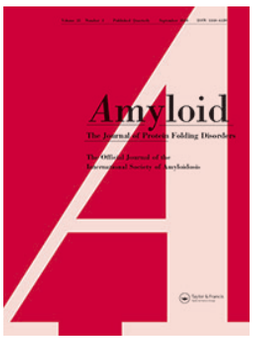

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ORIGINAL ARTICLE

Hereditary transthyretin amyloidosis: baseline characteristics of patients in the NEURO-TTR trial

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ABSTRACT

Background: Hereditary transthyretin (ATTRm) amyloidosis is a rare, progressive and fatal disease with a range of clinical manifestations.

Objective: This study comprehensively evaluates disease characteristics in a large, diverse cohort of patients with ATTRm amyloidosis.

Methods: Adult patients ($N = 172$) with Stage 1 or Stage 2 ATTRm amyloidosis who had polyneuropathy were screened and enrolled across 24 investigative sites and 10 countries in the NEURO-TTR trial (www.clinicaltrials.gov, NCT01737398). Medical and disease history, quality of life, laboratory data, and clinical assessments were analyzed.

Results: The NEURO-TTR patient population was diverse in age, disease severity, TTR mutation, and organ involvement. Twenty-seven different TTR mutations were present, with Val30Met being the most common (52%). One third of patients reported early onset disease (before age 50) and the average duration of neuropathy symptoms was 5.3 years. Symptoms affected multiple organs and systems, with nearly 70% of patients exhibiting broad involvement of weakness, sensory loss, and autonomic disturbance. Over 60% of patients had cardiomyopathy, with highest prevalence in the United States (72%) and lowest in South America/Australasia (33%). Cardiac biomarker NT-proBNP correlated with left ventricular wall thickness ($p < .001$). Quality of life, measured by Norfolk QoL-DN and SF-36 patient-reported questionnaires, was significantly impaired and correlated with disease severity.

Conclusions: Baseline data from the NEURO-TTR trial demonstrates ATTRm amyloidosis as a systemic disease with deficits in multiple organs and body systems, leading to decreased quality of life. We report concomitant presentation of polyneuropathy and cardiomyopathy in most patients, and early involvement of multiple body systems.

Abbreviations: Echo: echocardiogram; eGFR: estimated glomerular filtration rate; EU: Europe; GI: gastrointestinal; GLS: global longitudinal strain; Hgb: hemoglobin; HP: heat as pain; HRDB: heart rate with deep breathing; hsCRP: high-sensitivity C-reactive protein; LLN: lower limit of normal; LV: left ventricle; mNIS +7: modified neuropathy impairment score; NCV: nerve conduction velocity; NIS: neuropathy impairment score; NSC: neuropathy symptoms and change score; NYHA: New York Heart Association; NT-proBNP: n-terminal pro-hormone brain natriuretic peptide; P/C: protein/creatinine ratio; PCS: physical component summary; PND: polyneuropathy disability score; QoL: quality of life; RBC: red blood cells; SA: South America/Australasia; TP: touch-pressure; TTR: transthyretin; ULN: upper limit of normal; US: United States

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Transthyretin; amyloidosis; polyneuropathy; cardiomyopathy; quality of life

Introduction

Transthyretin (TTR) is a 55 kDa protein composed of four identical subunits, primarily synthesized in the liver and secreted into the plasma as a transporter of thyroxine (T4) and retinol (vitamin A). Hereditary transthyretin (ATTRm) amyloidosis is a rare, systemic, autosomal dominant genetic disorder caused by mutations in the TTR gene with an estimated 50,000 patients world-wide [1]. These mutations destabilize the normal tetrameric structure of TTR causing

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Supplemental data for this article can be accessed here.

dissociation into free monomers. The free monomers aggregate into insoluble fibrils which deposit as amyloid on tissues in multiple organs, including the peripheral nervous system, gastrointestinal (GI) tract, heart, kidneys, and eyes, resulting in local cell damage. Historically, the disease has been described as either a polyneuropathy when peripheral nerves are predominantly involved or a cardiomyopathy when the heart is predominantly affected. However, ATTRm amyloidosis is a single, systemic disease presenting with a wide spectrum of manifestations that frequently includes both nerve and heart involvement [2].

A major clinical manifestation of ATTRm amyloidosis is intractable peripheral sensorimotor and autonomic neuropathy. Sensory neuropathy first presents in the lower extremities, with paresthesia and hypoesthesia followed by neuropathic pain of the feet [3]. Gastrointestinal symptoms of diarrhea and/or profound constipation often follow, reflecting autonomic dysfunction [4,5]. Motor neuropathy results in mobility impairment. In patients with cardiomyopathy, TTR amyloid fibrils infiltrate the myocardium, which can result in diastolic dysfunction progressing to restrictive cardiomyopathy and heart failure [6]. A subset of patients will develop renal deposits, often with microalbuminuria as the initial presentation occurring within 3–5 years of disease onset, and progressing to renal failure ~5 years after microalbuminuria [7,8]. Life expectancy ranges from 3 to 15 years, with poorer prognosis when cardiomyopathy is present [1,9]. The most common causes of death are malnutrition and cachexia, renal failure, or cardiac disease [10].

There are over 100 amyloidosis-associated TTR mutations identified. One of the most common mutations, Val30Met, accounts for ~50% of ATTRm amyloidosis worldwide [11]. The prevalence of this mutation, however, varies greatly depending on geographical region [12,13]. The age of polyneuropathy symptom onset has been described as bimodal, with one peak in the third to fourth decade of life, and the other in the sixth decade [14]. Age of onset is also associated with the underlying TTR gene mutation and the geographical origin of the patient [15,16]. Individuals with TTR gene mutations that are predominantly associated with late onset disease (after age 50), have more cardiac abnormalities and a shorter overall life expectancy than those diagnosed with early onset disease (before age 50) [12,17,18].

This study describes the baseline demographic and disease characteristics of participants enrolled in NEURO-TTR, an international randomized, placebo-controlled 15-month phase 3 clinical trial evaluating the efficacy and safety of inotersen in patients with ATTRm amyloidosis [19]. Inotersen is a second-generation antisense oligonucleotide (ASO) inhibitor of both mutant and wild type TTR production [20].

The depth of information from the NEURO-TTR trial provides the opportunity to describe the disease manifestations of this rare disease in a relatively large ATTRm cohort from endemic and non-endemic regions of the world. Disease characteristics were assessed by genotype, geographical region, disease onset and duration, quality of life (QoL), and symptom manifestation.

Methods

Trial design and oversight

NEURO-TTR was a randomized, placebo-controlled, phase 3 trial designed to evaluate the efficacy and safety of inotersen in patients with ATTRm polyneuropathy and conducted at 24 sites worldwide from March 2013 to November 2017. The protocol was approved by each site's institutional review board or independent ethics committee. The trial was performed in compliance with the Declaration of Helsinki (2002) and Good Clinical Practice guidelines. All participants provided written informed consent before enrollment. Screening and baseline evaluations were performed during a 6-week screening and baseline assessment period.

Participants

Male and female participants 18–82 years of age with Stage 1 or 2 ATTRm amyloidosis, a Neuropathy Impairment Score (NIS) between 10–130, TTR mutation by genotyping, and biopsy-confirmed amyloid deposits were eligible to participate in the NEURO-TTR trial. Key exclusion criterion included New York Heart Association (NYHA) Class III and higher, previous liver transplant, presence of diabetes mellitus or other causes of neuropathy, platelets $<125 \times 10^9$/L, proteinuria >1.0 g/24 hours, and estimated glomerular filtration rate (eGFR) calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EIP) equation as <60 mL/min/1.73 m^2 (platelet and GFR criteria were modified by protocol amendment).

Baseline data collected

The collected data included demographics, geographic location (United States (US), Europe (EU), South America/Australasia (SA)), TTR mutation, disease and medical history, cardiac echocardiogram (Echo), NYHA classification and clinical laboratory tests. Baseline ATTRm disease status was measured using Coutinho Staging (Stage 1, walk unaided; Stage 2, walk with aid; Stage 3, wheelchair or bedbound) [21], polyneuropathy disability score (PND; Stage I, sensory disturbances in limbs without motor impairment; Stage II, difficulty walking without the need of a walking aid; Stage III, one stick or one crutch required for walking; Stage IV, two sticks or two crutches needed); and the modified neuropathy impairment score (mNIS +7) which consists of two components, the NIS composite score and the modified +7 composite score of physiological tests as previously described [22]. Nerve conduction, quantitative sensation testing (touch-pressure (TP) and heat as pain (HP) [22–26]) and heart rate with deep breathing (HRDB) were part of the modified +7. Two mNIS +7 assessments were performed prior to the first dose of study drug. In order to

assess the fraction of participants that had demyelinating features to their neuropathy, the proportion of participants with fibular nerve conduction velocities <39m/s, a fibular amplitude >1.0 mV and fibular nerve motor distal latency >5.6 ms were determined. Patient-perceived QoL was collected using the Norfolk QoL-DN and SF-36 patient-reported questionnaires [27,28]. Presence of neuropathy symptoms was also measured with the Neuropathy Symptoms and Change Score (NSC) score which is a physician administered questionnaire of the patient's neuropathy symptoms [29]. The NSC captures the presence and severity of four broad neuropathy symptom categories (muscle weakness (W), sensory loss (S), positive neuropathic sensory symptoms, including pain (P) and autonomic symptoms (A)).

Statistical analysis

This baseline characteristics study was performed on all eligible randomized participants. Statistical significance was determined by analysis of variance (ANOVA) and two-sample *t*-test with two-sided significance level of 0.05. Software utilized for the analyses was SAS version 9.3 or higher (SAS Institute, Cary, NC, USA).

Results

Demographics and disease baseline characteristics

The trial enrolled and dosed 172 participants with ATTRm amyloidosis across 10 countries; United States ($n = 82$), Portugal ($n = 24$), France ($n = 15$), Italy ($n = 8$), UK ($n = 6$), Spain ($n = 4$), Germany ($n = 3$), Brazil ($n = 22$), Argentina ($n = 7$) and New Zealand ($n = 1$). The study population was diverse with respect to age, TTR mutation, disease severity by neurological assessments and QoL, and organ involvement, reflecting the broad heterogeneity of patients with ATTRm amyloidosis worldwide (Table 1). The mean age of the cohort was 59 years, ranging from 27–81 years. The oldest and youngest patients both had Val30Met mutation, demonstrating diversity of presentation even within the same mutation. Approximately 60% of patients had prior treatment with TTR stabilizers (tafamidis or diflunisal) and 40% were treatment-naïve. One third of the cohort reported early onset of disease symptoms, and the average duration of neuropathy symptoms was 5.3 years. The mNIS +7 score ranged from 11–175 with about 1/3 of patients reporting no ambulatory disability (PND score I), 1/3 with some walking impairment (PND score II) and 1/3 requiring either one or two walking aids (PND score III–IV). Over 60% of patients had concomitant cardiomyopathy.

The mean ages across geographical regions were 65, 57 and 49 years for US, EU and SA respectively, with the US subgroup significantly older than either EU or SA (vs. EU, $p<.001$ and vs. SA, $p<.001$). The proportion of early onset patients varied by geographical region with over 60% of patients from SA with early onset disease (US 16%; EU 40%; SA 63%). The use of TTR stabilizers corresponded with standard of care and availability of these treatments in the different geographical regions. Previous treatment with tafamidis was more prevalent in the EU population (43 [72%] patients), while the majority of those treated with diflunisal were from the US (41 [50%] patients). SA patients, by contrast, were largely treatment-naïve (21 [70%] patients). Presence of cardiomyopathy was high in US patients (77%), compared with EU (57%) and SA (37%). Despite these differences, disease stage, duration of symptoms, and mean mNIS +7 scores were similar across geographic regions.

Comparing early versus late onset patients, late onset patients had shorter disease duration, were mostly males, and had a high frequency of cardiomyopathy. Early onset patients were more likely to be Stage 1, Val30Met, with lower PND scores and no cardiomyopathy. Patients who were previously treated with TTR stabilizers had longer disease duration compared to treatment-naïve patients, but were otherwise similar in age and disease stage and onset (Table 1).

A total of 27 TTR mutations were represented in this study population (Supplemental Figure S1). The most common mutation was Val30Met, occurring in 52% of patients, followed by Thr60Ala (13%), Leu58His (6%), Ser77Tyr (5%), and Phe64Leu (5%). Of the mutations with ≥5% prevalence in NEURO-TTR, patients with Phe64Leu were the oldest, all male, had the lowest frequency of cardiomyopathy, while patients with Ser77Tyr were the youngest, mostly female, and had the highest frequency of cardiomyopathy (Table 2). Comparing the Val30Met patients to the non-Val30Met patients, overall non-Val30Met patients were older with a higher frequency of late onset disease and cardiomyopathy. The prevalence of the Val30Met mutation was highest in SA (93%), with the US having the most genotypic diversity (26% Val30Met with 20 other genotypes represented), while diversity in the EU varied depending on country. Portugal had almost exclusively Val30Met whereas other countries had more diversity in TTR mutations.

Disease manifestations

Sensory, motor and autonomic neuropathy

Clinical baseline sensory, motor, and autonomic neuropathy status was assessed by the NSC score and mNIS +7 composite score. Components of each score were examined relative to TTR mutation. At baseline, 70% of patients exhibited symptoms associated with all four neuropathy categories, and another 19% exhibited muscle weakness plus two other categories (W-S-P, W-P-A, and W-S-A). These data suggest a broad involvement of weakness, sensory loss and autonomic disturbance. Similar results were seen when analyzed by mutation status (Table 2), noting that Thr60Ala and Ser77Tyr mutations had a higher percentage of patients with autonomic involvement. When the symptom category was examined by PND status, it showed approximately 50% of PND I patients already had a pan-modality of symptoms and this increased to 70% in PND II and almost 90% in PND III/IV (Supplemental Figure S2).

Table 1. Demographics and baseline characteristics by region, disease onset and prior treatment with TTR Stabilizers.

		Region			Disease onset			Prior treatment
	Total ($n = 172$)	US ($n = 82$)	Europe ($n = 60$)	SA ($n = 30$)	Early ($n = 56$)	Late ($n = 116$)	Stabilizers ($n = 99$)	Naïve ($n = 73$)
Age, yrs								
Mean (SD)	59.2 (13.0)	64.6 (8.7)	57.0 (13.8)	48.6 (13.9)	43.8 (9.3)	66.6 (6.3)	59.8 (13.2)	59.7 (13.0)
Min–Max	27–81	40–78	27–81	28–73	27–70	53–81	28–81	27–78
Sex								
Male, *n* (%)	118 (69%)	61 (74%)	39 (65%)	18 (60%)	33 (59%)	85 (73%)	65 (66%)	53 (73%)
mBMI[a], kg/m^2 g/L								
Mean (SD)	1025 (228)	1050 (245)	1008 (210)	986 (213)	989 (235)	1041 (224)	1034 (222)	1012 (238)
Min–Max	573–1752	573–1752	685–1687	669–1405	669–1710	573–1752	573–1752	630–1710
BP, mm Hg								
Mean, SBP / DBP	121 / 76	122 / 76	123 / 78	116 / 72	115 / 74	124 / 77	123 / 77	118 / 74
TTR, mg/dL								
Mean (SD)	21.5 (5.6)	20.5 (5.4)	21.7 (5.4)	23.7 (6.3)	22.0 (5.7)	21.2 (5.6)	22.5 (4.9)	20.1 (6.2)
Min–Max	8.6–39.7	9.6–35.3	8.6–33.4	11.8–39.7	11.8–39.7	8.6–33.4	9.8–35.3	8.6–39.7
TTR Mutation, *n* (%)								
Val30Met	89 (52%)	21 (26%)	40 (67%)	28 (93%)	40 (71%)	49 (42%)	52 (53%)	37 (51%)
Prior Treatment, *n* (%)								
Tafamidis	53 (31%)	3 (4%)	43 (72%)	7 (23%)	28 (50%)	25 (22%)	53 (54%)	0 (0%)
Diflunisal	49 (28%)	41 (50%)	6 (10%)	2 (7%)	6 (11%)	43 (37%)	49 (49%)	0 (0%)
None	73 (42%)	39 (48%)	13 (22%)	21 (70%)	22 (39%)	51 (44%)	0 (0%)	73 (100%)
Disease stage[b], *n* (%)								
Stage 1	116 (67%)	57 (70%)	38 (63%)	21 (70%)	44 (79%)	72 (62%)	68 (69%)	48 (66%)
Stage 2	56 (33%)	25 (30%)	22 (37%)	9 (30%)	12 (21%)	44 (38%)	31 (31%)	25 (34%)
Disease onset[c], *n* (%)								
Early	56 (33%)	13 (16%)	24 (40%)	19 (63%)	56 (100%)	0 (0%)	34 (34%)	22 (30%)
Duration of symptoms[d], yrs								
Mean (SD)	5.3 (4.4)	5.7 (5.7)	5.3 (2.7)	4.3 (2.4)	6.6 (5.4)	4.7 (3.7)	5.9 (4.3)	4.6 (4.4)
Presence of CM[e], *n* (%)	108 (63%)	63 (77%)	34 (57%)	11 (37%)	20 (36%)	88 (76%)	60 (61%)	48 (66%)
NYHA[f], *n* (%)								
I	111 (65%)	55 (67%)	33 (55%)	23 (77%)	45 (80%)	66 (57%)	67 (68%)	44 (60%)
II	61 (35%)	27 (33%)	27 (45%)	7 (23%)	11 (20%)	50 (43%)	32 (32%)	29 (40%)
NT-proBNP, pg/mL								
Mean (SD)	910 (1916)	1023 (1470)	1027 (2693)	362 (467)	354 (569)	1181 (2256)	665 (1103)	1254 (2640)
Min–Max	11–19084	31–7198	11–19,084	21–1743	11–2499	21–19,084	11–7387	21–19,084
mNIS +7								
Mean (SD)	78 (38)	71 (36)	88 (33)	76 (47)	79 (38)	77 (37)	81 (36)	73 (39)
(Min–Max)	(11–175)	(11–175)	(30–169)	(13–160)	(13–160)	(11–175)	11–175	13–165
NSC[g], *n* (%)								
W-P-S-A	120 (70%)	55 (67%)	46 (77%)	19 (63%)	40 (71%)	80 (69%)	68 (69%)	52 (71%)
W-S-P	18 (10%)	10 (12%)	5 (8%)	3 (10%)	6 (11%)	12 (10%)	15 (15%)	3 (4%)
W-P-A	11 (6%)	6 (7%)	3 (5%)	2 (7%)	1 (2%)	10 (9%)	3 (3%)	8 (11%)
W-S-A	5 (3%)	2 (2%)	2 (3%)	1 (3%)	3 (5%)	2 (2%)	3 (3%)	2 (3%)
S-P-A	3 (2%)	0 (0%)	2 (3%)	1 (3%)	2 (4%)	1 (1%)	1 (1%)	2 (3%)
Other	15 (9%)	9 (11%)	2 (3%)	4 (13%)	4 (7%)	11 (9%)	9 (9%)	6 (8%)
PND, *n* (%)								
I	55 (32%)	35 (43%)	10 (17%)	10 (33%)	16 (29%)	39 (34%)	26 (26%)	29 (40%)
II	61 (35%)	22 (27%)	28 (47%)	11 (37%)	28 (50%)	33 (28%)	42 (42%)	19 (26%)
III	45 (26%)	21 (26%)	18 (30%)	6 (20%)	9 (16%)	36 (31%)	28 (28%)	17 (23%)
IV	11 (6%)	4 (5%)	4 (7%)	3 (10%)	3 (5%)	8 (7%)	3 (3%)	8 (11%)

[a]Modified BMI (mBMI) adjusts for serum albumin
[b]Stage I does not require assistance with ambulation, Stage II requires assistance with ambulation
[c]Disease symptoms before age 50 (calculated by age minus years with symptoms).
[d]Duration of symptoms calculated as time between symptom onset and ICF date.
[e]Presence of cardiomyopathy was defined by a diagnosis of TTR cardiomyopathy at study entry and/or by the following criteria: interventricular wall thickness of $\geq$13 mm on transthoracic ECHO at baseline as ascertained by a central reader, no known history of persistent hypertension $\geq$150 mmHg within 12 months prior to screening.
[f]Study excluded NYHA III and higher
[g]Neuropathy Symptom and Change (NSC) domain categories: Muscle Weakness (W), Sensory Loss (S), Positive Neuropathy Sensory Symptoms (P), Autonomic (A). Other includes combinations of 2 domains (P-A, W-A, W-P, S-P) or a single domains (A).

Severity of muscle weakness and sensation loss as assessed by the NIS muscle weakness component and TP quantitative sensation (large fiber) showed roughly equal deficits across the mutation groups. However, HP sensation (small fiber) assessment showed that early onset Val30Met patients had a statistically significant increased deficit versus the late onset Val30Met patients, Thr60Ala, or other mutations combined (data not shown). These data suggest that early onset Val30Met patients have a higher prevalence of small fiber deficits versus late onset Val30Met patients. Examination of nerve conduction showed only three patients with demyelinating features present in the fibular nerve. This is consistent with demyelinating neuropathy being uncommon in ATTRm with polyneuropathy. The

Table 2. Baseline characteristics by TTR mutation (> =5% prevalence in NEURO-TTR).

	Total ($n = 172$)	Val30Met ($n = 89$)	Non-Val30Met ($n = 83$)	Thr60Ala ($n = 22$)	Leu58His ($n = 10$)	Ser77Tyr ($n = 9$)	Phe64Leu ($n = 8$)
Region[a]							
United States	82 (48%)	21 (24%)	61 (73%)	19 (86%)	10 (100%)	2 (22%)	7 (88%)
Europe	60 (35%)	40 (45%)	20 (24%)	2 (9%)	0 (0%)	6 (67%)	1 (13%)
South America	30 (17%)	28 (31%)	2 (2%)	1 (5%)	0 (0%)	1 (11%)	0 (0%)
Age, yrs							
Mean (SD)	59.2 (13.0)	55.5 (15.2)	63.1 (8.8)	65.8 (6.5)	65.3 (7.8)	62.4 (4.0)	69.4 (4.7)
Min–Max	27–81	27–81	40–78	52–78	52–75	55–68	59–74
Sex							
Male, *n* (%)	118 (69%)	63 (71%)	55 (66%)	17 (77%)	6 (60%)	5 (6%)	8 (100%)
mBMI[b], kg/m^2 g/L							
Mean (SD)	1025 (228)	1003 (209)	1047 (246)	1081 (305)	1206 (177)	1005 (205)	1036 (157)
Min–Max	573–1752	573–1687	630–1752	630–1710	883–1450	817–1388	775–1257
BP, mmHg							
Mean, SBP/DBP	121 / 76	120 / 75	123 / 76	123 / 76	133 / 81	122 / 80	123 / 78
TTR, mg/dL							
Mean (SD)	21.5 (5.6)	22.9 (5.3)	20.0 (5.6)	20.3 (5.8)	24.2 (5.8)	19.4 (4.9)	18.9 (3.4)
Min–Max	8.6–39.7	11.8–39.7	8.6–35.3	9.6–30.1	15.2–35.3	10.6–26.4	13.5–23.7
Prior Treatment, *n* (%)							
Tafamidis	53 (31%)	40 (45%)	13 (16%)	0 (0%)	1 (10%)	4 (44%)	0 (0%)
Diflunisal	49 (28%)	14 (16%)	35 (42%)	11 (50%)	7 (70%)	2 (22%)	4 (50%)
None	73 (42%)	37 (42%)	36 (43%)	11 (50%)	2 (20%)	3 (33%)	4 (50%)
Disease stage[c], *n* (%)							
Stage 1	116 (67%)	59 (66%)	57 (69%)	17 (77%)	5 (50%)	7 (78%)	5 (63%)
Stage 2	56 (33%)	30 (34%)	26 (31%)	5 (23%)	5 (50%)	2 (22%)	3 (38%)
Disease onset[d], *n* (%)							
Early onset	56 (33%)	40 (45%)	16 (19%)	3 (14%)	2 (20%)	0 (0%)	0 (0%)
Duration of symptoms[e], yrs							
Mean (SD)	5.3 (4.4)	5.3 (3.9)	5.4 (4.9)	5.4 (4.4)	6.0 (7.5)	4.6 (2.5)	5.2 (2.1)
Presence of CM[f], *n* (%)	108 (63%)	43 (48%)	65 (78%)	20 (91%)	4 (40%)	9 (100%)	3 (38%)
NYHA[g], *n* (%)							
I	111 (65%)	67 (75%)	44 (53%)	10 (45%)	9 (90%)	3 (33%)	5 (63%)
II	61 (35%)	22 (25%)	39 (47%)	12 (55%)	1 (10%)	6 (67%)	3 (38%)
NT-proBNP, pg/mL							
Mean (SD)	910 (1916)	829 (2332)	997 (1343)	1563 (1689)	192 (125)	641 (509)	234 (174)
Min–Max	11–19,084	11–19,084	31–7036	83–7036	37–432	207–1615	31–495
mNIS +7							
Mean (SD)	78 (38)	81 (39)	74 (36)	63 (32)	83 (37)	77 (28)	97 (34)
Min–Max	11–175	11–175	14–169	14–160	35–131	45–115	67–165
NSC[h], *n* (%)							
W-P-S-A	120 (70%)	61 (69%)	59 (71%)	15 (68%)	5 (50%)	6 (67%)	7 (88%)
W-S-P	18 (10%)	10 (11%)	8 (10%)	1 (5%)	3 (30%)	0 (0%)	1 (13%)
W-P-A	11 (6%)	2 (2%)	9 (11%)	4 (18%)	0 (0%)	3 (33%)	0 (0%)
W-S-A	5 (3%)	5 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
S-P-A	3 (2%)	3 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Others	15 (9%)	8 (9%)	7 (8%)	2 (9%)	2 (20%)	0 (0%)	0 (0%)
PND Score, *n* (%)							
I	55 (32%)	19 (21%)	36 (43%)	12 (55%)	3 (30%)	2 (22%)	2 (25%)
II	61 (35%)	40 (45%)	21 (25%)	5 (23%)	2 (20%)	5 (56%)	3 (38%)
III	45 (26%)	23 (26%)	22 (27%)	4 (18%)	4 (40%)	2 (22%)	1 (13%)
IV	11 (6%)	7 (8%)	4 (5%)	1 (5%)	1 (10%)	0 (0%)	2 (25%)

[a]US: Ala97Ser, Asp38Ala, Glu54Ser, Glu89Gln, Gly67Arg, Ile107Val, Ile84Ser, Leu58His, Lys35Thr, Lys79Asn, Phe33Leu, Phe64Leu, Pro24Ser, Ser50Arg, Ser77Tyr, Thr49Ala, Thr59Lys, Thr60Ala, Tyr114Cys, Val30Met, Val122Ile; Europe: Ala109Ser, Glu61Lys, Glu89Gln, Glu89Lys, Gly47Ala, Ile107Phe, Phe64Leu, Ser77Phe, Ser77Tyr, Thr49Ala, Thr60Ala, Val30Met; SA: Ser77Tyr, Thr60Ala, Val30Met.

[b]Modified BMI (mBMI) adjusts for serum albumin

[c]Stage I does not require assistance with ambulation, Stage II requires assistance with ambulation

[d]Disease symptoms before age 50 (calculated by age minus years with symptoms).

[e]Duration of symptoms calculated as time between symptom onset and ICF date.

[f]Presence of cardiomyopathy was defined by a diagnosis of TTR cardiomyopathy at study entry and/or by the following criteria: interventricular wall thickness of ≥13 mm on transthoracic ECHO at baseline as ascertained by a central reader, no known history of persistent hypertension ≥150 mmHg within 12 months prior to screening.

[g]Study excluded NYHA III and higher

[h]Neuropathy Symptom and Change (NSC) domain categories: Muscle Weakness (W), Sensory Loss (S), Positive Neuropathy Sensory Symptoms (P), Autonomic (A). Other includes combinations of 2 domains (P-A, W-A, W-P, S-P) or single domains (A).

reduction in nerve conduction velocity (NCV) was mild in those patients with an average fibular NCV of 37.0 ± 0.5 m/s and distal motor latency (DML) of 6.0 ± 0.3 ms.

Presence of autonomic neuropathy, as reported by patients, was consistent with the NSC results. Over 50% of patients had a history of gastrointestinal manifestations, which are generally attributed to deficits in the autonomic nervous system. GI amyloidosis or symptoms, such as recurring diarrhea, constipation, defecation urgency, GI mobility disorder, or GI hypomotility were reported in 80 (47%)

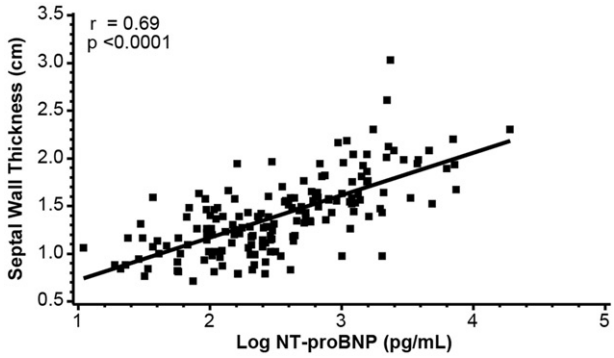

Figure 1. Relationship of NT-proBNP to LV Septal Wall Thickness.

patients; nausea, vomiting, or early satiety in 23 (13%) patients; and unintentional weight loss (abnormal loss of weight, weight decreased, cachexia, decreased appetite) in 31 (18%) patients. In addition, 61 (35%) patients reported a history of either orthostatic hypotension, sexual dysfunction, urinary tract infection (UTI), urinary retention/hesitation/incontinence or neurogenic bladder.

Cardiomyopathy and conduction abnormalities

Sixty three percent of patients had cardiomyopathy, including 19% with implanted pacemakers, and 21% reporting a history of cardiac blocks, atrial fibrillation and arrhythmia. An analysis of ECGs collected at baseline showed ~40% of patients with abnormalities; the most common being left axis deviation/inferior infarct pattern, first degree heart block, and anterior infarct pattern. Interestingly, low-voltage was less common in this population (observed in about 10% of the patients) compared with previous reports (25% Italian ATTRm amyloidosis population) [30].

Levels of N-terminal pro-hormone brain natriuretic peptide (NT-proBNP) correlates with LV dysfunction and can be used for early detection of cardiac impairment in patients with ATTRm amyloidosis [31]. NT-proBNP tracked with cardiac involvement and varied considerably by mutation. Val30Met early onset had lower levels versus Val30Met late onset, consistent with a higher degree of cardiomyopathy in the latter group. Thr60Ala, known to have significant cardiac involvement [13,32], showed one of the highest average NT-proBNP levels. There was a positive correlation between increased NT-proBNP concentrations and increased left ventricle (LV) wall thickness ($p<.0001$) (Figure 1). LV mass and NT-proBNP also showed strong positive correlations with global longitudinal strain (GLS) ($p<.0001$, $r=0.61$; and $p<.0001$, $r=0.56$ respectively), with increases in each parameter corresponding to increased or worse GLS (data not shown).

Laboratory abnormalities

Laboratory tests were assessed by geography, disease onset, and prior treatment (Supplemental Table S1), and TTR mutation (Supplemental Table S2). Low red blood cells (RBC) and hemoglobin (Hgb) levels were noted in many patients, with 41 (24%) and 49 (28%) patients below LLN at baseline, respectively. There were 32 (19%) patients with baseline high-sensitivity C-reactive protein (hsCRP) values above the upper limit of normal (ULN, 3 mg/L) with the mean value of 3.8 mg/L. Creatine kinase (CK) and high-sensitivity C-reactive protein (hsCRP) were higher in Val30Met and early onset patients.

Mild to moderate renal impairment and proteinuria as assessed by laboratory tests was common, especially in Val30Met patients. The mean eGFR was 88.4 mL/min/1.73 m^2, with 14 of 172 (8%) of patients below the LLN at baseline (normal range >60 mL/min/1.73 m^2), and lower mean eGFR in subgroups associated with older patients (i.e., US and late onset). The mean urine albumin/creatinine (A/C) ratio at baseline was 51.6 mg/g (normal range 0–16 male, 0–24 female) and mean urine protein/creatinine (P/C) ratio was 187.5 mg/g (normal range <200), with 59 of 172 (34%) patients above the ULN for one or both ratios. 41 of 59 patients who had A/C or P/C ratio > ULN, and 6 of 10 patients with >500 mg urine protein/24-hr had the Val30Met mutation.

Other assessments

Based on medical history, 34% of patients had a history of carpal tunnel syndrome and 14% of patients had ocular involvement based on the reported terms.

Quality of life characteristics

Patient-reported outcomes were used to assess patient-perceived burden of disease. Baseline QoL was evaluated though two instruments, the Norfolk QoL-DN and SF-36 questionnaires. The Norfolk QoL-DN assesses patient perception of symptoms associated with specific nerve fiber damage and has been validated in patients with ATTRm polyneuropathy [27]. The SF-36 is a more general questionnaire to measure overall health status, with the physical component summary (PCS) indicating overall physical functioning, bodily pain and general physical health [28].

The mean baseline Norfolk QoL-DN and SF-36 (PCS domain) scores were substantially worse in the NEURO-TTR cohort compared with controls. The Norfolk QoL-DN score was 48.4 ± 27.2 compared with 2.6 ± 5.0 for healthy volunteers (larger scores reflect worse QoL) [27]. The average SF36 PCS score was 36.3 ± 9.1 compared with 50 reported for the general US population (lower scores reflect worse QoL) [28]. There was a good correlation between severity of disease as measured by neuropathy instruments (mNIS +7 and PND score) and the patients QoL (Figure 2). Increased (worse severity) scores for Norfolk QoL-DN significantly correlated with increased mNIS +7 scores ($r=0.54$, $p<.001$). Likewise, increased walking disability corresponded to decreased (worsening of severity) SF-36 PCS scores (ANOVA overall $p<.001$; PND I vs II, $p=.016$; PND II vs III, $p=.011$; and PND III vs IV, $p=.221$). Subgroup analysis by TTR mutation and symptom onset (Val30Met early onset, Val30Met late onset, Thr60Ala, or

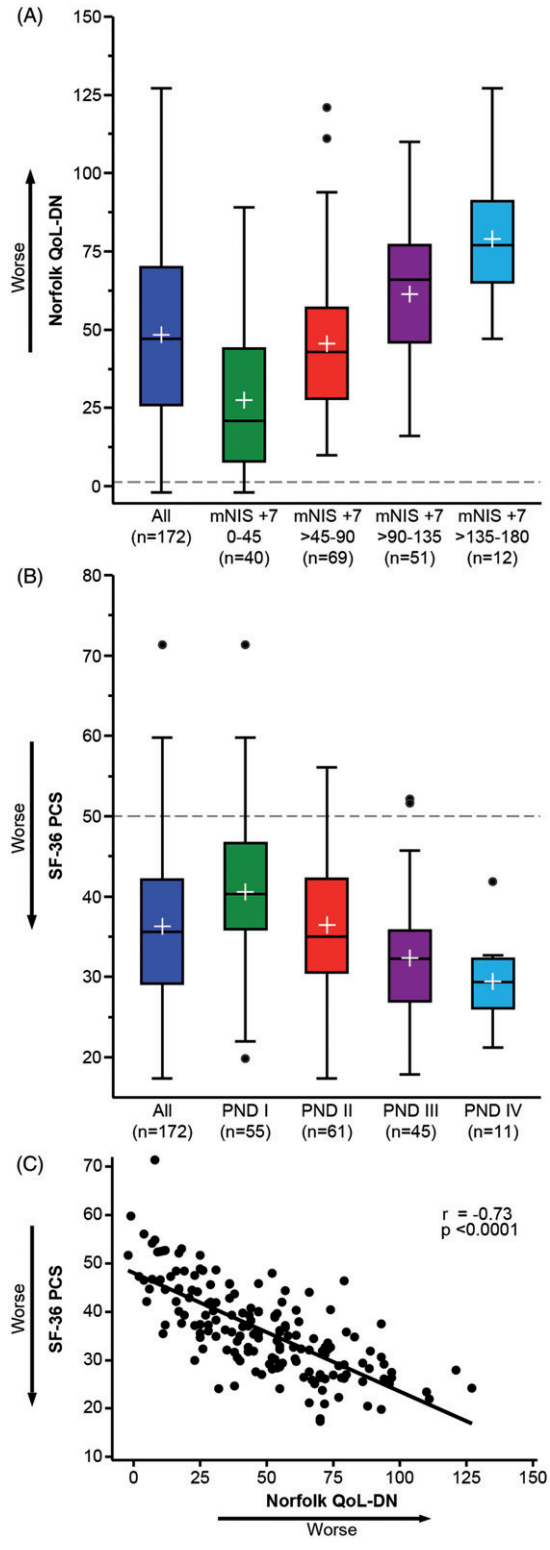

Figure 2. Quality of Life Corresponds to ATTRm Disease Severity (A) mNIS +7 composite score increases with worsening Norfolk QoL-DN score. The dotted horizontal line refers to the average Norfolk QoL-DN score in healthy volunteers ($n = 12$) [27]. (B) PND stage advances with worsening SF-36 PCS. The dotted horizontal line refers to the average SF-36 PCS score in the general US population [28]. (C) Norfolk Total Score increases with worsening SF-36 PCS.

others) showed similar trends as the cohort. Finally, there was a positive correlation between baseline Norfolk QoL-DN and SF-36 PCS baseline scores ($r = 0.73$, $p < .001$), with worse Norfolk QoL-DN scores associated with worse SF-36 PCS scores.

Discussion

Hereditary transthyretin amyloidosis is a rare, progressive, and fatal disease that presents with a wide spectrum of clinical manifestations, making natural history difficult to accurately describe. Diagnosis can be easily overlooked as patients often present with common and non-specific symptoms, and there is limited data on the natural history of ATTRm amyloidosis [33].

The NEURO-TTR study population reflects the broad heterogeneity of ATTRm. Detailed neurologic, cardiac, QoL and lab assessments enabled a detailed analysis of baseline characteristics in this cohort of 172 diverse ATTRm patients. Substantial differences in disease characteristics were noted between patients grouped by either region, mutation or age of onset. This heterogeneity is consistent with previous regional, site-specific and disease registry reports [12,32,34–37] and highlights the challenges of diagnosis.

Even though many of the patients had early stage neuropathy, nearly 70% of patients already had broad involvement of strength, sensation (both large and small fiber involvement), and autonomic disturbance. The neuropathy was primarily an axonal neuropathy with little demyelination being present. Besides neuropathy, there was significant cardiovascular, GI, renal and ocular involvement. Approximately 60% of patients had cardiomyopathy, many with heavy amyloid burden in the heart as measured by LV wall thickness. Autonomic dysfunction was broadly seen, but especially prevalent in the Thr60Ala patients. Renal impairment is common in patients with ATTRm amyloidosis, both as a consequence of cardiac impairment and amyloid deposits in the kidney [8]. In the NEURO-TTR cohort, renal amyloidosis was noted to be common in patients with the Val30Met mutation.

Hereditary ATTR is associated with a substantial disruption in activities of daily living, including employment rates and work productivity. In this study two distinct QoL measurements were used to quantitate the impact of ATTRm amyloidosis. Both instruments showed that in this disease, QoL was significantly impaired and there was a good correlation between the Norfolk QoL-DN and SF-36 PCS scores. Both instruments showed a decreased QoL correlated with clinical progression of polyneuropathy. These data correlate with previous reports that showed an association between Norfolk QoL-DN and NIS [22,27]. Caregivers also experience a large impact on QoL, with increased fatigue and mental health burden [1]. It will be important to further explore the impact of QoL not only on patients with ATTRm amyloidosis, but also on their caregivers.

Data reported here was limited by the NEURO-TTR study design, and the respective eligibility criteria. While the cohort was diverse in a number of key demographic parameters and disease characteristics, it excluded pre-symptomatic and severe ATTRm amyloidosis patients, such as Stage

3 patients and patients with NYHA III and higher, and was represented predominantly by Stage 1 patients.

Collectively, the medical history data and clinical and lab assessments performed at baseline in the NEURO-TTR study demonstrate a significant impact of ATTRm amyloidosis on multiple body systems and organs, as well as a correlation between worsening QoL and disease progression. This underscores the importance of a holistic approach to management.

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