

**National Consensus on the Diagnosis, Treatment, Monitoring, and Prevention of
Hereditary Transthyretin Amyloidosis**

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National Consensus on the Diagnosis, Treatment, Monitoring, and Rehabilitation of Hereditary Transthyretin Amyloidosis

Today, 23.04.2026 we, the undersigned specialists, have reached a consensus on the diagnosis, treatment, follow-up, and prevention of transthyretin familial amyloidosis

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Abbreviations used:

AD - Autosomal dominant
ACE inhibitor - Angiotensin-converting enzyme inhibitor
ARB - Angiotensin receptor blocker
GI tract - Gastrointestinal tract
DL - Distal latency
ECG - Electrocardiography
EMB - Endomyocardial biopsy
EMG - Electromyography
ENG - Electroneurography
EchoCG - Echocardiography
ECG - Electrocardiogram
CM - Cardiomyopathy
LEP - Laser-evoked potentials
LV - Left ventricle
LAHB - Left anterior hemiblock
MRI - Magnetic resonance imaging
LHT - Orthotopic liver transplantation
PNS - Peripheral nervous system
SCA - Senile cardiac amyloidosis
SNAP - Sensory nerve action potential
SMAP - Summarized muscle action potential
CV - Conduction velocity
ATTR - Transthyretin amyloidosis
TTR - Transthyretin
FAC - Familial amyloid cardiomyopathy
FAP - Familial amyloid polyneuropathy
EF - Ejection fraction
hATTR – Hereditary transthyretin amyloidosis

I. Introduction to hereditary transthyretin amyloidosis (hATTR).

Amyloidoses are a broad spectrum of diseases caused by changes in protein structure, whereby destabilization of the quaternary structure and subsequent breakdown into free monomers cause a normally soluble tetrameric protein to form insoluble extracellular fibrillar deposits, leading to organ dysfunction. All types of amyloids contain one main fibrillar protein that determines amyloid type, as well as smaller components. Over 20 different fibrillar proteins in humans have been associated with amyloidosis, each with a distinct clinical presentation. One such protein that forms human amyloid fibrils is transthyretin (Ando Y. et al. 2005)

TTR acts as a transport protein for thyroxine in plasma. TTR also transports retinol (vitamin A) through its interaction with retinol-binding protein. It circulates as a tetramer composed of four identical subunits. TTR can be found in plasma and cerebrospinal fluid. It is synthesized primarily in the liver, the choroid plexus of the brain, and, to a lesser extent, in the retina. The *TTR* gene is located on the long arm of chromosome 18 and contains 4 exons and 3 introns.

Systemic amyloidoses are designated with the capital letter A (for amyloid), followed by the abbreviation for the chemical nature of the fibrillar protein. For example, TTR amyloidosis is abbreviated as ATTR, and amyloidosis involving the deposition of immunoglobulin light chains is abbreviated as AL (Saraiva M. et al., 1984; Connors L. et al., 2003; Ando Y. et al., 2005).

The classification of identified genetic variants is of paramount importance for molecular genetic testing and its interpretation. The assessment of the pathogenicity of a given genetic variant must be based on scientific evidence and follow standardized nomenclature and rules. In this regard, the terms “mutation” and “polymorphism,” which have been widely used to date, have been replaced by a classification of genetic variants that distinguishes five categories: pathogenic, likely pathogenic, variant of uncertain clinical significance, likely non-pathogenic, and non-pathogenic (Richards S. et al., 2015; Nykamp K. et al., 2017).

II. Pathophysiology.

Both *TTR* with a normal sequence (wild type) and pathogenic genetic variants can cause amyloidosis. Wild-type *TTR* can cause cardiac amyloidosis in older adults, known as senile cardiac amyloidosis (SCA). When it was found that SCA is accompanied by microscopic deposits in many other organs, the alternative name senile systemic amyloidosis was proposed. Currently, both terms are used.

Pathogenic genetic variants in the *TTR* gene accelerate the formation of *TTR* amyloid and are the most important risk factor for the development of clinically significant ATTR. More than 140 amyloidogenic *TTR* variants cause systemic hereditary amyloidosis (Rowczenio D. 2014; Hou X. et al. 2007; Benson M.D., Kincaid JC, 2007; Parman Y. et al., 2016)

It is standard practice to name *TTR* variants according to the latest version of the international nomenclature <http://www.HGVS.org/varnomen>. An older version of the nomenclature does not account for a short signal sequence of 20 amino acids in the N-terminal domain within the protein structure, which leads to a shift in amino acid numbering when describing genetic variants in the *TTR* gene. Currently, a combined nomenclature is used, in accordance with Mutations in Hereditary Amyloidosis – [http://amyloidosismutations.com/main_menu.html].

Most variants that cause hereditary *TTR* are rare, but some are common in certain population groups. To date, the following variants in the *TTR* gene have been identified in Bulgarian patients:

- **c.148G>A, p.Val50Met (Val30Met)** is the first pathogenic variant in the *TTR* gene to be identified worldwide. This amino acid substitution is due to a missense variant in the *TTR* gene. This pathogenic variant is found worldwide and is the best-studied *TTR* variant, serving as the prototype for hATTR with a mutant sequence. The disease among relatives of index patients with *TTR* p.V30M is called FAP, as early symptoms present as peripheral neuropathy, but these patients actually have systemic amyloidosis, with multiple deposits, often involving the heart, gastrointestinal tract, the eyes, and other organs.
- The **c.200G>A; p.Gly67Glu (Gly47Glu)** pathogenic genetic variant was first identified in patients of Italian descent. The disease typically has an earlier onset,

a more aggressive course, and a short survival time. Subsequently, Bulgarian patients of Roma origin who carry this genetic variant have also been described (Pelo E. et al., 2002)

- The **c.214T>C, p.Ser72Pro (Ser52Pro)** pathogenic genetic variant was first described in British patients. Currently, it has also been identified in one Bulgarian family (Stangou, A. et al., 1998).
- The **c.290C>T, p.Ser97Phe (Ser77Phe)** genetic variant was first described in France. In Bulgaria, it has been identified in several families with a founder effect from the village of Vakarel. The leading phenotypic manifestations are polyneuropathy and cardiomyopathy (Planté-Bordeneuve V. et al., 1998).
- The **c.325G>C, p.Glu109Gln (Glu89Gln)** genetic variant was first described in Italy. It has been identified in a large number of Bulgarian patients. Most of the families are from the Kyustendil and Blagoevgrad districts, which is emerging as an endemic region in Bulgaria. The leading phenotypic manifestation is polyneuropathy, often accompanied by restrictive cardiomyopathy and gastrointestinal disorders (Almeida M. et al., 1992).
- The **c.220_221delGAinsCT, p.Glu74Leu (Glu54Leu)** genetic variant is new to Bulgaria and was first identified in a family from the city of Targovishte. It has been reported in patients with hereditary ATTR from Sweden, Belgium, and the United Kingdom (Hellman U et al., 2019). It is associated with severe cardiac and milder neurological involvement.

Currently, over 130 TTR variants are known, with varying geographic distribution, degrees of amyloidogenicity, and organ predisposition (Connors L. et al., 2003; Saraiva M. et al., 1984; Palha J. et al., 2000; Peterson S. et al., 2001).

Currently, there is one known endemic region in Bulgaria, located in the southwestern part of the country (Figure 1).

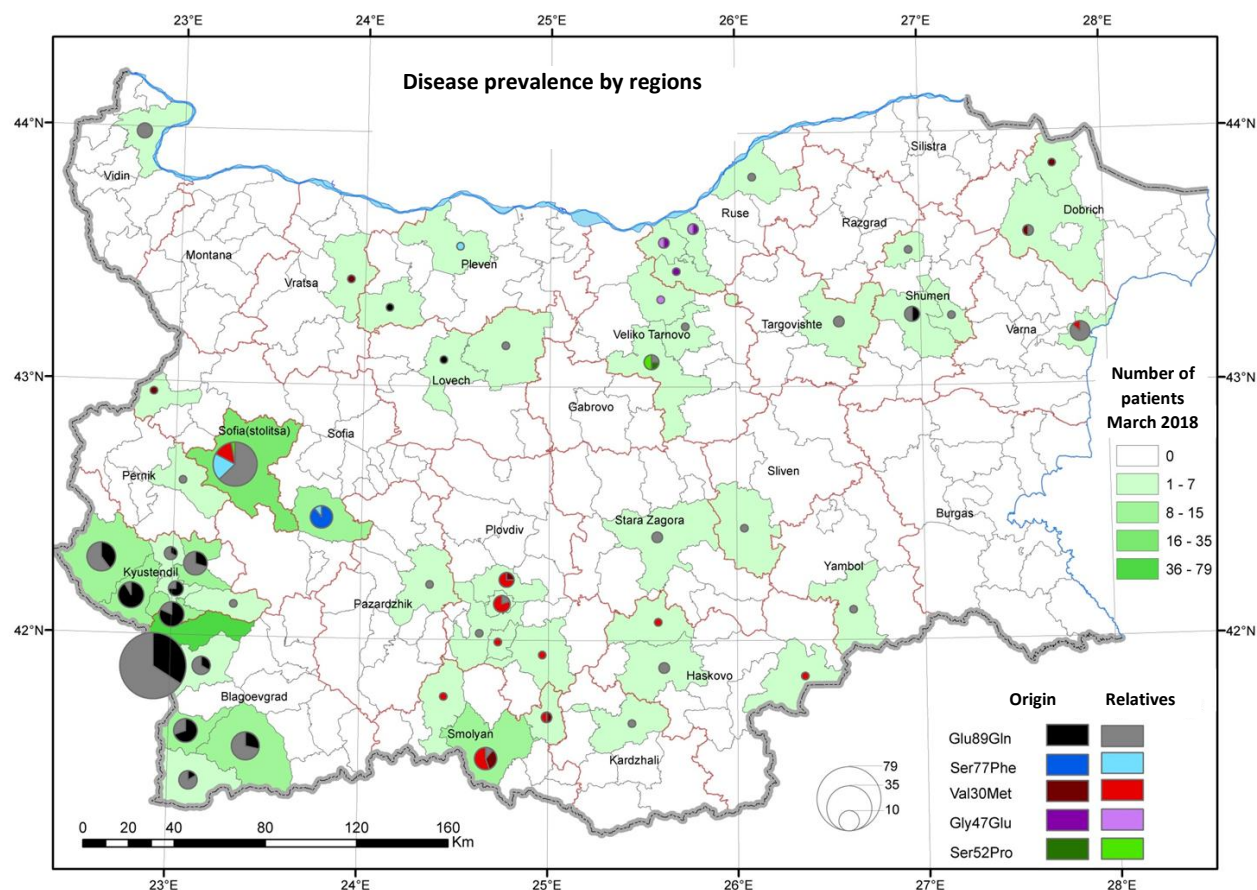


Figure 1. Geographic distribution of carriers of pathogenic variants of the TTR gene within Bulgaria.

To date, the efforts of the interdisciplinary team have led to the identification of 443 carriers of pathogenic TTR variants, including patients and their relatives (some of whom still show no clinical signs of the disease). Figure 1 shows the distribution of the individual pathogenic variants. The genetic diagnostic approach in Bulgaria begins with testing for the most common mutations according to geographic region. If the targeted screening yields a negative result, a comprehensive analysis of the coding sequence of the TTR gene is performed. On this basis, mutant variants are distinguished from wild-type TTR.

Mutation	Total	% of confirmed
Glu89Gln	326	73,59
Val30Met	57	12,87
Ser77Phe	38	8,58
Gly47Glu	8	1,81
Ser52Pro	2	0,45
Glu54Leu	7	1,58
Glu86Asp	2	0,45
Val30Met/Glu89Gln – double heterozygote	1	0,23
Thr80Ala	1	0,23
Cys30Arg	1	0,23
All confirmed	443	

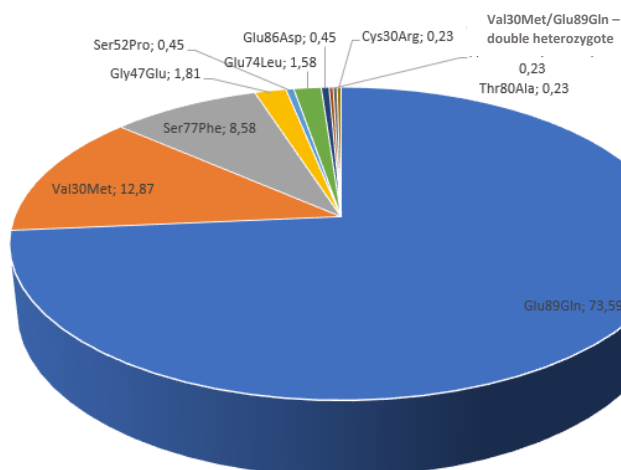


Figure 2. Distribution of pathogenic mutations in the Bulgarian population.

III. Genetic aspects.

1. Genetic aspects of hereditary ATTR.

Hereditary ATTR is traditionally considered a monogenic autosomal dominant disorder, yet is far more complex. The greatest body of evidence is available for the p.Val30Met variant, but more in-depth studies have also become available for other variants, such as p.Glu89Gln, now referred to as the “Bulgarian mutation.” The following characteristics have been observed:

- Variations in the age of onset of the first symptoms: The typical age of onset among carriers of p.Val30Met in Portugal, Brazil, and Japan is in the third to fourth decade of life. However, there are cases of late onset (such as in Sweden), in which the onset of the disease occurs in the fifth to sixth decade of life. In Bulgaria, carriers of this pathogenic variant are characterized by late onset.
- Variable penetrance of the disease: in Portugal and Japan, more than 90% of p.Val30Met carriers develop symptoms by middle age. However, in Sweden, the penetrance of the disease is only 2%, and some p.Val30Met homozygous individuals remain asymptomatic.

- Some atypical Portuguese and Japanese relatives follow the Swedish pattern of late onset with low penetrance.
- Some patients without a family history of amyloidosis have asymptomatic relatives who are carriers of the p.Val30Met variant.
- The disease develops earlier in men than in women. For the “Bulgarian” p.Glu89Gln mutation, the difference in age of onset between the two sexes is about 4.5 years, with earlier onset again observed in males.
- In some cases, the age of onset of the first symptoms is earlier in subsequent generations. This phenomenon is called anticipation, and its cause is unclear. It is possible that epigenetic factors, in addition to genetic ones, may be involved.

Anticipation, incomplete penetrance, and sporadic cases in relatives with unaffected carriers of the pathogenic allele have also been described for other pathogenic variants of the TTR gene.

2. Transthyretin-associated amyloidosis with wild-type TTR

In contrast to the mutant TTR, the senile form, which lacks pathogenic genetic variants (wild type), is associated with the aging process, typically in the seventh and eighth decades of life. These cases may be asymptomatic or have minimal clinical impact. On the other hand, other adult patients with wild-type TTR develop extensive, symptomatic, and even fatal cardiac ATTR. The factors leading to wild-type ATTR are unknown, with likely involvement of other genetic and epigenetic factors. The clinical manifestations of severe SSA are similar to those observed in familial ATTR and in cardiac amyloidosis of the immunoglobulin light chain (AL) deposition type.

IV. Epidemiology of hereditary transthyretin amyloidosis.

1. Global epidemiology of hereditary transthyretin amyloidosis.

Hereditary transthyretin amyloidosis was first described in northern Portugal, in the Póvoa de Varzim region, by the Portuguese neurologist Mário Corino da Costa Andrade in 1952 (Andrade, C., 1952), but was subsequently identified in many other countries (Planté-Bordeneuve V. et al., 2007). As of today, it has been detected in 36 countries.

Until the gene was sequenced in 1983 (Dwulet F., Benson M., 1983; Sasaki H. et al., 1985), it was known as “Portuguese disease.” Later, the genetic mutation causing the disease—Val30Met—was identified (Saraiva, M. et al., 1984). In the 1960s and 1970s, two other large endemic areas were discovered in Japan (1968) and Sweden (1976), with patients harboring the same mutation (Ikeda, S. et al., 2002; Saraiva M. et al., 1984).

Transthyretin amyloid polyneuropathy is a rare disease with a global prevalence of 1 in 10,186. The highest prevalence is found in Portugal (Sousa A. et al., 1995), followed by Sweden (Holmgren G. et al., 2004) and Japan (Kato-Motozaki Y. et al., 2008). The prevalence in the disease-endemic regions of Portugal is 1 in 1,108 (Sousa A. et al., 1995).

The prevalence in the United States (Benson M. et al., 2000) is 1/100,000, but only among the white population. A variety of mutations is observed in the United States due to the multiethnic origin of the population. A total of 34 different mutations have been identified according to the THAOS (Transthyretin Amyloid Outcome Survey) registry, with the most common being Val122Ile (45.3%), Thr60Ala (20.4%), and Val30Met (6.0%), while the Ser77Tyr mutation is also observed (Maurer M. et al., 2016). The average age of onset is approximately 63 years. It is believed that Val122Ile, which is characteristic of African Americans and leads to familial amyloid cardiomyopathy, originates from West Africa (Pinto M. et al., 2017).

The prevalence in Japan (Kato-Motozaki Y. et al., 2008) is 0.87–1.1 per 1,000,000 inhabitants. It is most prevalent in Nagano—12 per 1,000,000—followed by Kumamoto at 10.20 per 1,000,000 and then Ishikawa at 3.8 per 1,000,000.

Val30Met is also prevalent in the Balearic Islands (the city of Mallorca has a Center for Transthyretin Amyloidosis), which are part of Spain. The prevalence is 5/100,000 in Mallorca and 1/100,000 in Menorca. In Cyprus, the prevalence of the disease is 43.34/1,000,000, and the incidence is 0.69/100,000.

Significant genetic heterogeneity has also been observed in the French population (Adams D. et al., 2012). Twenty-nine different mutations have been identified, with Val30Met *being* the most common (62%), followed by Ser77Tyr (11.8%) and Phe77 (6.2%).

In Italy, a variety of mutations have been identified (Rapezzi C. et al., 2006). Of particular interest is the Glu89Gln mutation, identified in Sicily, which is the most common mutation in our country (Mazzeo A. et al., 2015).

Seven mutations have been identified in Turkey, three of which are also found in our patients: Val30Met, Glu89Gln, Gly47Glu, Gly53Glu, Glu54Gly, Thr49Ser, and Glu54Lys. The first three are also found in Bulgaria (Bekircan-Kurt CE. et al., 2015; Durmuş-Tekçe H. et al., 2016).

Five different mutations have been identified in Israel: Ser77Tyr, Phe33Leu, Gly6Ser, Val32Ala, Val30Met, and Ser77Tyr, Val30Met.

In Slovenia, the mutations Val122Ala, Val30Ala, Ile107Phe, and Asp38Asn have been identified. In Romania, Glu54Gln has been reported.

The international THAOS registry was established in December 2007. As of July 1, 2018, there were just over 4,000 patients with TTR-FAP amyloidosis worldwide from 18 centers (<https://www.ttr-fapconnection.com/treatment-center-finder>), including Bulgaria. According to the registry, common mutations include Val30Met (73.3%), Val122Ile (4.4%), Glu89Gln (2.1%), Leu111Met (1.9%), Thr60Ala (1.7%), Ser77Tyr (1.6%), and Phe64Leu (1.5%). Most patients (82.6%) are of Caucasian descent (Coelho T., 2013).

2. Epidemiology of hereditary transthyretin amyloidosis in Bulgaria.

Bulgaria is among the countries with a high number of reported cases of ATTR. The most common TTR pathogenic variant among Bulgarian patients is Glu89Gln, but other, less common variants have also been observed: Val30Met, Ser77Phe, Gly47Glu, Ser52Pro, Glu54Leu, Glu66Asp, and Thr60Ala.

As a result of a ten-year selective screening program among a high-risk population of patients and relatives of genetically verified affected individuals, out of a total of 1,408 people tested, 445 carriers of *TTR* mutations (280 affected and 165 asymptomatic) were identified in Bulgaria, belonging to 147 families, distributed as follows:

- **Glu89Gln** - 107 families
- **Val30Met** – 18 families
- **Ser77Phe** – 14 families
- **Glu54Leu** – 3 families

- **Gly47Glu** – 2 families
- **Ser52Pro** – 1 family
- **Glu66Asp** – 1 family
- **Thr60Ala** – 1 family

All affected individuals exhibit a mixed phenotype with variable age of onset and rate of progression depending on the mutation. A program for regular follow-up of asymptomatic carriers over the past 8 years has allowed for the detection of the transition to symptomatic patients and the initiation of timely treatment.

For Glu89Gln, the founder effect has been established, with the approximate age of the mutation's origin dating back 850 generations. Families harboring the various mutations have a specific region of origin, as follows:

- **Epidemiology of Glu89Gln**

Glu89Gln is the most common TTR mutation in Bulgaria (73.48%).

The clinical phenotype in the group of patients with Glu89Gln is mixed, affecting the peripheral nerves, the heart, and the gastrointestinal system.

Patients with this mutation originate exclusively from southwestern Bulgaria, from Dupnitsa to Petrich. The cluster spans 200 km and is located along the border with the Republic of North Macedonia. The regions of Kyustendil and Blagoevgrad are primarily affected. Due to internal migration, patients with this mutation are also found in Northeastern and Central Northern Bulgaria—the regions of Varna, Shumen, Targovishte, Dobrich, Veliko Tarnovo, and Ruse. They are less frequently found in the regions of Lovech, Pleven, Gabrovo, as well as in Southeastern and Southern Bulgaria—the regions of Haskovo, Kardzhali, Stara Zagora, Sliven, Pazardzhik, Plovdiv, and Smolyan.

In the Kyustendil region, cases originate from the municipalities of Kyustendil, Bobov Dol, Boboshevo, Dupnitsa, Kocherinovo, and Nevestino. In the Blagoevgrad region, cases originate from the municipalities of Blagoevgrad, Sandanski, Simitli, Strumyani, and Petrich.

- **Epidemiology of Val30Met**

Patients with this mutation have been identified in the regions of Blagoevgrad, Varna, Plovdiv, Smolyan, Pernik, and the Sofia region. Overall, the distribution of patients with this mutation across the country is more scattered. One family originates from the Xanthi region of Greece. Members of families affected by Val30Met live in the Plovdiv and Asenovgrad areas.

This mutation also exhibits a certain degree of endemicity. The Smolyan region is the smaller cluster. The larger one likely covers all of Southern Bulgaria and is confined to the cities of Smolyan, Haskovo, Svilengrad, Stara Zagora, Plovdiv, and Blagoevgrad. It cannot be ruled out that the endemic foci of Glu89Gln and Val30Met may overlap along the course of the Struma River, suggesting the possibility of more than one compound heterozygote for both mutations.

Bulgarian citizens, members of affected families, with symptoms of the disease and undergoing treatment with Vindaquel, as well as asymptomatic carriers live in the United Kingdom, France, Germany, Italy, Spain, Portugal, Canada, the United States, and the United Arab Emirates.

- **Epidemiology of Ser77Phe**

All patients with this mutation originate from the village of Vakarel, but from different neighborhoods. One patient with this mutation is from the village of Venkovets, located 32 km from the village of Vakarel. A second patient with the same mutation is from the village of Karapoltsi/Elin Pelin municipality. The distance between these two villages and Vakarel suggests the possibility of migration.

This mutation has the highest frequency in the French population (the southwestern region—the Toulouse area). Historically, the mutation may have been introduced during the Middle Ages by French crusaders, but this must be proven through haplotype analysis. There is another hypothesis that it was brought to France by the Bogomils after their expulsion from Bulgaria during the Middle Ages.

- **Epidemiology of Ser52Pro**

The family with Ser52Pro originates from the municipality of Elena. A carrier of this mutation lives in Sofia.

- **Epidemiology of Gly47Glu**

The families with Gly47Glu originate from the Ruse and Veliko Tarnovo regions and belong to the Roma ethnic group, specifically among the group of Muslim Roma known as the Milet. The first family is from the village of Tsenovo, the second from the town of Polski Trambesh. The same mutation was first described in Italy in a large family spanning four generations, as well as among Muslims from the city of Xanthi, Greece.

- **Epidemiology of Glu54Leu**

The first family, spanning three generations, was identified in Targovishte, but they are migrants from the Blagoevgrad region. The second family is from Blagoevgrad.

- **Prevalence and incidence of hereditary transthyretin amyloidosis.**

“Prevalence” – number of newly diagnosed cases per year / total population (at-risk population).

“Prevalence” – total number of genetically confirmed cases / total population.

To calculate these two indicators, data from the National Statistical Institute on the distribution of the population by place of origin—municipalities and regions—were used (www.nsi.bg).

Glu89Gln

The incidence of new cases for the Blagoevgrad region is 1 per 7,300 people, and for the Kyustendil region, 1 per 8,500. For the two regions combined, the incidence of new cases amounts to 1 per 7,636.

The prevalence of the disease in the Blagoevgrad region is 1 in 200 people, and in the Kyustendil region, it is 1 in 3,900 people. For both regions combined, the prevalence is 1 in 4,150 people.

Val30Met

The incidence of new cases in the Smolyan region is 1 per 9,000 people.

The prevalence of the disease in the Smolyan region is 1 in 3,200. Patients and carriers with Val30Met who do not originate from municipalities in the Smolyan region are not included in the calculations.

Ser77Phe

The incidence rate of new cases in the municipality of Ihtiman is 1 per 1,600 people, and the prevalence is 1 per 850 people.

The data obtained indicate the presence of endemic areas of varying sizes for the individual mutations—Glu89Gln, Ser77Phe, and Val30Met. The incidence and prevalence of the three mutations, based on the patients' place of origin, meet the criteria for endemic areas (<https://www.recognizingttr-fap.com/epidemiology>)—between 1 per 1,000 and 1 per 10,000.

For the country, with a population of 6.839 million (December 31, 2022, National Statistical Institute of the Republic of Bulgaria), the average incidence of new cases for all mutations and clinically diagnosed patients over a ten-year observation period amounts to an average of 1 in 54,500. The prevalence of the disease averages 1 in 28,700.

The data are highly dynamic and change every month as new patients and asymptomatic carriers are diagnosed. This causes both indicators to trend upward.

The number of identified carriers over the past 7 years is 122.

V. Neurological involvement in hereditary transthyretin amyloidosis.

Peripheral nerve involvement is one of the main clinical manifestations of the disease. It presents as:

- *Progressive distal sensory-motor polyneuropathy syndrome* (Plante-Bordeneuve V., Gerard S., 2011). It is characterized by hypo/areflexia in all four limbs, more pronounced in the lower limbs, and involvement of all types of somatosensory perception (touch, pain, temperature, joint-muscle, vibration, and two-dimensional spatial sense) without a strictly defined sequence in our patients. In most cases, sensory disturbances are the first manifestation, but changes in reflexes may also be the initial presentation. Sensory disturbances range from postural instability on the Romberg test to pronounced ataxia, including locomotor ataxia. The onset begins in the lower extremities with subjective hypoesthesia, paresthesia, and dysesthesia. Often, pain in the same areas that is resistant to standard analgesics is added to the hypoesthesia. Symptoms in the arms usually appear somewhat later, within months, rarely after more than a year. Much less frequently, symptoms

in the arms develop almost simultaneously with those in the legs. As the disease progresses, atrophy of the distal muscle groups develops, followed shortly thereafter by atrophy of the proximal muscle groups, accompanied by peripheral paresis/paralysis. Visible fasciculations may also be present.

Involvement of the cranial nerves is considered an unusual clinical manifestation. The bulbar cranial nerves are primarily affected, which can lead to vocal cord paresis (Plante-Bordeneuve V., 2007). It is accepted that bulbar palsy is rare and presents with pharyngeal areflexia, dysarthria, dysphagia, tongue atrophy and fasciculations, and denervation of the tongue and extremities, reflecting amyloid deposition in peripheral nerve structures in Val30Met (Ikeda K., et al., 1998). Cranial nerve damage and predominantly dysarthria have also been observed in our patients with Glu89Gln.

- *Carpal tunnel syndrome.* It is considered a common manifestation of the disease (Sekijima Y., 2015; Kana T. et al., 2010). It is due to amyloid deposition in the carpal ligament and direct compression of the median nerve by amyloid deposits (Suhr O. et al., 2003). It is usually bilateral, more pronounced on one side. The clinical picture is typical of carpal tunnel syndrome: painful hypoesthesia with paresthesia, hyperesthesia with dysesthesia in the wrists and fingers, which is briefly relieved by shaking the hands. Clinically, it is indistinguishable from that caused by other etiologies. Symptoms are more pronounced at night. The symptoms progress over time. It progresses to classic polyneuropathic syndrome after a variable period of time—ranging from six months to over 20 years—during which it is the sole manifestation of the disease (Kana T. et al., 2010). In our patients, this interval averages 6 years. They have often undergone surgery—unilateral or bilateral. After surgery, symptoms frequently recur.
- *Effects on the autonomic nervous system.* Manifestations of this impairment include (Plante-Bordeneuve V. and P. Kerschen, 2013) diarrhea resulting from damage to and a reduction in the number of Cajal cells, which act as the “pacemaker” of the intestines; urinary disorders—retention and incontinence; erectile dysfunction in men (often cited as an early symptom), and in recent years, sexual dysfunction in women; disturbances in sweat secretion manifesting as

profuse sweating or dry skin in advanced stages; and arterial hypotension—in more severe cases with signs of collapse. This list also includes disturbances in ocular tear secretion—usually reduced, rarely increased at the onset—as well as increased tear film instability.

Classic transthyretin neuropathy is axonal, dependent on nerve length, the so-called length-dependent small fiber neuropathy, described by Andrade in early-onset Val30Met (Andrade C., 1952). Initially, unmyelinated and thin myelinated nerve fibers are affected, leading to disturbances in temperature and pain sensation, beginning in the lower extremities (Conceição I. et al., 2015), while the sense of touch and deep sensory perception remain intact. Paresthesias, dysesthesia, allodynia, hyperalgesia, or spontaneous, burning pain in the legs are observed. This is described as so-called sensory dissociation. Not all patients experience pain (Plante-Bordeneuve V., Gerard S., 2011). At this stage, reflexes are usually preserved. Axonal degeneration progresses proximally and reaches the arms after an average period of four to five years. Within a few years, the thicker myelinated sensory and motor fibers are also affected, leading to impaired sense of touch, proprioception, two-dimensional spatial perception, and vibration sense; a motor deficit develops, which impairs gait. Trophic lesions of the feet resembling perforations, as well as neurogenic arthropathies, may be observed.

The clinical phenotype in our patients does not strictly follow this type of sensory dissociation. It is mixed, regardless of which of the five mutations is identified (Glu89Gln, Ser77Phe, Val30Met, Gly47Glu, Ser52Pro): there is involvement of the peripheral nervous system and the heart; in more advanced cases, the gastrointestinal tract is also affected. Glu89Gln has a distinctly mixed phenotype (Coelho T. et al., 2013) with a mean age of onset of 52 years and slightly earlier in men; Ser77Phe also has a mixed clinical phenotype, but with a mean age of onset of 58 years; the mean age of onset for Val30Met is the highest—68 years; the phenotype is mixed, but with polyneuropathy predominating over cardiomyopathy. The median survival of patients is 8 years. Renal involvement is observed in Ser52Pro and Gly47Glu. Gly47Glu has a median age of onset of 30 years for men and 35 for women; the disease begins with gastrointestinal involvement and mild polyneuropathy. The median survival for Gly47Glu is 5 years.

Mutations with other presentations have also been described worldwide: an exclusively cardiac phenotype in Val122Ile without polyneuropathy, an oculoleptomeningeal type (Tyr69His, Ser64, Ala36Pro, Val30) (Benson MD, J.C. Kincaid, 2007; Salvi F. et al., 2012) or leptomeningeal type (Plante-Bordeneuve V., S. Gerard, 2015). Leptomeningeal amyloidosis due to the mutations Asp18Gly, Ala25Thr, Tyr114Cys, is associated with cerebral amyloid angiopathy. It is characterized by amyloid deposits in the media and adventitia of medium- and small-sized arteries, arterioles, and rarely in the cerebral veins. Clinical manifestations include cerebral infarction, cerebral hemorrhages, hydrocephalus, varying degrees of cerebral dysautonomia, ataxia, spastic paralysis, epileptic seizures, and dementia. Leptomeningeal amyloidosis is observed in patients with Val30Met and in transplant recipients—the choroid plexus continues to produce mutant transthyretin despite liver transplantation (Sekijima Y., 2015).

Polyneuropathy has several clinical stages (Coutinho P. et al., 1980)

Stage I	Sensory and motor neuropathy confined to the lower extremities. Severely impaired temperature and pain sensation with relatively preserved proprioceptive sensation (sensory dissociation). Mild motor impairment. Preserved independent gait without assistive devices.
Stage II	Gait with assistance or assistive devices. Neuropathy progresses, affecting the upper limbs and trunk. Amyotrophy in the upper and lower limbs. Moderate motor deficit.
Stage III	Terminal stage. Confined to a wheelchair or bed. Severe sensory, motor, and autonomic neuropathy in all limbs.

A newer, modified scale for neuropathy has been developed, taking into account the degree of disability in patients (Ando Y. et al., 2013):

I. Sensory disturbances, but preserved ability to walk
II. Difficulty walking, but no need for assistive devices
III A. Use of a single cane or crutch when walking

III B. Need for two canes or two crutches when walking
IV. The patient is confined to a wheelchair or bedridden

Nerve conduction examination

The primary diagnostic method for neuropathies, providing information on thick myelinated fibers, but an insensitive method when thin myelinated fibers are affected.

When examining nerve conduction in patients with a mixed clinical phenotype and non-Val30Met mutations from non-endemic regions, various changes are observed in most parameters of the nerves studied: prolonged distal latencies of the median, ulnar, and tibial nerves to varying degrees (Koike H. et al., 2008; Koike H. et al., 2011; Escolano-Lozano F. et al., 2018), reduced amplitude of motor responses from the median and ulnar nerves, reduced conduction velocity along the sensory fibers of the median and ulnar nerves, and frequent absence of sensory response from the sural nerve (Koike H., 2009). At the time of diagnosis, the reduction in the amplitudes of motor and sensory responses of the nerves of the lower extremities is more pronounced (Koike H. et al., 2012).

Nerve conduction studies of the tibial, median, and sural nerves reveal axonal neuropathy with elements of demyelination, as evidenced by prolonged distal latencies (Koike H. et al., 2008; Louise-Laure M. et al., 2015). The combination of prolonged distal motor latency of the median nerve with axonal features of polyneuropathy is emphasized (Luigetti M. et al., 2013). In our patients, prolonged distal latency along the motor fibers of both median nerves is characteristic, more often combined with an absence of sensory responses from the same nerves. A possible explanation is damage to the myelin sheath by amyloid, associated with segmental demyelination (Plante-Bordeneuve V. et al., 2007).

Electrophysiological parameters correlate with the clinical stage of disease and the duration of polyneuropathy. It is generally accepted that sensory potentials disappear more rapidly than motor potentials (when examining the ulnar and tibial nerves). A linear relationship has been found between disease duration and the deterioration of the examined parameters of the respective nerves (motor response from the m. abductor digiti minimi, conduction velocity along motor and sensory fibers of the ulnar nerve, distal latency of the ulnar and tibial nerves) (Kodaira M. et al., 2011). To assess the progression of neuropathy in transthyretin amyloid polyneuropathy, the motor potential is considered

the most sensitive parameter. The mutation does not affect the parameters of the nerve conduction study, but some indicators deteriorate with age (amplitude and conduction velocity along the sensory fibers of the median nerve, amplitude of the sensory response along the peroneal and sural nerves (Lefaucheur J. et al., 2013).

Cerebrospinal fluid testing

Changes in cerebrospinal fluid are important from a differential diagnostic perspective to avoid a misdiagnosis of chronic inflammatory demyelinating polyneuropathy. In patients with transthyretin amyloid polyneuropathy, changes in the protein profile may be observed regardless of the mutation. Most often, these are related to total cerebrospinal fluid protein. It may be elevated, though not as markedly as in chronic inflammatory demyelinating polyneuropathy (Conceicao I. et al., 2016). Most authors report mild to moderate hyperproteinorachia with a normal cell count, i.e., protein-cell dissociation (Cappellari M. et al., 2011; Koike H. et al., 2012). In isolated cases, high values have also been reported—7.23 g/L (C. Douglass, 2007) and exceptionally high values of 2.20 g/dL (Pelo E. et al., 2002). In the latter case, there was also an elevated albumin index (cerebrospinal fluid albumin to serum albumin).

Changes in the CSF are not limited to elevated total protein but also include a compromised blood-brain barrier. In patients with leptomeningeal transthyretin amyloidosis, an elevated CSF/serum albumin concentration ratio has been observed. (Nakamura M. et al., 2005). Histopathologically, in these cases, marked amyloid deposits have been identified in the leptomeninges, the vessels, and the parenchyma of the spinal and cerebral cord, which, according to the authors, is the cause of the compromised blood-cerebrospinal fluid and blood-brain barriers.

VI. Cardiac impairment in hereditary transthyretin amyloidosis.

Cardiac impairment is characterized by the development of restrictive cardiomyopathy, manifesting as heart failure and rhythm and conduction disturbances (Rapezzi C, 2010; Rodney H. Falk, 2005, 2010; Frederick L. Ruberg, 2012).

Common initial symptoms include precordial heaviness and palpitations. The onset of heart failure is preceded by an asymptomatic period of varying duration, during which

there is progressive amyloid deposition in the interstitial space. In addition to the myocardium of the left and right ventricles, amyloid accumulation is also observed in the atria, heart valves, and perivascularly in small vessels; the conduction system is also involved. The manifestations of heart failure may be masked by reduced physical activity due to peripheral polyneuropathy. Rhythm and conduction disturbances are common—atrial flutter, atrioventricular block, and sinoatrial block. In some cases, implantation of a permanent pacemaker is necessary. Atrial flutter increases the risk of embolic stroke.

VII. Gastrointestinal (GI) Tract Involvement in Transthyretin Familial Amyloidosis.

Gastrointestinal involvement in hereditary TTPA manifests clinically with symptoms from the gastrointestinal tract (GIT), and these are common.

More than half of patients with a confirmed mutation (63%) have at least one GI symptom. Symptoms arise from the upper GI tract (esophagus, stomach, and duodenum), the lower GI tract (small and large intestine), or both the upper and lower GI tracts simultaneously. Consequently, gastrointestinal symptoms in hereditary TTPA are highly variable. These include: difficulty swallowing or food getting stuck in the esophagus (dysphagia); heaviness or bloating in the upper abdomen or the entire abdomen; early (rapid) satiety during meals (after eating small amounts of food); nausea; vomiting; bowel rumbling; abdominal gas (meteorism or flatulence); constipation; diarrhea; alternating constipation and diarrhea; fecal incontinence (spontaneous leakage of bowel contents/stool).

The most commonly observed symptoms are early satiety and changes in bowel habits—alternating between constipation and diarrhea, followed by constipation, diarrhea, nausea, and vomiting. These are also associated with weight loss. Fecal incontinence is less common, and, most rarely, patients report belching; symptoms associated with gastroesophageal reflux and abdominal pain.

Gastrointestinal symptoms are more common in carriers of the Val30Met mutation and when the disease manifests at an earlier age—before 50 years (Wixner J. et al., 2014).

Typically, symptoms arise after changes in the nervous system and/or heart have

already occurred, but they can also be the first manifestation of the disease before the onset of peripheral polyneuropathy (Wixner J. et al., 2014; Conceição I., 2016). Although diarrhea is characteristic of the late stages, it can also be the first, earliest symptom. Once it occurs, it persists and is often accompanied by an urgent need to defecate, fecal incontinence, and severe malnutrition. The frequency and severity of gastrointestinal symptoms increase with the duration of the disease. In the late stages, symptoms are frequent or daily and severe. New symptoms appear and existing complaints worsen, leading to malnutrition and progressive weight loss.

The presence of gastrointestinal symptoms is associated with more severe disease, a poorer quality of life, and reduced survival. This is why it is important for them to be thoroughly assessed, monitored, and treated (Wixner J. et al., 2014; Conceição I., 2016; Sekijima Y., 2015).

Physical examination of the abdomen reveals no specific changes—a distended abdomen due to gas, slightly increased or delayed peristalsis. Palpation pain is often absent. No abnormalities may be detected despite severe symptoms. In more advanced disease, signs of malnutrition come to the fore.

Changes in the gastrointestinal tract are due to amyloid deposition in the gastrointestinal mucosa with impaired function and regulation of the autonomic and enteric nervous systems, a reduction in gastrointestinal neuroendocrine cells and interstitial Cajal cells (Yoshimatsu S et al., 1998; Ikeda S. et al., 2009; Wixner J. et al., 2013). This leads to numerous generalized disturbances in the motor function of the esophagus and stomach (impaired accommodation and delayed emptying), changes in defecation (delayed or accelerated passage of intestinal contents), small intestinal bacterial overgrowth, malabsorption of bile acids, essential nutrients (fats, proteins), fat- and water-soluble vitamins, electrolytes, trace elements, and others, and protein loss through the gastric and/or intestinal mucosa (Yoshimatsu S. et al., 1998; Ikeda S. et al., 2009; Wixner J. et al., 2013).

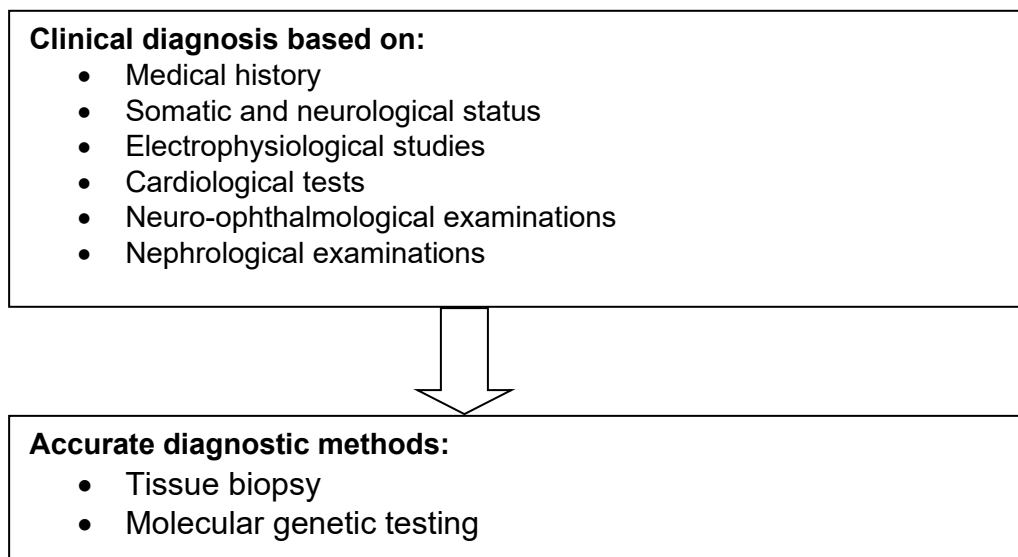
VIII. Diagnosis of hereditary transthyretin amyloidosis.

Early and accurate diagnosis of hereditary ATTR remains a challenge for clinicians due to the disease's high clinical heterogeneity (Adams D. et al., 2016; Conceição I. et al.,

2016).

The diagnostic process for hereditary ATTR involves two main stages:

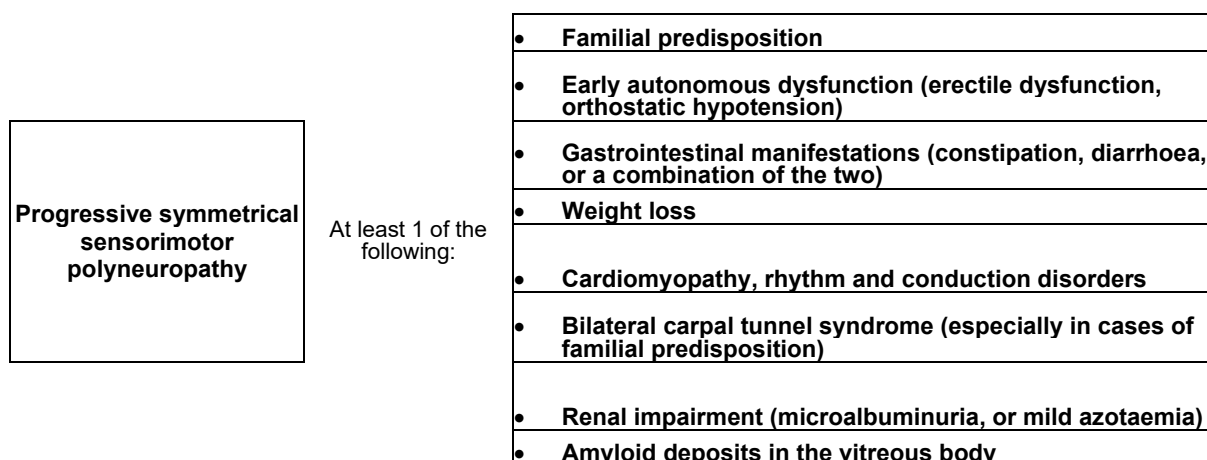
- Clinical diagnosis with additional confirmatory tests
- Accurate diagnostic methods: tissue biopsy and molecular genetic testing



It is important to distinguish diagnostic approaches for individuals with a known family history from those in so-called endemic regions among patients without a known family history (Adams D. et al., 2016; Conceição I. et al., 2016).

In patients with a family history or a presymptomatic genetic defect, symptoms such as peripheral neuropathy, autonomic dysfunction, and rhythm and conduction abnormalities warrant further testing to determine the clinical onset of the disease (see additional tests).

In patients without a known family history, **the main clinical manifestations** that warrant testing for hereditary ATTR are (Figure 3):



Additional manifestations:

- **Rapid progression**
- **Lack of response to other therapies**

Figure 3. Potential symptoms suggestive of a diagnosis of hereditary ATTR.

It is important to refer patients whose clinical manifestations are suggestive of hereditary ATTR for **additional testing**:

1. Neurological examinations:

- The PND (polyneuropathy disability) assessment, presented in Table 1.

Table 1. PND score for the severity of peripheral nerve involvement in hereditary TTRA

0	No symptoms
I	Sensory symptoms, no changes in gait
II	Altered gait, possible without the use of an assistive device
IIIA	Gait is possible with one assistive device
IIIB	Gait is possible with two assistive devices or a walker
IV	Independent walking is impossible; confined to a wheelchair or bed

- Assessment of the stage of hereditary ATTR—is important for evaluating the possibility of pathogenetic therapy (Table 2)

Table 2. Assessment of the stage of hereditary ATTR (Coutinho P. et al., 1980).

I	Gait is not impaired; mild sensory, motor, or autonomic disturbances in the lower extremities
II	Independent walking is possible only with an assistive device; moderately severe involvement of the lower and upper limbs and trunk

III Independent walking – impossible; the patient uses a wheelchair or is bedridden

- Neuropathy Impairment Score (NIS) – see Appendix 1

PND assessment, hereditary TTA stage, and NIS are performed prior to treatment initiation and to determine the effect of therapy in already diagnosed patients.

Electroneurography (ENG) and electromyography (EMG) showing axonal sensorimotor polyneuropathy with more severe involvement of the peripheral nerves of the lower extremities and of sensory fibers relative to motor fibers. The following combinations are possible:

- Reduced amplitude (A) of SNAP and SOMP with normal conduction velocity (CV) and distal latencies (DL)
- Normal A and DL with reduced VC
- Reduced A and VC with normal DL
- DLs are rarely prolonged, with the exception of the DL of the median nerve, which may be prolonged up to twice the normal value, even in the absence of clinical manifestations of carpal tunnel syndrome.

In the early stages of the disease, given the predominant involvement of small unmyelinated nerve fibers, nerve conduction studies (NCS) may be normal, necessitating the following tests:

The sympathetic skin response reflects the activation of sweat glands in response to an electrical impulse or loud noise. It is recorded from the palms and soles. The amplitude is assessed, which is a marker of preserved integrity of the sudomotor nerve fibers. In patients with hereditary ATTR, the amplitude is reduced or there is no response (Conceição I. et al., 2014; Escolano-Lozano F. et al., 2017)

The Sudoscan sudomotor test uses direct low-intensity stimulation ($\leq 4V$) and reverse iontophoresis to measure local conductivity between the chloride ions in sweat secretion and the device's nickel electrodes. The electrochemical conductivity (EEC) of the skin, mediated by unmyelinated C-fibers, is measured in microSiemens (μS). A value of 67 μS is considered normal for the feet. When EC of the skin of the feet is below 66 μS ,

the sensitivity of this method for confirming impaired autonomic regulation is 76%, and the specificity is 85% (Castro J. et al., 2016).

The Quantitative Sudomotor Axon Response Test (QSART) assesses sudomotor function, but due to the long duration of the test, it is not widely used in routine practice.

Heart rate variability (HRV) is assessed during deep breathing and the Valsalva maneuver, but it primarily evaluates parasympathetic function. It is not applicable in patients with a permanent pacemaker (Escolano-Lozano F. et al., 2017).

Quantitative Sensory Testing (QST) – evaluates vibration sensation, the cold temperature threshold, and the heat-pain threshold. In patients with hereditary ATTR, an elevated threshold is observed in all three modalities at the 95th percentile (Escolano-Lozano F. et al., 2017).

Laser-evoked potentials (LEP) – a CO₂ laser stimulator is used to stimulate the sole of the foot (wavelength 10.6 µm; intensity 15–20 W, duration 15 ms). The interstimulus interval varies between 10 and 20 s. The N2–P2 ERP complex is recorded using subcutaneous needle electrodes at the vertex (Cz) and frontal reference (Fpz). The peak latency and amplitude of the N2–P2 complex are assessed. In symptomatic patients, a prolonged N2 latency is observed.

1. Tests to assess cardiac involvement.

The diagnosis of hereditary transthyretin amyloid cardiomyopathy is based on: family history, clinical data, ECG, laboratory and imaging methods, molecular genetic analysis, and histological examination with amyloid typing in unclear cases. It is essential to rule out light-chain and “wild-type” transthyretin amyloidosis, the other two most common types of amyloid cardiomyopathy, which require different management and have a different prognosis. (Rodney H. Falk, 2010; González-López E., 2015, 2017; Rapezzi, C. et al., 2009)

- **ECG changes** most commonly include a pathological Q wave (pseudo-infarction pattern), left bundle branch block (LBBB), low voltage in the peripheral leads, and first-degree atrioventricular (AV) block. Less commonly, left and right bundle branch block, atrial flutter, and a permanent pacemaker rhythm are recorded. A specific but not

mandatory sign is low ECG voltage in the presence of evidence of left ventricular hypertrophy on imaging studies (O'Donnell E, 2013; Dungu, J. et al., 2010).

- **Transthoracic echocardiography** is the primary imaging modality (Mohty, D. et al., 2013, Gertz MA 2005, Quarta CC 2014, Bellavia D 2007, 2008, Lindqvist P 2006, Lindqvist P 2016, Fadel B 2013, Agha AM 2018, Phelan D). Echocardiographic changes include: left ventricular (LV) wall thickness greater than 12 mm, as well as:

- Normal or reduced LV dimensions
- Diastolic dysfunction of varying severity—ranging from impaired relaxation through a pseudonormal appearance to restrictive LV filling
- Preserved (>50%) or, in advanced stages, reduced ejection fraction
- Early impairment of longitudinal systolic function
- Basal-apical gradient of longitudinal strain with relatively preserved apical deformation in STE.
- Left atrial dilatation
- Dilation of the right atrium and inferior vena cava
- Thickening of the valve leaflets, usually with mild to moderate regurgitation
- Thickening of the interatrial septum
- Small pericardial effusion (in approximately 50% of patients)

- **Magnetic resonance imaging (MRI)**

Provides information on the structure and function of the heart and the tissue characteristics of the myocardium. Late gadolinium enhancement has a characteristic appearance in cardiac amyloidosis with 80% sensitivity and 90% specificity (Fontana, M. et al. 2014, 2015, Maceira, AM et al. 2009, Vogelsberg H, 2008).

The following MRI findings are characteristic of amyloid cardiomyopathy:

- Gadolinium contrast enhancement: Gadolinium exhibits an interstitial distribution and is retained longer in segments with amyloid accumulation, leading to late contrast enhancement. This can be diffuse or patchy, more commonly subendocardial, but may also be intramural or transmural

- T1 mapping is a quantitative method for measuring myocardial signals. In native T1 mapping (without contrast), a significantly prolonged T1 relaxation time is observed, in contrast to other cardiomyopathies with a similar phenotype, such as hypertrophic cardiomyopathy and Fabry disease. This method can be used in patients with severe renal impairment, in whom the use of contrast is contraindicated. T1 time may be prolonged before the onset of LV hypertrophy, and this can be used for earlier diagnosis of the disease.
- MRI allows for the calculation of extracellular volume, which is increased in amyloidosis. This enables early diagnosis, tracking of amyloid accumulation, and monitoring of treatment response.

2. Nuclear medicine

For ATTR cardiomyopathy, a validated non-invasive (biopsy-free) diagnostic algorithm based on ^{99m}Tc bone scintigraphy has been established, provided that light-chain amyloidosis is definitively ruled out (Figure 4) (Gillmore, JD , et al. 2016, Glaudemans, AW et al. 2009, 2014, Rapezzi, C. et al. 2011, Bokhari, S. et al. 2013, 2014). It has been established that ^{99m}Tc-3,3-diphosphono-1,2-propanedicarboxylic acid (^{99m}Tc-DPD), ^{99m}Tc-pyrophosphate (^{99m}Tc-PYP), and ^{99m}Tc-hydroxymethylene diphosphonate (^{99m}Tc-HMDP)—isotopes used in bone scintigraphy—accumulate in the myocardium of patients with transthyretin cardiac amyloidosis, both hereditary and “wild-type.” A scale has been introduced for the visual assessment of isotope uptake in the myocardium (0 – no uptake in the myocardium, normal uptake in the bones; 1 – mild uptake in the myocardium and normal uptake in the bones; 2 – moderate accumulation in the myocardium and reduced accumulation in the bones; 3 – marked accumulation in the myocardium with minimal or no accumulation in the bones) (Perugini, E. et al 2005). The detection of grade 2–3 isotope accumulation in the myocardium has a very high degree of sensitivity and specificity, approaching 100% for transthyretin amyloid cardiomyopathy. SPECT-CT is mandatory to rule out a false-positive result when the radiopharmaceutical is visualized in the left ventricular cavity rather than in the myocardium. Genetic analysis is mandatory to distinguish hereditary from wild-type transthyretin amyloidosis. A

negative result rules out transthyretin cardiomyopathy and points to another type of cardiomyopathy or another type of cardiac amyloidosis. In a small percentage of patients with light-chain amyloidosis, isotope accumulation is also observed, but to a significantly lesser extent—Perugini stages 1–2—which makes it mandatory to rule out this rapidly progressive disease with an extremely poor prognosis and distinct treatment requirements.

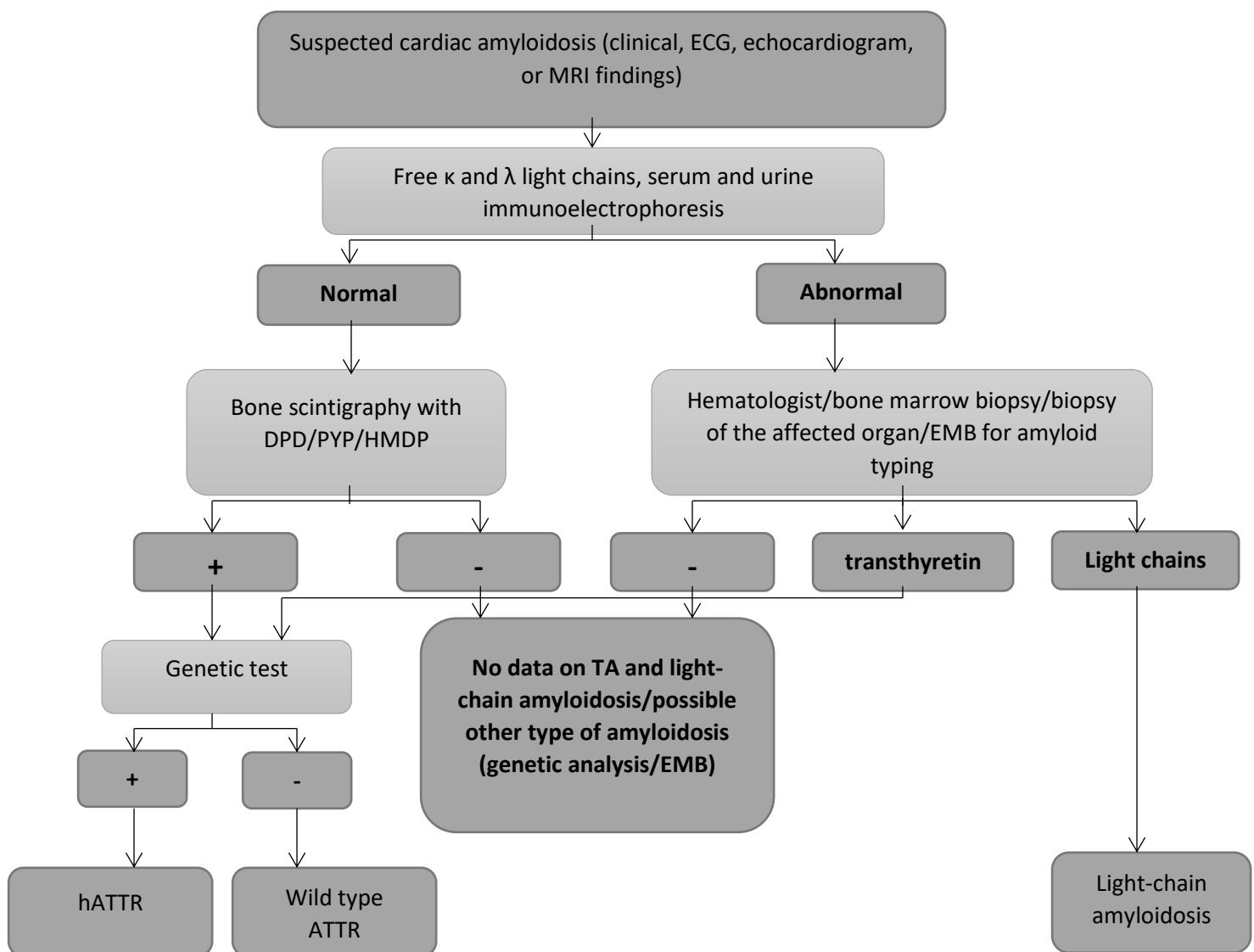


Figure 4. Diagnostic algorithm for patients suspected of having cardiac amyloidosis. ECG – electrocardiogram; Echocardiography – echocardiography; MRI – magnetic resonance imaging; EMB – endomyocardial biopsy; DPD – 3,3-diphosphono-1,2-

propanedicarboxylic acid; HDMP – hydroxymethylene diphosphonate; PYP – pyrophosphate; TTR – transthyretin amyloidosis.

- **Cardiac biomarkers.**

BNP, NT-proBNP, and troponin are cardiac biomarkers that become elevated in heart failure and myocardial necrosis, respectively. The mechanism behind the increase in BNP and NT-proBNP is elevated left ventricular filling pressure, while elevated troponin results from cell death. These biomarkers provide information on the severity of cardiac involvement, have prognostic significance, and can be used for disease staging (Panagiota Kyriakou1 2018, González-López E, 2017, Krinsten 2014).

- **Holter ECG monitor.**

A 24- to 48-hour recording of the heart rhythm is necessary in the presence of relevant symptoms or once a year to detect asymptomatic atrial fibrillation or significant conduction abnormalities.

- **Early diagnosis and monitoring of carriers.**

Cardiac symptoms usually appear after an asymptomatic period of varying duration, during which significant amyloid infiltration of the heart occurs. Suspicion of amyloid cardiomyopathy usually arises during an echocardiographic examination performed due to nonspecific complaints or when the disease is suspected based on symptoms from the peripheral or autonomic nervous system. Identifying carriers of the transthyretin gene mutation in affected families through genetic screening and their follow-up allows for the detection of the earliest signs of the disease. The starting age and frequency of follow-up for carriers regarding cardiac involvement are determined in accordance with generally accepted guidelines. The following cardiac tests are recommended: ECG, echocardiography, NT-proBNP, MRI, and bone scintigraphy when cardiac involvement is suspected (Conceição I. et al., 2019).

Table 3. Necessary tests for the diagnosis and follow-up of symptomatic patients and carriers of a genetic mutation associated with ATTR.

		ECG	Echocardiogram	MRI	Bone scintigraphy	NT-proBNP	hs-TN	Holter ECG	Blood/urine
Patients	Diagnosis	+	+	+/-	+	+	+	+	+
	Tracking	+	+	+/-	-	+	+	+	+
Carriers	Diagnosis	+	+	+/-	+	+	+	+/-	+/-
	Tracking	+	+	+/-	+/-	+	+	+/-	+/-

+ recommended; - not recommended; +/- at the discretion of the physician for certain patients

ECG – electrocardiogram, Echocardiography – echocardiography, MRI – magnetic resonance imaging; NT-proBNP, N-terminal pro-brain natriuretic peptide, hsTn – high-sensitivity troponin

Criteria for progression of ATTR-CM.

One criterion from each group is the minimum requirement for the presence of ATTR-CM progression (P. Garcia-Pavia et al. European Journal of Heart Failure (2021), doi:10.1002/ejhf.2198):

Clinical and functional criteria	Laboratory biomarkers	Imaging and ECG criteria
Increased number of hospitalizations for HF and/or diuretic dose	30% increase in NT-proBNP (300 pg/ml cut-off)	2 mm increase in LV wall thickness
Upgrade in NYHA class	30% increase in troponin	Increase in the degree of diastolic dysfunction
5–10-point decrease in KCCQ quality of life, 10% decrease in EQ-5D	Worsening on the NAC scale	Change in systolic function ($\geq 5\%$ decrease in EF, ≥ 5 ml decrease in SV, $\geq 1\%$ increase in GLS)
Decrease of 30–40 m on the 6-minute walk test		Newly developed conduction abnormalities

HF – heart failure, KCCQ – Kansas City Cardiomyopathy Questionnaire, EQ-5D – EuroQol five dimensions, 6MWT – 6-min walk test, NAC – UK National Amyloidosis Centre, NT-proBNP – N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; QoL, quality of life, EF – ejection fraction, GLS – global longitudinal strain.

3. Gastroenterological examinations.

To determine the cause of gastrointestinal symptoms and rule out other conditions, upper or lower endoscopy with biopsy, computed tomography or magnetic resonance enterography, and enteroclysis may be performed. Endoscopic and histological changes are mild and nonspecific. Crucial for the diagnosis is the detection of amyloid deposits in the mucosa of the gastrointestinal tract via histological examination using polarized light microscopy and Congo red staining or through immunohistochemical typing (Yoshimatsu S. et al., 1998; Ikeda S. et al., 2009). This is particularly important for early diagnosis in cases where gastrointestinal symptoms are the first, early manifestation of the disease. A characteristic feature of hereditary ATTR is the presence of a small amount of amyloid in the submucosa with marked deposition around nerve fibers, whereas in primary and secondary amyloidosis, the deposition is massive and predominantly around the vessels (Yoshimatsu S. et al., 1998; Ikeda S. et al., 2009).

To definitively establish the diagnosis, genetic and/or histopathological testing is required.

4. Genetic testing

Its purpose is to identify the specific mutation in the *TTR* gene. This allows for the differentiation between the two forms of transthyretin amyloidosis—hereditary and sporadic. Amyloidogenic pathogenic variants in the *TTR* gene are most commonly single nucleotide substitutions in the protein-coding DNA sequence, which lead to a change in the encoded amino acid. The detection of such genetic defects using PCR and direct Sanger sequencing is extremely successful. Identification of the genetic defect and genetic counseling in cases of hereditary transthyretin amyloidosis are of paramount importance for the screening and detection of asymptomatic carriers in affected families. Early genetic testing of patients with suspected transthyretin amyloidosis in many cases significantly shortens the time to diagnosis and leads to the timely initiation of specific therapy.

5. Histopathological examination

The goal is to identify amyloid deposits in various tissues, such as the salivary glands, subcutaneous adipose tissue, mucosa of the gastrointestinal tract, peripheral nerves, or other affected organs (heart, kidneys). Biopsies are most commonly taken from abdominal subcutaneous adipose tissue or a salivary gland, followed by biopsies of the sural nerve and rectal mucosa. The biopsied tissue is stained with Congo red and examined under a polarizing microscope to visualize the extracellular amyloid deposits, which appear bright green. After establishing a diagnosis of amyloidosis by Congo red staining, the specific type of amyloid must be determined by immunohistochemical examination using an antiserum against TTR. Control antisera against other types of amyloid precursors, including immunoglobulin light chains and amyloid A protein, should also be used to confirm the specific substance.

Histopathological examination should be performed in carriers of a mutation in the *TTR* gene who have been diagnosed presymptotically, but who, during follow-up, exhibit subjective symptoms of peripheral nervous system involvement and in whom PNS involvement, as assessed by other neurological tests such as ENG, cutaneous

sympathetic response, quantitative sensory testing (QST), laser-evoked potentials, quantitative sudomotor axon test, and Sudoscan sudomotor test, is inconclusive.

To determine when an asymptomatic carrier of a TTP gene mutation becomes symptomatic, the individual must exhibit symptoms that can be associated with the disease (polyneuropathy, autonomic dysfunction, wasting syndrome, arrhythmia, rhythm and conduction abnormalities), as well as abnormalities in 2 or more of the objective instrumental tests and/or amyloid deposits in the tissue biopsy (Figure 5). A negative biopsy is not an exclusion criterion in the presence of clinical manifestations and abnormal electrophysiological and autonomic tests.

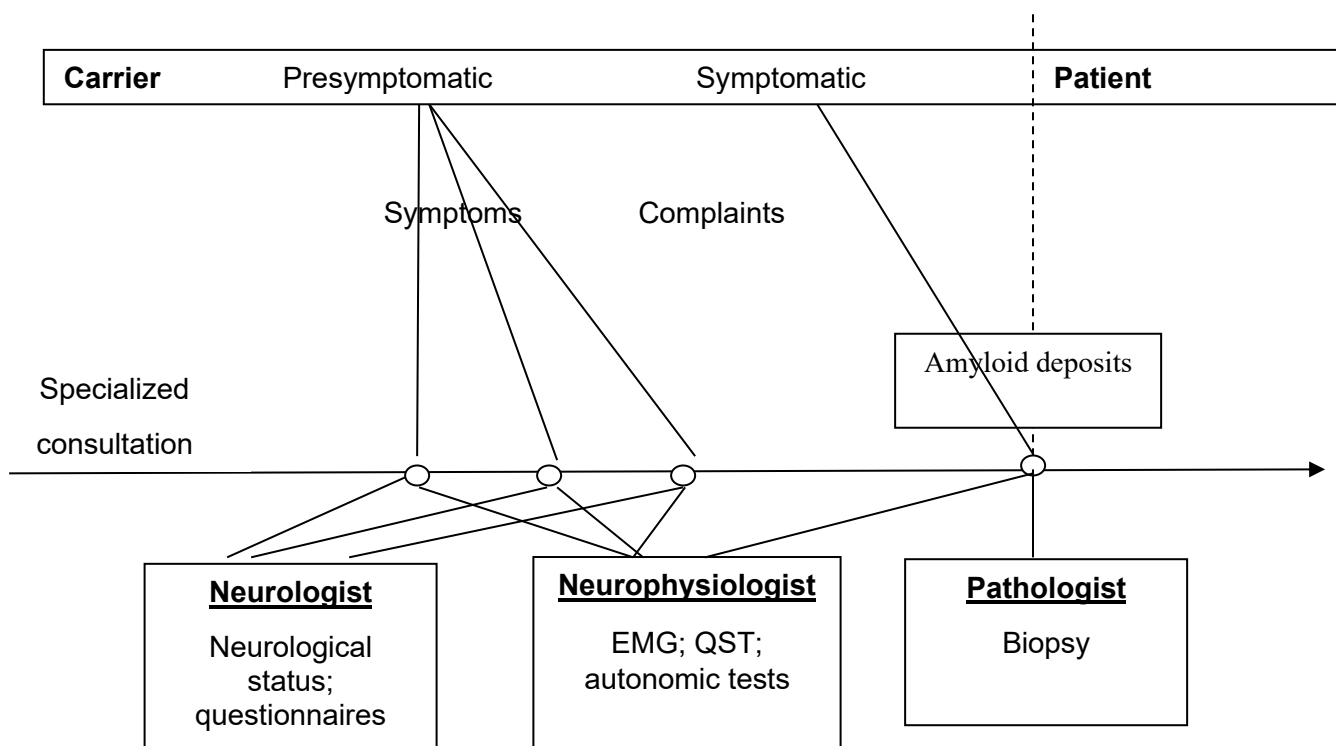


Figure 5. Key elements of the diagnostic process for determining when an asymptomatic carrier becomes a patient who should begin treatment.

Diagnostic methods for monitoring patients with hereditary ATTR and assessing the effect of therapy. A baseline assessment of the patient is performed before starting

therapy. Subsequently, the patient's condition and the effect of treatment are monitored every 6 months using the following tests:

- **Neurological:**
 - Neurological status
 - Functional assessments (gait; PND; stage of hereditary ATTR; NIS)
 - Quantitative Sensory Testing (QST)
 - Autonomic symptoms (urinary disorders, erectile dysfunction, orthostatic hypotension)
 - Sympathetic skin response
 - Sudoscan sudomotor test
 - Electroneurography
- **Cardiological:**
 - ECG
 - Echocardiography
 - 99mTc-PYP/DPD/HMDP scintigraphy
 - Cardiac magnetic resonance imaging
 - Cardiac biomarkers (NT-proBNP, hs-Tn)
 - Holter ECG
- **mBMI (modified body mass index)**
- **Consultation with a gastroenterologist**
- **Neuro-ophthalmological evaluation**
- **Assessment of renal function and microalbuminuria**

IX. Differential diagnosis

1. **Peripheral nerve involvement in ATTR should be differentiated from that seen in (Ando Y. et al., 2013):**
 - Chronic inflammatory demyelinating polyneuropathy
 - Idiopathic axonal neuropathy
 - Diabetic polyneuropathy
 - Toxic polyneuropathies (most commonly of ethyl alcohol origin)

- Deficiency polyneuropathies (following preceding diarrhea)
- Vitamin B₁₂ deficiency
- Charcot–Marie–Tooth disease
- Hereditary sensory and autonomic polyneuropathies
- Fabry disease
- Other forms of hereditary amyloidosis (gelsolin, apolipoprotein A1)
- AL amyloidosis—in monoclonal gammopathies

Table 4 presents the main differences between ATTR, diabetic sensorimotor polyneuropathy, and AL amyloidosis.

2. Cardiac involvement in:

- Sarcomeric hypertrophic cardiomyopathy
- Fabry disease
- Other rare cardiomyopathies with a hypertrophic phenotype
- Hypertensive cardiomyopathy
- LV hypertrophy in aortic stenosis

3. Gastrointestinal manifestations should be distinguished from those seen in:

- Chronic ulcerative colitis
- Crohn's disease
- Chronic gastroduodenitis
- Whipple's disease
- Gluten enteropathy, lymphoma, and other organic diseases of the small intestine
- Colon cancer, other organic diseases of the colon
- Irritable bowel syndrome
- Other forms of gastrointestinal amyloidosis.

Table 4. Key differences between ATTR, diabetic sensorimotor polyneuropathy, and AL amyloidosis.

	Diabetic polyneuropathy	AL amyloidosis	ATTR
Clinical manifestations	Polyneuropathy affecting small nerve fibers, subsequently involving the thick fibers, sensory loss, neuropathic pain, tendon areflexia	Symmetrical predominantly sensory PNP (pain and temperature) Carpal tunnel syndrome - 25% Late-onset motor involvement	Polyneuropathy affecting small nerve fibers, subsequently involving large fibers Progressive distal-to-proximal weakness Carpal tunnel syndrome Tendon areflexia
Systemic manifestations	Nephropathy, retinopathy	Macroglossia Purpura Nephrotic syndrome Multiple myeloma, paraproteinemia	Cardiomyopathy Rhythm and conduction disorders Nephropathy Ocular involvement
Autonomic manifestations	+/-	+/-	++
Precursor protein	-	AL amyloid	TTR amyloid
Progression	Slow progression over 5–20 years	Rapid progression	Progresses over 1–10 years
Family history	-	-	+/-
Cerebrospinal fluid protein	Normal	Normal	Normal/Elevated
Effects on the CNS	+/-	-	-

ENG	Axonal sensorimotor	Axonal sensorimotor	Axonal sensorimotor
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X. Pathogenetic treatment of hereditary transthyretin amyloidosis

Orthotopic liver transplantation (OLT)

Because TTR is predominantly produced in the liver, orthotopic liver transplantation was introduced in 1990 as a rational therapy for hereditary TTR amyloidosis by preventing the production of mutant transthyretin. Until 2011, this was the only treatment option for TTR amyloidosis. Liver transplantation has proven most effective in patients with the Val30Met mutation, with early-onset polyneuropathy and no cardiac involvement.

Long-term follow-up of transplanted patients shows regression of amyloid deposits and a five-year survival rate of 77% among patients. At the same time, there is evidence that the progression of neuropathy and/or cardiomyopathy may continue in some patients after HCT due to deposition of wild-type transthyretin. In a multinational study involving 362 patients with hereditary TTR amyloidosis, approximately 33% of patients progressed within 3–5 years (PND score worsened) after SCT. LHT does not alter TTR production in the eyes and brain; therefore, central nervous system and ocular manifestations are not affected. Like any transplant, LT has limited application due to insufficient organ availability and risks associated with hepatic artery thrombosis, drug toxicity, and malignancies related to prolonged immunosuppression.

Because most of the mutations identified in the Bulgarian population have a mixed clinical phenotype (neurological and cardiological), liver transplantation is not considered an effective therapeutic method for Bulgarian patients. The only mutation, Val30Met, which leads to a predominantly neurological phenotype of TTR amyloidosis, has a late onset in our patients, which is why they are not suitable for OLT.

In November 2011, the European Medicines Agency (EMA) issued a positive opinion on the first medicinal product—Tafamidis (Vindaqel)—for the treatment of transthyretin amyloidosis in adult patients with stage 1-associated symptomatic polyneuropathy (ATTR-PN) to delay peripheral neurological impairment.

Tafamidis (Vindaqel) is a selective TTR stabilizer. Tafamidis binds to TTR at the thyroxine binding sites, stabilizing the tetramer and slowing its dissociation into monomers, the rate-limiting step in the amyloidogenic process. Tafamidis binds with negative cooperativity to both thyroxine-binding sites of the native tetrameric form of transthyretin, thereby preventing its dissociation into monomers. **Inhibition of TTR tetramer dissociation is the basis for the use of Tafamidis to slow disease progression in patients with stage 1 ATTR-PN.**

Each capsule contains 20 mg of micronized Tafamidis meglumine, equivalent to 12.2 mg of Tafamidis. It is taken orally, once daily.

The main study of Tafamidis meglumine in patients with stage 1 ATTR-PN is an 18-month, multicenter, randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of 20 mg tafamidis meglumine once daily in 128 patients with ATTR-PN with a Val30Met mutation, primarily in stage 1 of the disease; 126 of the 128 patients did not routinely require walking assistance. Eighty-six of the 91 patients who completed the 18-month treatment period were subsequently enrolled in an open-label extension study, where all received 20 mg of tafamidis meglumine once daily for an additional 12 months.

After 18 months of treatment, most patients treated with Tafamidis meglumine responded to therapy according to the NIS-LL assessment (a change of less than 2 points on the NIS-LL). Secondary endpoints indicate that treatment with Tafamidis meglumine resulted in fewer neurological function impairments and an improvement in nutritional status (mBMI) compared with placebo. In the open-label extension study, the degree of change on the NIS-LL over the 12 months of treatment was similar to that observed in patients who were randomized and treated with Tafamidis during the preceding 18-month double-blind period.

The effects of Tafamidis were evaluated in patients with ATTR-PN with a non-Val30Met mutation in a supportive open-label study in 21 patients and in a post-marketing observational study in 39 patients. Based on the results of these studies, the mechanism of action of Tafamidis, and the results regarding TTR stabilization, Tafamidis meglumine is expected to have a positive effect in patients with stage 1 ATTR-PN caused by mutations other than Val30Met.

The clinical program for Tafamidis includes 13 controlled and uncontrolled studies, as well as two non-interventional observational studies. Treatment with the drug halts the progression of neuropathy in 60% of patients after 18 months of treatment, with no disease progression observed in over 50% of patients after 30 months (Coelho T. et al., 2010). Long-term use of Tafamidis for a period of over 6 years is well tolerated (Barroso F., 2017). No life-threatening adverse drug reactions or deaths have been observed.

According to the 2022 Consensus Recommendations for the Treatment and Follow-up of Hereditary ATTR, the evaluation of long-term treatment with Tafamidis (up to 6 years) in Portuguese stage 1 patients with the Val30Met mutation shows that patients with early-stage disease (NIS 10) and female patients had the best response to therapy, with sustained and complete disease stabilization. In all studies with Tafamidis 20 mg, no serious safety signals were observed.

In Bulgaria, treatment with Tafamidis 20 mg for patients with stage 1 ATTR-PN was reimbursed in 2013, when treatment began for the first 10 patients with hereditary TTR-PN in the first stage of peripheral neuropathy.

Between 2013 and 2022, 162 patients with stage 1 ATTR-PN began treatment with Tafamidis. 68 of them discontinued treatment: 20 died, 45 experienced disease progression, and 3 refused treatment. All deceased patients had advanced restrictive cardiomyopathy and heart failure. Some of them died suddenly from cardiac complications, while others died from a stroke. 30% of patients did not progress and remain in stage 1 of polyneuropathy after 60 months of treatment. 62% remain in stage 1 of polyneuropathy after 48 months of treatment. A more sustained effect of treatment with Tafamidis is observed in patients with the Val30Met mutation, whereas in patients with the Glu89Gln mutation, despite a delay in disease progression, progression is observed more frequently, necessitating discontinuation of treatment. The treatment effect is weakest and disease progression is fastest in patients with the Glu47Gln mutation. Early initiation of treatment is of critical importance for the outcomes and efficacy of therapy. Most patients in whom disease progression is observed are diagnosed more than 4 years after disease onset.

Patients with ATTRv often present with a mixed phenotype. The mixed phenotype is characterized by varying degrees of neurological and cardiac involvement. Cardiac involvement largely determines the poor prognosis in these patients.

In February 2020, the European Medicines Agency issued a positive opinion on Tafamidis 61 mg for the treatment of wild-type or hereditary transthyretin amyloidosis in adult patients with cardiomyopathy (ATTR-CM).

Tafamidis binds competitively to both thyroxine-binding sites of the native tetrameric form of transthyretin, thereby preventing its dissociation into monomers.

Inhibition of TTR tetramer dissociation is the basis for the use of Tafamidis 61 mg in patients with ATTR-CM.

Each capsule contains 61 mg of micronized Tafamidis. It is taken orally, once daily.

In patients with ATTR-CM, the efficacy of Tafamidis has been evaluated in two completed **clinical trials** (one controlled Phase III trial and one open-label Phase II trial), as well as in two corresponding clinical trial extensions. A total of 530 patients with ATTR-CM were enrolled, which represents a large number for a clinical program for a rare disease. The efficacy of Tafamidis 61 mg was demonstrated in a multicenter, international, double-blind, placebo-controlled, randomized, 3-arm study (ATTR-ACT) in 441 patients with ATTRv-CM or ATTRwt-CM.

The results show that patients treated with Tafamidis 61 mg had a significantly better composite endpoint of all-cause mortality and frequency of cardiovascular hospitalizations compared to placebo after a 30-month treatment period ($p=0.0006$). Better results were also observed in terms of all-cause mortality (30%, $p=0.026$) and the rate of cardiovascular hospitalizations (32%, $p<0.0001$). Functional capacity was improved at 6 months compared to placebo and was maintained through 30 months. Tafamidis 61 mg reduced the deterioration of parameters during the 6-minute walk test ($p<0.0001$) and of overall health status based on a questionnaire used in patients with cardiomyopathies ($p<0.0001$) (Kansas City Cardiomyopathy Questionnaire).

Results are better in patients with NYHA Class I or II, supporting the conclusion that Tafamidis 61 mg should be initiated as early as possible in the course of the disease, when the clinical benefit regarding disease progression is greatest (Maurer MS, 2018). A

64.4% reduction in all-cause mortality compared to placebo was observed in NYHA Class I patients, 39.6% in NYHA Class II patients, and 16.2% in NYHA Class III patients.

For the patient's convenience, the Tafamidis 61 mg free acid capsule has been developed, which is comparable to a dose of Tafamidis meglumine 80 mg (4 capsules x 20 mg) and has the same mechanism of action and biological effect (Lockwood PA. et al., 2020).

The clinical trial of **Tafamidis at two different dosages—20 mg and 80 mg**—was conducted globally in patients with ATTRv-CM and ATTRwt-CM over a 30-month period. The primary efficacy endpoint showed significant differences between the 264 patients who received Tafamidis and the 177 patients who received placebo ($p < 0.001$). Tafamidis was associated with lower overall mortality compared to placebo: 78 (264) or 29.5% versus 76 (177) or 42.9% and a lower rate of hospitalizations related to cardiovascular disease, with a relative risk ratio of 0.68 (0.48 versus 0.70 per year). At month 30, treatment with Tafamidis was associated with a lower percentage decrease in 6-minute walk test (6-MWT) distance ($p < 0.001$) and a lower percentage decline in KCCQ-OS score ($p < 0.001$).

The Long-term Extension Study (LTE) compares Tafamidis meglumin 20 mg (Tafamidis 12.2 mg) with Tafamidis meglumin 80 mg (Tafamidis 61 mg). Analysis of the results from the main study and the LTE regarding mortality favors the higher dose—a 30% relative risk reduction at month 51 compared to the lower dose. Tafamidis at both dosages has a safety profile comparable to placebo. (Damy T. et al., 2021)

When comparing patients across different NYHA classes, NYHA Class III patients showed lower mortality compared to patients on placebo. NYHA Class III patients demonstrated an increased risk of hospitalization. This is likely due to longer survival, as these patients require more frequent hospitalizations.

When comparing the two doses (20 mg vs. 80 mg) in terms of efficacy, the higher dose showed a greater effect on mortality. No safety signals were reported with either dose. The 2022 consensus recommendations confirm that Tafamidis 61 mg (80 mg Tafamidis meglumin) is an approved treatment option for ATTRv-CM and ATTRwt-CM.

In conclusion, Tafamidis 61 mg is a medicinal product with efficacy proven in clinical trials, a favorable safety profile, and ease of use for both patients and healthcare

professionals. It is the first approved treatment for ATTRv-CM and ATTRwt-CM. Tafamidis 20 mg is a recommended treatment option for patients with early-stage ATTR-PN.

3. Patisiran (Ompatro)

Patisiran is a small interfering RNA that binds to coding regions of the TTR messenger RNA, specifically suppressing hepatic transthyretin synthesis. A clinical trial conducted at 44 centers in 19 countries involving 225 patients (148 receiving intravenous treatment with Patisiran (0.3 mg/kg body weight) and 77 on placebo over 18 months showed that Patisiran improves multiple clinical manifestations of hereditary transthyretin amyloidosis (D. Adams et al., 2018). After 18 months of treatment, 56% of patients on Patisiran showed improvement on the mNIS+7 scale compared to 4% of patients on placebo. Patisiran also demonstrated an effect on walking speed and adjusted BMI. Analysis of the APOLLO clinical trial shows that Patisiran reduces left ventricular wall thickness, global longitudinal strain, the cardiac biomarker NT-ProBNP, and adverse cardiovascular events. These results suggest that Patisiran may halt the progression of cardiac manifestations and even improve them (Adams D. et al., 2018; Solomon S. et al., 2018; Kristen A. et al., 2019).

In the group of patients treated with Patisiran, an 81% reduction in serum transthyretin levels was observed, regardless of age, sex, and genotype.

The clinical study demonstrates that lowering transthyretin levels may be an effective therapeutic approach. It was found that Patisiran significantly improves the sensory-motor and autonomic symptoms of polyneuropathy in patients with hereditary transthyretin amyloidosis. Additionally, treatment with Patisiran significantly improves quality of life, walking ability, nutritional status, and activities of daily living. In 8% of the Patisiran group, a transition from assisted to unassisted walking was observed.

In the placebo group, the disease has a rapid, progressive course, with worsening symptoms and reduced functional activity.

Regarding the safety of the medication, mild to moderate adverse reactions were observed in 20% of patients receiving Patisiran, with their frequency decreasing over time,

and in 10% of patients on placebo. The most common adverse reactions are peripheral edema, diarrhea, nausea, and infusion-related reactions. Mortality is similar in both groups.

Treatment with Patisiran was approved by the EMA and FDA in August 2018 and is indicated for the treatment of stage 1 and stage 2 peripheral neuropathy.

To date, there have been no direct comparative clinical trials evaluating the efficacy of treatment with Patisiran and Tafamidis. A recent indirect comparison of the efficacy of treatment with Patisiran and Tafamidis has been conducted and published, applying Bucher's standard pairwise method for endpoints used in the APOLLO and Tafamidis Fx-005 clinical trials: change from baseline in the Neuropathy Impairment Score-lower limbs (NIS-LL), the Norfolk QoL-Diabetic Neuropathy (QoL-DN) questionnaire, the NIS-LL response, and mBMI compared to placebo. Analysis of data for patients with stage 1 PAD treated with Patisiran suggests a better therapeutic effect compared to patients treated with Tafamidis for all endpoints, with a significant improvement in the mean change in NIS-LL and QoL-DN at 18 months (Plante-Bordeneuve V. et al., 2019).

4. Vutrisiran (Amvuttra)

Vutrisiran is a double-stranded small interfering RNA (siRNA) that specifically targets variant and wild-type transthyretin (TTR) messenger RNA (mRNA). Through a natural process called RNA interference (RNAi), Vutrisiran causes the catalytic degradation of TTR mRNA in liver hepatocytes, leading to a reduction in serum levels of both variant and wild-type TTR protein.

Vutrisiran is indicated for the treatment of hereditary transthyretin-related amyloidosis (hATTR) in adult patients with stage 1 or stage 2 polyneuropathy, as well as for the treatment of wild-type or hereditary transthyretin amyloid cardiomyopathy.

Vutrisiran 25 mg solution for injection, in a pre-filled syringe

Subcutaneous administration. The recommended dose is 25 mg, administered by subcutaneous injection once every 3 months.

With repeated administration of 25 mg once every 3 months, the mean reduction in serum TTR after 9 and 18 months of treatment was 83% and 88%, respectively.

Reductions in TTR were observed regardless of genotype (V30M or non-V30M), prior use of a TTR stabilizer, weight, sex, age, or race.

The efficacy of Vutrisiran was evaluated in a global, randomized, open-label clinical trial (HELIOS-A) in adult patients with hATTR amyloidosis with polyneuropathy. Patients were randomized in a 3:1 ratio to receive 25 mg of Vutrisiran (N=122) subcutaneously once every 3 months or 0.3 mg/kg of Patisiran (N=42) intravenously once every 3 weeks. The treatment period in the study lasted 18 months with two analyses at Month 9 and Month 18. Efficacy assessments are based on data from the Vutrisiran arm of the study compared to an external placebo group (the placebo arm from the Phase 3 APOLLO study), comprising a similar population of patients with hATTR amyloidosis with polyneuropathy.

Although treatment with Vutrisiran and Patisiran uses different RNA delivery platforms, these demonstrate comparable reductions in serum transthyretin, as well as comparable efficacy in improving polyneuropathy, as measured by mNIS+7 and the 10-minute walking test, and in quality of life improvement, as measured by the Norfolk QOL-DN at 18 months. An advantage of treatment with Vutrisiran over Patisiran is that it is administered subcutaneously every 3 months, whereas Patisiran is administered intravenously via a 4-hour infusion with cardiac monitoring every 3 weeks in a healthcare facility.

Regarding the safety of the medication, during the 18-month treatment period in HELIOS-A, the most common adverse reactions reported in patients treated with Vutrisiran were pain in the extremities (15%) and arthralgia (11%). Other common adverse reactions include dyspnea and injection site reactions such as bruising, erythema, pain, itching, and warmth. These were mild, transient, and did not lead to discontinuation of treatment.

Treatment with Vutrisiran in patients with hATTR-associated polyneuropathy was approved by the EMA on September 15, 2022. The European Medicines Agency (EMA) approved Vutrisiran for the treatment of cardiomyopathy associated with wtATTR or hATTR amyloidosis on June 9, 2025, based on data from the HELIOS-B clinical trial.

HELIOS-B is a global, randomized, double-blind, placebo-controlled clinical trial in adult patients with ATTR-CM. Patients were randomized in a 1:1 ratio to receive 25 mg of Amvuttra subcutaneously once every 3 months or the corresponding placebo. At baseline, 40% of patients had received treatment with Tafamidis. Treatment assignment was stratified according to Tafamidis use at baseline, the type of ATTR disease (wtATTR or hATTR amyloidosis), as well as disease severity and age at baseline (NYHA Class I or II and age < 75 years versus all others).

Among patients who received Amvuttra at baseline, the median age was 77 years (range 45 to 85 years) and 92% were male. Eighty-five percent (85%) of patients were of European descent, 7% were Black or African American, and 6% were of Asian descent. Eighty-nine percent (89%) of patients had wtATTR amyloidosis, and 11% had hATTR amyloidosis. According to the NYHA classification of heart failure (HF), 15% of patients were in Class I, 77% in Class II, and 8% in Class III, with ATTR disease stage 1 or 2 according to the NAC. Patient demographics and baseline disease characteristics were similar across treatment groups.

The primary efficacy endpoint was a composite of all-cause mortality and recurrent cardiovascular events (cardiovascular hospitalizations and emergency department visits due to heart failure [CHF]) during double-blind treatment lasting up to 36 months, assessed in the overall population and in the monotherapy population (defined as patients who did not receive tafamidis at baseline).

The use of Amvuttra resulted in a significant reduction in the risk of all-cause mortality and recurrent cardiovascular events compared with placebo in the overall population and in the monotherapy population by 28.2% and 32.8%, respectively. Approximately 77% of all deaths in HELIOS-B were related to cardiovascular disease. The incidence of both cardiovascular-related and non-cardiovascular-related deaths was lower in patients treated with Amvuttra compared to placebo.

Of the total number of cardiovascular events, 87.9% were cardiovascular hospitalizations, and 12.1% were visits due to heart failure. Both components of the primary composite endpoint individually contributed to the treatment effect in the overall population and in the monotherapy population.

In the analysis of the secondary endpoint of all-cause mortality based on data through Month 42, which included survival data for all patients from the double-blind period and for an additional maximum of 6 months, Amvuttra resulted in a 35.5% reduction in the risk of death compared with placebo in the overall population (hazard ratio: 0.645; 95% CI: 0.463; 0.898; p=0.0098) and a 34.5% reduction in the monotherapy population (hazard ratio: 0.655; 95% CI: 0.440; 0.973; p=0.0454).

The results of the subgroup analysis of the primary composite endpoint favor Amvuttra in all pre-specified subgroups in the overall population and in the monotherapy population. In the subgroup of patients receiving Tafamidis as primary treatment, Amvuttra resulted in a 21.5% absolute reduction in the risk of all-cause mortality and recurrent CV events compared with placebo (hazard ratio: 0.785; 95% CI: 0.511; 1.207).

5. Acoramidis

The European Medicines Agency (EMA) approved Acoramidis for the treatment of cardiomyopathy associated with wtATTR or hATTR amyloidosis on February 11, 2025

Acoramidis is an oral, highly selective, low-molecular-weight, near-complete (>90%) TTR stabilizer for the treatment of ATTR-CM.

The efficacy and safety of Acoramidis were evaluated in one Phase 2 study and one Phase 3 study.

In a Phase 2 study in patients with ATTR-CM with NYHA functional class II to III (n=49), treatment with acoramidis twice daily resulted in:

- An increase in serum TTR from baseline to Day 28 of 36% at a dose of 400 mg twice daily and 50% at a dose of 712 mg twice daily, indicating stabilization of TTR.
- Serum TTR levels within the normal range in all participants and for both dose levels by day 28, regardless of baseline TTR or the presence of vATTR-CM or wtATTR-CM
- TTR stabilization, measured ex vivo on Day 28, >90% for both dose levels, with similar stabilization rates for vATTR-CM and wtATTR-CM
- A lower incidence of adverse reactions in the active treatment arms compared with the placebo arm (acoramidis 356 mg: 63%; acoramidis 712 mg: 69%; placebo: 88%)

The efficacy and safety of Acoramidis were evaluated in the Phase 3 ATTRIBUTE-CM study. ATTRIBUTE-CM was a multicenter, international, randomized, double-blind,

placebo-controlled clinical trial conducted in 632 participants with wild-type or variant (hereditary or de novo) ATTR-CM and NYHA Class I–III heart failure. Participants were randomized in a 2:1 ratio to receive Acoramidis 712 mg (n=421) or matching placebo (n=211) twice daily for 30 months. Treatment assignment was stratified according to whether participants had variant ATTR-CM (ATTRv-CM) or wild-type ATTR-CM (ATTRwt-CM) and according to baseline disease severity, i.e., NT-proBNP level and renal function as determined by eGFR. Patients with an eGFR <15 mL/min/1.73 m² were excluded from the study. Treatment with tafamidis was permitted only after the first 12 months of the study had elapsed.

In the Phase 3 ATTRibute-CM study in patients with ATTR-CM and NYHA functional class I to III (n=632), treatment with acoramidis 712 mg twice daily for 30 months resulted in:

- A significant improvement in the primary endpoint, consisting of all-cause mortality, hospitalizations for cardiovascular disease, change from baseline in NT-proBNP and change from baseline in 6MWD, compared with placebo treatment (p<0.0001)
- Significant improvement in a number of secondary endpoints, including HF-related hospitalizations, change from baseline in 6MWD, and change from baseline in the total KCCQ score
- Reduction in hospitalizations related to cardiovascular disease, such that the NNT for preventing one cardiovascular-related hospitalization (CVD) per year was 5, and the NNT for preventing one death or first CVD event over 2.5 years was 7
- Near normalization of 30-month survival compared with age-matched controls in the U.S. (81% vs. 85%, respectively) and of CVH compared with hospitalizations in the broader U.S. Medicare population

A similar incidence of treatment-emergent adverse events (TEAEs) as that observed in the placebo arm (acoramidis 712 mg: 98.1%; placebo: 97.6%), with a lower incidence of fatal TEAEs than in the placebo arm (Acoramidis 712 mg: 14.3%; placebo: 17.1%)

Results from the ATTRibute-CM extension (AG10-304)

AG10-304 is a Phase 3, multicenter, open-label extension study enrolling adult patients (aged 18 to 90 years) with symptomatic ATTR-CM who have completed 30 months of treatment in the ATTRibute-CM study. Patients receive Acoramidis 712 mg twice daily.

The primary endpoint of the AG10-304 study is to assess the long-term safety and tolerability of Acoramidis. Secondary endpoints include all-cause mortality, cardiovascular hospitalization (CVH), cardiovascular mortality (CVM), change in distance on the 6-minute walk test (6MWT) from baseline, and change in the Kansas City Cardiomyopathy Questionnaire (KCCQ-OS) score from baseline.

A total of 389 patients were enrolled in the AG10-304 study, including 263 patients who received acoramidis in the ATTRibute-CM study and 126 patients who received placebo in the ATTRibute-CM study.

A summary of the clinical results for all-cause mortality or cardiovascular hospitalization for the OLE study shows a 48% relative risk reduction for patients who received acoramidis, from baseline in the ATTRibute-CM study through month 12 of the OLE compared to patients who switched from placebo to acoramidis at the start of the OLE.

At month 42, patients who received continuous treatment with acoramidis experienced a greater reduction in all-cause mortality (ACM) or cardiovascular hospitalization (CVH) compared to patients who switched from placebo to acoramidis. Continuous treatment with acoramidis was associated with a 33.7% relative risk reduction (RRR) in all-cause mortality (ACM) (HR: 0.64; 95% CI: 0.47–0.88), a 41.0% RRR in first cardiovascular hospitalization (CVH) (HR: 0.53; 95% CI: 0.41–0.69), and a 33.9% RRR in the composite endpoint of all-cause mortality or first cardiovascular hospitalization (HR: 0.57; 95% CI: 0.46–0.72).

The medications approved by regulatory authorities for the treatment of patients with hereditary ATTR amyloidosis with stage I polyneuropathy and cardiomyopathy are Tafamidis 61 mg and Vutrisiran.

For patients with stage II polyneuropathy and cardiomyopathy, treatment with Vutrisiran alone is approved.

For patients with hereditary ATTR amyloidosis with cardiomyopathy, the approved medications are Tafamidis 61 mg, Vutrisiran, and Acoramidis.

6. Inotersen (Tegsedi) – a treatment based on reducing transthyretin

Inotersen is an antisense nucleotide therapy that suppresses the expression of wild-type and mutant forms of transthyretin. The treatment has been approved by the European Medicines Agency and the FDA for the treatment of stage 1 and stage 2 polyneuropathy. This approval is based on the results of the NEURO-TTR clinical trial in patients with hereditary TTR amyloidosis. Patients treated with Inotersen showed significant improvement compared to patients who received placebo, as assessed by mNIS+7 and the Norfolk QoL-DN. No improvement in cardiac parameters was observed in the subpopulation of patients with cardiac involvement. Regarding safety, Inotersen is associated with an increased risk of thrombocytopenia and glomerulonephritis, requiring increased monitoring for early detection of these side effects (Kristen A. et al., 2019).

6. Eplontersen (Wainzua) 45 mg (56 mg/ml - 0.8 ml) solution for injection x 1 pre-filled syringe (glass)

The medicinal product Eplontersen (Wainzua) is indicated for the treatment of hereditary transthyretin-mediated amyloidosis (ATTRv) in adult patients with stage 1 or 2 polyneuropathy, administered independently at home using an easy-to-use autoinjector.

A systematic review of the scientific literature regarding the efficacy, therapeutic efficacy, and safety of Wainzua (eplontersen) in patients with ATTR-PN in PubMed using the keyword “eplontersen” as of December 2024, which identified 25 publications. The identified studies were analyzed according to the inclusion and exclusion criteria.

The NEURO-TTRansform trial is the only study identified in the literature review that provides clinical evidence of the efficacy and safety of eplontersen in patients with ATTR-PN.

Eplontersen demonstrated a consistent and significant reduction in serum TTR levels over time compared to open-label placebo through week 85. Eplontersen demonstrated a consistent, significant reduction in the progression of neuropathic

impairment compared to open-label placebo, as measured by the composite neuropathic impairment score using the modified Neuropathy Impairment Score +7 (mNIS+7).

A greater proportion of patients in the Eplontersen group showed improvement or no change in the PND score, compared to the open-label placebo group, in which a greater proportion of patients worsened.

The results from the 10MWT suggest improved mobility for patients treated with Eplontersen compared to baseline.

When assessed based on modified mBMI, patients receiving Eplontersen showed improvement. Treatment with Eplontersen led to improvements in autonomic dysfunction relative to baseline values of the Heart Rate Deep Breathing Score compared to the external placebo group. At week 66, more than half of the patients treated with Eplontersen (57.6%) showed improvement (a negative change from baseline) in the total Norfolk QoL-DN score, compared with 20.0% of the external placebo-treated patients.

Eplontersen is well tolerated and has a favorable safety profile. The most common adverse reactions during treatment with Eplontersen are decreased vitamin A levels (16% of patients) and vomiting (9% of patients).

8. Diflunizal

Diflunizal is a generic, nonsteroidal anti-inflammatory drug used as a tetrameric stabilizer. Although it is not approved for use in hereditary ATTR, it is used in some countries. Similar to Tafamidis, a 24-month clinical trial demonstrated that Diflunizal slows the progression of neuropathy (measured by NIS+7) compared to placebo. Only 48% of the patients enrolled in the study completed it, and discontinuation was primarily due to disease progression, with a mean change in NIS+7 of +8.2 over two years. Treatment with Diflunizal in patients with cardiac involvement did not significantly reduce left ventricular wall thickness or left ventricular longitudinal strain compared to placebo. Side effects reported include cases of impaired renal function and thrombocytopenia. Treatment is contraindicated in cases of heart and kidney failure. Since cardiac

involvement is common in hereditary ATTR, the use of Diflunizal is limited (Kristen A. et al., 2019).

Treatment of hereditary transthyretin amyloidosis is prescribed and monitored by rare disease treatment committees at registered expert centers, which must include neurologists and cardiologists with proven experience in the diagnosis, treatment, and monitoring of the disease.

X. Summary and prospects in the treatment of ATTR amyloidosis according to current recommendations, 2022.

Guidelines and new directions in the therapy and monitoring of ATTRv amyloidosis (Ando et al., 2022)

The potential for treating ATTR amyloidosis lies in its pathophysiology. The three therapeutic pillars are: TTR knockout, TTR stabilization, and amyloid removal.

Recommendations for the treatment of hereditary TTR amyloidosis (2022 recommendations from the International Amyloidosis Society):

Ideally, ATTR amyloidosis should be treated as soon as the disease is diagnosed to achieve the best therapeutic response and prevent significant organ damage. The lack of direct comparative studies between effective therapies makes it difficult to select the best therapy.

- Patients with isolated neuropathy should be treated with either tafamidis (in early stage 1 of the disease), diflunisal, or with eplontersen, patisiran, vutrisiran, or inotersen (in stages 1 and 2), at the physician's discretion and in accordance with local health authority guidelines.

Level of evidence II, grade of recommendation A

- Patients with a mixed phenotype, neuropathy, and cardiomyopathy (defined as evidence of cardiac involvement on echocardiography with a left ventricular septal wall thickness at end-diastole > 12 mm), may be treated with tafamidis 61 mg and vutrisiran.

Level of evidence II, grade of recommendation A

However, in patients with a mixed phenotype, physicians may prescribe a gene silencer based on rationale and indirect, albeit not officially confirmed, evidence of benefit.

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Level of evidence II, grade of recommendation A

- Patients with isolated cardiomyopathy should be treated with tafamidis 61 mg or Acoramidis

Level of evidence II, grade of recommendation A

- Multidisciplinary supportive care is essential

Level of evidence IV, grade of recommendation D

The combination of TTR stabilizers and gene silencers is expected to be synergistic. However, there are no trials supporting this combination, and given the high cost of most of these drugs, it is recommended that the efficacy of the combination be investigated in a controlled clinical trial.

Several Phase III trials are nearing completion, and new drug approvals with new indications are expected soon. This will change therapeutic options and recommendations.

Taking into account internationally approved guidelines for the treatment of hereditary transthyretin amyloidosis, we recommend:

Patients with pure neuropathy should be treated with either Tafamidis 20 mg (in early stage 1 of the disease) or with Eplontersen, Patisiran, Vutrisiran, or Inotersen gene silencers (in stages 1 and 2) at the discretion of the Hereditary Transthyretin Amyloidosis Treatment Committee.

Patients with a mixed phenotype, neuropathy, and cardiomyopathy (defined as evidence of cardiac involvement on echocardiography with a left ventricular septal wall thickness at end-diastole > 12 mm) may be treated with Tafamidis 61 mg and Vutrisiran.

Patients with isolated transthyretin amyloid cardiomyopathy may be treated with Tafamidis 61 mg, Acoramidis, and Vutrisiran.

XI. Symptomatic treatment.

• Symptomatic treatment for neurological involvement.

Symptomatic treatment for neurological involvement aims to address the neuropathic pain present in some patients, as well as the paresthesias, which are more common. Anticonvulsants such as Gabapentin (at a dose of 1,200 mg to 2,400 mg) and

Pregabalin (at a dose of 150 mg to 300 mg) or antidepressants such as Duloxetine (at a dose of 30 mg to 90 mg) are used. The daily dose varies depending on the severity of symptoms and individual tolerance. Medications must be titrated slowly and administered with extreme caution due to arterial hypotension, the risk of collapse, and the presence of diarrhea.

Symptomatic treatment of neuropathic manifestations aims to improve patients' quality of life.

- **Symptomatic treatment for cardiac involvement.**

Diuretics are essential medications in the treatment of heart failure, but they must be used with caution in cardiac amyloidosis due to the frequently accompanying severe hypotension resulting from low cardiac output combined with autonomic nervous system dysfunction and hypovolemia. Maintaining optimal fluid balance is essential. The most commonly used combination is a loop diuretic with an aldosterone antagonist. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are poorly tolerated by patients for the same reason. Data from real-world clinical practice show that SGLT2 inhibitors are well tolerated and produce good results in patients with ATTR-HF with signs of heart failure. Beta-blockers may be used with caution, taking into account their negative inotropic effect, as well as the fact that slowing the heart rate may lead to a reduction in cardiac output, since tachycardia is a compensatory mechanism for reduced stroke volume. An exception is high-frequency atrial fibrillation and EF <40%, in which case beta-blockers may be administered. Calcium channel blockers and digitalis glycosides bind to amyloid fibrils, which may lead to increased accumulation and toxicity. Digitalis glycosides may be used with caution in some cases of poorly controlled atrial fibrillation. Patients with atrial flutter are indicated for anticoagulant therapy to prevent ischemic stroke regardless of the CHA₂DS₂-VASc score due to the increased risk of embolism. Ablation may be considered as a therapeutic option in patients with atrial flutter and ATTR-CM, but its use should be individualized based on symptom severity and cardiac function. Amiodarone may be used for ventricular and supraventricular arrhythmias. Involvement of the conduction system often necessitates implantation of a permanent pacemaker. At this stage, there is no evidence that implanting a cardioverter-

defibrillator improves survival or prevents sudden cardiac death, as it most often occurs as a result of electromechanical dissociation (González-López, E., 2017; Kristen, A.V. et al., 2008; Joseph P. Donnelly 2017; Maurer, M.S. et al., 2017; Rubinow, A., 1981, Bouhour, JB 1986, Lin, G. et al., 2013, Kojima, T. et al., 2012).

- **Diet and symptomatic treatment for gastrointestinal symptoms.**

For all symptoms of the digestive system, it is recommended to eat slowly and calmly, chewing food thoroughly, with 3 main meals and 2 or more snacks. It is advisable to avoid spicy foods and seasonings, fried foods, and very coarse or hard foods (e.g., quince), as well as foods that trigger symptoms.

In cases of upper GI symptoms, frequent intake of small portions of food in the form of soups and purees is recommended. Fatty foods and meats, as well as alcohol, carbonated beverages, coffee, and tea, should be avoided. Light and easily digestible foods are recommended, such as rice, clear broths, milk and dairy products (in the absence of lactose intolerance), especially low-fat yogurt, fish, and whole-grain foods (whole-grain bread). Water and other fluids should be consumed frequently in small sips. It is important to determine which beverages the patient tolerates better—hot or cold—and to increase their intake. In cases of nausea and vomiting, eating toast and baked potatoes, as well as ginger or chamomile tea, can be helpful.

In cases of diarrhea, it is additionally recommended to avoid dairy products and foods high in fat and fiber, as well as very sweet foods. You can eat bananas, salty foods, and drinks such as boiled rice, boiled potatoes, toast, carrots, roasted chicken (skinless), rice water with salt, chicken soup with salt, and vinegar.

For constipation, increased intake of fluids and fiber is recommended to ensure the necessary stool volume—8–10 glasses of water; a varied diet with increased intake of fiber-rich foods and juices—fresh vegetables and fruits, prunes, spinach, whole-grain foods with bran, which can be consumed according to tolerance due to developing flatulence, abdominal bloating, or pain. Fatty and low-fiber foods such as cheeses, meats, and sweets should be limited, as well as the use of medications that cause constipation.

Treatment of gastrointestinal symptoms in hereditary ATTR is symptomatic and often unsatisfactory. It targets the primary complaints and aims to control symptoms and improve quality of life.

For symptoms of the upper gastrointestinal tract, medications are prescribed that accelerate or enhance motility (prokinetics), antiemetics (anti-nausea medications), e.g., sodium citrate, dopamine (D_2) antagonists—metoclopramide, domperidone, itoprid hydrochloride (3–4 times daily in standard doses). These have a mild effect on gastric emptying but often provide good symptomatic relief. Motilin agonists (e.g., erythromycin) have a good effect on gastric emptying but a lesser effect on symptoms. It should be noted that some of them prolong the QT interval. Drugs that reduce or bind gastric acid are used—antisecretory agents (proton pump inhibitors—omeprazole, pantoprazole, esomeprazole—20–40 mg/day or lansoprazole 30 mg/day); H_2 blockers such as ranitidine 300 mg/day or famotidine 20–40 mg/day, alginates, and antacids (3–4 times daily in standard doses).

All medications used to treat diarrhea can be used—primarily loperamide, though it is ineffective in many cases. Codeine may also be used in some cases. In severe cases, treatment with somatostatin may be administered. In milder cases, hydrasec and tanakan may be administered in standard doses, along with simethicone and dimethicone for gas, and absorbents such as activated charcoal and clay-containing preparations (humus, kaolin), antisecretory agents—buscolizine, papaverine, duspethaline, opioid agents in standard doses, bismuth preparations—bismuth subsalicylate—up to 8 times daily at 30 ml or colloidal bismuth 4 times daily at 120 mg. For both prevention and treatment of diarrhea, the use of probiotics is recommended - for example, Enterol 2 tablets twice daily. In moderate and severe cases, empirical antibiotic therapy is administered with ciprofloxacin or norfloxacin, metronidazole or tinidazole, as well as with non-absorbable medications such as rifaximin—in standard doses, along with fluids and electrolytes.

For constipation, various laxatives can be taken—those that increase stool bulk (fiber, psyllium), softening the stool (surfactants—sodium or calcium docusate, phosphosoda enema), stimulating peristalsis (contact laxatives—senna, bisacodyl, olive oil, prunes, rose; ispaghula, husk, cascara), osmotic laxatives (lactulose, mannitol, glycerin, polyethylene glycol), probiotics, and others, or enemas with ready-made

solutions. The most appropriate treatment is the regular intake of lactulose in a standard dose of 15–30 ml to 60 ml once daily in the morning, psyllium, and polyethylene glycol. Osmotically active substances, e.g., polyethylene glycol, which, according to a meta-analysis, has a better effect and fewer side effects compared to lactulose. Sodium picosulfate is also used. With the exception of lactulose, laxatives should be used with caution due to the risk of dependence, increased fluid and electrolyte loss, or impaired nutrient absorption.

Correction of malabsorption involves replacement therapy with pancreatic enzymes (Creon 20–40,000 IU daily with meals) and fat-soluble vitamins (A, E, D, and K), particularly vitamin D and calcium, water-soluble B vitamins, especially B12, electrolytes, magnesium, high-calorie, protein-rich enteral foods, and human albumin infusion in cases of severe hypoalbuminemia. Enteral feeding, including via a nasogastric tube and gastrostomy, is preferred over parenteral feeding.

Due to the increased risk of osteoporosis and bone fractures, most often resulting from malabsorption, in addition to vitamin D and calcium, intravenous administration of bisphosphonates once a year is recommended. Oral treatment with bisphosphonates should be administered with caution due to the risk of damage to the esophageal mucosa resulting from motility disorders.

Antibiotics are used to treat small intestinal bacterial overgrowth: short courses of doxycycline or metronidazole, and probiotics. For longer-term use, non-absorbable antibacterial agents (such as rifaximin) are preferred.

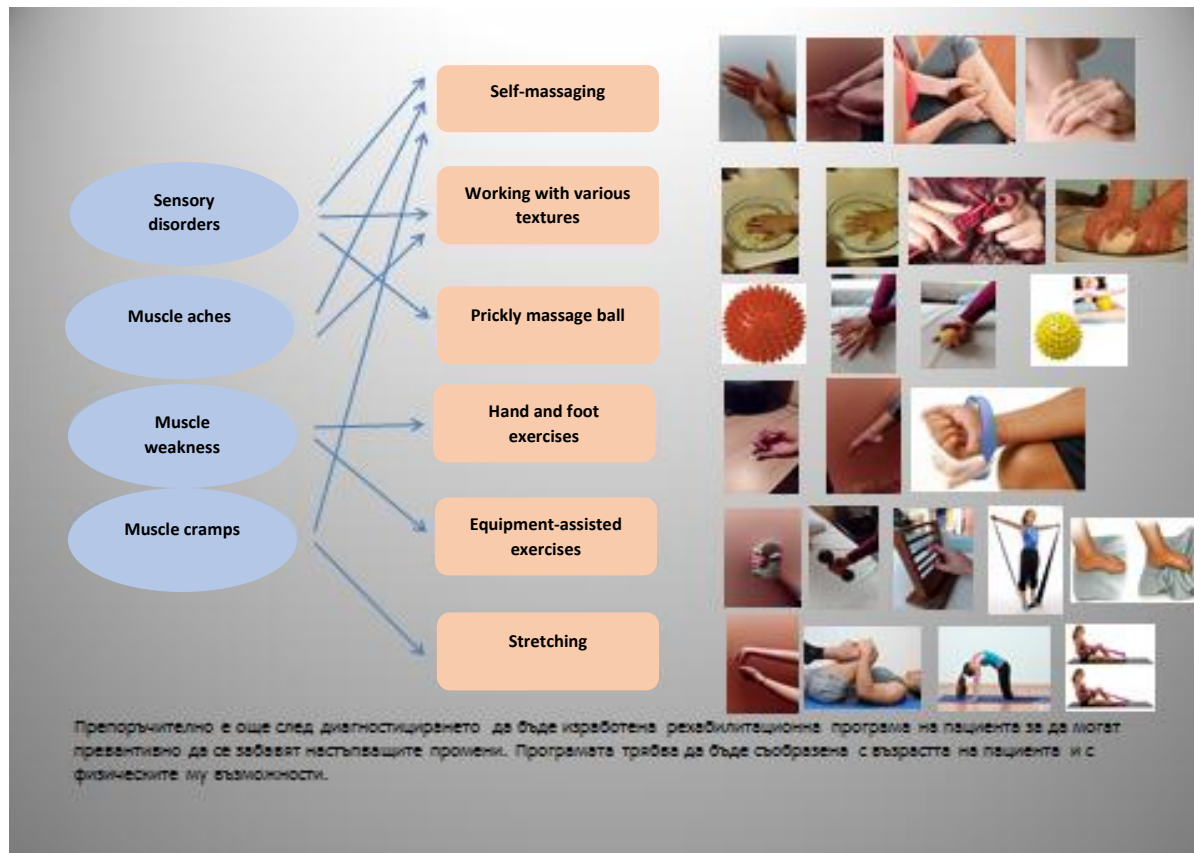
Bile acid malabsorption requires treatment with bile acid sequestrants (e.g., cholestyramine) with or without a reduced dietary intake of lipids.

Patients with hereditary ATTR may also have adrenal insufficiency, leading to nausea, vomiting, weakness, and orthostatic hypotension. A good response to hydrocortisone replacement therapy is usually observed.

XII. Rehabilitation in patients with transthyretin familial amyloidosis.

One of the most important recommendations for improving overall physical condition is regular and steady walking. It is recommended to walk slowly and steadily in

a calm and quiet place with fresh air (in a park or outdoors) for 5–10 minutes, 3–5 times a week. The duration of the walk should be increased by 1–2 minutes per week.



The main rehabilitation activities that patients can perform on their own at home are:

- Self-massage
- Various fabrics—working with them improves sensitivity and does not require much energy
- Spiky massage ball
- Exercises for the palms and soles—improves blood flow and enhances sensitivity
- Exercises with equipment—significantly improve strength and increase the number of muscle fibers used
- Stretching – allows for fuller utilization of muscle fibers and improves recovery.

XIII. Selective screening program to test for TTR gene mutations among at-risk families with the aim of identifying carriers, conducting dynamic monitoring, and initiating early treatment for symptomatic carriers.

The target group of the screening program consists of the first-degree relatives of diagnosed patients from 147 affected families: brothers, sisters, children, and cousins who are at risk of developing the disease. During the screening program, genetic counseling is provided to family members, along with detailed information about the disease for all family members, specifically: detailed explanations of what the disease is, why it occurs, how it can be inherited, what genetic testing entails, what it means to be a carrier, how often a carrier should be monitored, and when treatment is initiated.

All participants are consulted in person; their results are communicated both verbally and in writing, and the significance of these results is explained to them in detail, while strictly adhering to the principle of confidentiality regarding the results obtained.

Between 2014 and 2025, more than 1,408 individuals were examined, and 280 patients with hereditary ATTR were diagnosed from 147 affected families. More than 165 asymptomatic carriers were identified

XIV. Follow-up of asymptomatic carriers.

The tests and examinations used to monitor carriers of TTR mutations should be determined based on the expected phenotypic manifestations of the specific mutation. Different mutations have different clinical manifestations, so it is important to use the most appropriate tests for the carriers.

Follow-up should begin 10 years before the predicted age of disease onset. The frequency of follow-up should be increased for those mutations associated with a rapidly progressive course. Carriers should be educated to recognize the early clinical signs associated with specific mutations. Follow-up of asymptomatic carriers is conducted by a multidisciplinary team (neurologist, cardiologist, gastroenterologist, ophthalmologist, nephrologist) at a Center of Excellence for the disease.

1. Identification of symptoms and their association with the diagnosis of hereditary TTR amyloidosis (Conceicao I. et al., 2019):

Neuropathic symptoms*Positive sensory symptoms*

Spontaneous pain

Burning pain

Sharp pain

Paroxysmal pain

Provoked pain

Allodynia

Paresthesias

Restless legs syndrome

Negative sensory symptoms

Sensory deficits

Autonomic symptoms

Sexual dysfunction

Impaired bladder function

Constipation alternating with diarrhea

Orthostatic hypotension

Dizziness and syncope

Unexplained weight loss

Early satiety

Cardiac symptoms

Dyspnea on exertion and at rest

Orthopnea

Syncope

Neuropathic signs*Small fiber damage*

Loss of pain sensation

Loss of temperature sensation

Allodynia

Hyperalgesia

Damage to thick fibers

Loss of tactile, joint-muscle, and vibratory sensation

Absent tendon reflexes

Muscle weakness

Bilateral carpal tunnel syndrome

Ocular symptoms and signs

Vitreous opacity with gradual vision loss

Glaucoma

Cardiac signs

Signs of left ventricular failure:

Pulmonary edema

Signs of right ventricular failure:

leg

edema

Atrial flutter, sinoatrial block,

atrioventricular block

Palpitations

Pulmonary edema

Increased thickness of the left and right ventricles,
Decreased global strain with preserved apical deformation.
Cardiac fixation observed on bone scintigraphy

Possible associated symptoms

Paresthesias

Restless legs syndrome

Early satiety

Dizziness

Palpitations

Episodic diarrhea

Leg swelling

Definitely related symptoms

Spontaneous and provoked pain

Sensory deficits

Constipation alternating with diarrhea

Orthostatic hypotension

Unexplained weight loss

Shortness of breath during exertion and
at rest

Orthopnea and syncope

Clinical follow-up includes a neurological examination, including the Neuropathy Impairment Score (NIS), electroneurography, cutaneous sympathetic response, and quantitative sensory testing. Tests to assess autonomic function include postural blood pressure monitoring, the sudomotor test, and heart rate variability.

Relevant cardiac tests include electrocardiography to assess heart rhythm, echocardiography, biomarkers of cardiac function, cardiac magnetic resonance imaging, and bone scintigraphy.

The identification of amyloid deposits in biopsies also aids in the diagnosis of symptomatic ATTR. Tissue biopsies can be obtained from salivary glands, abdominal adipose tissue, or clinically affected organs.

2. Minimum criteria for establishing the onset of the disease.

A diagnosis of symptomatic hereditary ATTR should be made in the presence of at least one objective symptom or sign definitively associated with the onset of symptomatic ATTR, or at least one likely-related symptom plus one abnormal result from confirmatory tests, or two abnormal results from confirmatory tests in the absence of clinical symptoms (Conceicao I. et al., 2019)

The minimum criteria for establishing symptomatic disease can be used when monitoring carriers from known affected families and ensure the earliest possible diagnosis and early initiation of treatment. Once the mutation responsible for the disease is identified through genetic testing, the age of disease onset can also be predicted, taking into account the type of mutation, the typical age of onset of the clinical phenotype, and the age of onset in affected family members (Conceicao I. et al., 2019)

Follow-up of affected patients and asymptomatic carriers is provided at the Expert Center for Diagnosis, Treatment, Rehabilitation, and Prevention of Hereditary ATTR at Alexandrovskaya University Hospital.

Appendix 1. Neuropathy Impairment Score (NIS)

1. Assessment of muscle weakness:

0 = normal	3.25 - movement against gravity
1 = 25% weakness	3.50 - movement with gravity removed
2 = 50% weakness	3.75 - single muscle contraction
3 = 75% weakness	4.0 - paralysis

	Right								Left							
Cranial nerves	0	1	2	3	3.25	3.50	3.75	4	0	1	2	3	3.25	3.50	3.75	4
oculomotor nerve																
abducens nerve																

Facial weakness																	
Palatal weakness																	
Weakness of the tongue																	
Muscle weakness																	
Respiratory																	
Neck flexion																	
Abduction of the shoulder joint																	
Elbow flexion																	
m. brachioradialis																	
Extension of the elbow																	
Wrist flexion																	
Extension of the wrist																	
Fingers flexion																	
Spreading of the fingers																	
Thumb abduction																	
Hip flexion																	
Extension at the hip																	
Knee flexion																	

Extension at the knee joint																	
Ankle flexion																	
Extension of the ankle joint																	
Flexion of the big toe																	
Extension of the big toe																	

2. Assessment of tendon reflexes

0 = normal; 1 = diminished; 2 = absent

	Right			Left		
Biceps	0	1	2	0	1	2
triceps						
radial						
knee						
Achilles						

3. Sensory assessment

0 = normal; 1 = hypesthesia; 2 = anesthesia

	Right			Left		
Thumb	0	1	2	0	1	2
Touch						
Pain						
Vibration sensation						

Joint-muscle sensation						
Big toe						
Touch						
Pain						
Vibratory sensation						
Musculoskeletal sensation						