.001
eGFR corrected for race (ml/min)	56±21	61±19	0.025
Bilirubin (µmol/L)	17±10	20±12	0.095
Albumin (g/L)	44±4	42±4	0.010
NT-proBNP (pg/ml)	3119 (1985, 5892)	3687 (2136, 5954)	0.420
Trop T (µg/L)	0.06 (0.02, 0.31)	0.08 (0.03, 0.4)	0.006
Atrial fibrillation	126 (61)	29 (33)	<0.001
Broad QRS (QRS≥120ms)	66 (32)	14 (16)	0.003
Pacemaker	25 (12)	7 (8)	0.311

Table 4.2: Patient characteristics at diagnosis. Data are presented as mean+/-SD for parametric variables, as number (% of group), or as presented as median (interquartile range). A P value of less than 0.01 was considered statistically significant. ACEi, Angiotensin Converting Enzyme inhibitor; ARB, Angiotensin II Receptor Blocker; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association; TropT, Troponin T.

4.4.2 Multi-modality cardiac assessment

A summary of the results of the multi-modality imaging is displayed in Table 4.3. Transthoracic echocardiography revealed that the majority of the measures of left ventricular systolic function (LVEF, global longitudinal strain, mitral annular plane systolic excursion (MAPSE), S' tissue Doppler imaging (TDI) and fractional shortening), were significantly worse in the ATTR-V122I group at diagnosis despite similar degrees of wall thickness and LV mass. Right ventricular systolic function as assessed by tricuspid annular plane systolic excursion (TAPSE) and tissue Doppler was reduced similarly in both groups. There was evidence for greater diastolic dysfunction (isovolumic relaxation time (IVRT) and mitral valve deceleration time (MV Dec Time)) in the ATTR-V122I group and a restrictive filling pattern (E/A >2) was found more commonly in the ATTR-V122I group (62% vs. 35%; $P<0.001$). This may have been confounded by the greater proportion of patients with AF in the ATTR-wt group making a diagnosis of restrictive filling more challenging given the absence of an A wave in the mitral inflow pattern. Left atrium (LA) diameter was greater in the ATTR-wt group but LA size by area was the same in both groups.

^{99m}Tc DPD scintigraphy was performed in 129 (63%) patients in the ATTR-wt group and 46 (53%) patients in the ATTR-V122I amyloidosis group (P=0.15). There was a significant difference in the DPD grade between the two groups ($P<0.001$); DPD grade 3 occurred in a greater proportion of patients in the ATTR-V122I group (43% vs. 11%; $P<0.001$) and DPD grade 2 in a greater proportion of the ATTR-wt group (57% vs. 87%; $P<0.001$) with very few patients having DPD grade 1 uptake (ATTR-V122I 0% vs. ATTR-wt 2%; P=0.567).

CMR imaging was performed at the NAC in 61 patients in the ATTR-wt group and 19 patients in the ATTR- V122I group. In addition to these scans performed at the NAC, some patients had a CMR performed and reported by their local referring hospital but these scans were not

Patient Imaging results	ATTR-wt n=206	ATTR-V122I n=87	*P*
Echocardiogram results			
LVEF Simpson's biplane (%)	47±11	41±10	<0.001
Global longitudinal strain (%)	-11.0±3.5	-9.2±5.1	0.001
MAPSE (cm)	0.8±0.3	0.7±0.3	0.003
TAPSE (cm)	1.4±0.5	1.4±0.4	0.454
Lat S' TDI (m/s)	0.06±0.02	0.05±0.01	0.001
Sep S' TDI (m/s)	0.04±0.01	0.04±0.01	0.002
RV S' TDI (m/s)	0.11±0.07	0.09±0.03	0.025
RV wall thickness (cm)	0.7±0.2	0.7±0.2	0.339
IVSd (cm)	1.7±0.2	1.7±0.2	0.772
LVPWd (cm)	1.6±0.2	1.7±0.2	0.596
LVIDd (cm)	4.5±0.5	4.3±0.5	0.004
LVIDs (cm)	3.4±0.6	3.4±0.6	0.917
FS (%)	24±8.2	21±7.4	0.001
2D LA diam (cm)	4.6±0.6	4.4±0.5	0.002
LA area (cm sq)	27.9±6.0	26.4±4.7	0.035
RA area (cm sq)	26.0±7.1	25.7±6.0	0.718
E/A	2.2±1.1	2.5±0.9	0.099
Restrictive filling pattern (E/A >2)	72 (35)	54 (62)	<0.001
IVRT (ms)	88±26	78±22	0.005
MV E'	0.05±0.02	0.05±0.01	0.022
MV DecT (ms)	188±51	169±52	0.005
E/e'	17.0±6.7	17.4±7.5	0.646
LV mass (g)	323±86	305±69	0.087
LV mass cor. for BSA (g/m²)	109±28	106±25	0.463
^{99m}Tc DPD scintigraphy	**n=129**	**n=46**	<0.001
Grade 1	3 (2)	0	0.567
Grade 2	112 (87)	26 (57)	<0.001
Grade 3	14 (11)	20 (43)	<0.001
CMR Imaging	**n=61**	**n=19**	
LV ejection fraction (%)	54±13	45±14	0.016
LV mass (g)	249±64	245±65	0.874
LV mass-indexed (g/m^2)	146±29	141±31	0.127
ECV	0.55±0.13	0.62±0.13	0.004
Native myocardial T1 (ms)	1085±62	1087±46	0.879

Table 4.3: Patient imaging results at diagnosis. Data are presented as mean+/-SD or as number (% of group). A P value of less than 0.01 was considered statistically significant. ^{99m}Tc DPD scintigraphy, ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; CMR, cardiac magnetic resonance; ECV, Extra-Cellular Volume; FS, fractional shortening; IVRT, isovolumic relaxation time; IVSd, Interventricular septal thickness in diastole; LA, left atrium; LV, left ventricular; LVIDd, Left Ventricular Internal Dimension In diastole; LVIDs, Left Ventricular Internal Dimension In Systole; LVPWd, Left ventricular posterior wall thickness in diastole; MAPSE, Mitral annular plane systolic excursion; RV, right ventricular; TAPSE, tricuspid annular plane systolic excursion; TDI, tissue Doppler imaging.

included in these results. In total, CMR was performed in a total of 147 (71%) patients in the ATTR-wt group, and 61 (70%) patients in the ATTR-V122I group. CMR imaging demonstrated a trend for a greater degree of left ventricular systolic impairment in the ATTR-V122I group (P=0.016) and similar degrees of increased LV mass in both groups as identified by transthoracic echocardiography. Interestingly, CMR also revealed a higher extracellular volume (ECV) in the ATTR-V122I group despite similar T1 values in both groups.

4.4.3 Changes with time

Data were available for 136 patients reassessed at six months and twelve months following diagnosis at the NAC; 93 patients in the ATTR-wt group and 43 patients in the ATTR-V122I group. Repeated measures ANOVA was performed to assess for changes in characteristics over time. The results of this are presented in Table 4.4 and Table 4.5.

Serum trop T levels were not examined in this assessment due to a large proportion of incomparable values as a result of the assay change described previously.

There was a decline in eGFR over time in both groups. The only other parameter that changed significantly with time was log NT-proBNP in the ATTR-V122I group. There was a trend for an increase in log NT-proBNP in the ATTR-wt group. Selected pairwise comparisons can be seen in Figure 4.1.

4.4.4 Survival analyses

The median duration of follow-up was 1.94 years (IQR 1.17, 2.65) (ATTR-wt 1.93 years; IQR 1.20, 2.86 and ATTR-V122I 1.96 years; IQR 1.16, 2.51). There were 79 (40%) deaths in the ATTR-wt group and 50 (58%) deaths in the ATTR-V122I group (P=0.005). Overall unadjusted

	ATTR-wt n=93			
	Baseline	**6 month**	**12 month**	***P***
Diastolic BP (mmHg)	74 (11)	73 (10)	71 (10)	0.421
Systolic BP (mmHg)	122 (17)	121 (15)	119 (16)	0.380
Log NT-proBNP(pg/ml)	2.52 (0.33)	2.58 (0.35)	2.60 (0.37)	0.011
eGFR (ml/min)	61 (21)	56 (20)	54 (19)	<0.001
Albumin (g/L)	44 (4)	44 (3)	44 (3)	0.155
Bilirubin (µmol/L)	16 (11)	18 (10)	17 (10)	0.804
NYHA Class	2.0 (0.6)	2.2 (0.6)	2.1 (0.6)	0.052
IVSd (cm)	1.7 (0.2)	1.7 (0.2)	1.7 (0.2)	0.704
LVPWd (cm)	1.6 (0.2)	1.6 (0.2)	1.7 (0.5)	0.238
LVEF (%)	48 (11)	47 (12)	48 (11)	0.087
GLS (%)	-11.2 (3.6)	-11.2 (3.7)	-11.0 (3.7)	0.514
MAPSE (cm)	0.8 (0.3)	0.8 (0.3)	0.7 (0.3)	0.192
Lat S' TDI (m/s)	0.06 (0.02)	0.06 (0.01)	0.05 (0.01)	0.302
Sep S' TDI (m/s)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.896
TAPSE (cm)	1.4 (0.5)	1.4 (0.5)	1.4 (0.50)	0.330
RV S' TDI (m/s)	0.12 (0.14)	0.10 (0.03)	0.11 (0.13)	0.453
2D LA diam (cm)	4.6 (0.6)	4.6 (0.6)	4.6 (0.5)	0.763
E/A	2.2 (1.1)	2.5 (1.0)	2.4 (1.3)	0.935
MV DecT (ms)	188 (62)	190 (58)	182 (40)	0.579
IVRT (ms)	87 (28)	85 (22)	88 (27)	0.655
E/e'	16 (5)	17 (6)	18 (7)	0.031

Table 4.4: Repeated measures ANOVA for ATTR-wt group. Data are presented as mean+/-SD or as number (% of group). A P value < 0.01 was considered statistically significant. ATTR-wt, Wild-type transthyretin amyloidosis; BP, blood pressure; eGFR, estimated glomerular filtration rate; GLS, global longitudinal strain; IVRT, isovolumic relaxation time; IVSd, Interventricular septal thickness in diastole; LVEF, left ventricular ejection fraction; LVPWd, Left ventricular posterior wall thickness in diastole; MAPSE, mitral annular plane systolic excursion; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association; TAPSE, tricuspid annular plane systolic excursion; TDI, tissue Doppler imaging.

	ATTR-V122I n=43			
	Baseline	**Six month**	**Twelve month**	***P***
Diastolic BP (mmHg)	73 (11)	74 (12)	71 (13)	0.644
Systolic BP (mmHg)	117 (18)	119 (18)	116 (19)	0.794
Log NTproBNP (pg/ml)	2.56 (0.30)	2.58 (0.37)	2.67 (0.33)	0.002
eGFR (ml/min)	63 (19)	61 (21)	52 (18)	<0.001
Albumin (g/L)	43 (3)	43 (4)	42 (4)	0.258
Bilirubin (µmol/L)	19 (11)	23 (14)	25 (17)	0.043
NYHA Class	2.2 (0.6)	2.3 (0.8)	2.5 (0.5)	0.387
IVSd (cm)	1.7 (0.2)	1.7 (0.2)	1.7 (0.2)	0.585
LVPWd (cm)	1.7 (0.2)	1.7 (0.2)	1.7 (0.2)	0.816
LVEF (%)	43 (10)	40 (11)	42 (14)	0.221
GLS (%)	-10.3 (3.3)	-9.9 (3.6)	-9.4 (3.1)	0.221
MAPSE (cm)	0.7 (0.2)	0.7 (0.3)	0.6 (0.2)	0.545
Lat S' TDI (m/s)	0.05 (0.01)	0.05 (0.01)	0.05 (0.01)	0.400
Sep S' TDI (m/s)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.559
TAPSE (cm)	1.4 (0.4)	1.3 (0.4)	1.3 (0.5)	0.123
RV S' TD I (m/s)	0.10 (0.03)	0.09 (0.03)	0.09 (0.03)	0.073
2D LA diam (cm)	4.3 (0.5)	4.4 (0.5)	4.4 (0.5)	0.771
E/A	2.3 (0.8)	2.5 (0.8)	2.5 (0.8)	0.570
MV DecT (ms)	170 (47)	170 (43)	172 (53)	0.916
IVRT (ms)	79 (22)	80 (24)	82 (19)	0.909
E/e'	18 (9)	17 (5)	17 (5)	0.459

Table 4.5: Repeated measures ANOVA for ATTR-V122I group. Data are presented as mean+/-SD or as number (% of group). A P value of less than 0.01 was considered statistically significant. ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; BP, blood pressure; eGFR, estimated glomerular filtration rate; GLS, global longitudinal strain; IVRT, isovolumic relaxation time; IVSd, Interventricular septal thickness in diastole; LVEF, left ventricular ejection fraction; LVPWd, Left ventricular posterior wall thickness in diastole; MAPSE, mitral annular plane systolic excursion; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association; TAPSE, tricuspid annular plane systolic excursion; TDI, tissue Doppler imaging.

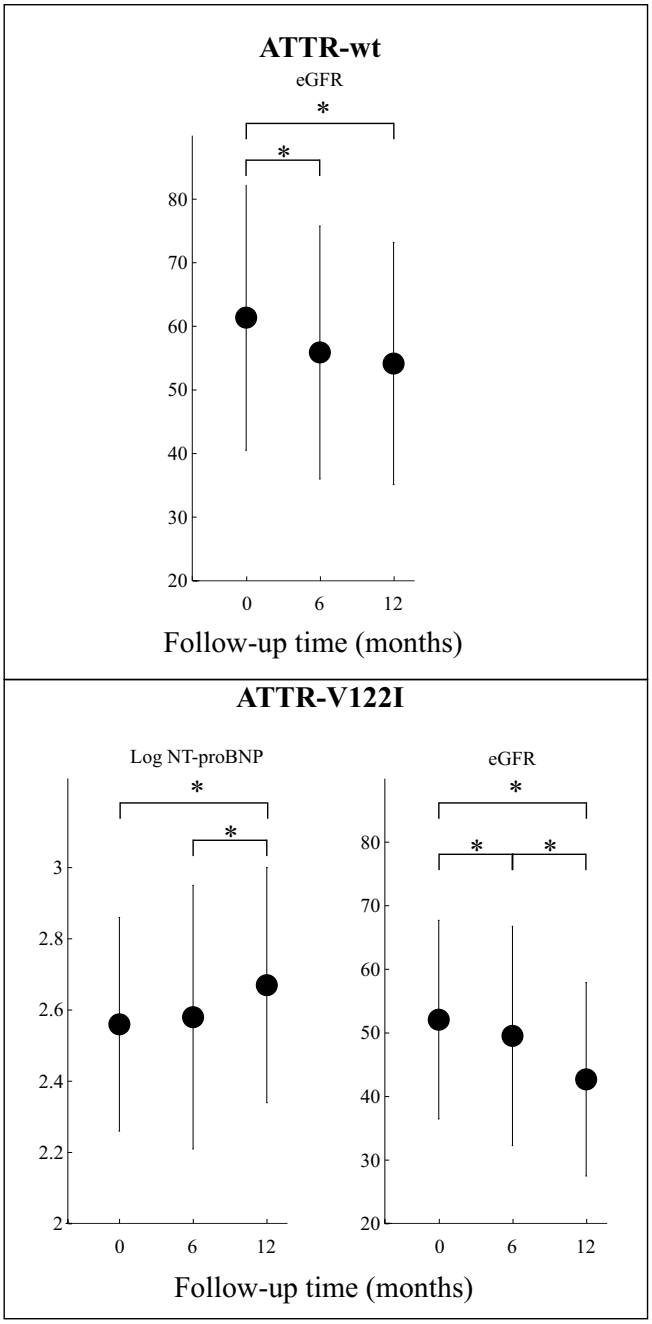


Figure 4.1: Pairwise comparisons of covariates that change with time over 12 months. Time 0 is baseline data at diagnosis. * A P value < 0.01 was considered statistically significant. ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide. eGFR measured in ml/min, NT-proBNP measured in pg/ml.

median survival for ATTR-wt group was 3.3 years (95% CI 2.9-3.8) and in ATTR-V122I 2.4 years (95% CI 2.2-2.7), log rank P=0.004 (Figure 4.2).

Univariate survival analyses of clinically relevant baseline characteristics and echocardiogram

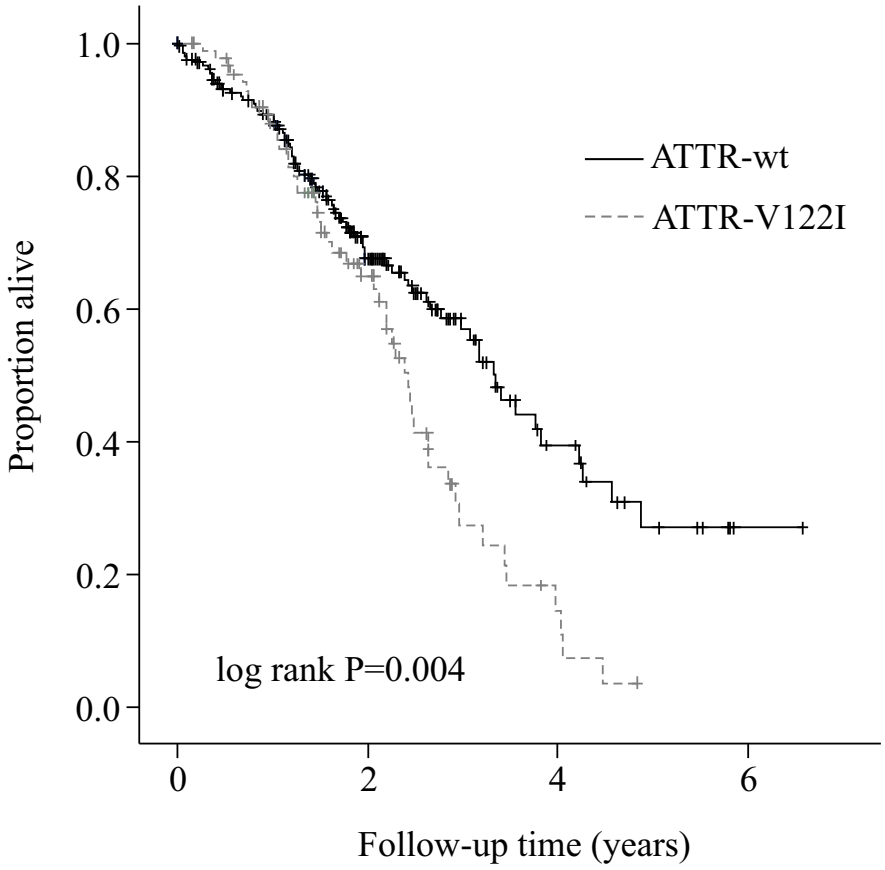


Figure 4.2: Overall (unadjusted) survival is superior in the ATTR-wt group compared to the ATTR-V122I group. ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis.

results are shown in Table 4.6. CMR and DPD data were not included because this would limit the number of patients that could be included in this analysis as a limited number of patients underwent these investigations. These analyses combine both ATTR groups but investigates the effect of TTR type by including it as a variable in the analyses.

All variables were considered as continuous variables except NYHA and NT-proBNP. NT-proBNP was considered as a categorical variable in univariate analysis with a grouping value for two categories of less than or equal to 4400pg/ml (n=118; 64% of patients), or greater than 4400 pg/ml (n=105; 36% of patients). An NT-proBNP cut-off of 4400pg/ml was calculated using receiver operator curve analysis. Univariate factors that were related to survival at a

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	***P***	**HR (95% CI)**	***P* value**
ATTR type [a]	1.7 (1.2-24)	0.004		
NYHA [b]		<0.001		0.001
NYHA II	4.9 (1.5-15.7)	0.007	3.6 (1.1-11.7)	0.031
NYHA III	13.6 (4.1-44.4)	<0.001	5.7 (1.7-19.1)	0.005
NYHA IV	61.9 (14.4-266.8)	<0.001	23.4 (4.4-125.9)	
Hypertension	0.9 (0.6-1.3)	0.653		
Diabetes Mellitus	0.8 (0.4-1.5)	0.475		
Ischaemic heart disease	1.1 (0.7-1.8)	0.563		
Cerebrovascular disease	0.8 (0.4-1.4)	0.376		
Atrial fibrillation	1.2 (0.8-1.6)	0.539		
NTproBNP>4400pg/ml [c]	3.0 (2.1-4.3)	<0.001	1.9 (1.2-3.0)	0.006
LVEF by Simpson's Biplane (%)	0.958 (0.940-0.975)	<0.001	0.969 (0.949-.0991)	0.005
Systolic BP (mmHg)	0.971 (0.959-0.983)	<0.001	0.980 (0.966-0.995)	0.008
eGFR (ml/min)	0.978 (0.968-0.987)	<0.001		
Age (years)	1.068 (1.035-1.102)	<0.001	1.083 (1.041-1.127)	<0.001
Bilirubin (µmol/L)	1.029 (1.014-1.045)	<0.001	1.024 (1.005-1.044)	0.012
GLS (%)	1.113 (1.051-1.178)	<0.001		
Albumin (g/L)	0.935 (0.894-0.978)	0.004		
TAPSE (cm)	0.538 (0.354-0.818)	0.004		
Broad QRS [d]	1.670 (1.134-2.459)	0.009		
Hb (g/dL)	0.870 (0.778-0.973)	0.015		
BMI	0.962 (0.916-1.010)	0.122		
MAPSE (cm)	0.588 (0.299-1.154)	0.123		
IVRT (ms)	0.998 (0.990-1.005)	0.525		
LVIDd (cm)	1.110 (0.792-1.555)	0.544		
Gender [e]	1.094 (0.646-1.851)	0.738		
E/e'	1.003 (0.982-1.025)	0.781		
IVSd (cm)	1.109 (0.506-2.432)	0.796		
LVPWd (cm)	1.064 (0.467-2.426)	0.882		
MVDecTime (ms)	1.000 (0.997-1.003)	0.909		

Table 4.6: Univariate and multivariate survival analyses of baseline characteristics and echocardiogram results. a Reference group is ATTR-wt, b Reference category is NYHA I, c Reference category is ≤4400pg/ml, d Reference category is narrow QRS (<120ms), e Reference category is female. ATTR, Amyloid transthyretin; BMI, body mass index; BP, blood pressure; CI, confidence interval; eGFR, estimated glomerular filtration rate; GLS, global longitudinal strain; Hb, haemoglobin; HR, hazard ratio; IVRT, isovolumic relaxation time; IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; LVIDd,left ventricular internal dimension in diastole; LVPWd, Left ventricular posterior wall thickness in diastole; MAPSE, mitral annular plane systolic excursion; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association; TAPSE, tricuspid annular plane systolic excursion.

significance level of less than 0.005 were considered in multivariate modelling. This strict cut-off was used to ensure that there were at least ten events for each co-variate used in the multivariate model. The result of the best multivariate model is displayed in Table 4.6.

Baseline patient factors independently related to survival in the multi-variate model were NYHA class, NT-proBNP greater than 4400pg/ml, LVEF, systolic blood pressure, age, and serum bilirubin level. NYHA functional class II was associated with 3.6 fold worse survival compared with NYHA I. Similarly for NYHA classes III and IV, these were associated with 5.7 and 23.7 fold worse survival respectively compared to NYHA class I (although notably the confidence interval for NYHA IV compared with NYHA I was broad in view of small numbers in these groups). An NT-proBNP value of >4400pg/ml was associated with 1.9 fold worse survival. An increase in LVEF by 1% was associated with 0.97 fold risk of death, implying that a 5% increase in LVEF would be associated with a 15% better survival. An increase in systolic blood pressure of 1mmHg was associated with a 0.98 fold risk of death suggesting that a 5mmHg higher systolic blood pressure is associated with 10% better survival. An increase in age by 1 year was associated with a 1.1 fold poorer survival. A 1 unit increase in serum bilirubin was associated with a 1.02 fold poorer survival.

In the multivariate model displayed in Table 4.6, TTR type was not predictive of survival and this is displayed in the survival curves adjusted for the other co-variates in the model (Figure 4.3).

In view of the imbalance in group sizes (ATTR-wt n=206, vs. ATTRV122I n=87) contributing to the multivariable model, a sensitivity analysis for the model was performed. The two ATTR types were separately assessed to investigate whether the model remained applicable for both groups. Table 4.7 shows this for the ATTR-wt group and Table 4.8 shows this for the V122I group.

	Multivariate Analysis	
	HR (95% CI)	***P***
NYHA [b]		0.005
NYHA (2)	3.701 (0.867-15.798)	0.077
NYHA (3)	7.336 (1.566-34.363)	0.011
NYHA (4)	22.462 (2.786-181.104)	0.003
NTproBNP>4400pg/ml [c]	2.102 (1.159-3.812)	0.015
LVEF (%)	0.951 (0.925-0.978)	<0.001
Systolic BP (mmHg)	0.983 (0.966-1.001)	0.059
Age (years)	1.076 (1.019-1.136)	0.008
Bilirubin (µmol/L)	1.019 (0.994-1.044)	0.148

Table 4.7: Multivariate survival model of baseline characteristics for the ATTR-wt group with the same co-variates as in main multivariate model. b Reference category is NYHA I. c Reference category is ≤4400pg/ml. BP, blood pressure; CI, confidence interval; HR, hazard ratio; LVEF, Left Ventricular Ejection Fraction; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association.

	Multivariate Analysis	
	HR (95% CI)	***P***
NYHA [b]		0.442
NYHA (2)	2.119 (0.268-16.731)	0.476
NYHA (3)	2.134 (0.258-17.637)	0.482
NYHA (4)	12.877 (0.570-290.88)	0.108
NTproBNP>4400pg/ml [c]	1.842 (0.736-4.610)	0.192
LVEF (%)	1.013 (0.972-1.055)	0.550
Systolic BP (mmHg)	0.972 (0.944-1.001)	0.055
Age (years)	1.091 (1.009-1.179)	0.029
Bilirubin (µmol/L)	1.052 (1.014-1.092)	0.07

Table 4.8: Multivariate survival model of baseline characteristics for the ATTR-V122I group with the same co-variates as in main multivariate model. b Reference category is NYHA I. c Reference category is ≤4400pg/ml. BP, blood pressure; CI, confidence interval; HR, hazard ratio; LVEF, Left Ventricular Ejection Fraction; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association.

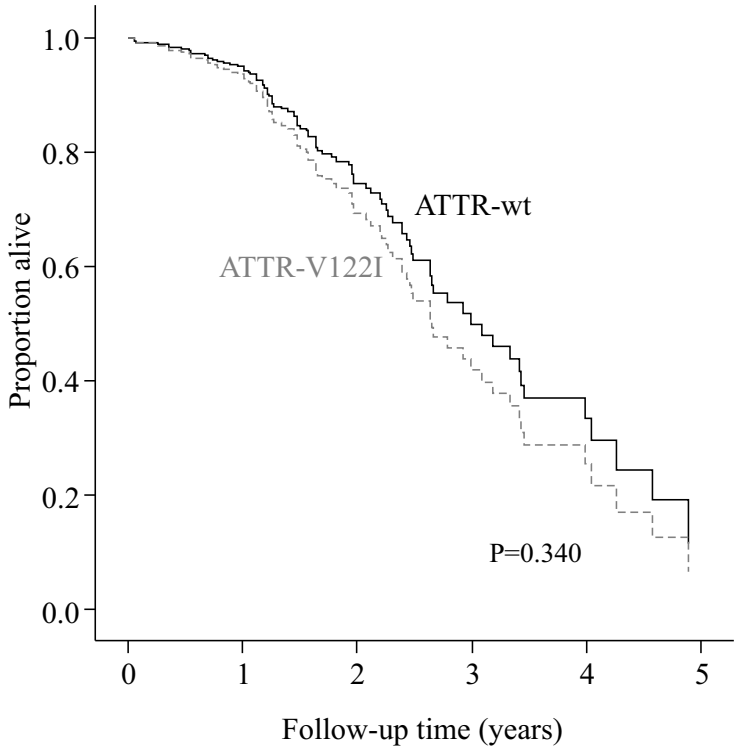


Figure 4.3: Survival curves for ATTR-wt and ATTR-V122I amyloidosis adjusted for age, NYHA class, left ventricular ejection fraction, N-terminal-pro B-type Natiuretic Peptide, systolic blood pressure, and serum bilirubin. ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis.

Since the independent predictors of survival that were significant in the multivariable model did not appear as suitable for either ATTR-wt or ATTR-V122I when considered separately, new models were developed separately for the two ATTR types. Table 4.9 shows this for the ATTR-wt group and Table 4.10 shows this for the ATTR-V122I group.

The best multi-variable model for the ATTR-wt group was one in which all the original variables remained except systolic blood pressure. The best multi-variable model for the ATTR-V122I group only included systolic blood pressure and serum bilirubin with all others variables removed (NYHA, NT-proBNP, age, LVEF).

Cause of death

The cause of death for patients who died during the follow-up period were obtained from death certificate data supplied by the national registry and are summarised in Figure 4.4.

	Multivariate Analysis	
	HR (95% CI)	***P***
NYHA [b]		0.004
NYHA (2)	4.205 (0.986-17.931)	0.052
NYHA (3)	7.717 (1.663-35.813)	0.009
NYHA (4)	27.898 (3.480-223.636)	0.002
NTproBNP>4400pg/ml [c]	2.022 (1.138-3.593)	0.016
LVEF (%)	0.952 (0.927-0.979)	<0.001
Age (years)	1.080 (1.025-1.138)	0.004
Bilirubin (µmol/L)	1.024 (1.000-1.049)	0.048

Table 4.9: New multivariate survival model of baseline characteristics for ATTR-wt group. b Reference category is NYHA I. c Reference category is $\leq$4400pg/ml. CI, confidence interval; HR, hazard ratio; LVEF, Left Ventricular Ejection Fraction; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association.

	Multivariate Analysis	
	HR (95% CI)	***P***
Systolic BP (mmHg)	0.970 (0.946-0.994)	0.015
Bilirubin (µmol/L)	1.044 (1.016-1.072)	0.002

Table 4.10: New multivariate survival model of baseline characteristics for ATTR-V122I group. BP, blood pressure; CI, confidence interval;HR, hazard ratio.

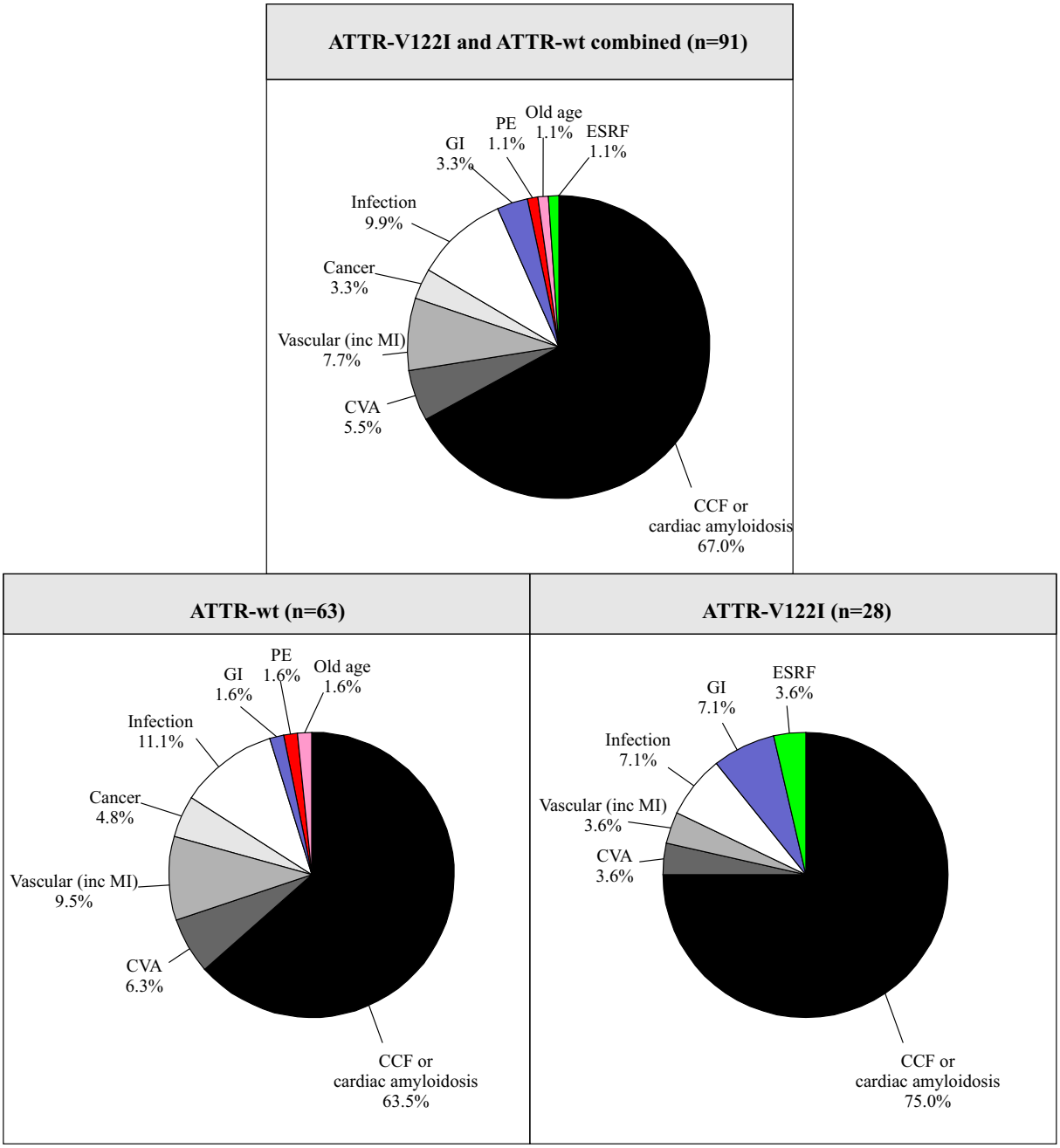


Figure 4.4: Cause of death from death certificates for patients with cardiac ATTR-wt and cardiac ATTR-V122I amyloidosis. ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; CCF, congestive cardiac failure; CVA, cerebrovascular accident; ESRF, end stage renal failure; GI, gastrointestinal; MI, myocardial infarction; PE, pulmonary embolism.

Congestive cardiac failure or cardiac amyloidosis was the primary cause of death for approximately two thirds (67%) all of patients who died. Separated by TTR type, cardiac amyloidosis or congestive cardiac failure was the primary cause of death in 75% of the ATTR-wt group and 63.5% of the ATTR-V122I group.

4.5 Limitations

The data in this study have been gathered retrospectively from a single specialist centre in the UK. This is likely to result in inherent selection biases in the patient population. Although the size of the study is the largest reported to date including the range of findings from different cardiac assessments, the size remains modest and the follow-up period was of intermediate length. Although serum troponin T may have an important role in multi-variate modelling, these data could not be adequately assessed in this study due to a change in assay during the follow-up period for this study.

4.6 Discussion

The main findings from data presented in this chapter are:

1. Patients with ATTR-V122I cardiac amyloidosis present at a younger age but with a more severe heart failure phenotype compared to those with ATTR-wt cardiac amyloidosis.

2. Patients with ATTR-V122I cardiac amyloidosis have a worse prognosis than those with ATTR-wt cardiac amyloidosis

3. The majority of patients with ATTR-wt and ATTR-V122I cardiac amyloidosis die from heart failure.

4. Predictors of survival in ATTR-wt and ATTR-V122I cardiac amyloidosis include parameters related to heart failure severity.

5. Over a 12 month follow-up there was no significant progression of disease that could be identified on cardiac imaging or biomarkers which may be relevant when planning clinical trials.

Patients with ATTR-V122I cardiac amyloidosis were younger than those with ATTR-wt

(75.3 +/- 5.8 years vs. 77.3 +/- 6.2 years, P=0.008) and the vast majority were of African or West Indian descent while the majority of patients with ATTR-wt cardiac amyloidosis were Caucasian. Patients with ATTR-V122I were more likely to present as an emergency, while those with ATTR-wt cardiac amyloidosis were more likely to be identified as an outpatient. Potential explanations for this difference include the possibility that ATTR-V122I cardiac amyloidosis has a more rapid onset or that ethnic differences in the populations mean healthcare is utilised differently between the populations.

Patients with cardiac amyloidosis are often considered to have preserved systolic function, however both groups had reduced overall left ventricular systolic function at diagnosis by transthoracic echocardiography and CMR imaging at the time of diagnosis. Notably, all the standard markers of left ventricular systolic function were significantly worse in the ATTR-V122I group (LVEF, longitudinal myocardial velocity by tissue Doppler assessment, MAPSE and GLS percentage). Furthermore, a greater proportion of patients in the ATTR-V122I group had grade 3 DPD uptake, and ECV by CMR was also greater in the ATTR-V122I group despite similar native myocardial T1 values. The reasons for these findings were not investigated here but possible hypotheses include a greater amyloid burden in ATTR-V122I or differences in the direct myocardial effect of variant and wild-type transthyretin leading to different myocyte responses. Native T1 is a composite signal from the interstitium and myocardial cells and thus reflects the amyloid load, fibrosis and particularly myocardial oedema. The ECV reflects myocardial amyloid load and fibrosis. Thus similar T1 values may reflect similarities in myocardial oedema, while differences in ECV may reflect a greater amyloid burden in patients with ATTR-V122I despite similar LV wall thickness and mass. If this is the case, it could be as a direct consequence of the variant TTR or even that the myocardial cell response to the variant TTR is greater cell hypertrophy triggering more

fibrosis. A differential effect on the heart from the two types of TTR may be supported by the fact that despite patients with ATTR-V122I having a more severe heart failure phenotype on imaging, the prevalence of a broad QRS was significantly greater in the ATTR-wt population.

In this study, patients with inherited transthyretin amyloidosis of the ATTR-V122I type were younger, however overall unadjusted survival was significantly worse than in the ATTR-wt group (overall unadjusted median survival for the ATTR-wt group was 3.3 years [95% CI 2.9-3.8] and in the ATTR-V122I group 2.4 years [95% CI 2.2-2.7], log rank P=0.004). This contrasts to the finding in Chapter 3 and is most likely to be the result of small sample size and under-powering in the previous chapter. The median survival of each group was similar in both chapters and there was a trend for worse surival in the ATTR-V122I group in Chapter 3 but it did not reach statistical significance. In a multivariate model of survival there was no difference in survival between the two TTR groups when adjusted for NYHA, NT-proBNP, age, LVEF, systolic blood pressure, eGFR and serum bilirubin. Thus although overall survival is shorter from diagnosis in the ATTR-V122I group this is likely to be a result of more advanced disease features at diagnosis.

It was not possible in this study to exclude delayed presentation to medical services as a possible contributing factor for the differences at diagnosis, although the symptom onset period prior to presentation was similar in the two groups and does not support this hypothesis. Access to the healthcare services is also unlikely to explain this difference, as in the UK, all citizens are entitled to free central government funded care, but there may be cultural differences that could contribute.

The factors independently related to survival may have been anticipated given the nature of this condition which has a predominant heart failure phenotype. The hypothesis that outcomes in these two groups of patients with cardiac transthyretin amyloidosis are related to the severity of

cardiac involvement is also supported by the cause of death data which shows that the majority of patients die from heart failure. A noteable non-cardiac predictor of survival is bilirubin. This is in keeping with other studies that have also shown a relationship between liver function tests and outcomes, albeit in different patient cohorts [138, 139] and may reflect hepatic congestion. There were few significant changes identified for either TTR group over twelve months follow-up. Estimated GFR was the only parameter that significantly changed over twelve months in both groups and may reflect increasing diuretic use, impaired renal perfusion or simply increasing age. There were trends for increasing NT-proBNP in both groups, increasing E/e' in the ATTR-wt group and increasing serum bilirubin in the ATTR-V122I group. The lack of significant change over 12 months is a particularly important finding as it highlights the difficulty that exists in identifying a clinically useful disease marker which could serve as a surrogate for disease severity and response to any current or future treatment options.

In this study, I identified some simple markers used in everyday clinical practice that may aid more accurate prognostication for patients with these two forms of cardiac transthyretin amyloidosis. This may be useful but the data presented here identify a key limitation of this methodology. Interestingly, although one multi-variate model identified seven co-variates that were independently related to survival when data for both groups were combined, separate multi-variable models with fewer co-variates in each were actually more applicable when the groups were considered separately. This may reflect the differential contribution to the model of the two different sized populations examined. This finding is important when interpreting any multivariate modelling for the assessment of patients with cardiac transthyretin amyloidosis if different types of TTR are combined in to a single group. Combining groups is tempting for statistical analyses due to the key effect of increased sample size and thus statistical power, however in the preceding chapter (Chapter 3) and this (Chapter 4) differences amongst the TTR

types have been clearly demonstrated in various characteristics including patient characteristics and investigation findings. Recently, two staging systems have been proposed for patients with cardiac ATTR amyloidosis. Grogan et al [63] studied patients with ATTR-wt amyloidosis and multivariate predictors of survival included age, LVEF, pericardial effusion and cardiac biomarkers. A staging system was developed using threshold values for NT-proBNP and troponin T. These findings were similar to my own presented in this chapter but they did not investigate prognostic markers in hereditary forms of ATTR amyloidosis. The second proposed staging system combined patients with hereditary and wild-type ATTR amyloidosis (Gilmore et al [140]). In this study, multivariate predictors of survival included eGFR, NT-proBNP, ECOG performance status, systolic BP and LVEF. A staging system was created using threshold values for NT-proBNP and eGFR. Combining the different types of ATTR amyloidosis may be an oversimplification and limit clinical utility, particularly in light of the differences that have been noted between the different forms of ATTR amyloidosis identified in this thesis. As an example, for patients with stage III disease in this latter proposed staging system, the median survival of patients with ATTR-V122I amyloidosis is almost half that in patients with ATTR-wt [17.7 months (95% CI 11.5-22.3 months) vs. 32.7 months (95% CI 23.4-37.0 months)].

Finally, I identified cardiac disease as the main cause of death in patients with these two types of cardiac ATTR amyloidosis. This finding was confirmed in a study published after the undertaking of this thesis in patients with ATTR-wt cardiac amyloidosis [141] but has not been described in patients with ATTR-V122I cardiac amyloidosis.

4.7 Conclusions

This study demonstrates the key similarities and differences in the baseline characteristics and overall survival from diagnosis of the two commonest cardiac ATTR amyloidosis types. These

results suggest that the cardiac transthyretin amyloidoses should be considered as separate entities particularly when assessing disease progression and the impact of disease-modifying treatments on disease trajectories. Outcomes in these two forms of cardiac ATTR amyloidosis appear to be poorer than previous estimates. Simple clinical, biochemical and transthoracic echocardiographic data assessed at diagnosis can provide prognostic information but larger prospective studies are needed to confirm these findings.

Chapter 5

Investigation of ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid Scintigraphy in Cardiac Amyloidosis

5.1 Introduction

Various radioactive isotope tracers used for the purposes of bone scintigraphy demonstrate a high sensitivity (>99%) for the detection of cardiac ATTR amyloidosis [33]. These tests are, however, less specific than originally thought with cardiac uptake also detected in individuals with cardiac AL amyloidosis and those with cardiac ApoA1 amyloidosis [77]. This contradicts the earlier report of 100% accuracy for the distinction between cardiac ATTR and AL aetiologies in the seminal study by Perugini et al. [132]. This was a small study of 25 patients with cardiac amyloidosis and 10 controls and it is now clear that approximately one third to one half of patients with cardiac AL amyloidosis have evidence of cardiac uptake by ^{99m}Tc DPD scintigraphy [75, 76]. International centres utilise different tracers, selected based on availability and familiarity. ^{99m}Tc DPD is used in much of Europe, except France and the Netherlands where hydroxymethylene diphosphonate (HMDP) is used. PYP is used in the US.

It is believed that these tracers are similarly sensitive and specific for the detection of ATTR amyloid.

Two small studies have suggested that the Perugini grade of cardiac uptake by ^{99m}Tc DPD scintigraphy correlates with the severity of disease in ATTR cardiac amyloidosis and is predictive of outcomes. Rapezzi et al studied 63 patients with hereditary ATTR [30]. Forty of these patients had cardiac involvement defined on echocardiography criteria (these were not confirmed on EMB). Correlation was demonstrated between cardiac uptake and LV wall thickness, LV ejection fraction. Furthermore, DPD grade was predictive of outcomes when major adverse cardiovascular events (MACE) was used as the primary outcome. Kristen et al. [74] studied 36 patients with ATTR-wt and ^{99m}Tc DPD uptake correlated with disease severity as assessed by MAPSE and septal thickness. Furthermore, in this study it was suggested that DPD grade was predicitive of mortality. However, it should be noted this result was found when patients with DPD grade 2 or 3 were combined (n=25) and compared to those with DPD grade 1 (n=6) so this result should be interpreted with caution.

The largest study investigating the value of ^{99m}Tc DPD scintigraphy in ATTR amyloidosis focused on its use as a prognostic tool. This study was published during the writing of this thesis and studied 602 patients with ATTR amyloidosis (377 patients with ATTR-wt amyloidosis and 255 patients with ATTR-m amyloidosis). There was no prognostic value of ^{99m}Tc DPD scintigraphy in terms of survival.

There are no published data investigating the relationship between echocardiographic and clinical findings with ^{99m}Tc DPD scintigraphy using the Perugini grading system for the three most common types of cardiac ATTR amyloidosis in the UK (cardiac ATTR-wt, ATTR-V122I and ATTR-T60A amyloidosis). This would in part address the question of whether DPD grade is related to disease severity. Furthermore, the prognostic utility of ^{99m}Tc DPD scintigraphy in

the assessment of individuals with cardiac AL amyloidosis has not been investigated beyond the earliest small studies.

5.2 Aims

- To investigate the correlation between DPD grade and disease severity in cardiac ATTR amyloidosis.
- To investigate the correlation between DPD grade and disease severity in cardiac AL amyloidosis.
- To evaluate if DPD grade is related to prognosis in cardiac AL amyloidosis.

5.3 Methods

5.3.1 Patients

Two patient cohorts were examined in this study. The first cohort had cardiac ATTR amyloidosis and underwent ^{99m}Tc DPD scintigraphy as part of their evaluation. The second patient cohort had a diagnosis of cardiac AL amyloidosis and underwent ^{99m}Tc DPD scintigraphy during their diagnostic evaluation on clinical grounds. All patients were identified from the NAC electronic database. Demographic and clinical data were collected.

5.3.2 Investigations

All patients underwent TTR gene sequencing, transthoracic echocardiography, and ^{99m}Tc DPD scintigraphy and ^{123}I SAP scintigraphy as previously described in the general methods, Chapter 2. Scoring of cardiac uptake by ^{99m}Tc DPD scintigraphy was performed as per the visual scoring

method described previously Perugini et al. for all patients. ^{123}I labelled SAP scintigraphy to assess whole body amyloid load, was performed in all patients on a separate visit. Blood samples were taken for haematological and biochemical evaluations. Urine analysis for protein and monoclonal immunoglobulin was performed.

The diagnosis of AL amyloidosis was confirmed in all cases with a tissue biopsy demonstrating characteristic birefringence on Congo red staining. Typing of AL amyloidosis was confirmed by immunohistochemical staining with appropriate antibodies and by exclusion of hereditary amyloidosis by genetic sequencing where features pointed to this as a significant possibility. Measurement of serum FLCs was carried out using the Freelite assay (The Binding site, Birmingham, United Kingdom). Cardiac disease stage for patients with AL amyloidosis was determined by Mayo staging (21).

5.3.3 Statistical analyses

Differences in echocardiographic, cardiac biomarkers, and functional capacity (NYHA for patients with cardiac ATTR amyloidosis, and NYHA and ECOG for patients with cardiac AL amyloidosis) between DPD grades were assessed with one way ANOVA tests for continuous variables and Chi square tests for categorical variables. Independent samples T tests were performed in pairwise comparisons for continuous variables reaching statistical significance in the ANOVA tests. In view of multiple testing, a P value, with a Bonferroni-type correction, of less than 0.01 was considered statistically significant. Survival analyses were performed using the Kaplan Meier method and compared using the log rank test. An ordinal logistic regression analysis was performed to investigate the relationship between patient characteristics and the grade of uptake by ^{99m}Tc DPD scintigraphy.

5.4 Results

5.4.1 Characteristics of patients with cardiac ATTR based on visual DPD grading

Two hundred and seventy-five patients with cardiac ATTR amyloidosis were included. Comparisons between patients with different DPD grades are displayed in Table 5.1 and Table 5.2.

Pairwise comparisons of continuous variables reaching statistical significance ($P<0.01$) across the DPD grades are displayed in Figure 5.1 and Figure 5.2. Pairwise comparisons of variables trending towards statistical significance ($P<0.05$) are also displayed for illustrative purposes.

Patients with higher grades of uptake by ^{99m}Tc DPD scintigraphy (grades 2 or 3 versus grade 1) were older, have greater LV wall thickness, higher LV filling pressures (E/e'), higher serum NT-proBNP and Troponin T, and higher serum bilirubin levels. Although not reaching statistical significance, there was a trend for patients with a higher grade of uptake to have a lower systolic blood pressure (P=0.035), lower LVEF (P=0.024), and shorter 6MWT (P=0.035).

Visual grading of uptake by ^{99m}Tc DPD scintigraphy appeared to be related to the transthyretin genotype; the majority of individuals with grade 2 uptake had wild-type TTR genotype and the majority of individuals with grade 3 uptake had the V122I genotype. Other than in the wild-type group and 'others', grade 1 uptake was rare.

The majority of differences in the variables was between DPD grade 1 vs DPD grade 2 and DPD grade 1 vs DPD grade 3. There appear to be few differences in characteristics between DPD grade 2 and DPD grade 3.

Pairwise comparisons of categorical variables reaching statistical difference between the

	DPD 1 n=24		DPD 2 n=189		DPD 3 n=62		*P*
	n	**%**	**n**	**%**	**n**	**%**	
TTR Genotype:							
WT	12	50.0%	137	72.5%	19	30.6%	<0.001
V122I	1	4.2%	32	16.9%	25	40.3%	
T60A	1	4.2%	16	8.5%	11	17.7%	
V30M	2	8.3%	2	1.1%	0	0.0%	
Others	8	33.3%	2	1.1%	7	11.3%	
Gender:							
Female	8	33.3%	18	9.5%	17	27.4%	<0.001
Male	16	66.7%	171	90.5%	45	72.6%	
NYHA:							
I	5	31.2%	18	9.9%	4	7.1%	0.067
II	10	62.5%	130	71.8%	37	66.1%	
III	1	6.2%	31	17.1%	15	26.8%	
IV	0	0.0%	2	1.1%	0	0.0%	
	n	**Mean (SD)**	**n**	**Mean (SD)**	**n**	**Mean (SD)**	
Age (yrs)	16	70.6 (12.1)	189	76.4 (6.8)	62	74.3 (8.3)	0.004
BMI	13	26.9 (3.4)	162	26.6 (6.1)	51	24.8 (4.3)	0.127
Systolic BP (mmHg)	15	135.4 (22.7)	186	112.9 (37.0)	59	117.3 (18.0)	0.035
6MWT (m)	12	381.3 (136.9)	70	300.1 (122.6)	27	269.2 (117.6)	0.035

Table 5.1: TTR genotype, demographic and functional characteristics of patients with cardiac ATTR based on visual DPD grading. A P value of less than 0.01 was considered statistically significant. Categorical variables are presented as proportions of the DPD group, and continuous variables are presented as mean and standard deviation. Statistical comparisons made with Chi square for categorical variables and one way ANOVA for continuous variables. 6MWT, six minute walk test; BMI, body mass index; BP, blood pressure; NYHA, New York Heart Association; TTR, transthyretin; WT, wild-type.

	DPD 1 n=24		DPD 2 n=189		DPD 3 n=62		*P*
	n	**Mean (SD)**	**n**	**Mean (SD)**	**n**	**Mean (SD)**	
Hb (g/dl)	24	13.1 (1.3)	189	13.4 (1.4)	62	13.0 (1.4)	0.068
eGFR (ml/min)	24	62.4 (24.3)	189	54.2 (15.9)	62	53.2 (16.1)	0.059
Albumin (g/L)	24	43.8 (2.3)	189	42.9 (3.0)	62	42.5 (2.3)	0.126
Bilirubin (µmol/L)	24	9.6 (4.4)	189	17.7 (9.4)	62	15.4 (9.5)	<0.001
Troponin T (µg/L)	19	0.057 (0.054)	157	0.085 (0.063)	40	0.111 (0.070)	0.008
NT-proBNP (pg/ml)	24	1872.9 (2222.6)	189	4840.2 (4098.4)	62	5106.3 (4502.6)	0.002
Log NT- proBNP (pg/ml)	24	2.8 (0.7)	189	3.6 (0.3)	62	3.6 (0.4)	<0.001
LVEF (%)	24	53.8 (12.1)	189	47.9 (11.8)	62	46.0 (11.6)	0.024
IVSd (mm)	15	12.9 (3.1)	186	16.8 (2.3)	58	17.0 (2.6)	<0.001
LVPWd (mm)	15	12.1 (3.2)	186	16.3 (2.2)	58	16.7 (2.5)	<0.001
E/e'	14	11.3 (4.9)	184	17.1 (7.0)	55	19.9 (8.2)	<0.001

Table 5.2: Blood result and echocardiographic characteristics of patients with cardiac ATTR amyloidosis based on visual DPD grading. A P value of less than 0.01 was considered statistically significant. Categorical variables are presented as proportions of the DPD group, and continuous variables are presented as mean and standard deviation (SD). Statistical comparisons made with Chi square for categorical variables and one way ANOVA for continuous variables. eGFR, estimated glomerular filtration rate; Hb, haemoglobin; IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; LVPWd, Left ventricular posterior wall thickness in diastole; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide

different DPD grades are displayed in Figure 5.3. Gene type was different in all comparisons.

Survival curves for combined TTR genotypes, separated based on grade of DPD uptake is illustrated in Figure 5.4. This illustrates that there was no significant difference in survival between the different DPD grades (log rank, P=0.402) consistent with other published data [76].

5.4.2 DPD grade comparing wild-type and hereditary cardiac transthyretin amyloidosis

The population of cardiac transthyretin amyloidosis was comprised of: wild-type (n=168), V122I (n=58), T60A (n=28), V30M (n=4) and Others (n=17). These last two groups made up a small proportion of cases and were excluded for subsequent analyses. Comparison of the features of the three main different TTR aetiologies can be seen in Table 5.3.

There was a significant difference in DPD grade across the TTR aetiologies (Figure 5.5). Over 80% of patients with ATTR-wt were DPD grade 2 while the hereditary TTR populations were

	WT n=168		V122I n=58		T60A n=28		*P*
	n	**%**	**n**	**%**	**n**	**%**	
DPD Grade							<0.001
1	12	7.10%	1	1.70%	1	3.60%	
2	137	81.50%	32	55.20%	16	57.10%	
3	19	11.30%	25	43.10%	11	39.30%	
NYHA class							0.555
1	17	10.80%	3	5.40%	3	12.50%	
2	113	72.00%	37	66.10%	17	70.80%	
3	26	16.60%	15	26.80%	4	16.70%	
4	1	0.60%	1	1.80%	0	0.00%	
Gender							<0.001
Female	14	8.3%	15	25.9%	8	28.6%	
Male	154	91.7%	43	74.1%	20	71.4%	
	Mean (SD)		**Mean (SD)**		**Mean (SD)**		
Age (years)	77.9 (6.3)		75.5 (6.5)		70.3 (4.4)		<0.001
IVSd (mm)	1.68 (0.26)		1.68 (0.21)		1.65 (0.28)		0.847
LVPWd (mm)	1.63 (0.24)		1.64 (0.23)		1.63 (0.31)		0.976
E/e'	17.3 (7.6)		16.5 (5.9)		20.7 (7.4)		0.059
LVEF (%)	48.7 (11.7)		41.9 (10.8)		52.0 (10.4)		<0.001
Systolic BP (mmHg)	113.1 (39.5)		116.9 (17.8)		116.1 (13.3)		0.738
6MWT (m)	304.4 (132.5)		248.7 (112.9)		306.2 (101.9)		0.297
Troponin T (µg/L)	0.082 (0.050)		0.084 (0.046)		0.120 (0.127)		0.030
NTProBNP (pg/ml)	4709.4 (4392.9)		5742.3 (3507.5)		3331.3 (3025.5)		0.035
Log NT-proBNP (pg/ml)	3.52 (0.39)		3.67 (0.29)		3.35 (0.44)		0.001
Bilirubin (µmol/L)	16.6 (9.0)		19.9 (11.4)		13.7 (4.9)		0.010
Hb (g/dl)	13.4 (1.4)		12.8 (1.4)		13.4 (1.0)		0.010
eGFR (ml/min)	54.1 (14.4)		45.0 (12.3)		64.3 (19.9)		<0.001
Serum albumin (g/L)	43.3 (2.6)		41.5 (3.4)		42.7 (2.1)		<0.001

Table 5.3: Comparison of features of ATTR-wt versus ATTR-V122I and ATTR-T60A cardiac amyloidoses. Continuous variables are described as mean (standard deviation) and compared using a one way ANOVA. Categorical data are presented as proportions and compared with Chi square test. 6MWT, six minute walk test; eGFR, estimated glomerular filtration rate; Hb, haemoglobin;IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; LVPWd, Left ventricular posterior wall thickness in diastole; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association; WT, wild-type.

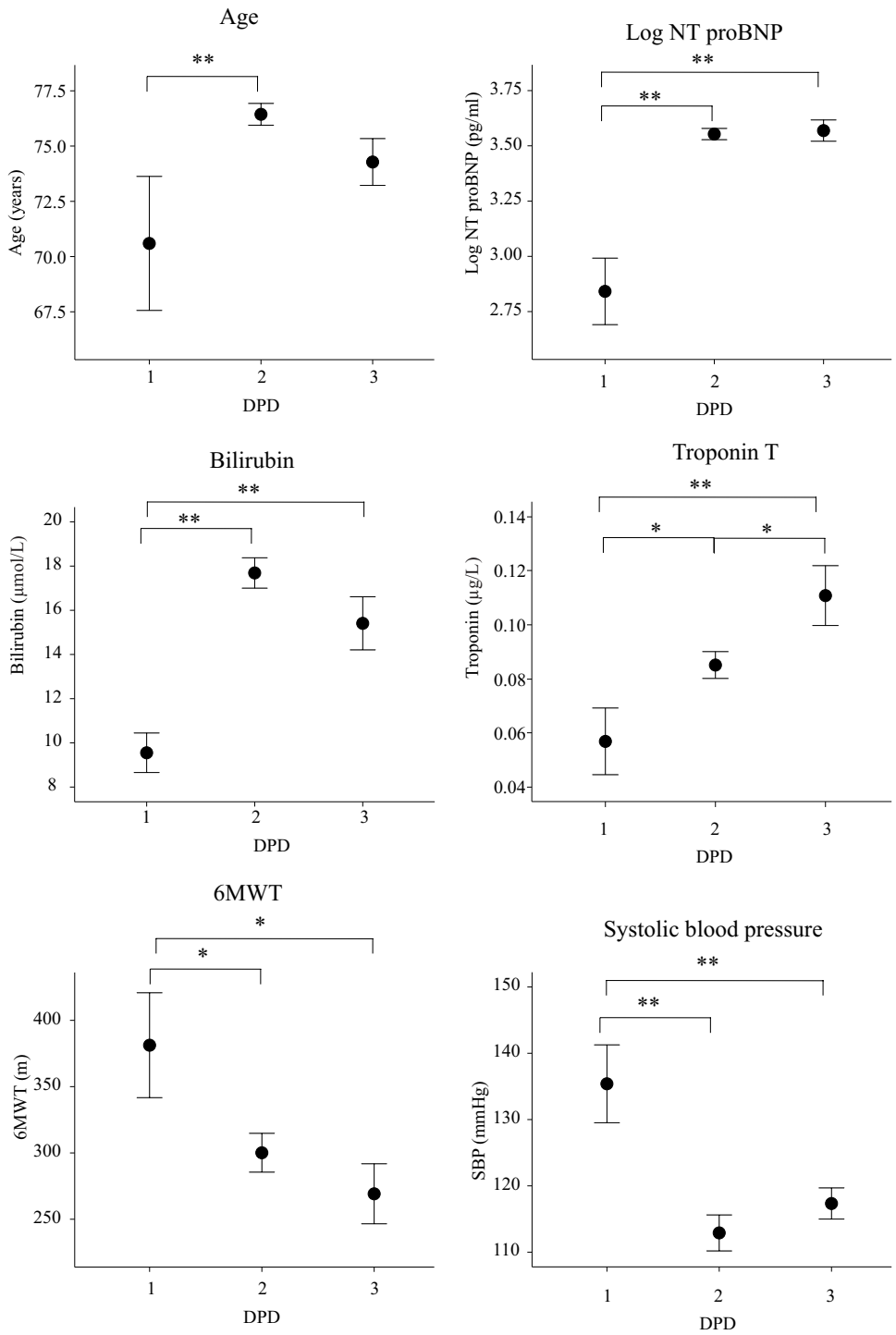


Figure 5.1: Pairwise comparisons of continuous variables (age, serum bilirubin and cardiac biomarkers and 6MWT) between different DPD grades in patients with ATTR amyloidosis. *indicates difference between populations to a significance of $P<0.05$ ** indicates significant difference between populations to a significance level of $P<0.01$. 6MWT, six minute walk test; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide.

DPD grade 2 in 55-57% of cases and DPD grade 3 in 39-48% of cases. This is despite similarities of LV wall thickness, cardiac biomarkers and other characteristics.

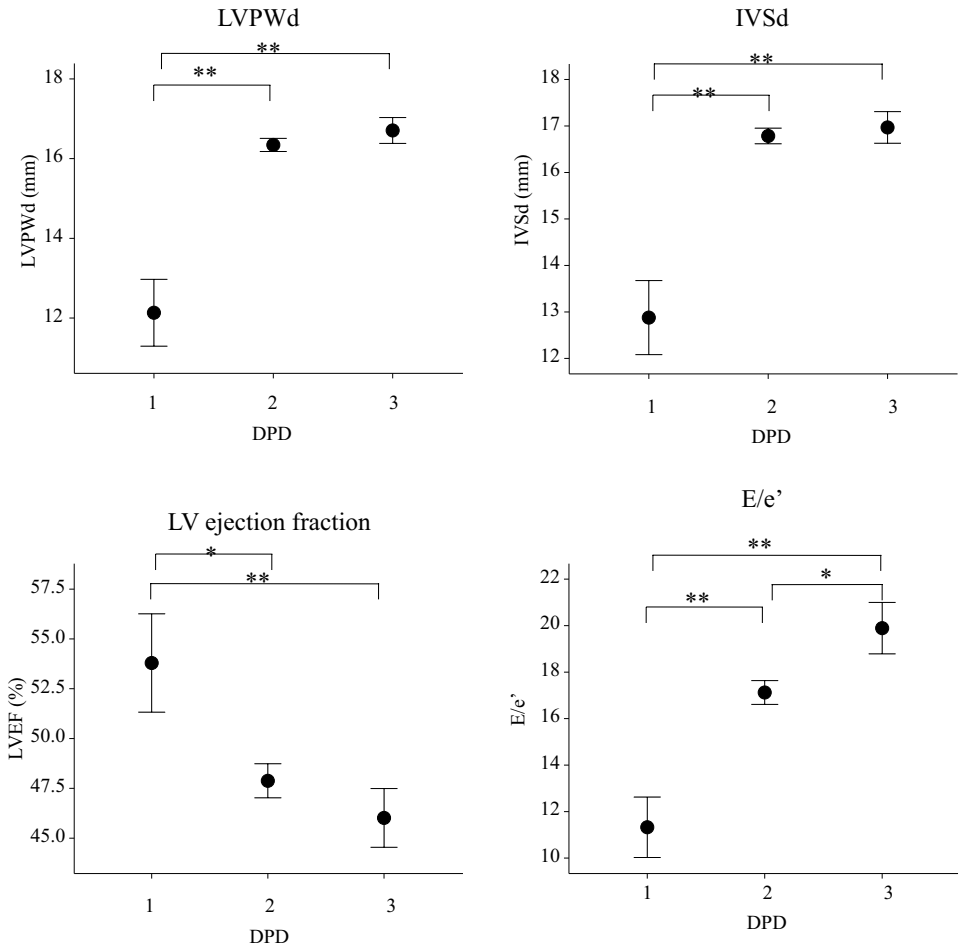


Figure 5.2: Pairwise comparisons of continuous variables (echocardiogram characteristics) between different DPD grades in patients with ATTR amyloidosis. *indicates difference between populations to a significance of $P<0.05$. ** indicates significant difference between populations to a significance level of $P<0.01$. IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; LVPWd, Left ventricular posterior wall thickness in diastole.

An ordinal logistic regression analysis was performed to investigate the relationship between patient characteristics and the grade of uptake by DPD scintigraphy (Table 5.4).

In this analysis, V122I TTR genotype was a significant predictor of grade of uptake by ^{99m}Tc DPD scintigraphy. Patients with the V122I TTR genotype were more than 5 times more likely than patients with the wild-type TTR genotype to have a higher DPD score. Other factors that were significantly related to increasing DPD grade were serum Troponin T and NT-proBNP levels and E/e', however LV septal wall thickness was not.

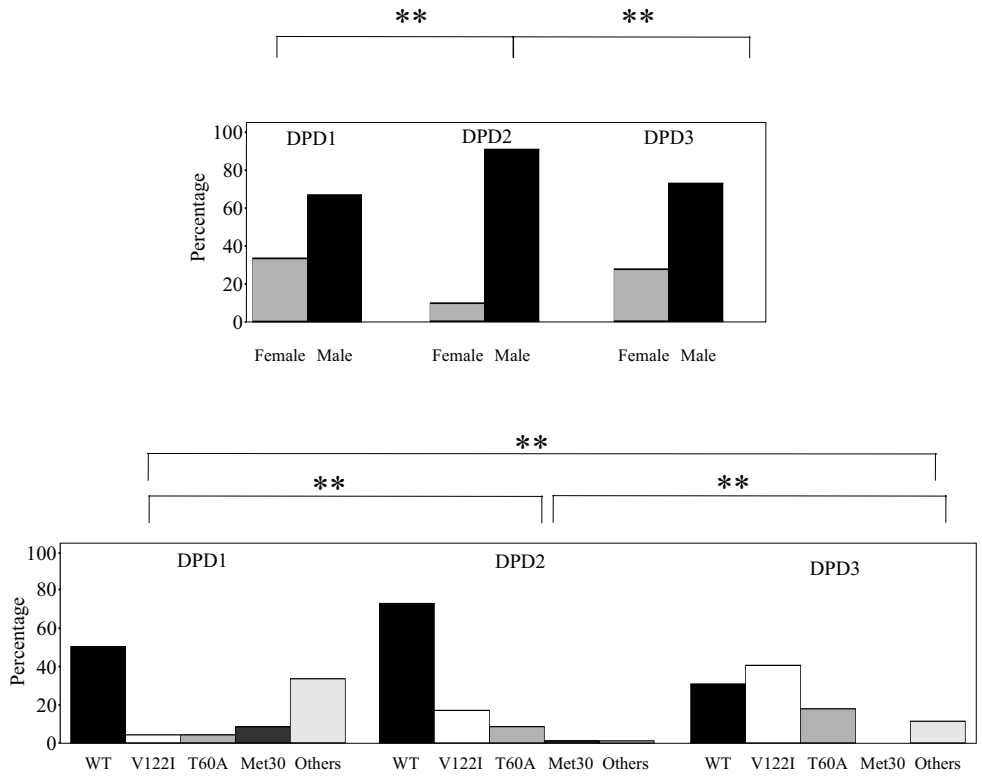


Figure 5.3: Pairwise comparison of categorical variables (gender and TTR genotype) between different DPD grades in patients with ATTR amyloidosis. **indicates statistical significance to P<0.05 for pairwise testing by Chi square.

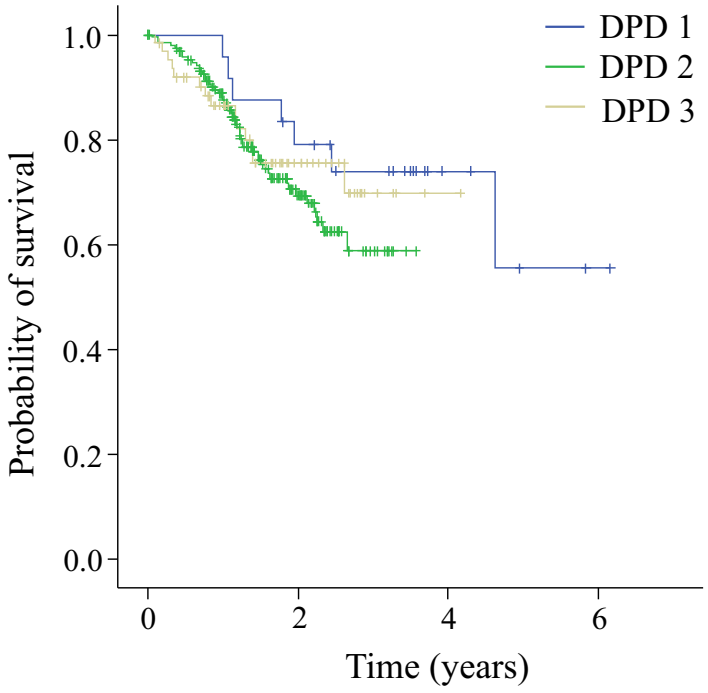


Figure 5.4: Kaplan Meier survival curves by DPD grade (all TTR genotypes combined)

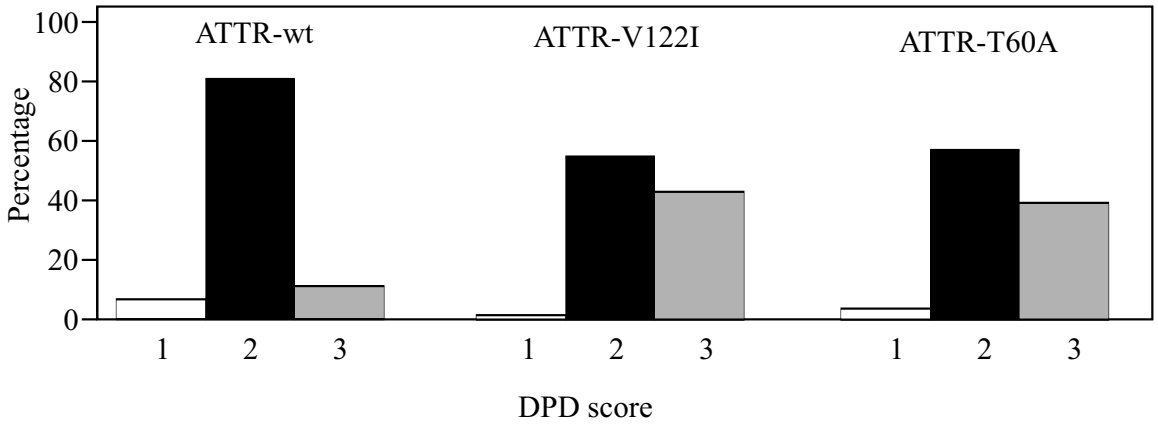


Figure 5.5: DPD grade differences between types of ATTR amyloidosis. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis.

5.4.3 DPD grade in cardiac amyloid light chain amyloidosis

^{99m}Tc DPD scintigraphy was performed in 98 patients with cardiac AL amyloidosis. Of these, 40 patients had evidence of cardiac uptake on DPD scintigraphy and 58 patients did not. Few patients with cardiac AL cardiac amyloidosis showed grade 2 (n=7) or grade 3 (n=2) DPD uptake, so for this analysis DPD grades 1, 2 and 3 were combined and classified as DPD positive. This group was compared to those with DPD grade 0 described as DPD negative (see Table 5.5).

Patients who were DPD positive had higher values of serum Troponin T [0.120μg/L (0.054-0.217) vs 0.071μg/L (0.041-0.117; P=0.007)] and NT-proBNP [8854 pg/ml (3423-14455) vs. 3843 pg/ml (2603-7913); (P=0.008)]. There were no other significant differences between DPD positive and DPD negative patients.

Kaplan Meier survival curves (unadjusted survival) of patients with cardiac AL amyloidosis based on DPD positivity is displayed in Figure 5.6.

There was no significant difference in survival between patients who were DPD positive compared to DPD negative (log rank, P=0.868).

	Estimate	OR	*P*	95% CI for estimate
Age (years)	-0.044	0.96	0.135	-0.102-0.014
Gender (male)	0.458	1.58	0.386	-0.578-1.494
IVSd (mm)	0.064	1.07	0.419	-0.091-0.219
Trop T (µg/L)	6.022	412.40	0.037	0.370-11.674
Log NT -proBNP (pg/ml)	1.547	4.70	0.003	0.519-2.575
E/e'	0.068	1.07	0.006	0.019-0.117
Bilirubin (µmol/L)	-0.011	0.99	0.551	-0.048-0.026
TTR gene type (wild-type is reference)				
ATTR-V122I	1.663	5.28	<0.001	0.755-2.571
ATTR-T60A	0.354	1.42	0.597	-0.956-1.664
ATTR-V30M	-0.588	0.56	0.695	-3.531-2.354
Others	-0.815	0.44	0.468	-3.019-1.388

Table 5.4: Ordinal logistic regression of predictors for DPD grade. ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-V30M, Amyloidosis associated with the V30M transthyretin gene mutation; CI, confidence interval; IVSd, Interventricular septal thickness in diastole; OR, odds ratio; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; TTR, transthyretin.

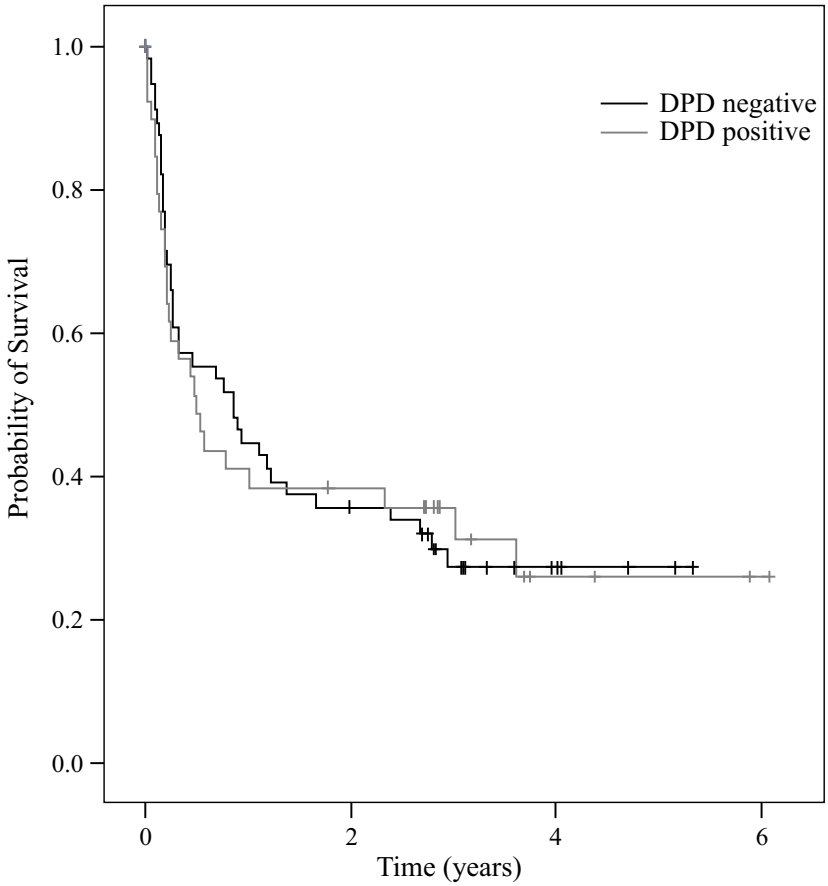


Figure 5.6: Survival curves for patients with cardiac AL amyloidosis based on DPD positivity

		DPD Negative (n=58)	DPD Positive (n=40)	*P*
Age (years)		71.9 (65.6-79.4)	69.8 (62.9-75.3)	0.124
Gender	**Male**	(49) 84.5%	(33) 82.5%	0.502
	Female	(9) 15.5%	(7) 17.5%	
IVSd (cm)		1.5 (1.3-1.6)	1.5 (1.4-1.7)	0.108
LVPWd (cm)		1.5 (1.3-1.6)	1.5 (1.4-1.6)	0.122
LVEF (%)		52.5 (43.8-58.0)	49.5 (44.0-58.0)	0.753
SBP (mmHg)		113.0 (101.0-128.3)	112.0 (103.5-121.0)	0.571
NT pro-BNP (pg/ml)		3843 (2603-7913)	8854 (3423-14455)	0.008
TropT (µg/L)		0.071 (0.041-0.117)	0.120 (0.054-0.217)	0.007
eGFR (ml/min)		62.5 (46.8-100.0)	59.0 (35.3-78.0)	0.281
Bilirubin (µmol/L)		10 (7-15)	13 (9-17)	0.014
Albumin (g/L)		40.0 (34.0-43.0)	39.5 (34.3-43.8)	0.548
Haemoglobin (g/L)		12.7 (11.4-13.8)	12.8 (11.7-14.1)	0.477
24hr urine protein		0.59 (0.19-1.66)	0.44 (0.10-1.38)	0.360
IgA		1.15 (0.58-1.83)	1.20 (0.50-1.60)	0.688
IgG		6.65 (4.70-8.50)	7.20 (5.10-11.80)	0.215
IgM		0.40 (0.20-0.80)	0.40 (0.30-1.05)	0.211
FLC ratio		0.09 (0.04-0.27)	0.05 (0.02-5.29)	0.423
Baseline dFLC		336.90 (131.80-687.40)	406.15 (161.80-624.45)	0.473
FLC lambda		226.00 (53.08-450.00)	218.00 (38.65-586.25)	0.948
FLC kappa		19.45 (13.05-38.28)	21.05 (8.70-109.50)	0.928
Monoclonal protein type	**IgG**	(19) 33.3%	(12) 30.0%	0.320
	IgA	(7) 12.3%	(1)2.5%	
	IgM	(5)8.8%	(4) 10.0%	
	Light chain only	(26)45.6%	(23) 57.5%	
Involved light chain	**Kappa**	(11) 19.3%	(10) 25.0%	0.355
	Lamda	(46) 80.7%	(30) 75.0%	
BJP class	**Unknown**	(2) 3.4%	(3) 7.5%	0.179
	Kappa	(5) 8.6%	(9) 22.5%	
	Lambda	(34) 58.6%	(19) 47.5%	
	None	(17) 29.3%	(9) 22.5%	
Amyloid load by SAP scintigraphy	**None**	(37) 66.1%	(28) 70.0%	0.699
	Small	(3) 5.4%	(4) 10.0%	
	Moderate	(10) 17.9%	(5) 12.5%	
	Large	(6) 10.7%	(3) 7.5%	
Mayo stage	**1**	(2) 3.6%	(0) 0.0%	0.446
	2	(8) 14.5%	(5) 12.5%	
	3	(45) 81.8%	(35) 87.5%	
NYHA class	**1**	(4) 7.3%	(1) 2.7%	0.672
	2	(34) 61.8%	(21) 56.8%	
	3	(16) 29.1%	(14) 37.8%	
	4	(1) 1.8%	(1) 2.7%	
ECOG	**0**	(12) 20.7%	(11) 27.5%	0.674
	1	(19) 32.8%	(11) 27.5%	
	2	(20) 34.5%	(11) 27.5%	
	3	(7) 12.1%	(7) 17.5%	

Table 5.5: Comparison in patients with cardiac amyloid light chain amyloidosis between those who are DPD negative (DPD grade 0) vs. DPD positive. A P value of <0.01 was considered statistically significant. Continuous variables are presented as median (IQR) and compared using Mann Whitney U tests. Categorical variables are presented as (n) % of group and compared using Chi square tests. BJC, Bence Jones Protein; dFLC, difference between involved and uninvolved free light chains; ECOG, Eastern Cooperative Oncology Group; eGFR, estimated glomerular filtration rate; FLC, free light chain; Ig, immunoglobulin; IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; LVPWd, Left ventricular posterior wall thickness in diastole; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association; SBP, systolic blood pressure.

5.5 Limitations

It should be noted that the finding of grade 1 DPD uptake was uncommon and so the sample size on which to base these findings was small. It was not possible to use an alternative quantitative methodology for describing DPD uptake as no such method has been validated. In the studies in patients with cardiac AL amyloidosis, no details were gathered related to chemotherapy regimens which may have been related to overall survival. However, in general standard best practice regimens are advised by the NAC to local treating physicians.

5.6 Discussion

The main findings from data presented in this chapter are:

1. Higher grade DPD uptake ($\geq$2) in patients with ATTR cardiac amyloidosis is associated with features suggestive of a more severe cardiac phenotype.

2. DPD grade in patients with AL cardiac amyloidosis is only weakly associated with a more severe cardiac phenotype.

3. DPD grade is not predictive of prognosis in either ATTR or AL cardiac amyloidosis.

In patients with ATTR cardiac amyloidosis a higher grade of DPD uptake was associated with a more severe cardiac phenotype. This is reflected in a greater NT-proBNP, troponin, IVSd, LVPWd and E/e' to a significance value of $<$0.01. To a lesser significance value of $<$ 0.05 patients with a higher DPD grade also had a lower LVEF, systolic BP and shorter distance on the 6MWT. These differences between the DPD grades was predominantly due to differences

between DPD grade 1 and DPD grade 2 or DPD grade 1 and DPD grade 3. There was very little difference between DPD grades 2 and 3. This is particularly relevant because only the minority of patients are in DPD grade 1. In this study, only 24 out of 275 patients were DPD grade 1 and in the largest study of this kind only 5% of patients were DPD grade 1 [76]. As such, this limits the usefulness of this test in the assessment of cardiac disease severity. Refinement of the technique to create more grades could improve the ability of the test to detect differences in cardiac severity. Alternatively, it may be that patients referred to the NAC already have more advanced disease and if more screening was applied then more mild presentations may be identified which could increase the proportion of patients in DPD grade 1. However, it seems unlikely that DPD scintigraphy would ever add value in terms of predicting severity of cardiac phenotype given all the other modalities available to assess cardiac involvement. These data do, however, support the idea that whatever the mechanism of DPD uptake in the heart is, that it is related to the amount of amyloid in the heart.

DPD grade may be affected by TTR type with ATTR-V122I being more likely to have a higher DPD grade than wild-type even in an ordinal regression that corrects for other measures of the more severe cardiac phenotype in ATTR-V122I. This is not a clinically useful finding as genetic testing can distinguish between the different types of ATTR amyloidosis but the fact that TTR genotype is related to the DPD grade of uptake could be interpreted as supporting the hypothesis that the mechanism by which amyloid deposition is demonstrated by scintigraphy is directly linked to amyloid composition [142].

Although the cohort of patients with cardiac AL amyloidosis in this study was a sizable, only a small number with higher grades of uptake (2 and 3) were identified; this necessitated combining groups for meaningful statistical analyses into those who were DPD positive compared to DPD negative. Patients who were DPD positive had higher NT-proBNP and

troponin T but there was no difference in other measures of cardiac involvement.

DPD grade is not predictive of prognosis in either ATTR or AL cardiac amyloidosis. This is consistent with a large study in ATTR cardiac amyloidosis [76] but is the first time this has been studied in AL cardiac amyloidosis. It may be expected that if a higher DPD grade is associated with a more severe cardiac phenotype and it is the cardac phenotype that leads to death in this population, that the higher DPD grade would also be associated with a worse prognosis. However, this is not the case in this study nor in the largest study investigating this. The initial study that suggested DPD grade was predictive of mortality, did so by comparing 6 patients who were DPD grade 1 to 19 patients who were grade 2 or 3 [74]. Not only is this a small study, combining DPD grades 2 and 3 in this study has shown that there may be a better survival in patients with DPD grade 1, nevertheless, this is a very small population (only 5% of patients in the largest study were in DPD grade 1 [76]). Patients may present late to the NAC and it may be with screening for the disease that more mild DPD grade 1 cases may be identified, but it seems unlikely that DPD scintigraphy will ever add value to the existing prognostic markers. It continues to be useful as a diagnostic tool.

5.7 Conclusions

Visual scoring of uptake in ^{99m}Tc DPD scintigraphy described with the Perugini scoring system appears to correlate with disease severity in UK patients with cardiac ATTR amyloidosis but does not appear to be a useful prognostic tool. A significant limitation is the small number of symptomatic patients with grade 1 uptake. The grade of DPD uptake for some cardiac ATTR types may be related to TTR genotype but this requires further investigation in a larger group of patients. Cardiac uptake by ^{99m}Tc DPD scintigraphy in cardiac AL amyloidosis is related to higher serum cardiac biomarker levels (Trop T and NT-proBNP) but does not appear to be a

useful prognostic tool.

Chapter 6

A Real World Study of the Safety and Tolerability of Diflunisal for Cardiac Transthyretin Amyloidosis and Investigation of Disease-modifying Effect

6.1 Introduction

Diflunisal is a NSAID; a salicylate derivative and non-selective cyclo-oxygenase inhibitor with a long half-life. *In vivo* studies have found that diflunisal, stabilises the TTR tetramer which may prevent mis-folding monomers from forming amyloid deposits in the heart [120]. In a recent phase 3 clinical trial of patients with FAP reported by Berk et.al. [121], the authors suggest that diflunisal is a well-tolerated treatment conferring some benefit in slowing polyneuropathy. However, diflunisal was discontinued by a significant number of patients and most patients had minimal cardiac involvement at enrolment. Furthermore, disease progression was reported as the main reason for discontinuation which raises concerns about both efficacy and tolerability of diflunisal.

It is conceivable that patients with cardiac ATTR amyloidosis tolerate difflunisal differently compared to patients with FAP. As an NSAID it poses an obvious potential problem in patients with heart failure in whom the general advice is avoidance of NSAIDs due to associated fluid retention and risks to renal function. In one small single-arm open-label study in 2012 of 12 patients with cardiac ATTR amyloidosis diflunisal was reported to be reasonably well-tolerated accepting some renal decline at 1 year follow-up [122]. Thus, although diflunisal may benefit early stage neuropathy in FAP, there is currently no strong evidence of benefit in ATTR cardiomyopathy.

At the NAC, highly selected patients (in view of the risk of adverse events) with a diagnosis of cardiac ATTR amyloidosis were offered difunisal on compassionate use grounds in view of the limited therapeutic options.

6.2 Aims

- To assess the safety and tolerability of diflunisal in patients with cardiac ATTR amyloidosis.
- To investigate if treatment with diflunisal in cardiac ATTR amyloidosis has a disease-modifying effect by assessment of impact on patient characteristics and investigations findings.

6.3 Methods

I conducted a retrospective, observational cohort study to assess the safety and tolerability of diflunisal, followed by a retrospective matched cohort study to investigate for possible disease-modifying effect of diflunisal. All patients who had been treated with diflunisal were

identified from pharmacy dispensing records from the Royal Free Hospital from 2009 to 2014. Transthyretin gene sequencing was performed in all patients. Only patients with transthyretin amyloid cardiomyopathy (ATTR-wt, ATTR-V122I or ATTR-T60A) were included. Medical records were reviewed to establish treatment duration and to record other relevant clinical information for each patient. All patients treated with diflunisal underwent detailed evaluation at 6 monthly intervals or as clinically indicated with clinical, haematological, biochemical, and echocardiographic assessment. Data pertaining to the assessment of safety and tolerability for patients treated with diflunisal, were gathered from the documented clinical assessment on retrospective review of the medical records.

All patients offered treatment with diflunsal were advised about the possible risks and benefits of the treatment during a clinical consultation and were agreeable to commencing treatment. All patients in the diflunisal group were prescribed 250mg oral diflunisal to be taken twice daily with food. All patients were co-prescribed a proton pump inhibitor if not taking this already. Reason for discontinuation was recorded in all patients, as was the duration of treatment.

To investigate for potential disease modifying effects, patients who were treated with diflunisal and continued treatment for 12 (+/- 2 months), designated the 'diflunisal' group, were compared to patients assessed over the same time period who were not treated with diflunisal, designated the 'not treated' group. Only patients in whom baseline and 12 month data (+/- 2 months) were available were included in this analysis. Following the preliminary analysis, frequency matching was performed between the 'not treated' group and the 'diflunisal' group to control for possible confounding baseline differences and factors related to prognosis that were identified in the previous investigations in this thesis. Patients with cardiac ATTR amyloidosis treated with oral diflunsal were matched with a patients in the in the 'not treated' group for age, gender, systolic blood pressure, LVEF, eGFR, LV wall thickness, NYHA class, serum NT-proBNP

and bilirubin levels. This established two groups for comparison ('control' and 'diflunisal'). Baseline and 12 month (+/- 2 months) follow-up data were analysed.

6.3.1 Statistical analysis

Baseline characteristics are presented as median and interquartile ranges. Data were compared with Mann-Whitney test for continuous variables and Chi-square or Fisher's exact test for categorical variables. A P value of <0.05 was considered significant in statistical analyses.

6.4 Results

6.4.1 Diflunisal tolerability

Diflunisal was dispensed to 82 patients during the study period; 36 (43.9%) patients with cardiac ATTR-wt amyloidosis, 32 (39%) patients with cardiac ATTR-T60A amyloidosis, and 14 (17.1%) patients with cardiac ATTR-V122I amyloidosis. Table 6.1 illustrates the time periods for which patients received diflunisal and the reasons for discontinuing treatment.

Of those who received diflunisal, 30 (36.6%) patients discontinued treatment within 6 months, 14 (17.1%) patients took diflunisal for 12-24 months and 24 (29.2%) patients took it for 24 months or more. Thus in total, fewer than half of all patients with cardiac ATTR starting treatment with diflunisal (46.3%), continued diflunisal for at least 12 months and were included in the subsequent analysis.

The side effects of diflunisal in the majority of cases were deterioration of heart failure symptoms (13.4%), gastrointestinal (GI) symptoms (11%) or deterioration of renal function (6.1%). In four (4.9%) patients who discontinued diflunisal before completing 6 months of treatment, it was not possible to ascertain the reason for cessation. In 25.6% of patients the

	Total number of patients (n=82)				
	<6months (n=30)	**6-12 months (n=14)**	**12-24 months (n=14)**	**> 24 months (n=24)**	**Total that discontinued**
Reason for discontinuation:					
Gastro-intestinal side effects	9 (11.0)				9 (11)
Did not tolerate – (unclear reason); stopped by patient	4 (4.9)				4 (4.9)
Worsening renal function	3 (3.7)	1 (1.2)	1 (1.2)		5 (6.1)
Thrombocytopenia	1 (1.2)				1 (1.2)
Worsening heart failure symptoms	6 (7.3)	1 (1.2)	4 (4.9)		11 (13.4)
Died	1 (1.2)	1 (1.2)	2 (2.4)		4 (4.9)
Development of atrial fibrillation	2 (2.4)	8 (9.8)	3 (3.7)	8 (9.8)	21 (25.6)
Commenced on DAPT post MI/PCI	1 (1.2)	1 (1.2)	1 (1.2)		3 (3.7)
Recruited to clinical trial	1 (1.2)	2 (2.4)	3 (3.7)		6 (7.3)
Supply problems	2 (2.4)			2 (2.4)	4 (4.9)

Table 6.1: Time periods patients were treated with diflunisal and reasons for discontinuation. Data are presented as n (% of total). DAPT, Dual anti-platelet therapy; MI, myocardial infarction; PCI, percutaneous coronary intervention.

		Not treated N=103	Diflunisal N=38	*P*
Age (years)		76.1 (71.4-79.4)	69.0 (65.0-73.0)	<0.001
Gender (male)		87%	92%	0.432
Systolic BP (mmHg)		117.0 (108.0-128.0)	120.0 (112.0-136.5)	0.114
IVSd (cm)		1.70 (1.60-1.83)	1.60 (1.60-1.90)	0.688
LVPWd (cm)		1.62 (1.56-1.76)	1.70 (1.55-1.95)	0.216
LVEF (%)		48.0 (38.8-55.1)	53.0 (45.5-59.0)	0.031
Hb (g/dL)		13.4 (12.2-16.7)	14.2 (13.3-14.8)	0.110
eGFR (ml/min)		59.0 (44.0-71.0)	74.0 (60.8-90.0)	<0.001
NTproBNP (pg/ml)		2644.2 (1974.7-4779.9)	1580.6 (953.4-3004.4)	<0.001
Bilirubin (µmol/L)		14.0 (9.5-21.0)	11.0 (8.5-16.0)	0.030
History of AF at baseline		57%	14%	0.027
NYHA (% in each classification)	**1**	8.6%	16.7%	0.122
	2	72.4%	77.8%	
	3	19.0%	5.6%	

Table 6.2: Comparison of baseline characteristics of patients with cardiac ATTR amyloidosis who were treated with diflunisal compared to those who were not. Continuous variables are described as median (interquartile range) and categorical variables are described as proportions. Statistical comparisons are made with Mann Whitney U test or Chi square test. AF, atrial fibrillation; BP, blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; LVPWd, Left ventricular posterior wall thickness in diastole; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association.

development of AF and need for anticoagulation prompted the cessation of diflunisal treatment. In a significant proportion of cases the development of AF was associated with a clinical deterioration (worsening heart failure) often necessitating the addition of or dose increase in diuretics.

Three (3.7%) patients in the diflunisal group required coronary revascularisation during treatment; one patient with a non-ST elevation myocardial infarction and two others with symptomatic stable angina.

6.4.2 Baseline characteristics of patients who tolerated diflunisal for at least 12 months

The baseline characteristics of the 38 patients who took diflunisal for at least 12 months are presented in Table 6.2 alongside 103 patients who were 'not treated' with diflunisal and for whom 12 month follow-up data were available.

Of note the patients in the diflunisal group were younger [69.0 years (65.0-73.0) vs. 76.1 years (71.4-79.4); P<0.001], had greater LVEF [53.0% (45.5-59.0) vs. 48.0% (38.8-55.1) P=0.031] and eGFR [74.0ml/min (60.8-90.0) vs. 59.0ml/min (44.0-71.0); P<0.001]. The diflunisal group also had lower serum NT-proBNP [1580.6 (953.4-3004.4) vs. 2644.2 (1974.7-4779.9); P<0.001] and bilirubin levels [11.0 (8.5-16.0) vs. 14.0 (9.5-21.0); P=0.030). As expected, a smaller proportion of patients in the diflunisal group had AF prior to commencing treatment (14% vs. 57%; P=0.027).

All patients were in sinus rhythm at the start of treatment with diflunisal with the exception of one patient who, having considered the risks and benefits, requested to commence treatment with diflunisal despite already receiving oral anti-coagulation treatment for stroke risk reduction.

6.4.3 Matched 'diflunisal' and 'not treated' groups baseline and follow-up characteristics

After matching patients in the 'diflunisal' and 'not treated' groups, the baseline characteristics of the study population are described in Table 6.3.

There were no statistically significant differences at baseline between these two matched cohorts of the key characteristics identified in earlier works of this thesis which may be related to prognosis. However, the disparity in the prevalence of AF remained and could not be corrected for (AF: controls 69% vs. 0% in matched diflunisal group; P<0.001). Furthermore, there were trends for patients in the diflunisal group to be younger, have greater LVEF and lower NT-proBNP.

Comparison of these matched cohorts at 12 month follow-up is presented in Table 6.4 and the delta values for change at 12 months are presented in Table 6.5.

		Control N=22	Diflunisal N=23	*P*
Age (years)		69.9 (68.3-71.4)	67.0 (64.0-72.0)	0.166
Gender (male)		95%	91%	0.577
Systolic BP (mmHg)		118.0 (110.8-133.5)	115.0 (112.0-133.0)	0.937
IVSd (cm)		1.77 (1.60-1.93)	1.60 (1.60-1.90)	0.172
LVPWd (cm)		1.75 (1.60-1.93)	1.70 (1.60-2.00)	0.458
LVEF (%)		47.3 (41.8-51.8)	53.0 (47.0-58.0)	0.071
Hb (g/dL)		13.8 (13.4-15.1)	14.3 (13.3-14.8)	0.708
eGFR (ml/min)		67.0 (55.3-79.3)	77.0 (61.0-86.0)	0.305
NTproBNP (pg/ml)		2381.5 (1580.6-3693.4)	1906.9 (1203.5-2932.4)	0.173
Bilirubin (μmol/L)		19 (11-23)	12 (10-16)	0.126
History of AF at baseline		15 (68%)	0 (0%)	<0.001
NYHA (% in each classification)	**1**	26.7%	13.6%	0.103
	2	60.0%	86.4%	
	3	13.3%	0.0%	

Table 6.3: Comparison of baseline characteristics of matched controls and diflunisal group. Continuous variables are described as median (interquartile range) and categorical variables are described as proportions. Statistical comparisons are made with Mann Whitney U test, Chi square test, or Fisher's exact test. AF, atrial fibrillation; BP, blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; LVPWd, Left ventricular posterior wall thickness in diastole; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association.

		Control N=22	Diflunisal N=23	*P*
Systolic BP (mmHg)		120.0 (110.8-131.8)	120.0 (110.0-128.0)	0.811
IVSd (cm)		1.79 (1.68-1.98)	1.70 (1.60-2.00)	0.294
LVPWd (cm)		1.77 (1.62-1.94)	1.50 (1.70-2.00)	0.444
LVEF (%)		46.0 (36.0-52.0)	50.0 (45.0-55.0)	0.074
Hb (g/dL)		14.3 (13.2-15.8)	14.4 (13.2-15.0)	0.879
eGFR (ml/min)		60.0 (39.5-70.5)	65.0 (53.0-82.0)	0.177
NTproBNP (pg/ml)		2567.9 (2034.0-5329.1)	1864.5 (1381.4-4424.0)	0.062
Bilirubin (μmol/L)		20 (10-29)	12 (9 -17)	0.045
NYHA (% in each classification)	**1**	11.8%	13.6%	0.982
	2	70.6%	68.2%	
	3	17.6%	18.2%	

Table 6.4: Comparison of characteristics of matched controls and diflunisal treated patients at 12 months. BP, blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; LVPWd, Left ventricular posterior wall thickness in diastole; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide; NYHA, New York Heart Association.

	Control N=22	**Diflunisal N=23**	***P***
ΔSystolic BP (mmHg)	0.0 (-8.3-9.8)	-2.0 (-10.0-9.0)	0.658
ΔIVSd (cm)	0.00 (-0.03-0.07)	0.00 (0.00-0.10)	0.597
ΔLVPWd (cm)	0.02 (-0.03-0.10)	0.00 (0.00-0.10)	0.264
ΔLVEF (%)	-4.0 (-11.1-2.8)	-2.0 (-7.0-2.0)	0.733
ΔHb (g/dL)	0.3 (-0.2-1.2)	0.0 (-0.4-0.7)	0.404
ΔeGFR (ml/min)	-8.0 (-18.5- -3.5)	-10.0 (-18-0.0)	0.832
ΔNTproBNP (pg/ml)	748.7 (-61.3-1685.7)	143.8 (-279.1-1116.7)	0.458
ΔBilirubin (µmol/L)	1 (-3-11)	0 (-4 -2)	0.447

Table 6.5: Comparison of characteristics of matched controls and diflunisal treated patients at 12 months presented as delta values. Data are displayed as median (IQR). BP, blood pressure; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; IVSd, Interventricular septal thickness in diastole; LVEF, Left Ventricular Ejection Fraction; LVPWd, Left ventricular posterior wall thickness in diastole; NT-proBNP, N-terminal-pro B-type Natiuretic Peptide.

At 12 months, there was no statistical difference between the two cohorts other than serum bilirubin which became statistically different following an increase in controls (median increased from 19 to 20 μmol/L)) but remained unchanged in the diflunisal group. This was a subtle difference and was not significant when examining the delta change in each group. There were no other differences identified between between those who received diflunisal for 12 months with those who had not.

6.5 Limitations

This was a retrospective study of individuals at the NAC treated with diflunisal with inherent selection bias since the decision to prescribe diflunisal required that the clinician felt the patient was a suitable candidate for the treatment. Frequency matching of the cohorts was undertaken due to the demonstrable baseline differences between the groups to form more similar groups which, although satisfied statistical tests of no differences still suggested important trends to differences.

6.6 Discussion

The main findings from data presented in this chapter are:

1. Diflunisal is poorly tolerated in patients with cardiac ATTR amyloidosis with more tham half of patients stopping the drug before 12 months of treatment.

2. There was no evidence of efficacy of difunisal in cardiac ATTR amyloidosis in this retrospective study.

Although this is a small, retrospective study, it is the largest study to date in patients with cardiac ATTR amyloidosis who have been treated with diflunisal. Although reported to be well-tolerated in patients with FAP, in this study of patients with cardiac ATTR amyloidosis this was not the case. By 12 months, 44/82 (54%) patients discontinued diflunisal; 25 due to side effects and 19 for other reasons. At six months, GI side-effects were the most common reason for diflunisal discontinuation and occurred in 9/82 (11%) of patients. However, no patient was noted to suffer a minor or major gastro-intestinal bleed during the treatment period. At the end of the total study period, the main reasons for discontinuation were the development of AF and need for anticoagulation in 25.6% of patients, worsening heart failure (commonly due to increased fluid retention) in 13.4%. and worsening renal function in 6.1%.

Patients at the NAC are advised to stop diflunisal if they develop AF due to the increased risk of bleeding with the concomitant use of anti-coagulants and NSAIDs. This is a departmental policy in view of the known general literature on this subject and the unproven benefit of diflunisal, however we are aware that some other international centres may have less stringent policies due to the limited therapeutic options for these patients. The risk of concomitant treatment with anti-coagulants and NSAIDs in this particular population of patients remains

unknown.

Non-steroidal anti-inflammatory use is known to be associated with an increased risk of thrombotic events particularly in patients with pre-existing cardiovascuIar disease. In this study, one patient suffered a non-ST elevation myocardial infarction (MI) during diflunisal treatment (ie. less than 1% of the cohort). No patient suffered a stroke whilst taking diflunisal. Analysis of the matched diflunisal and control cohorts showed no significant differences in the patient characteristics at 12 month follow-up except for a serum bilirubin which was not statistically significantly different between groups at baseline but may have constituted a type II error at baseline assessment due to the small cohort sizes. There were no significant differences in delta values between the diflunisal and not treated groups.

6.7 Conclusions

This study did not demonstrate any benefit of diflunisal in cardiac ATTR amyloidosis. The main limitation of this study is the retrospective nature and the selection bias this introduces since diflunisal is only given to a select group of patients. A more suitable approach to address this question would be to conduct a prospective randomised study. However, since the drug is poorly tolerated even in highly selected patients, it is doubtful that it can have a significant impact on the management of cardiac ATTR amyloidosis.

Chapter 7

A Phase 2 Open-Label Study of Revusiran in Patients with Transthyretin Cardiac Amyloidosis. The UK National Amyloidosis Centre Experience and Insights for Future Clinical Trial Designs

7.1 Introduction

The prognosis of patients with cardiac ATTR amyloidosis is poor. In general, the management of patients with cardiac ATTR amyloidosis is conservative. Patients are advised to restrict salt and fluid intake. Medical therapies are predominantly comprised of diuretics to improve symptoms caused by excess fluid accumulation. There have been no studies examining the use of therapies such as ACEis, beta-blockers or mineralocorticoid receptor antagonists which have a prognostic role in heart failure with reduced ejection fraction. Other medical or implantable cardiac device therapies may include those to treat arrhythmia or electrical conduction disease with standard indications for their use.

RNA interference is a potential treatment strategy as discussed in the introduction, Chapter

1. Revusiran is an siRNA which targets transthyretin mRNA particularly in the liver. An open-label phase 2 clinical trial of repeated administration of subcutaneous doses of revusiran (ALN-TTRSC-002) in patients with cardiac ATTR amyloidosis was conducted (NCT01981837). This was a multicentre study with the NAC being the UK centre.

The primary objective of the clinical trial was to evaluate the safety and tolerability of multiple doses of revusiran in patients with ATTR cardiac amyloidosis. The study design and protocol were conceived and written by Alnylam Pharmaceuticals Inc. Preliminary data from the open label extension study have been reported [143].

In my role as a sub-investigator, I identified patients and performed clinical assessments during the recruitment phase. During the clinical testing phase of the study, I performed follow-up clinical and echocardiographic assessments, reviewed results of serological tests and reported adverse event (AE)s.

The patient selection criteria represent an important factor in the design and execution of a clinical trial, and can determine how widely applicable the results of the trial will be in clinical practice. Strict enrolment criteria may be scientifically sound but can make recruitment challenging and may make the results of the clinical trial difficult to apply to the wider disease population. Conversely, if the enrolment criteria are too broad then the study population may become a heterogenous group in whom it becomes difficult to prove a treatment effect.

In this chapter, and independently of the trial Sponsor, my own original work reported here assessed the impact of the patient selection criteria on the UK recruitment to the trial and the potential applicability of the results of this study to the UK cardiac ATTR amyloidosis population with important insights for future trial design. With the Sponsor's permission, and for completeness, I have also summarised the trials methodology, key results, and reported the results for the patients recruited from our centre to provide a context for my findings.

7.2 Aims

- To describe the UK experience of patient recruitment in an open-label phase 2 clinical trial of subcutaneous revusiran in patients with cardiac ATTR amyloidosis.

- To report the baseline characteristics of the patients I recruited

7.3 Methods

The methods section is divided into two sections. The first briefly describes details of the main phase 2 clinical trial devised by Alnylam. The second section describes my role in patient recruitment and the investigations I undertook to understand the impact of the enrolment criteria on patient recruitment with analyses performed independently of the Sponsor and which forms the main focus of this chapter.

7.3.1 Main phase 2 clinical trial study design and protocol (reproduced with permission from Alnylam)

This was an open-label, multi-centre phase 2 clinical study. There were four study centres; the NAC in the UK and three others in the USA (Boston, New York, Cleveland). Patients were recruited between December 2013 and March 2014.

Patient were screened (Days -28 to -1), treated (Days 0 to 35) and followed-up (Days 42 to 90), thus patient participation was for approximately 16 weeks. Screening assessments were performed over several visits. Each patient underwent 14 study visits at the study site over the treatment and follow-up periods. Patients received a total of 10 subcutaneous (SC) doses of revusiran. Dosing was once daily for the first five consecutive days and once-weekly thereafter.

See Figure 7.1 for study schematic.

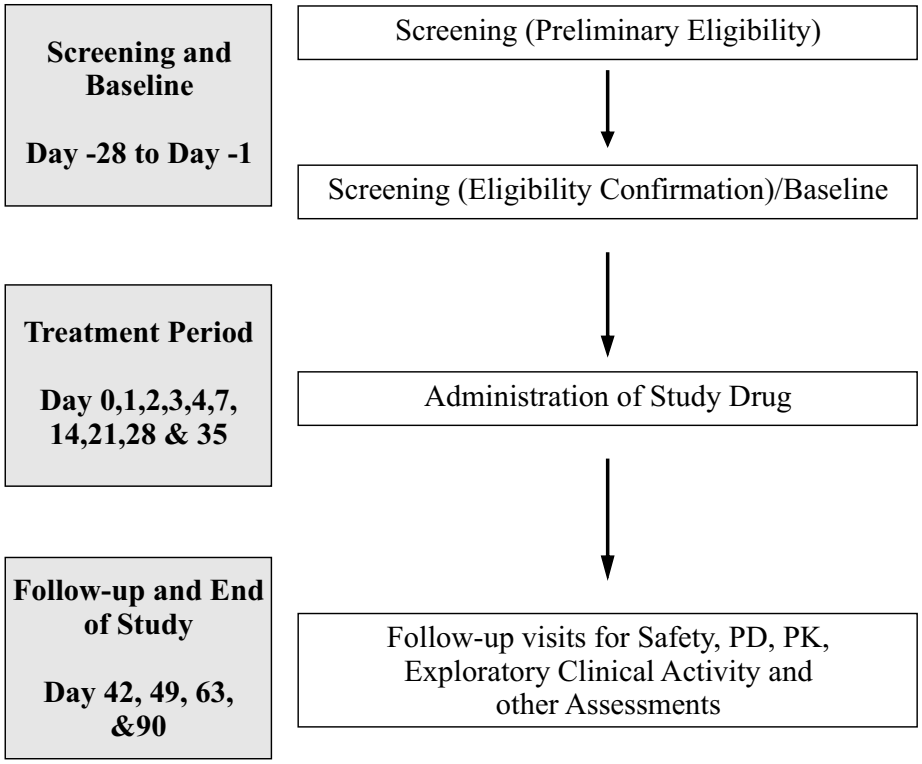


Figure 7.1: Schematic of Phase 2 open-label study of revusiran in patients with transthyretin cardiac amyloidosis. PD, pharmacodynamics; PK, pharmacokinetics.

At the study outset patients were to receive a total of 10 SC doses of revusiran 7.5 mg/kg. However, after the inclusion of three patients in the 7.5 mg/kg group, data from the revusiran Phase I study (NCT01814839) demonstrated similar TTR level reductions at 5.0 mg/kg with fewer and less severe injection site reactions. Based on these data, the protocol for this Phase 2 study was amended for all remaining patients to receive 5.0 mg/kg and the sample size increased from 12 to 15 patients. An additional protocol amendment was made at a later stage when it became apparent that most of the patients recruited had wild-type ATTR amyloidosis rather than hereditary forms. The amendment was to subsequently only include patients with a TTR mutation.

Key enrolment criteria are displayed in Table 7.1. The enrolment target for our centre was 10 patients.

Inclusion criteria	Exclusion criteria
Biopsy proven ATTR amyloidosis with imaging evidence of cardiac involvement	New York Heart Association class IV
Male or female aged 18-80 years	eGFR <30 mL/min/1.73m^2
Stable on heart medications (no hospitalisations and stable medications for 4 weeks prior to screening)	Uncontrolled hypertension, ischaemic heart disease or significant cardiac arrhythmia.
Neutrophil count ≥1500 cells/mm^3 Platelet count ≥100,000 cells/mm^3 Haemoglobin ≥10 g/dL	Previous organ transplantation, hepatitis C, hepatitis B or HIV.
AST and ALT ≤2.5 x ULN Bilirubin <34.2 µmol/L	Known intolerance to SC injections
Karnofsky performance status at least 60%	Vitamin A levels below the normal range
6 minute walk test at least 150m	Untreated thyroid disease
	Metastatic cancer within five years of screening

Table 7.1: Enrolment criteria for the phase 2 clinical trial of revusiran. ALT, alanine aminotransferase; AST, aspartate transaminase; ATTR, transthyretin amyloidosis; eGFR, estimated glomerular filtration rate; HIV, human immunodeficiency virus; SC, subcutaneous; ULN, upper limit of normal.

Primary outcome	The proportion of subjects experiencing adverse events, serious adverse events and study drug discontinuation (up to 63 days)
Secondary outcomes	Pharmacokinetics of revusiran (up to 90 days) Transthyretin levels (% lowering) (up to 90 days)
Exploratory outcomes	N-Terminal pro B-type natriuretic peptide Troponin I 6MWT EQ-5D-5L KCCQ Echocardiography and CMR

Table 7.2: Predefined outcomes for the phase 2 clinical trial of revusiran. 6MWT, six minute walk test; CMR, cardiac magnetic resonance; EQ-5D-5L, EuroQol-5 Dimensions-5 levels; KCCQ, Kansas City cardiomyopathy questionnaire.

The pre-defined outcomes for the trial are described in Table 7.2. The primary outcome was safety. The safety review committee convened after every three patients enrolled had received a minimum of five doses of revusiran and completed safety assessments 48 hours post-dose.

Secondary and exploratory outcomes

The pharmacodynamic effect of revusiran included assessment of serum TTR and vitamin A levels. TTR levels were measured by enzyme-linked immunosorbent assay method. Investigators at each site performed the clinical and imaging assessments. A single US centre performed the imaging review (echocardiography and CMR). A single central laboratory in the US was responsible for the cardiac biomarker and vitamin A level testing. Three other central laboratories in the US were individually responsible for mutant TTR sample analysis, pharmacokinetic analyses, and a fourth separate central laboratory in Canada performed TTR sample analysis.

7.3.2 Assessment of the suitability of UK patients for trial recruitment

My role in the trial was to identify patients for recruitment who attended clinic at the NAC from the 1st of April 2013 to the 31st of December 2013. I approached eligible patients, explained the details of the trial, consented patients and was responsible for the follow-up visits and reporting AEs.

All patients with ATTR cardiac amyloidosis were assessed against the trial enrolment criteria (Table 7.1). For any patients with more than one visit during this period, fulfilment of selection criteria were based on the most recent data available from the electronic medical records. Baseline data of patient characteristics for patients I recruited were provided by Alnylam.

	ATTR-wt N=189	ATTR-V122I N=49	ATTR-T60A N=39	TOTAL N=277
Number of patients eligible for trial inclusion, n (%)	**48 (25)**	**19 (39)**	**19 (49)**	**86 (31)**
Excluded patients, n (%):	**141 (75)**	**30 (61)**	**20 (51)**	**191 (69)**
Age>80yrs, n (%)*	51 (27)	7 (14)	3 (7)	61 (22)
Abnormal LFTs, n (%)*	21 (11)	9 (18)	0	30 (11)
eGFR<30ml/min, n (%)*	10 (5)	8 (16)	0	18 (6)
Died before trial start, n (%)*	14 (7)	4 (8)	0	18 (6)
6MWT<150m, n (%)*	34 (18)	13 (27)	15 (38)	62 (22)
No histological confirmation of amyloid, n (%)*	84 (44)	16 (32)	8 (21)	108 (39)

Table 7.3: Patients under routine clinical assessment during 9 month recruitment period prior to clinical trial start (April-Dec 2013 inclusive). Percentages are of N patients for each group. * % of N for each group, thus some patients had multiple reasons for exclusion. 6MWT, six minute walk test; ATTR-T60A, Amyloidosis associated with the T60A transthyretin variant; ATTR-V122I, Amyloidosis associated with the V122I transthyretin variant; ATTR-wt, Wild-type transthyretin amyloidosis; eGFR, estimated glomerular filtration rate; LFT, liver function test.

7.3.3 Statistical analysis

Descriptive statistics for continuous variables are presented for baseline data as mean and standard deviation or as median and range. Categorical variables are presented as numbers and percentages.

7.4 Results

7.4.1 Patient screening and recruitment from the National Amyloidosis Centre, UK

Table 7.3 summarises the number of patients assessed during the recruitment period and the reasons for exclusion.

Overall, from a total of 277 patients, fewer than a third (31%) were eligible for trial participation. More than half the patients in each group met one or more of the exclusion

criteria and could not be considered for trial inclusion (ATTR-wt 75%, ATTR-V122I 61%, ATTR-T60A 51%). Some patients were excluded for more than one reason, so the total number of exclusions were more than the number of patients. The two most common reasons for exclusion in the ATTR-wt group were the absence of histological confirmation of amyloid (44%) and age >80years (27%). In the hereditary groups, the two most common reasons for exclusion were the absence of histological confirmation of amyloid (ATTR-V122I 32%; ATTR-T60A 21%) and 6MWT<150m (ATTR-V122I 18%; ATTR-T60A 27%).

From the 86 patients who met the enrolment criteria based on a review of clinical notes, 60 patients did not want to take part due to the burden of travelling to the NAC for frequent trial visits, 12 patients were ultimately screened out when they attended the formal screening visit and ten were recruited as this was the target for our centre.

7.4.2 Summary of key phase 2 trial findings (reproduced with the permission of Alnylam)

For completeness, the findings for baseline demographics and patient characteristics for patients recruited by me from the NAC are reproduced here. Summary AE data for the entire trial cohort are reported from a preliminary study report with the permission of Alnylam. Despite my contribution to this study and the presentation of these data, the following results in this section are not my own original work.

Characteristic	ATTR-m amyloidosis n=6		ATTR-wt amyloidosis n=4		Total n=10
	n		n		
Age (years)	6	68.5 (60-78)	4	70(65-78)	70 (60-78)
Male sex	6	5 (83)	4	4 (100)	9 (90)
Race White Black	6	 4 (67) 2 (33)	4	 4 (100) 0	 8 (80) 2 (20)
Genotype T60A V122I WT Other	6	 2 (33) 3 (50) 0 1 (17)	4	 0 0 4 (100) 0	 2 (20) 3 (30) 3 (40) 1 (10)
NYHA Class 1 2	6	 1 (17) 5 (83)	4	 0 4 (100)	 1 (10) 9 (90)
Karnofsky PS, 60/70/80/90	6	2/0/3/1	4	0/1/3/0	2/1/6/0
Concurrent TTR stabilizer use, n (%)	6	1	4	0	1
mBMI	6	1008.6 (934.4-1116.1)	4	1238.6 (706.1-1386.8)	1060.0 (706.1-1386.8)
6-MWT (m)	6	411.5 (270-568)	4	406.6 (289-487)	411.5(270-568)
KCCQ overall score	6	80.2 (62.5-95.8)	4	82.3 (71.9-95.8)	80.2 (62.5-95.8)
Echocardiogram					
IVS thickness (cm)	6	2.1(1.5-2.9)	4	1.7 (1.6-2.0)	1.9 (1.5-2.9)
LVEF (%)	6	38 (34-56)	4	54 (28-64)	54 (34-64)
GLS (%)	6	-10.0 (-8.8 to -11.8)	4	-11.06 (-10.4 to -11.18)	-10.67 (-8.82 to -11.79)
Cardiac MRI					
Left ventricular mass (g)	6	400 (245-467)	4	293(247-422)	336 (245-467)
Global myocardial ECV	6	0.59 (0.50-0.73)	3	0.58(0.53-0.59)	0.59 (0.50-0.73)
Cardiac biomarkers					
NT-proBNP (pg/ml)	6	4951 (1708-20199)	4	1716 (1357-4583)	3906 (1357-20199)
Troponin I (ng/ml)	6	0.22 (0.13-0.34)	4	0.10 (0.10-0.10)	0.15 (0.10-0.34)

Table 7.4: Baseline demographics and patient characteristics for patients recruited from the NAC (reproduced with the permission of Alnylam). Continuous variables are presented as median and range. Categorical variables are presented as number and percentage. 6MWT, 6-minute walk test; ECV, extracellular volume fraction; GLS, global longitudinal strain; IVS, intraventricular septum; KCCQ, Kansas City Cardiomyopathy Questionnaire; LV, left ventricular; mBMI, modified body mass index; NYHA, New York Heart Association.

Baseline demographics and patient characteristics of patients recruited from the National Amyloidosis Centre, UK

The baseline data for the patients I recruited are displayed in Table 7.4. There was an approximately even split between hereditary and wild-type patients. As expected the ATTR-wt group were older with a greater proportion of males compared with the hereditary group. Heart failure symptoms for both groups were predominantly mild (NYHA I or II) in keeping with good functional capacity as assessed by 6MWT. Echocardiographic parameters showed greater wall thickness and worse LVEF and GLS in the hereditary group, and CMR measured parameters showed increased LV mass despite similar ECV. Cardiac biomarkers were greater in the hereditary group.

Summary of safety and tolerability for entire trial cohort

Adverse events (AEs) were reported in 20/26 (77%) patients. AEs related to revusiran were observed in 9/26 (35%) patients. Three (12%) patients experienced a serious adverse event (SAE) one of which was related to the study drug. One patient with normal liver function test (LFT)s at baseline experienced a SAE of aspartate transaminase (AST) and alanine transaminase (ALT) elevation that was considered to be related to revusiran. In this patient AST and ALT levels were 4.2 upper limit of normal (ULN) and 3.2 x ULN, respectively, after 6 doses of revusiran 5.0 mg/kg. This event was not associated with any clinical symptoms, and resolved without treatment. The study drug was temporarily interrupted (no dosing on Day 21) and then resumed. There was no recurrence of LFT abnormalities in this patient on resumption of treatment. Two other SAEs were observed during the study (non-cardiac chest pain, and ICD implantation).

There were no deaths, and no AEs leading to discontinuation of treatment or withdrawal from

the study. Revusiran did not result in any significant changes in other laboratory chemistry, renal function or haematologic parameters (data not shown). There were no AEs related to vitamin A deficiency (data not shown).

7.5 Limitations

The Phase 2 clinical trial itself was limited by its small size and short duration of follow-up, thus limiting inferences about the potential beneficial or adverse effects of longer-term treatment. Further, separate analyses of the UK patients were not permitted by the Sponsor as it was not deemed relevant given the small subgroup sample that would be assessed, therefore I have only been permitted to report the baseline features of the patients recruited from the NAC and not the outcomes of the exploratory outcomes for these patients. The summary AE findings for the entire cohort and the patient characteristics for patients recruited from the NAC have been reproduced with the Sponsor's permission and are not my own original data.

7.6 Discussion

The main findings from data presented in this chapter are:

1. A third of patients met the eligibility criteria for enrolment in a phase 2 study of revusiran. The main exclusions were a lack of biopsy evidence for amyloid and age over 80 years.

2. There appeared to be differences in the baseline investigation findings between ATTR-wt and ATTR-m patients recruited from the UK.

In this chapter I have demonstrated that approximately one third of patients with cardiac TTR amyloidosis were eligible for a phase 2 study of the RNA silencing treatment, revusiran.

The implication of this is that that trial findings may only be applied to the minority of patients with the condition. Two simple changes in enrolment criteria could mitigate this issue. In particular the requirement for biopsy proven amyloidosis and the age cut-off (which is particularly relevant in patients with ATTR-wt cardiac amyloidois who are generally an elderly population). The requirement for histological evidence for amyloid has diminished in clinical practice as evidenced by the recent consensus paper for the non-invasive diagnostic criteria for cardiac ATTR amyloidosis [33]. A diagnosis of ATTR amyloidosis may be made following clinical assessment, if associated with the typical findings by standard cardiac imaging methods as discussed earlier. This change in clinical practice is likely to impact the inclusion criteria for future clinical trials in this disease. The patients with cardiac ATTR-wt amyloidosis described in this thesis had a mean age of approximately 78 years. As such, it is unsurprising that an age cut-off of 80 years resulted in the exclusion of a significant proportion of patients. For future trials, particularly those enrolling patients with cardiac ATTR-wt amyloidosis it would seem reasonable to remove the age cut-off as an inclusion criterion. Meeting inclusion criteria for functional capacity appears to also be particularly relevant to patients with the hereditary transthyretin amyloid cardiomyopathies and in particular to patients with ATTR-T60A amyloidosis, since these patients often have significant peripheral and autonomic neuropathy which plays a significant part in impairing exercise capacity in addition to the cardiac involvement. Another exclusion criterion not specifically examined in this work but of relevance to the feasibility of patient recruitment here, was the investigators assessment of the patient's ability to participate in, and complete the trial. This trial required frequent patient assessments and investigations over a short time period. The challenge posed by frequent and long trial assessments is a valid reason for patient and investigator reluctance for study participation.

Despite reaching its recruitment goal, this trial was challenging to recruit to. As highlighted previously, one of the later protocol amendments was to focus the inclusion criteria to only include additional patients with hereditary transthyretin cardiac amyloidosis and increase the sample size. This suggests that patients with wild-type cardiac ATTR amyloidosis were more readily recruited, and although by the end of the study there were similar numbers of patients in the two groups, the need to make this amendment is likely to reflect the more challenging recruitment of patients with hereditary ATTR amyloidoses. The findings in Table 7.3 show that the majority of patients with cardiac transthyretin amyloidosis in the UK have ATTR-wt amyloidosis, and although a greater proportion of the wild-type group did not meet the trial inclusion criteria, the overall eligible number was greatest in the wild-type group. Although not specifically examined here, it is also possible that ethic and cultural differences may also contribute patient interest in study participation. Furthermore, in the UK group a significant portion of the ATTR-T60A amyloidosis group resides in North-West Ireland (a reflection of their ancestral links), and the requirement of frequent travelling for trial participation may have negatively impacted the desire to participate. Equally, some patients eligible in the ATTR-V122I amyloidosis group spent prolonged periods of time abroad in their ancestral homelands and felt unable to participate in clinical trials.

As highlighted in Chapter 4, differences in characteristics amongst patients with different TTR genotypes must be taken into consideration at the design stage of any similar future trials in this area. The TTR genotype of patients has an impact on characteristics such as LVEF, LV mass and cardiac biomarkers which may be used as surrogate end-points and it is possible that neutral, positive, or negative changes in these surrogates may be misinterpreted if separate analyses by TTR genotype are not performed. Inevitably, this requires larger numbers of patients with each genotype to be studied when a drug reaches phase 2/3 status.

The Sponsor has reported that treatment with revusiran was associated with a marked reduction in plasma TTR levels, however, there were a large number of adverse events. Despite only a relatively small number of patients involved, there were concerns raised by the safety review committee about longer-term safety based on the findings of the open-label extension (NCT02292186) of this study which found that when patients continued treatment with revusiran there were reports of new-onset or progression of peripheral neuropathy. At that time the efficacy and safety of revusiran-mediated TTR reduction was also being explored in a Phase 3 study (ENDEAVOUR; NCT02319005) in patients with hereditary ATTR amyloidosis. Whilst no signal of new onset or worsening peripheral neuropathy was identified in the Phase 3 study, the data monitoring committee determined that the benefit-risk profile for revusiran no longer supported continued dosing. Dosing was discontinued in both the open-label extension and Phase 3 studies based upon the recommendation of the Phase 3 study data monitoring committee. Results from the ENDEAVOUR trial report that the study drug was discontinued early due to an imbalance in mortality observed between patients treated with revusiran and placebo. The Alnylam website reveals that the company has halted further development of the drug.

Chapter 8

Conclusions

The work in this thesis focuses on cardiac amyloidosis, in particular cardiac transthyretin amyloidosis. The studies I have undertaken contribute to a better understanding of the clinical characteristics and prognostic factors. I have also explored possible treatments for this disease, focusing on treatment tolerability and the impact of trial design on trial recruitment.

A systematic study of the electrocardiogram characteristics of patients with different cardiac transthyretin amyloidosis types has not been previously undertaken. Although the population studied in Chapter 3 was modestly sized and restricted to the three commonest cardiac transthyretin types, significant differences in the ECG characteristics of individuals related to ATTR amyloidosis type were identified. In particular, I identified differences in the prevalence of atrial arrhythmias and broad QRS at diagnosis despite similar left ventricular wall thickness. This has not been described previously and may be specific to the TTR mutation. Such evidence for differences between types of ATTR cardiac amyloidosis may have implications for the study of these patients and suggest differences in pathophysiology between the types of ATTR amyloidosis. A broad QRS was associated with a worse survival in patients with ATTR-wt cardiac amyloidosis. A broad QRS is also known to be a poor prognostic feature in other forms of heart failure, however, this was not the case in the hereditary forms of ATTR

cardiac amyloidosis. This could be due to different underlying pathophysiology or because the hereditary ATTR cardiac amyloidosis groups studied in this chapter were relatively small.

The two most common causes of ATTR cardiomyopathy are ATTR-wt and ATTR-V122I cardiac amyloidoses. Data presented in Chapter 4 reveals significant differences in phenotype between these types of ATTR cardiac amyloidosis. Patients with ATTR-V122I amyloidosis present at a younger age with a more severe phenotype and have worse survival. It is the severity of cardiac disease that appears to determine prognosis in all patients with ATTR cardiac amyloidosis. I demonstrated using cause of death data, that the majority of patients die from heart failure.

Bone scintigraphy has recently been demonstrated to permit the non-biopsy diagnosis of cardiac ATTR amyloidosis in selected individuals. In Chapter 5, I demonstrated that visual grading assessment of ^{99m}Tc DPD scintigraphy by the original Perugini system correlates with cardiac disease severity in ATTR cardiac amyloidosis. There were indications from my data that visual grading assessment may be related to TTR type but this hypothesis requires further investigation. Furthermore, although cardiac uptake in ^{99m}Tc DPD scintigraphic evaluation appears to be related to more severe cardiac disease in AL amyloidosis cardiomyopathy, it does not appear to be a helpful diagnostic or prognostic test in these individuals.

The search for effective treatments for cardiac ATTR amyloidosis is an ongoing one. The NSAID, diflunisal may have some disease-modifying effect in FAP but does not appear to be well-tolerated even in selected patients with transthyretin amyloid cardiomyopathy based on data presented in Chapter 6. Fewer than half of patients commencing treatment, continuing therapy for more than 6 months. Furthermore, in terms of efficacy, there were no significant differences between matched cohorts treated with diflunisal compared with those not treated with diflunisal after 12 months of follow-up.

In Chapter 7 I described the UK experience of a phase 2 study of revusiran. Approximately a third of patients were eligible for trial inclusion and there appeared to be baseline differences between the wild-type and hereditary groups recruited from the UK. There was a high rate of adverse events and ultimately this therapy has been withdrawn due to a higher mortality in the treatment arm of a subsequent phase 3 trial.

8.1 Future Work

Prospective longer-term evaluation of individuals with cardiac ATTR amyloidosis will be invaluable in establishing a greater understanding of this highly variable condition. Since commencing the studies in this thesis, the NAC in the UK has commenced a prospective longitudinal cohort study; TRansthyretin Amyloidosis: Neuropathy, Senility, Cardiomyopathy, Evaluation, Natural history and Diagnosis (TRANSCEND). This study is being undertaken to characterise the phenotype and natural history of all patients with ATTR amyloidosis in the UK. In addition, the THAOS study continues to collect international, longitudinal data which will provide valuable information in the coming years.

The prognosis of patients with a new diagnosis of cardiac AL amyloidosis appears to be well-predicted by cardiac biomarker (NT-proBNP and Troponin) assessment as originally described by the Mayo group. However much still remains to be uncovered. Amongst this includes the likelihood of response to chemotherapies, risk stratification for sudden cardiac death, and prognostic markers following successful chemotherapy treatment. The intriguing finding of cardiac uptake by ^{99m}Tc DPD scintigraphy in cardiac AL amyloidosis remains unclear and requires further investigation. Furthermore, the reason for the sensitivity of 99mTc DPD in cardiac ATTR amyloidosis and cardiac ApoA1 amyloidosis still remains unclear and requires further evaluation.

Patient selection criteria for clinical trials stongly affect the applicability of results to the wider population of patients with the condtion. Furthermore, these criteria and the trial design also have a major role in determing the ease with which patients can be recruited and thus potentially impact the success or failure of a clinical trial. A more pragmatic approach based on the recently published consensus document on the non-invasive diagnosis of cardiac tranthyertin amyloidosis [33] is likely to mean that from 2017, the absence of a histological diagnosis of the condition is less likely to be as significant a limitation to clinical trial recruitment if incorporated in to any future clinical trial design. Others selection criteria must be carefully rationalised and consideration may even need to be given to separate trials for the different cardiac ATTR sub-types given the potential for variety of clinical phenotype and outcomes.

One of the disease-specific therapeutics currently under investigation at an advanced stage in patients with ATTR cardiac amyloidosis is AG10 [144], an orally administered TTR stabiliser. Following completion of the promising phase 2 study in 49 patients with either ATTR-wt or ATTR-m amyloidosis [145], recruitment has recently begun for the phase 3 trial of AG10 (ATTRibute-CM trial, NCT 03860935). Study completion of ATTRibute-CM is estimated for 2022. Notably the maximum age for entry is 90 years.

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Appendices

Appendix A

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