Cardiac Transthyretin-derived Amyloidosis

Klaassen, Sebastiaan

DOI:
10.33612/diss.255251963

Summary, discussion and future perspectives

S.H.C. Klaassen

8

Summary

The aim of this thesis was to improve the diagnosis of cardiac ATTR in patients presenting with HFpEF and *TTR* gene mutation carriers. The first part focused on the early detection of cardiac involvement in patients affected by hereditary transthyretin amyloidosis (ATTRv). The second part described strategies to identify cardiac ATTR in the general HFpEF population.

In **chapter 2** we described a Dutch family with a novel TTR mutation and showed that this mutation was characterised mainly by heart failure at relative high age. The systemic nature of this type of ATTRv was reflected by the presence of bi-lateral carpal tunnel syndrome and the early signs of polyneuropathy. The systemic nature of ATTRv was further emphasized in **chapter 3**. Here, we demonstrated in the Groningen Amyloid Cohort (GAC) consisting of patients with proven ATTRv with different genotypes that cardiac involvement was present in ~50% of the patients at the time of diagnosing amyloidosis, irrespective of genotype or initial clinical presentation. We showed that cardiac involvement determined the survival of ATTRv patients. NT-proBNP was shown to be an effective and easy to use screening tool for the detection of cardiac involvement in ATTRv. In **chapter 4** we described the value of echocardiographic deformation imaging, i.e. global longitudinal strain (GLS), in a population consisting of individuals presenting at our centre because of familial screening for ATTRv. We demonstrated that a combination of GLS and NT-proBNP may detect cardiac pathology at an earlier stage than the bone scintigraphy, the current non-invasive gold standard for diagnosis of cardiac ATTR.

Part two of this thesis described studies on diagnosing cardiac ATTR in patients with HFpEF. In **chapter 5** we described the rationale for screening for the presence of ATTRwt in the general HFpEF population. The clinical hallmarks of ATTRwt were described, and we suggested that ATTRwt should be considered in elderly patients with HF and in which either a specific echocardiographic pattern is present or the medical history reveals bi-lateral carpal syndrome. A bone scintigraphy should be performed in these patients to finally diagnose ATTRwt. In **chapter 6** we investigated the prevalence of ATTRwt in HFpEF/HFmrEF patients. In this selected population we reported an ATTRwt prevalence of 5%. In addition, we demonstrated that patients with cardiac amyloidosis had a distinctly different biomarker pattern as compared to HFpEF/HFmrEF patients without cardiac amyloidosis. Serum levels of the three biomarkers GLB1, S100A4, and HGF were most distinctive and we demonstrated that this triplet was able to identify ATTRwt patients within a HFpEF/HFmrEF patient population. In **chapter 7** we described the interplay between ATTRwt or isolated atrial amyloidosis (IAA) and atrial fibrillation (AF) in patients with HFpEF. Both HFpEF and AF are very common diseases that occur often in combination and aggravate each other.

In conclusion, this thesis contributes to the awareness of early detection of cardiac involvement of ATTRv and to the identification of ATTRwt in patients with heart failure.

General discussion

Cardiac amyloidosis, both due to wild-type (ATTRwt) and hereditary (ATTRv) transthyretin-derived amyloidosis can cause heart failure with preserved ejection fraction (HFpEF) and is a potentially treatable disease[1,2,3,4]. Current treatment options for transthyretin amyloidosis are most beneficial when initiated at an early disease stage. Early diagnosis of cardiac transthyretin amyloidosis in TTR variant carriers or patients with HFpEF is thus of great importance. Clinicians should actively look for cardiac transthyretin-derived amyloidosis in TTR variant carriers and patients with HFpEF, as cardiac ATTR deposition is prevalent in both populations.

The early detection of cardiac involvement in ATTRv amyloidosis

ATTRv is a systemic disease, however it usually seems that only a single organ system is affected at the time of disease presentation. The primary manifestation at disease onset is a length-dependent peripheral neuropathy and autonomic nervous dysfunction[5,6]. Exercise intolerance or dyspnea on exertion frequently observed in these patients, is then explained as a consequence of neurological features such as muscle weakness and atrophy. However, subtle involvement of other organs systems such as the heart should be suspected at the onset of ATTRv, due to the systemic nature of this disease. How the disease develops depends among other things, on the *TTR* gene mutation[5]. Exercise intolerance and dyspnea on exertion, are also hallmarks of heart failure. Moreover, the cardiac related origin of these symptoms could be masked by the advanced neurologic disease. Thus, cardiac involvement is only diagnosed when actively looked for.

We demonstrated in **chapter 3** that cardiac involvement is present in half of the patients with ATTRv at the time of diagnosis, irrespective of genotype or the predominant affected organ. This finding is consisted with Rapezzi et al. who found mixed manifestations of almost all studied TTR genotypes[5]. They also found that the I68L genotype was exclusively associated with cardiac symptoms, but this genotype was not present in our population. In both the study of Rapezzi et al. and our study patients with cardiac involvement were older as compared to patients without cardiac involvement. In addition, this age difference between patients with cardiac involvement and no cardiac involvement was observed in **chapter 4** as well. In **chapter 3** we also demonstrated that the presence of cardiac involvement is of great influence on the mortality. Ruberg et al. also described that patients with cardiac involvement had a significant higher all-cause mortality than the patients without cardiac involvement[7]. In fact, cardiomyopathy can be the first manifestation in carriers of pathogenetic TTR variant[5,7]. These findings emphasize the importance of active screening for cardiac

involvement in ATTRv patients. We next investigated the optimal approach to detect cardiac involvement as clinical symptoms alone might not give a clear picture of the degree of organ involvement.

In the general population, N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a powerful marker to predict new-onset heart failure[8]. In **chapter 3** we proposed that NT-proBNP could be used for the detection of cardiac involvement in ATTRv patients as well. NT-proBNP appeared to be a good marker for cardiac involvement in patients with typical ECG and traditional echocardiographic abnormalities as was depicted by a ROC curve for NT-proBNP for cardiac involvement with an AUC of 0.905. NT-proBNP is an easy to use biomarker and therefore a good diagnostic tool for the detection of cardiac involvement by medical specialists other than cardiologists. Whether NT-proBNP is also useful for the detection of early cardiac involvement, is unknown.

In **chapter 4** we demonstrated the value of speckle-tracking echocardiography in TTR mutation carrier patients. Peak global longitudinal strain measurements in combination with conventional echo parameters and NT-proBNP were able to detect cardiac abnormalities in 50% of patients with a negative bone scintigraphy. Bone scintigraphy is currently seen as the non-invasive gold-standard for the detection of cardiac ATTR amyloidosis and was validated with excellent diagnostic sensitivity and specificity in patients which were both symptomatic and with distinct stigmata on echocardiography and/or cardiac magnetic resonance imaging for cardiac amyloidosis[9]. Thus the question arises whether bone scintigraphy is a suitable diagnostic modality for especially the early detection of cardiac involvement in ATTRv[10]. Our results suggest that speckle-tracking echocardiography, conventional echo parameters and NT-proBNP serum levels have high diagnostic sensitivity for the early detection of cardiac ATTRv.

These findings underline the importance of a multi-modular approach of ATTRv, as different imaging techniques and biomarkers might detect cardiac abnormalities in different (early) phases of disease[11,12]. Thus, it is recommended that all these modalities, including the bone scintigraphy, are used in standard patient care for the screening of early signs of cardiac amyloidosis.

Improvement of early diagnosis of cardiac ATTR in the general HFpEF population

For the clinical cardiologist it is important to identify patients with disease entities that require specific therapeutical interventions. Establishment of the prevalence of ATTR in HFpEF is therefore important in justification of screening for cardiac ATTR in HFpEF. We found a prevalence of 5% of ATTRwt in HFpEF and HFmrEF patients (chapter 6). This is a slightly lower prevalence than the 7 – 13% that was reported in a selected patient populations, thus the prevalence might depend on the selection criteria of patients[1,2,13]. In addition, in patients with severe aortic valve stenosis a prevalence of

ATTRwt of 16% was reported[13]. Thus it might be speculated that ATTR is present in around ~10% of the HFpEF patients, identifying ATTR as a significant underlying disease in HFpEF.

In **chapter 6**, the applicability of biomarker analyses for the screening of cardiac ATTR in HFpEF was described. We showed that biomarker profiles were significantly different between patients with cardiac amyloidosis as compared to HFpEF due to other causes. From this difference in biomarker profiles, we selected a biomarker triplet consisting of HGF, S100A4 and GLB1, which was best discriminatory between the presence of cardiac amyloidosis and without cardiac amyloidosis. Zhang et al. also found that HGF was elevated in ATTRwt cardiac amyloidosis as compared to all-cause LVH and patients with heart failure with reduced ejection fraction (HFrEF)[14]. Thus, our data suggest that determination of serum levels of the biomarker triplet (HGF, S100A4, GLB1) is helpful in the identification of ATTR patients in the general HFpEF population. However, additional prospective studies are needed to define the diagnostic power for cardiac ATTR of this triplet.

Our findings suggest that potential ATTR patients can be identified in the general HFpEF population on basis of a typical clinical presentation (as described in **chapter 5**), and biomarker profiling with the triplet (HFG, S100A4, GLB1). At this time it is recommended to confirm the diagnosis using bone scintigraphy[15]. As stated, prospective studies are needed to define the optimal diagnostic procedures especially with respect to the specificity of the biomarker triplet as well as to sensitivity of bone scintigraphy for early stage ATTR cardiomyopathy.

Future perspectives

The aim of this thesis was to improve the diagnosis of cardiac ATTR. In recent years the amyloidoses received increased attention at congresses and from medical specialists. Different types of cardiac amyloidosis are now increasingly being diagnosed, however the early diagnosis of cardiac amyloidosis remains difficult. Early diagnosis is important because there are progressively better treatments for different types of amyloidosis that are most beneficial when initiated in an early disease stage. This certainly applies to ATTR cardiomyopathy.

ATTRv, as compared to ATTRwt, is a very heterogenous disease and the presence of specific mutation does not solely predict the course of the disease. Also the early diagnosis of ATTRwt in the general HFpEF population remains a challenge. The diagnostic strategy, as proposed in this thesis, for both *TTR* gene mutation carriers and patients with HFpEF improve the early detection of ATTR cardiomyopathy if implemented in the daily clinical practise. Further studies should aim to improve the knowledge of

the (sub)clinical course of cardiac ATTR amyloidosis and to optimize this diagnostic strategy.

A multi-center prospective study is needed in asymptomatic *TTR* gene mutation carriers with different mutations in which the different diagnostic tools (clinical patient charts, serum biomarkers, ECG, traditional and speckle-tracking echocardiography bone scintigraphy/SPECT-CT, CMR) are performed at regular reasonably timed intervals. We suggest that at least in this study our proposed diagnostic biomarker triplet is included. Via this methodology an optimal screening strategy for the early development of cardiac ATTR amyloidosis could be established.

The identification of ATTRwt cardiomyopathy in HFpEF patients requires a different strategy as compared to the detection of amyloid cardiomyopathy in *TTR* gene carriers. In this thesis, the approach of screening for ATTR cardiomyopathy in the general patient population with HFrEF/HFpEF was described. However, it is important to make a preselection of patients at increased risk of having ATTRwt cardiomyopathy. In half of the patients presenting with ATTRwt cardiomyopathy, the medical history reveals carpal tunnel syndrome (CTS) years before presentation[16]. Other non-cardiac clinical manifestations of ATTRwt are spinal canal stenosis and biceps tendon ruptures. Especially CTS is of interest, because amyloid deposition in tenosynovial tissue is already present in 10% of patients over 60 years of age undergoing carpal release surgery[17]. We propose a study to prospectively follow patients with amyloid deposits in the tenosynovial tissue discovered during CTS release surgery, as we hypothesize that a significant part of these patients will develop ATTRwt cardiomyopathy in the following years. Secondly, treating these patients with TTR stabilizing drugs might prevent the development of ATTR cardiomyopathy. A prospective study should investigate the optimal screening strategy for ATTRwt in the "real life" cardiology patient. Especially in certain subgroups, because the prevalence of ATTRwt is increased in male patients with HFpEF, in patients with concomitant aortic valve stenosis, and in HFpEF patients in which the medical history reveals CTS as mentioned above. In this study our proposed biomarker triplet will be validated. In addition, the role of conventional diagnostic tools in early detection of cardiac amyloid compared to other causes of heart failure should be investigated[10], such as speckle tracking in TTE and in cardiac MRI the late gadolinium enhancement patterns[18] and the value of extra cellular volume[7,19]. Furthermore, the effect of different types of treatment options should be included in these two prospective studies.

Besides clinical diagnostic studies, it is important to increase the knowledge of the pathogenesis of cardiac ATTR amyloidosis. Especially the mechanisms underlying the amyloid nucleus formation and amyloid fibril aggregation in the heart. Recently, advanced cardio tissue techniques using pluripotent human stem cells have become available in our lab[20]. Using this engineered tissue we propose in-vitro studies with amyloidogenic TTR proteins and to induce stressful events to cardiac tissue in the

presence of monomeric and tetrameric TTR or TTRv proteins. Instead of engineered cardiac tissue, human cardiac cell line AC16[21] or a mouse model might be used to address these questions[22].

Moreover, future research might also direct to the physiological degradation of already deposited amyloid fibrils. It was demonstrated that the chaperone protein Clusterin, which is an important mediator in the degradation of ATTR, was significantly reduced in patient with ATTR amyloidosis[23,24]. Whether this is a causative finding in the development of especially ATTRwt amyloidosis needs to be determined, as chaperone proteins become less effective with increasing age.

References

1. González-López E et al. Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction. Eur Heart J. 2015;36:2585–2594.
2. Mohammed SF et al. Left ventricular amyloid deposition in patients with heart failure and preserved ejection fraction. JACC Heart Fail. 2014;2:113–122.
3. Maurer MS et al. Tafamidis Treatment for Patients with Transthyretin Amyloid Cardiomyopathy. N Engl J Med. 2018;379:1007–1016.
4. Adams D et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. N Engl J Med. 2018;379:11–21.
5. Rapezzi C et al. Disease profile and differential diagnosis of hereditary transthyretin-related amyloidosis with exclusively cardiac phenotype: an Italian perspective. Eur Heart J. 2013;34:520–528.
6. Conceição I et al. Early diagnosis of ATTR amyloidosis through targeted follow-up of identified carriers of TTR gene mutations*. Amyloid. Taylor & Francis; 2019;26:3–9.
7. Ruberg FL et al. Transthyretin Amyloid Cardiomyopathy: JACC State-of-the-Art Review. J Am Coll Cardiol. 2019;73:2872–2891.
8. Brouwers FP et al. Clinical risk stratification optimizes value of biomarkers to predict new-onset heart failure in a community-based cohort. Circ Hear Fail. 2014;7:723–731.
9. Gillmore JD et al. Nonbiopsy diagnosis of cardiac transthyretin amyloidosis. Circulation. 2016;133:2404–2412.
10. Pagourelias ED et al. Echo Parameters for Differential Diagnosis in Cardiac Amyloidosis: A Head-to-Head Comparison of Deformation and Nondeformation Parameters. Circ Cardiovasc Imaging. 2017;10:1–11.
11. Boldrini M et al. Multiparametric Echocardiography Scores for the Diagnosis of Cardiac Amyloidosis. JACC Cardiovasc Imaging. 2020;13:909–920.
12. Bella G Di et al. Usefulness of Combining Electrocardiographic and Echocardiographic Findings and Brain Natriuretic Peptide in Early Detection of Cardiac Amyloidosis in Subjects with Transthyretin Gene Mutation. Am J Cardiol. Elsevier Inc.; 2015;116:1122–1127.
13. Castano A et al. Unveiling transthyretin cardiac amyloidosis and its predictors among elderly patients with severe aortic stenosis undergoing transcatheter aortic valve replacement. Eur Heart J. 2017;38:2879–2887.
14. Zhang KW et al. Plasma Hepatocyte Growth Factor for Diagnosis and Prognosis in Light Chain and Transthyretin Cardiac Amyloidosis. JACC CardioOncology. Elsevier; 2020;2:56–66.
15. Garcia-Pavia P et al. Diagnosis and treatment of cardiac amyloidosis: a position statement of the ESC Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2021;1554–1568.
16. Nakagawa M et al. Carpal tunnel syndrome : a common initial symptom of systemic wild-type ATTR (ATTRwt) amyloidosis Carpal tunnel syndrome : a common initial symptom of systemic wild-type ATTR (ATTRwt) amyloidosis. 2016;23:58–63.
17. Sperry BW et al. Tenosynovial and Cardiac Amyloidosis in Patients Undergoing Carpal Tunnel Release. J Am Coll Cardiol. 2018;72:2040–2050.
18. Zhao L, Tian Z, Fang Q. Diagnostic accuracy of cardiovascular magnetic resonance for patients with suspected cardiac amyloidosis: A systematic review and meta-analysis. BMC Cardiovasc Disord. BMC Cardiovascular Disorders; 2016;16:1–10.
19. Martinez-Naharro A et al. Magnetic Resonance in Transthyretin Cardiac Amyloidosis. J Am Coll Cardiol. 2017;70:466–477.
20. Bliley JM et al. Dynamic Loading of Human Engineered Heart Tissue Enhances Contractile Function and Drives Desmosome-linked Disease Phenotype. bioRxiv. 2020;2020.05.25.111690.

21. Penchala SC et al. AG10 inhibits amyloidogenesis and cellular toxicity of the familial amyloid cardiomyopathy-associated V122I transthyretin. Proc Natl Acad Sci U S A. 2013;110:9992–9997.

22. Joel N. Buxbaum MD. Animal models of human amyloidoses: Are transgenic mice worth the time and trouble? FEBS Lett. 2009;583:2663–2673.

23. Greene MJ et al. Cooperative stabilization of transthyretin by clusterin and diflunisal. Biochemistry. 2015;54:268–278.

24. Greene MJ et al. Evidence for a functional role of the molecular chaperone clusterin in amyloidotic cardiomyopathy. Am J Pathol. Elsevier Inc.; 2011;178:61–68.