Cardiac Transthyretin-derived Amyloidosis

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Frequency of and Prognostic Significance of Cardiac Involvement at Presentation in Hereditary Transthyretin-Derived Amyloidosis and the Value of N-Terminal Pro-B-Type Natriuretic Peptide

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Abstract

Objective

The aim of this study is to assess the prevalence of cardiac involvement in hereditary transthyretin-derived (ATTRm) amyloidosis at the time of diagnosis and to determine the diagnostic and clinical value of N-terminal pro-B-type natriuretic peptide (NT-proBNP).

Patients and Methods

The University Medical Center Groningen is the national center of expertise for amyloidosis. All consecutive patients between 1994 and 2016 with ATTRm amyloidosis were followed prospectively. Baseline was set at the time of the first positive biopsy. All patients underwent a standard cardiac and neurologic work-up. Cardiac involvement was defined by otherwise unexplained left and/or right ventricular wall hypertrophy on cardiac ultrasound and/or advanced conduction disturbances.

Results

Seventy-seven patients had ATTRm amyloidosis and were included in the study. The TTR V30M mutation was present in 30 patients (39%). In both the V30M and the non-V30M groups, the neurologic presentation dominated (77% vs 51%), whereas cardiac presentation was infrequent (7% vs 15%). Clinical work-up showed that cardiac involvement was present at baseline in 51% of all patients irrespective of genotype and was associated with increased overall mortality (hazard ratio 5.95, 95% confidence interval 2.12 to 16.7), independent from clinical confounders. At a cutoff level of 125 ng/L, NT-proBNP had a sensitivity of 92% for establishing cardiac involvement.

Conclusion

Irrespective of the frequent noncardiac presentation of ATTRm amyloidosis, cardiac involvement is already present at diagnosis in half of the patients and is associated with increased mortality. NT-proBNP is a useful marker to determine cardiac involvement in this disease.

Introduction

Hereditary transthyretin-derived amyloidosis (ATTRm) is a rare protein-folding disease.[1,2] ATTRm amyloidosis usually presents with symptoms of a length-dependent polyneuropathy. At presentation, this disease usually seems to affect 1 single organ or tissue. However, because of the systemic nature of ATTRm amyloidosis, subtle involvement of multiple organ systems may already be present at the time of diagnosis. Limited data are available on the actual presence and the impact on prognosis of cardiac involvement in patients with ATTRm amyloidosis presenting with noncardiac phenotypes. Whereas N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a powerful marker to predict new-onset heart failure in the general population,[3] its role in identifying cardiac involvement in ATTRm amyloidosis is described in only few studies.[4,5] Therefore, the objective of this study was to investigate the prevalence of cardiac involvement at the time of diagnosis in patients presenting with ATTRm amyloidosis and the utility of NT-proBNP as a tool to detect cardiac involvement.

Patients and Methods

The University Medical Center Groningen is the national center of expertise for all types of systemic and localized amyloidosis in The Netherlands. Patients with proven or suspected amyloidosis are referred by different specialists, depending on the phenotype, for further diagnostic testing and treatment. All patients are followed prospectively in the Groningen Amyloidosis Cohort since 1985. For the purpose of the present study, data of all consecutive patients in this database between 1994 and 2016 with suspected ATTR amyloidosis were reviewed. Family members of patients with ATTRm are routinely referred to our center to be screened, pre-symptomatic, for the presence of a TTR mutation and of amyloid.

Patients were included only when the presence of amyloid was proven in a biopsy, subsequently typed as ATTR, and DNA analysis revealed a mutation in the *TTR* gene.[6] Patient charts were used to collect data on clinical characteristics, laboratory measurements, and electrocardiographic and echocardiographic measurements. Baseline was set at the time of the first biopsy containing amyloid. Measurements were considered to be performed at "baseline" if they took place within a window of 1 year previously or past the date of the positive biopsy. Patients with iatrogenic ATTR amyloidosis caused by a domino liver transplantation of a donor with ATTRm amyloidosis were excluded. The study conformed to the Principles of the Declaration of Helsinki and the local ethics committee.

Cardiac involvement was defined by the presence of structural myocardial wall abnormalities and/or conduction disturbances.[7] Structural abnormalities were defined as otherwise unexplained increased left (>11 mm) or right (>6 mm) ventricular wall thickness.[8] Right ventricular involvement, a common finding in systemic amyloidosis,

was included in this definition.[9] Conduction disturbances were defined as second or third-degree atrioventricular block, or hemi- or complete bundle branch block. Low-voltage electrocardiogram was defined by the Sokolow-Lyon index.[10]

The reason for performing the biopsy was retrieved and classified as the presenting phenotype. Furthermore, as part of specialized care, patients underwent cardiac investigations such as echocardiography, electrocardiography, ambulatory 24-hour electrocardiographic monitoring, and measurement of serum NT-proBNP using a standard immunoassay (Elecsys 2010; Roche Diagnostics),[11] and usually patients were referred to the cardiologist of the team. When the left ventricular ejection fraction had been measured as a range, the median value of that range was used for analysis. Neurophysiologic tests were performed to detect polyneuropathy, autonomic neuropathy (Ewing tests[12]), or thin fiber neuropathy. Vitreous opacities were documented by ophthalmologic examination. During follow-up, these tests were repeated when deemed necessary by the attending physician.

Data are presented as medians with interquartile ranges, means with standard deviations, or percentages depending on the variable. For testing significance of differences between the 2 groups, the $\chi 2$ test, the Kruskal-Wallis equality-of-populations rank test, or the 1-way analysis of variance was used where appropriate. The Pearson test was used for the correlation analyses. Kaplan-Meier curves were used for survival and analyzed using the log-rank test. Univariate and multivariate Cox proportional hazard regression models were used to calculate the hazard ratio of cardiac involvement on mortality. To determine the role of the biomarker NT-proBNP in the discrimination of patients with or without cardiac involvement, a receiver operating characteristic (ROC) curve was constructed. A 2-tailed p value of <0.05 was considered to be statistically significant. Analyses were conducted using STATA version 13 (StataCorp LP, College station, TX).

Results

In total, 155 patients with proven or suspected ATTR were referred to our center between 1994 and 2016. Thirty-five patients were excluded based on a negative biopsy, 12 patients were excluded based on missing biopsy data, 20 patients were excluded because a *TTR* gene mutation was lacking (wildtype variant), 10 patients were excluded because no sufficient clinical data were available, and 1 patient was excluded because of iatrogenic amyloidosis caused by domino liver transplantation. The remaining 77 patients were included for further analysis, including the 29 patients who underwent a liver transplantation during follow-up. In 30 of the 77 patients, the *TTR* V30M genotype was present, and the non-V30M group consisted of 47 patients. Nine different *TTR* gene mutations were present in the non-V30M group: Y114C (N = 23), V71A (N = 8), G47E (N = 7), G89K (N = 3), V122I (N = 2), V94A (N = 1), A45G (N = 1), A36P (N = 1), and S23N (N = 1).

Baseline characteristics are provided in *Table 1*. Symptoms of a peripheral neuropathy were the most frequent reason for performing the biopsy in both the V30M group (77%) and the non-V30M group (51%). Symptoms of a cardiomyopathy were much less frequently the reason for a biopsy in both the V30M (7%) and the non-V30M group (15%). During diagnostic work-up at baseline, peripheral neuropathy (78%) and autonomic dysfunction (57%) were frequently present. Symptoms of heart failure were absent in most patients.

At baseline, 39 (51%) patients met the criteria of cardiac involvement, and this did not differ between the 2 genotype groups. In the Supplement *Table S1* the baseline data is classified by cardiac involvement (*Table S1*) and also classified by cardiac reason for the biopsy (*Table S2*). All 9 (12%) patients with a cardiac reason for the biopsy had cardiac involvement. Of the remaining 68 (88%) patients with a noncardiac reason for biopsy, 30 (44%) patients had cardiac involvement, including 2 of 8 patients who were screened because of family history. Supplementary *Table S3* shows the results of logistic regression on cardiac involvement.

Table 1: Clinical characteristics at baseline

Variable	Total (n=77)	Non-V30M (n=47)	V30M (n=30)	P
Age at diagnosis (years + SD)	54 + 14	50.5 ± 11.4	56.3 ± 16.1	0.067
Age at death (years + SD)	61 + 10	58.4 ± 9.5	65.0 ± 9.2	0.067
Men	55.8%	46.8%	70%	0.024
Reason for a biopsy, signs of:				
Peripheral neuropathy	61.0%	51.1%	76.7%	0.025
Autonomic dysfunction	2.6%	2.1%	3.3%	0.750
Cardiomyopathy	11.7%	14.9%	6.7%	0.270
Ocular opacities	7.8%	10.6%	3.3%	0.240
CTS	3.9%	6.4%	0.0	0.160
Family history	10.0%	12.8%	6.7%	0.390
Miscellaneous	3.0%	2.1%	3.3%	0.746
Clinical status				
Peripheral neuropathy	77.6%	72.3%	86.3%	0.160
Autonomic dysfunction	57.1%	59.6%	53.6%	0.590
Gastro-intestinal complaints	32.0%	34.0%	28.6%	0.620
Cardiac involvement	51.0%	49.0%	53.0%	0.707
Ocular opacities	22.0%	31.9%	6.7%	0.009
CTS	19.7%	23.4%	13.8%	0.310
NYHA class				0.250
I	80.5%	87.2%	72.4%	0.063
II	13.0%	8.5%	20.7%	0.144
III	5.2%	4.3%	6.9%	0.642
IV	0	0	0	-
Blood pressure (mmHg)				
Peak Systolic	130.0 (115.0, 145.0)	130.0 (110.0, 140.0)	140.0 (120.0, 150.0)	0.043
End Diastolic	80.0 (70.0, 88.0)	80.0 (70.0, 88.0)	80.0 (70.0, 87.5)	0.900
Electrocardiographic findings				
Atrial fibrillation	2.8%	2.2%	3.8%	0.680
Low voltage QRS complexes	4.2%	4.3%	3.8%	0.920
Bundle branch block	13.9%	13.0%	15.4%	0.780
1st degree AV-block	11.7%	8.5%	16.6%	0.190

Table 1: Clinical characteristics at baseline

Variable	Total (n=77)	Non-V30M (n=47)	V30M (n=30)	P
2nd/3rd degree AV-block	0	0	0	-
Echocardiogram findings				
Septum wall thickness (mm)	11.0 (9.0, 13.0)	11.0 (9.0, 13.0)	11.0 (10.0, 14.0)	0.310
Left posterior wall thickness (mm)	10.0 (8.0, 12.0)	10.0 (8.4, 12.0)	9.0 (8.0, 12.0)	0.430
Right ventricular wall thickness (mm)	5.0 (4.0, 6.5)	5.0 (4.0, 6.0)	5.0 (4.0, 7.0)	0.820
LVEF (%)	60 (60, 60)	60.0 (60.0, 60.0)	60.0 (56.0, 60.0)	0.320
Laboratory findings				
NT-proBNP (ng/L)	202 (78, 646)	177 (67, 677)	210 (117, 595)	0.390
Creatinine (µmol/L)	83 (73, 93)	80 (66, 93)	85 (81, 94)	0.340
eGFR (ml/min * 1.73m2)	80.0 (70.0, 104.0)	79 (66.0, 97.0)	82.0 (74.0, 110.0)	0.140

Data are presented as medians with interquartile ranges, means with standard deviations, or percentages depending on the nature of the variable. CTS: carpal tunnel syndrome. eGFR: estimated glomerular filtration rate. NYHA: New York Heart Association functional classification of heart failure. LVEF: left ventricular ejection fraction.

Table 2: Cox regression analyses

		HR (95% CI)	P-value
Cardiac Involvement	Univariate	6.23 (2.49 - 15.6)	0.000
	Model 1	6.34 (2.20 - 18.3)	0.001
	Model 2	5.95 (2.12 - 16.7)	0.001

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, systolic pressure, eGFR, and V30M genotype

In *Figure 1A*, the serum levels of NT-proBNP and age at diagnosis are depicted for males and females. A correlation was present in both males and females. *Figure 1B* depicts the NT-proBNP serum levels and age at diagnosis for the V30M and non-V30M groups. The regression lines of both V30M and non-V30M are shown and seem to run parallel and nearby the 97.5% upper limit of controls.[13] *Figure 1C* depicts the NT-proBNP serum levels and age at diagnosis for patients with and without cardiac involvement. Levels were higher in the patients with cardiac involvement ($P < 0.001$).

In *Figure 2*, the ROC curve for NT-proBNP for cardiac involvement is shown; the area under the curve was 0.905. Based on this ROC curve, 2 cutoff levels were set: one with high sensitivity and another with high specificity. The sensitive cutoff level of 164 ng/L had negative predictive value 87.5%. The specific cutoff level of 365 ng/L had a positive predictive value of 93%. The 2 chosen cut-off levels are shown in *Figure 1C*. The literature-based cutoff level of 125 ng/L[14] yielded a sensitivity of 92% and a specificity of 65%.

Median follow-up was 6 years, and in this period, 33 (43%) patients died. Mean age at death was 61 years. The Kaplan Meier survival curve (*Figure 3A*) shows that no significant difference in mortality was observed between the V30M group and the non-V30M group. *Figure 3B* shows that patients with cardiac involvement at baseline had reduced survival during follow-up compared with patients without cardiac involvement. Patients with cardiac involvement had a 50% all-cause mortality of 4 years, whereas the 50% all-cause mortality was not reached in the patients without cardiac involvement.

Supplementary *Figure S1* shows the survival curves for patients with high and low NT-proBNP. A Cox survival analysis was performed to determine the hazard ratio of cardiac involvement on mortality (*Table 2*). Even when adjusted for age, sex, systolic pressure, renal function, and V30M genotype, cardiac involvement remained associated with increased mortality (model 2 in *Table 2*).

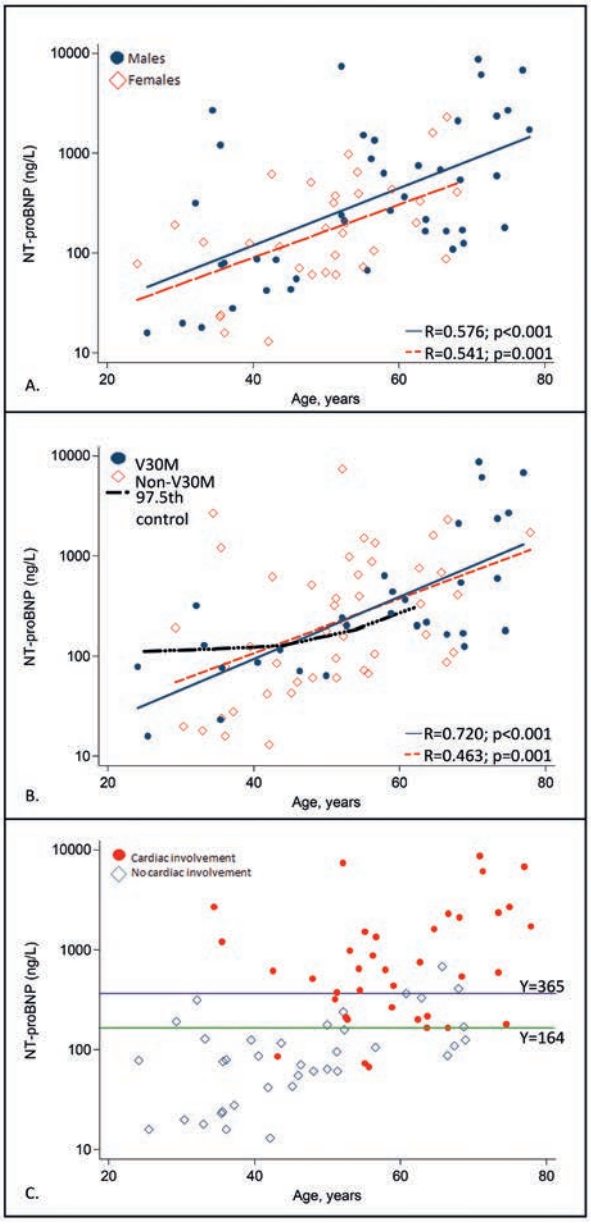


Figure 1. Log-transformed serum NT-proBNP values and age at diagnosis. (A) Males (filled circles) and females (open diamonds). Linear regression lines for males (solid line) and females (dashed line). (B) V30M (filled circles) and non-V30M (open diamonds) genotypes. Linear regression lines for V30M (solid line) and non-V30M (dashed line) genotypes. Inserted is the 97.5% upper limit (UL) of controls (dashed line). (C) Patients with cardiac involvement (filled circles) and patients without cardiac involvement (open diamonds). The 2 horizontal lines are the chosen cutoff values of NT-proBNP, 164 ng/L and 365 ng/L, respectively.

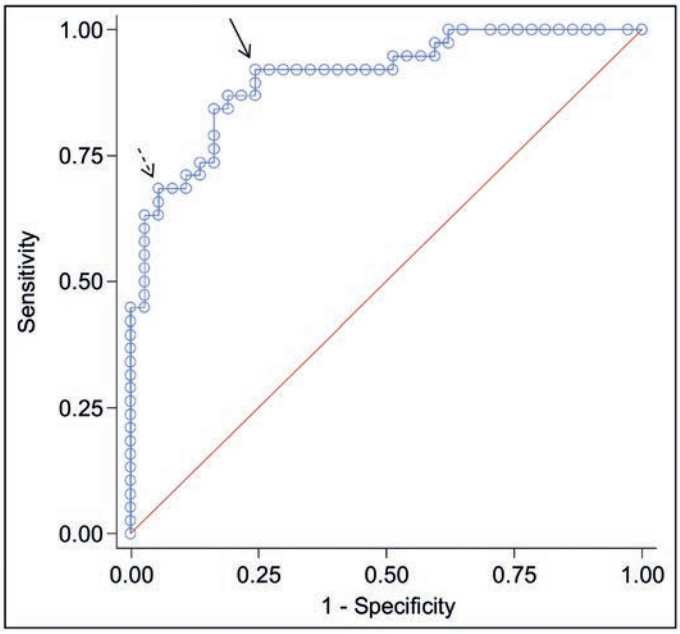


Figure 2. ROC curve of serum NT-proBNP for cardiac involvement. The 2 chosen cutoff values are shown with arrows: 164 ng/L, with sensitivity 92% and specificity 76% (solid arrow), and 365 ng/L, with sensitivity 92% and specificity 68% (dashed arrow).

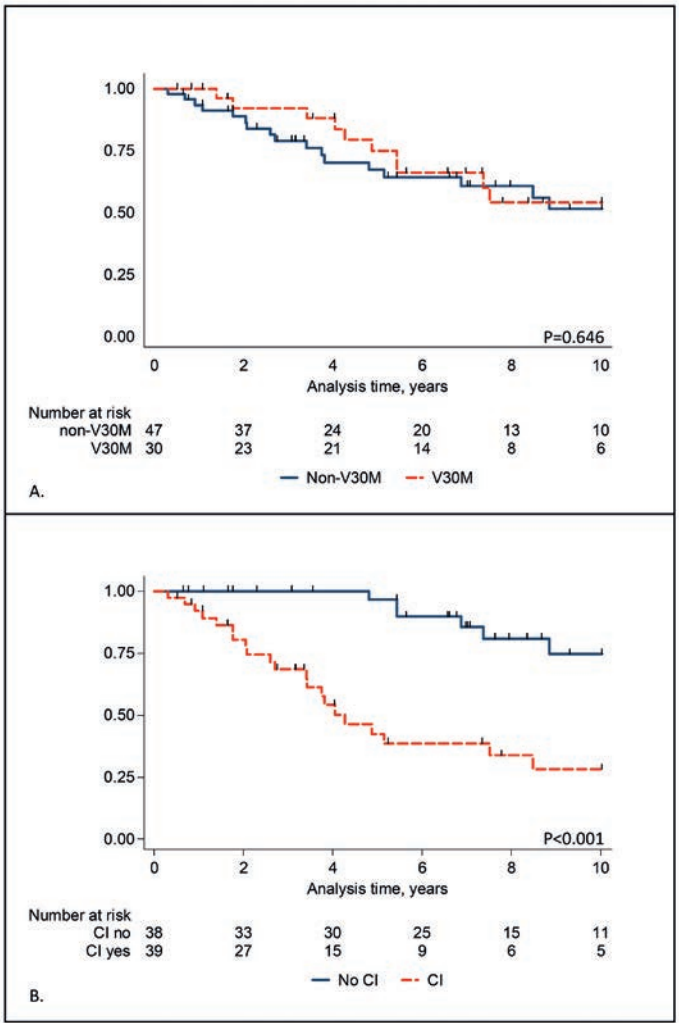


Figure 3. Kaplan-Meier survival curves after diagnosis. Censored values are shown as ticks. CI, cardiac involvement. (A) Patients with V30M (dashed line) and non-V30M (solid line) genotypes. (B) Patients with cardiac involvement (dashed line) and patients without cardiac involvement (solid line).

Discussion

This study shows that cardiac involvement is present in half of the patients presenting with ATTRm amyloidosis, regardless of initial clinical presentation and genotype (V30M vs non-V30M). Furthermore, the results indicate that cardiac involvement is associated with adverse outcomes, independent of other clinical characteristics. Lastly, NT-proBNP appears to be a useful marker in determining cardiac involvement in patients presenting with ATTRm amyloidosis. Although ATTRm amyloidosis is a heterogeneous disease,

most TTR mutations induce neurologic symptoms. It was therefore initially described as familial amyloid polyneuropathy.[15] However, *Rapezzi et al.* described a mixed presentation of both neurologic and cardiac symptoms in their Italian cohort of patients with ATTR amyloidosis comprising a sizeable number of mutations.[7] Because of these findings, we aimed to investigate the presence of cardiac involvement in our population of Dutch patients with ATTRm amyloidosis subdivided in V30M and non-V30M *TTR* genotypes.

Length-dependent peripheral neuropathy is typically seen in patients with ATTR amyloidosis and is characterized by numbness, spontaneous pain, muscle weakness, and atrophy. Similar to autonomic dysfunction, these peripheral neuropathic characteristics all contribute to a decreased performance and impaired exercise tolerance.[16] Exercise intolerance can already be explained by these neurologic limitations; however, one of the hallmarks of cardiac disease is also a decreased exercise tolerance.[17] Irrespective of phenotype, patients with ATTR amyloidosis do not challenge their exercise capacity and therefore cardiac symptoms may be camouflaged by concurrent neurologic symptoms, making cardiac involvement of amyloidosis a challenging feature to diagnose. More speculative is that autonomic dysfunction may cause lower filling pressures in the heart because of venous blood pooling, thereby masking underlying cardiac abnormalities.[18]

In only a small proportion of our population the reason for a biopsy was symptoms suggestive of a cardiomyopathy, their presenting symptoms were shortness of breath, edema, and/or signs of congestion. Typical amyloidosis related electrocardiogram findings, that is, low voltages and advanced atrioventricular blocks, were not prominent, and evident echocardiographic abnormalities were also absent in most of these patients at presentation.[19] Of note, the criteria of cardiac involvement used in this study were a little less stringent than those used by *Rapezzi et al.*[7] In our study, cardiac hypertrophy was defined as an interventricular septum or posterior wall thickness of >11 mm or when right ventricular hypertrophy (>6 mm) was present, whereas in the study of Rapezzi et al right ventricular dimensions were not included in the definition, and a thickness of 13 mm or greater was used for the left ventricle. In our opinion, especially the wall thickness cutoff criteria of our study are more in line with regard to the current echocardiography guidelines.[8] Irrespective of the noncardiac clinical presentation or the genotype, the heart was already affected at diagnosis in about half of our patients. If myocardial radiotracer uptake on bone scintigraphy had been used to detect cardiac involvement in this study, even more patients might have been identified.[20]

In recent years, the natriuretic peptide BNP and NT-proBNP biomarkers became available in heart failure. These natriuretic peptides predict outcome not only in the general population,[21] but also in patients with heart failure.[22] Several studies described the prognostic value of NT-proBNP on outcome in patients with ATTR amyloidosis, including a recent study using the THAOS Registry, but few studies described the diagnostic value of NT-proBNP of cardiac involvement in these patients.[4,5,23,24] The

NT-proBNP levels did not differ in the 2 genotype groups (V30M vs non-V30M), and the NT-proBNP levels correlated with age for both males and females in both genotype groups. This age-related increase of NT-proBNP in our study group was at a higher level than the well-known physiological age-dependent increase of NT-proBNP.[13] It therefore appears that cardiac involvement in ATTRm amyloidosis is not so much determined by genotype but by the age at disease onset.

The current findings emphasize the importance of NT-proBNP measurements in patients with ATTR amyloidosis, because increased serum levels of NT-proBNP reveal cardiac involvement.[14] In as many as half of our patients, cardiac involvement could be demonstrated at presentation, and our results indicate that NT-proBNP is a good diagnostic tool for medical specialists other than cardiologists to detect cardiac involvement.

Recognition of cardiac involvement is of great importance in the proper treatment of ATTR amyloidosis. This study shows that cardiac involvement predicts overall survival and confirms previous studies.[23,25] In the last few years, TTR tetramer-stabilizing treatment for ATTR amyloidosis became available for polyneuropathy.[26] A similar randomized clinical trial in patients with cardiomyopathy as disease manifestation will be completed in 2018.[27] Early identification of cardiac involvement is therefore mandatory to treat amyloidosis adequately, because late-stage cardiomyopathy probably will benefit less from treatment.[16]

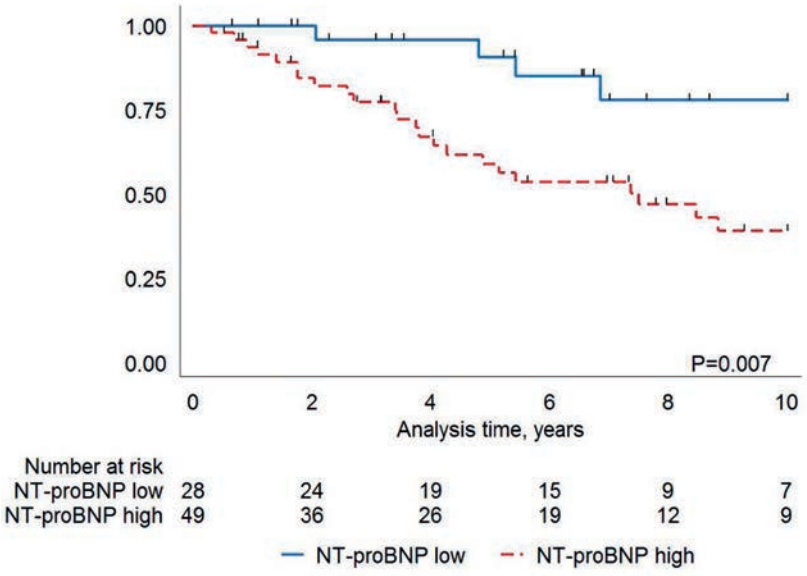
Limitations of our study are the relatively small population size and small number of mutations. Loss of renal function and cardiac rhythm abnormalities may cause elevated NT-proBNP levels, but severe renal function loss was not present, whereas only 2 patients had atrial fibrillation. Because of the long study period of more than 20 years, it was not possible to use more recent and advanced techniques to detect cardiac involvement, such as bisphosphonate scintigraphy, magnetic resonance imaging T1-myomapping, and strain measurement and speckle tracking by echocardiography.

In conclusion, cardiac involvement in ATTRm amyloidosis is not limited to certain genotypes or a specific clinical presentation and is of great influence on the overall survival. Measuring the cardiac biomarker NT-proBNP is useful in the early evaluation of cardiac involvement in ATTR amyloidosis.

Disclosures

The authors have no conflicts of interest to disclose.

Supplementary material:



Supplementary figure 1: Kaplan-Meier survival curves between patients with low values of NT-proBNP (solid line and ≤125 ng/L) and high values of NT-proBNP (dashed line and >125 ng/L). Censored values are shown as ticks.

Supplementary table 1: Clinical characteristics at baseline stratified for cardiac involvement

	No cardiac involvement (Endpoint)	Cardiac involvement (Endpoint)	P
	N= 38	N= 39	
Age at diagnosis (years ± SD)	46.1 ± 13.0	59.3 ± 10.9	<0.001
Age at death (years ± SD)	60.2 ± 11.3	60.8 ± 9.3	0.880
Men	47.4%	64.1%	0.140
Reason of biopsy, sign of:			
Peripheral neuropathy	60.5%	61.5%	0.930
Cardiomyopathy	0.0%	23.1%	0.002
Ocular opacities	10.5%	5.1%	0.380
Autonomic dysfunction	5.3%	0.0%	0.150
Family history	15.8%	5.1%	0.130
CTS	5.3%	2.6%	0.540

Supplementary table 1: Clinical characteristics at baseline stratified for cardiac involvement

	No cardiac involvement (Endpoint)	Cardiac involvement (Endpoint)	P
	N= 38	N= 39	
Clinical status			
Peripheral neuropathy	70.3%	84.6%	0.130
Autonomic dysfunction	52.6%	61.5%	0.430
Gastro-intestinal complaints	24.3%	39.5%	0.160
Ocular opacities, %	18.4%	25.6%	0.450
CTS	18.9%	20.5%	0.860
NYHA class			0.060
I	92.1%	71.1%	0.011
II	5.3%	21.1%	0.047
III	2.6%	7.9%	0.317
IV	0.0	0.0	-
Blood pressure (mmHg)			
Peak Systolic	130.0 (112.5, 145.0)	130.0 (115.0, 150.0)	0.660
End Diastolic	75.0 (70.0, 85.0)	80.0 (75.0, 90.0)	0.054
Electrocardiographic findings			
Atrial fibrillation	0.0%	5.6%	0.150
Low voltage QRS complexes	0.0%	8.3%	0.077
Bundle branch block	0.0%	27.8%	<0.001
1st degree AV-block	5.2%	17.9%	0.075
2nd/3rd degree AV-block	0.0	0.0	-
Echocardiogram findings			
Septum wall thickness (mm)	9.0 (8.0, 10.0)	13.0 (12.0, 16.0)	<0.001
Left posterior wall thickness (mm)	8.4 (7.3, 10.0)	12.0 (11.0, 15.0)	<0.001
Right ventricular wall thickness (mm)	4.0 (3.0, 5.0)	6.0 (5.0, 7.0)	0.016
LVEF (%)	60.0 (60.0, 60.0)	60.0 (60.0, 60.0)	0.490
Laboratory findings			
NT-proBNP (ng/L), median (IQR)	86.0 (43.0, 159.0)	621.5 (217.0, 1710.0)	<0.001
eGFR (ml/min * 1.73m2)	94.0 (77.0, 109.5)	78.0 (66.0, 90.0)	0.014
Creatinine (µmol/L)	84.0 (65.0, 93.0)	82.5 (75.0, 93.0)	0.780

Data are presented as medians with interquartile ranges, means with standard deviations, or percentages depending on the nature of the variable. CTS: carpal tunnel syndrome. eGFR: estimated glomerular filtration rate. NYHA: New York Heart Association functional classification of heart failure. LVEF: left ventricular ejection fraction.

Supplementary table 2: Clinical characteristics at baseline stratified for cardiac reason of biopsy

	Non cardiac presentation (n=68)	cardiac presentation (n=9)	p-value
Age at diagnosis (years ± SD)	51.5 ± 13.3	62.4 ± 12.8	0.024
Age at death (years + SD)	60.6 ± 9.1	60.8 ± 14.4	0.970
Men	52.9%	77.8%	0.160
Reason of biopsy, signs of:			
Peripheral neuropathy	69.1%	0.0%	<0.001
Autonomic dysfunction	2.9%	0.0%	0.600
Cardiomyopathy	0%	100.0%	0.002
Ocular opacities	8.8%	0.0%	0.350
CTS	4.4%	0.0%	0.520
Family history	11.8%	0.0%	0.280
Miscellaneous	3%		
Clinical status			
Peripheral neuropathy	77.6%	77.8%	0.990
Autonomic dysfunction	60.3%	33.3%	0.120
Gastro-intestinal complaints	30.3%	44.4%	0.390
Ocular opacities	22.1%	22.2%	0.990
CTS	17.9%	33.3%	0.280
NYHA class			<0.001
I	88.0%	33.3%	<0.001
II	9.0%	44.4%	0.003
III	3.0%	22.2%	0.014
IV			
Blood pressure (mmHg)			
Peak Systolic	130.0 (115.0, 150.0)	130.0 (130.0, 140.0)	0.670
End Diastolic	80.0 (70.0, 88.0)	85.0 (80.0, 85.0)	0.370
Electrocardiographic findings			
Atrial fibrillation	1.6%	12.5%	0.076
Low voltage QRS complexes	1.6%	25.0%	0.002
Bundle branch block	10.9%	37.5%	0.041
1st degree AV-block	7.8%	50.0%	<0.001
2nd/3rd degree AV-block	0	0	-
Echocardiogram findings			
Septum wall thickness (mm)	10.0 (9.0, 12.0)	16.0 (15.0, 20.0)	<0.001
Left posterior wall thickness (mm)	9.6 (8.0, 12.0)	15.5 (13.0, 17.0)	0.003
Right ventricular wall thickness (mm)	5.0 (4.0, 6.0)	7.0 (5.0, 8.0)	0.086

Supplementary table 2: Clinical characteristics at baseline stratified for cardiac reason of biopsy

	Non cardiac presentation (n=68)	cardiac presentation (n=9)	p-value
LVEF (%)	60.0 (60.0, 60.0)	53.0 (47.0, 60.0)	0.007
Laboratory findings			
NT-proBNP (ng/L)	173.5 (76.0, 513.0)	1601.0 (748.0, 2116.0)	0.006
Creatinine (µmol/L)	81.0 (70.0, 106.0)	78.0 (64.0, 82.0)	0.140
eGFR (ml/min * 1.73m2)	84.0 (73.0, 93.0)	77.5 (75.0, 80.0)	0.490

Data are presented as medians with interquartile ranges, means with standard deviations, or percentages depending on the nature of the variable. CTS: carpal tunnel syndrome. eGFR: estimated glomerular filtration rate. NYHA: New York Heart Association functional classification of heart failure. LVEF: left ventricular ejection fraction.

Supplement 3: Logistic regression and Odds ratio on cardiac involvement.

	Odds Ratio (95% CI)	P-value
Genotype, (non-V30M = base)	1.19 (0.48 - 2.98)	0.707
Age, yrs	1.09 (1.09 - 1.14)	0.000
Sex, (female gender = base)	1.98 (0.79 - 4.94)	0.141
ECG findings		
PR-interval	1.02 (0.99 - 1.03)	0.074
QRS-time	1.03 (0.99 - 1.06)	0.073
QTc-time	1.03 (1.01 - 1.04)	0.008
Laboratory findings		
NT-proBNP (per doubling)	3.36 (1.93 - 5.81)	0.000
eGFR	0.98 (0.96 - 1.00)	0.020
Creatinine	1.02 (0.95 - 1.09)	0.612

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