Cardiac Transthyretin-derived Amyloidosis

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Introduction

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I: Defining a specific cause of heart failure

Heart failure (HF) is a debilitating syndrome and a substantial burden on the Western health care system[1,2]. It is estimated that a person aged 50 has a 30% remaining lifetime risk of developing HF[3] and that in the elderly population (>70 years) the prevalence of HF is approximately 10% and increases further with advancing age[2]. The two year all-cause mortality of HF patients is 17%[4]. Historically heart failure is divided into two major entities, with both equal prevalence, defined by the left ventricular ejection fraction (LVEF): heart failure with reduced (<40%) ejection fraction (HFrEF) and heart failure with preserved (>50%) ejection fraction (HFpEF).

Different causative mechanisms can be distinguished in HF. Myocardial causes are numerous of which ischaemic heart disease is the most common, resulting in HFrEF and is characterized by cardiomyocyte loss. In addition, toxic damage, e.g. alcohol and medication and also metabolic derangements and genetic abnormalities are also disruptive to the myocardial tissue and can result in HFrEF. These mechanisms are quite-well understood and adequate treatment options are available for HFrEF[3]. Non-myocardial causes include abnormal loading conditions of the heart and arrhythmias[3].

In contrast to HFrEF, the causative mechanisms of HFpEF are less well understood. HFpEF is currently regarded as a final common manifestation of different diseases leading to increased diastolic stiffness of the myocardium that results in reduced filling capacity of the left ventricle. The restrictive filling capacity encountered in HFpEF is caused either by intrinsic cardiomyocyte alterations or by alteration in the extra-cellular matrix (ECM). These processes are considered to be not mutually exclusive but rather to be coexistent. HFpEF has a poor prognosis, and until recently treatment options were not available[5,6,7].

Cardiomyocyte alterations include both increased intrinsic cellular stiffness and hypertrophy. Increased cellular stiffness is caused by the interplay of older age, female sex and the presence of comorbidities such as obesity, diabetes mellitus, renal failure, lung disease and iron deficiency[8,9]. This interplay leads to accumulation of inflammatory cytokines, advanced glycosylation end products (AGEs) and, finally, to a pro-inflammatory state[8,10,11]. In this state, reactive oxygen species (ROS) are increased and disrupt the intra-cellular metabolism of proteins such as the cytoskeleton protein titin, which leads to increased cardiomyocyte stiffness[8,12,13]. Hypertrophy (in HFpEF) can be due to intracellular accumulation of sarcomeric protein components resulting from for instance chronic arterial hypertension and genetically determined hypertrophic cardiomyopathies as well as by ROS as a consequence of the above described pro-inflammatory state[8].

Alterations in the extra-cellular matrix (ECM) also occur due to ROS, which influence the composition of the ECM-proteins by TGF-β- and angiotensin-mediated pathways

acting on interstitial fibroblasts[8,14]. ROS induces the formation of collagen and a reduction in the formation of elastine, finally causing functional stiffening of the myocardium[8,14]. These processes manifest as "fibrosis" in endomyocardial biopsies[14,15].

As described above, a number of different entities are able to activate these pathways leading to HFpEF. Besides "these usual suspects", HFpEF can also be found in a group of other diseases, primarily due to the deposition of proteins in the ECM, such as the amyloidosis and other infiltrative cardiomyopathies[16]. Especially transthyretin-derived amyloidosis was described as a specific and significant cause of HFpEF[17]. Because transthyretin-derived amyloidosis has become a treatable disease[18,19], identification of cardiac transthyretin amyloidosis is of great interest in HFpEF patients and is therefore the focus of this thesis.

II: Amyloidosis; a group of diseases characterised by the deposition of misfolded and aggregated protein

Amyloidosis is the collective naming for disorders in which proteins misfold and aggregate leading to the formation and deposition of insoluble amyloid fibrils. Depending on the protein that the amyloid fibrils are composed of, different types of amyloid can be distinguished. Characteristic of amyloid fibrils is its repetitive structure of Beta-sheets, which makes amyloid fibrils rigid and relatively resistant to proteolysis. Interestingly, the strength and stiffness of the β-sheet structure is also recognized in the light weighted industrial fibre Kevlar (Dupont.com), which is the basis of bullet-proof vests. Deposition of amyloid fibrils between cells within organs and tissues leads to impaired functioning and eventually failure of these organs[20]. Deposition of amyloid fibrils within the myocardium typically causes HFpEF[12,16,17], because the deposition of fibrils leads to abnormal mechanical properties i.e. myocardial stiffness. In addition to the mechanical alterations, it has been shown that monomers or oligomers of amyloidogenic proteins like mutated transthyretin also have direct cytotoxic effects on cardiomyocytes[21,22]. Currently, a total of 36 proteins are known to potentially cause amyloidosis in humans[23]. The two most frequently occurring types of cardiac amyloidosis are transthyretin- (ATTR) and immunoglobulin light chain- (AL) amyloidosis[23].

Transthyretin-derived (ATTR) amyloidosis will be discussed in more detail below (III).

AL-amyloidosis arises from monoclonal plasma cell dyscrasias resulting in increased production of kappa or lambda light-chain immunoglobulins. AL-derived amyloid fibrils can deposit in almost any organ but most frequently deposit in heart, kidneys and peripheral nerves, leading not only to HF but also to renal failure and autonomic and peripheral neuropathy[24]. Cardiac involvement is one of the most important prognostic factors in systemic AL amyloidosis[25,26]. Overall survival in AL amyloidosis is improving

over time, in part due to earlier diagnosis and in part due to more effective therapies[27,28]. Notwithstanding its importance, AL amyloidosis is beyond the scope of this thesis.

AA-amyloidosis results from deposition of the acute phase protein Serum amyloid A and was in the past occasionally found in patients with chronic inflammatory conditions, e.g. not well treated rheumatoid arthritis[29]. Nowadays, this form of amyloidosis is rare as more effective anti-inflammatory drugs have become available. Patients with AA-amyloidosis were not included in our studies.

Finally, an organ-specific type of amyloidosis is isolated atrial amyloidosis (IAA), in which the amyloid deposition is confined to the atria of the heart. The precursor protein is atrial natriuretic peptide (ANP), a naturally occurring peptide hormone synthesised by atrial cardiomyocytes, mainly secreted in case of atrial stretch. IAA may manifest as atrial fibrillation[30].

III: Transthyretin-derived cardiac amyloidosis

The transthyretin (TTR) protein, is a negative acute phase protein produced mainly in the liver, but is also produced in small quantities in the retinal pigment epithelium of the eye and the choroid plexus in the ventricles of the brain31. TTR is a plasma transporter protein, mainly for thyroxine and retinol binding protein in complex with retinol[31]. It is a 55 kDa protein homo-tetrameric protein, in which the constituting monomers are organized in a dimer configuration by hydrophobic interaction and hydrogen-bonds in large corresponding β-sheet regions, resulting in a β-sheet rich pro-amyloidogenic protein as its tertiary structure. Two dimers are subsequently linked by hydrophobic and hydrogen bonds in smaller corresponding regions in the tales of dimer subunits, finally forming the homo-tetrameric configuration[32,33]. The monomeric TTR is a 127-polypeptide, encoded by TTR, a gene residing on chromosome 18. Although not fully understood, the degradation of TTR mainly takes place in the liver, muscle and skin and is believed to take the opposite way of the synthesis of TTR, i.e. from tetrameric to monomeric configuration[34]. In healthy individuals, the different configurations of the TTR protein are in a dynamic equilibrium between the tetrameric, the dimeric and the monomeric forms[35].

In amyloidosis, the balance of the different forms of the TTR protein is disrupted. The monomers are subjected to misfolding. When monomers become misfolded they aggregate into oligomers, which aggregates become insoluble polymers, and they finally form β-sheet rich ATTR amyloid fibrils[35,36]. The onset of amyloidosis is the formation of an amyloid nucleus in the extra-cellular matrix of the end-organ (figure 1)[37]. This nucleus acts as a seed for the aggregation and further lengthening of the amyloid fibrils[38,39]. Although it is widely accepted that the amyloid formation process is driven by the aggregation of misfolded monomers, precise mechanisms are not yet eluded[40,41].

Two forms of ATTR amyloidosis are distinguished based on the absence or presence of mutation in the encoding TTR gene. In the wild type form (ATTRwt) no mutation is present, whereas in the variant or hereditary form (ATTRv) a mutation in TTR causes an aberrant TTR protein, usually with a single amino-acid substitution due to a point mutation. ATTRv is a rare disease but it is endemic in certain areas especially Portugal, Sweden and the Afro-American population in the USA[42,43]. Over 120 mutations are known to cause this form and they have an autosomal dominant inheritance pattern[44]. The onset of ATTRv is in general at younger age (<50 years). In contrast to ATTRv, a high age of onset (>70 years) characterizes the ATTRwt form, formerly known as "senile systemic amyloidosis". Patients are predominantly male. The prevalence of ATTRwt is not well established as yet and was addressed in three studies, which were either post-mortem, single center, or comprised a select population[16,17,45].

The clinical presentation of patients with ATTRv and ATTRwt differs (figure 1). ATTRv is a heterogeneous disease where multiple organ systems are affected. The disease manifestation depends on the type of mutation. Most mutations present as a peripheral neuropathy, (formerly known as familial amyloid polyneuropathy), but some mutations present primarily as a cardiomyopathy[46]. Furthermore, involvement of other organs is seen, such as the eyes (particularly vitreous opacities), autonomic nerves leading to orthostasis, impotence, bladder dysfunction and gastrointestinal symptoms (diarrhea and constipation) and renal involvement[47]. Even within the same mutation, there is a substantial inter-individual heterogeneity in the clinical presentation 46. In contrast, patients with ATTRwt usually present with heart failure, arrhythmias and/or conduction disturbances and cardiac problems are often preceded in years by bilateral carpal tunnel syndrome (CTS) or spinal canal stenosis[48].

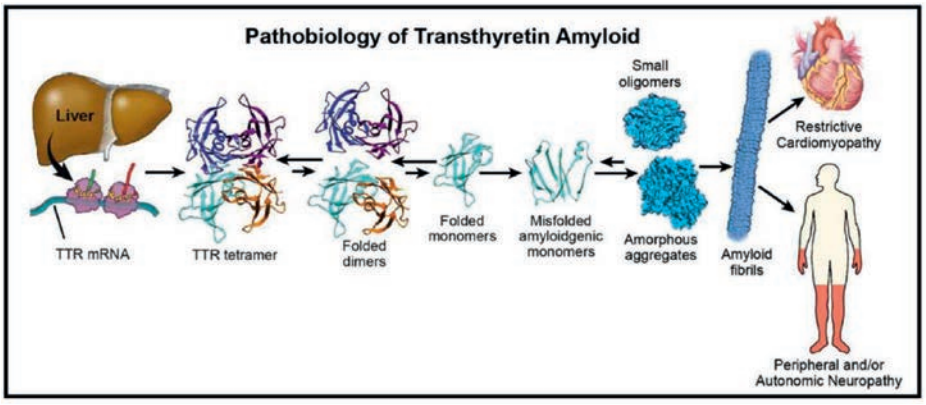

Figure 1. Pathobiology of transthyretin-amyloidosis, adapted[48].

Historically, the gold standard for diagnosis of cardiac amyloidosis is to detect amyloid deposits in a myocardial biopsy, but this is an invasive procedure carrying a small risk for the development of serious complications. A subcutaneous abdominal adipose tissue aspirate is a good screening alternative[49,50]. Amyloid deposits stained with Congo red show apple green birefringence, the red colour changes intro green, when investigated under polarized light. Currently, when clinical suspicion of ATTR cardiomyopathy is raised, based on specific findings upon transthoracic echocardiography, ECG and/or cardiac MRI, a bone scintigraphy instead of the previously used endocardial biopsy is recommended[51]. It was described that scintigraphy with ^{99m}technetium-hydroxymethylene diphosphonate, normally used to detect bone abnormalities, also allows for accurate diagnosis of cardiac ATTR amyloidosis[52]. The scintigraphy has a high sensitivity and specificity for cardiac amyloidosis and has thus become the new non-invasive gold-standard for diagnosis[51].

The median survival for symptomatic cardiac ATTR amyloidosis is around 3 years in both ATTRwt and ATTRv amyloidosis without treatment[48]. In recent years, multiple treatment options for ATTR became available. Treatment options are based on three different principles. The first treatment principle is lowering the production of the TTR protein by gene silencing. Both patisiran (small interfering RNA) and inotersen (antisense oligonucleotide) have shown to reduce the circulating levels of TTR, thereby halting disease progression and by which even slight improvement of clinical parameters was observed in part of the patients[19,53]. In addition, a novel approach using CRISPR-Cas9 technique to knock-out the *TTR* gene seems to persistently decrease the serum TTR protein concentration[54]. The second treatment principle is to stabilize the TTR tetramer, thus preventing the tetramer to dissociate in amyloidogenic monomers. The rate of disease progression was indeed reduced but not halted by tetramer stabilizers Tafamidis and AG10[18,55]. The last treatment principle is targeting the degradation of amyloid fibrils, with this purpose several antibodies are being investigated at present and is currently investigated in a phase-1 trial (NCT04360434).

IV: scope/aim of this thesis

The clinical diagnosis of cardiac ATTR is difficult and often missed, especially in the early stages of amyloid disease in the general HFpEF population. This is mainly because cardiac amyloidosis stigmata – such as low-voltage ECG despite hypertrophy, increased wall thickness on echocardiography and late gadolinium enhancement on cardiac MRI – are often not present in the early stages of cardiac amyloidosis. However, since the currently available treatments are most effective when applied in the early stages of disease, early identification of ATTR is paramount.

The first part describes three studies to improve the early detection of cardiac involvement in ATTRv. The second part aims to describe strategies to improve early diagnosis of cardiac ATTR in the general HFpEF population.

In part I, chapter two describes cardiac involvement in a new *TTR* gene mutation. Chapter three describes the prevalence of cardiac involvement in in a group of patients with ATTRv and the value of NT-proBNP in the detection of cardiac involvement. Chapter four describes the value of global longitudinal strain measurement for the early detection of cardiac disease in ATTRv carriers, compared to the gold standard bone scintigraphy.

In part II, chapter five the potential usefulness of screening for ATTR amyloidosis in the general HFpEF population is discussed. In chapter 6 to identify patients with ATTRwt in the general HFpEF population, which may aid in the early diagnosis of this disease in patients with HFpEF. In chapter 7, the role of amyloidosis in HFpEF and atrial fibrillation is described.

Amyloidosis Center of Expertise

This thesis is a result of decades of research and clinical experience with amyloidosis at the University Medical Centre Groningen. As head of the department of internal medicine of the University Medical Centre Groningen, prof. dr. E. Mandema started a research line primarily focused on amyloidosis in the early 1960's. In 1967 the first international symposium on Amyloidosis was hosted in Groningen. In the following decennia the diagnostic approach of amyloidosis was greatly improved, in addition to the better understanding and recognition of different subtypes of amyloidosis. In 1990, Dr. B.P.C. Hazenberg became the leading coordinator of the then called Groningen Unit for Amyloidosis Research and Development (GUARD), which resulted in the acknowledgement of the UMCG as the national center of expertise for amyloidosis in 2015.

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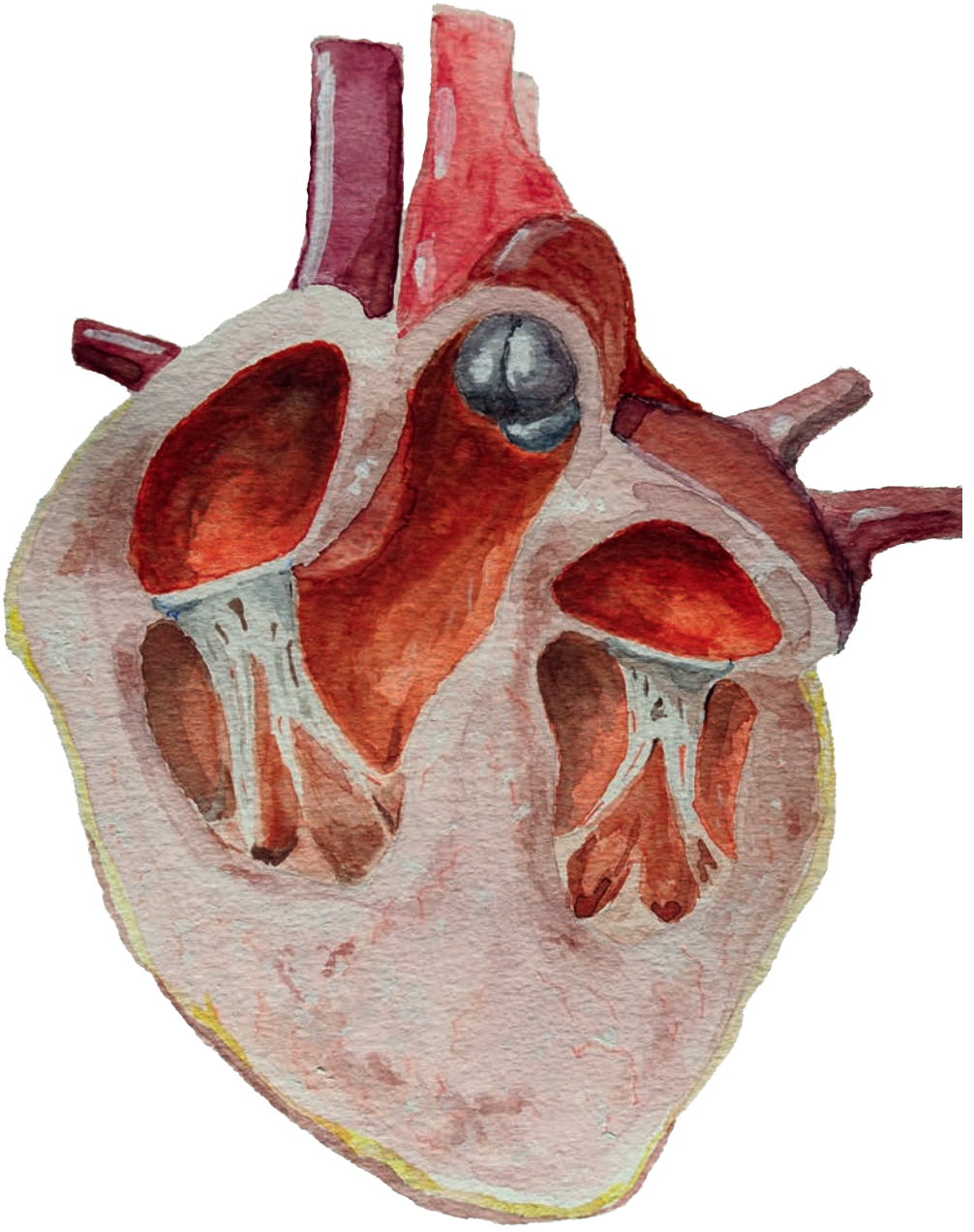

Part 1

Hereditary transthyretin-derived amyloidosis