National consensus on the diagnosis, treatment, follow-up, and prevention of Hereditary Transthyretin Amyloidosis

edited by

Acad. Prof. Dr. Ivan Milanov, M.D, Ph.D.

Prof. Ivailo Tournev, M.D, Ph.D.

Assoc. Prof. M. Gospodinova Ph.D

Sofia, 2023r.

At the initiative of:

Bulgarian Society of Neuromuscular Diseases

Bulgarian Society of Neurology

Bulgarian Society of Cardiology

The Bulgarian Society of Gastroenterology

National consensus on the diagnosis, treatment, follow-up, and prevention of hereditary transthyretin amyloidosis

Today, 14.09.2023 we the undersigned specialists have reached a consensus on the diagnosis, treatment, follow-up and prevention of transthyretin familial amyloidosis

A/ from the Bulgarian Society of Neurology

Acad. Prof. Ivan Milanov .....

Prof. Ivailo Tournev .....

Acad. Prof. L. Traykov .....

Prof. P. Atanasova .....

Prof. L. Haralanov .....

Prof. S. Cherninkova .....

Prof. D. Maslarov .....

Prof. K. Genov .....

Prof. A. Kaprelyan .....

Prof. I. Staikov .....

Assoc. Prof. D. Bogdanova .....

Assoc. Prof. T. Chamova .....

Assoc. Prof. V. Guergueltcheva .....

B/ from the Bulgarian Society of Cardiology

Assoc. Prof. V. Traykov .....

Assoc. Prof. M. Gospodinova .....

Prof. E. Kinova .....

Prof. I. Gruev .....

C/ From the Bulgarian Society of Gastroenterology

Prof. K. Antonov .....

D/ From the Bulgarian Society of Neuromuscular Diseases

Prof. A. Todorova .....

Prof. L. Angelova

Content

Abbreviations used: 4
Introduction.....5
Pathophysiology.....6
Genetic Aspects.....9
Worldwide Epidemiology.....10
Epidemiology for Bulgaria.....12
Neurological Impairment.....16
Cardiac Impairment.....22
Gastroenterological Impairment.....23
Diagnosing.....24
Differential Diagnosis.....39
Pathogenic Treatment.....41
Summary and Perspective.....49
Symptomatic Treatment.....51
Rehabilitation.....56
Selective Screening Program.....57
Monitoring for Asymptomatic Carriers.....57

Abbreviations used:

AD – Autosomal dominant

ACE inhibitor – Angiotensin converting enzyme inhibitor

ARB – Angiotensin receptor blocker

GIT – Gastrointestinal tract

DL – Distal latency

ECG – Electrocardiography

EMB – Endocardial biopsy

EMG – Electromyography

ENG – Electroneurography

EchoCG – Echocardiography

EChC – Electrochemical conduction

CMP – Cardiomyopathy

LEP – Laser evoked potentials

LV – Left ventricle

LAHB – Left anterior hemiblock

MRI – Magnetic resonance imaging

OLT – Orthotopic liver transplantation

PNS – Peripheral nervous system

PND - Polyneuropathy disability

SCA – Senile cardiac amyloidosis

SNAP – Sensory nerve action potential

CMAP – Compound muscle action potential

CV – Conduction velocity

TTRA – Transthyretin amyloidosis

TTR – Transthyretin

FAC – Familial amyloid cardiomyopathy

FAP – Familial amyloid polyneuropathy

EF – Ejection fraction

HTTRA – Hereditary transthyretin amyloidosis

ATTR-CM - Transthyretin amyloid cardiomyopathy

ATTR-PN - Transthyretin amyloid polyneuropathy

ATTRv - Hereditary transthyretin-related amyloid protein

ATTRwt - Wild type transthyretin-related amyloid protein

I. Introduction to Hereditary Transthyretin Amyloidosis (HTTRA)

Amyloidosis refers to an extensive group of diseases associated with changes in protein structures which cause the normally soluble protein tetramers, following destabilization of their quaternary structure with subsequent dissociation to free monomeric proteins, to form insoluble extracellular deposits of fibrils, thus resulting in organ dysfunction. All types of amyloids contain a major fibril protein which determines the type of amyloid and other, minor components. More than 20 diverse amyloidosis-related fibril proteins have been described in patients, with each type featuring different clinical presentations. One such protein, which makes up human amyloid fibrils, is transthyretin (Ando et al., 2005)

TTR acts as a transport protein for plasma thyroxine. TTR also transports retinol (vitamin A) by binding with the retinol-binding protein. It circulates as a tetramer comprising four identical subunits. TTR may be found in plasma and in cerebrospinal fluid. It is synthesized mostly in the liver and in the choroid plexus of the brain, and also in smaller amounts in plasma. The TTR gene is located on the long shoulder of chromosome 18 and contains 4 exons and 3 introns.

Systemic amyloidosis is designated by the capital letter A (for amyloid), followed by an abbreviation indicating the chemical nature of the fibril protein. Thus, for example, TTR amyloidosis is abbreviated as ATTR and amyloidosis with immunoglobulin light chain deposits is abbreviated to AL (Saraiva M. et al., 1984; Connors L. et al., 2003; Ando Y. et al. 2005).

Classifying the known genetic types is exceptionally important in molecular genetics assays and their interpretation. The pathogenicity evaluation of any of the genetic types should be based on scientific evidence and should follow the unified nomenclature and rules. In view of the above, the terms ‘mutation’ and ‘polymorphism,’ which are in extensive use at present, have been replaced by a classification of genetic

variants which distinguishes the following 5 categories: pathogenic, likely pathogenic, variants of unclear clinical significance, likely non-pathogenic, and non-pathogenic (Richards S. et al., 2015; Nykamp K. et al., 2017).

II. Pathophysiology

Both TTRs with normal sequences (wild type) and pathological genetic variants of TTR may cause amyloidosis. A TTR gene with a normal sequence can cause cardiac amyloidosis in the elderly, which is known as senile, or wild-type, cardiac amyloidosis (SCA). After it was established that SCA was accompanied by microscopic deposits in multiple other organs, an alternative name, senile systemic amyloidosis, was proposed. Both terms are in use today.

The TTR pathological genetic variants can speed up the process of TTR amyloid formation and are the most important risk factor for the development of clinically significant ATTR. There are over 130 amyloidogenic TTR variants which can cause systemic hereditary amyloidosis (Rowczenio D. 2014; Hou X.et al. 2007; Benson MD, Kincaid JC, 2007; Parman Y. et al., 2016).

TTR variants have conventional designations which follow the most recent version of the international nomenclature http://www.HGVS.org/varnomen. An older version of the nomenclature failed to distinguish a shorter signal sequence of about 20 amino acids in the N-terminal domain of the protein structure, leading to a misplacement of amino acid numbering in the description of the genetic variants of the TTR gene. Currently, a combined nomenclature is used consistent with the Mutations in Hereditary Amyloidosis [http://amyloidosismutations.com/main_menu.html].

Most variants causing hereditary TTRA are rare, but some are more common among certain groups in the human population. By now, the following variants of the TTR gene have been identified in Bulgarian patients:

- **c.148G>A, p.Val50Met (Val30Met)** is the first pathological variant discovered in the TTR gene. This amino acid replacement is due to a missense variant in the TTR gene. Said pathological variant occurs globally and is the best studied TTR variant serving as the XTTRA prototype with a mutant sequence. Among relatives of patients indexed with the TTR p.V30M, the disease is known as FAP because

the early symptoms present as peripheral neuropathy; in fact, those patients have systemic amyloidosis with multiple deposits, often involving the heart, the gastrointestinal tract, the eyes, and other organs.

- **c.200G>A; p.Gly67Glu (Gly47Glu)** pathological variant was first identified in patients of Italian origin. Usually, the disease has an early onset, a more aggressive course and short survival periods. Recently, Bulgarian patients of Roma origin have been reported to be carriers of this genetic variant (Pelo E. et al., 2002)
- **c.214T>C , p.Ser72Pro (Ser52Pro)** pathological variant was first reported in British patients. By now, it has been reported in one Bulgarian family (Stangou, A. et al., 1998).
- **c.290C>T, p.Ser97Phe (Ser77Phe)** genetic variant was first reported in France. In Bulgaria, it was identified in several families with an ancestor effect from the village of Vakarel. The prevalent phenotypic presentations include polyneuropathy and cardiomyopathy (Planté-Bordeneuve V. et al., 1998).
- **c.325G>C, p.Glu109Gln (Glu89Gln)** pathological variant was first reported in Italy. It has been identified in many Bulgarian patients. Most patients are from the area of Kyustendil and Blagoevgrad regions, which appear to be endemic regions in Bulgaria. The prevalent phenotypic presentation is polyneuropathy, often concomitant with restrictive cardiomyopathy and gastrointestinal disorders (Almeida M. et al., 1992).
- **c.220_221delGAinsCT, p.Glu74Leu (Glu54Leu)** genetic variant is new for Bulgaria and was discovered for the first time in a family from the city of Targovishte. It has been reported in patients with hereditary TTRA from Sweden, Belgium and the UK (Hellman U et al., 2019). It is associated with severe cardiac and milder neurological involvement.

So far, more than 130 TTR variants have been identified. These display different geographic distribution, amyloidogenicity potential and organ predisposition (Connors L. et al., 2003; Saraiva M. et al., 1984; Palha J. et al., 2000; Peterson S. et al., 2001).

As of today, there is one known endemic region in the southwest of Bulgaria (Figure 1).

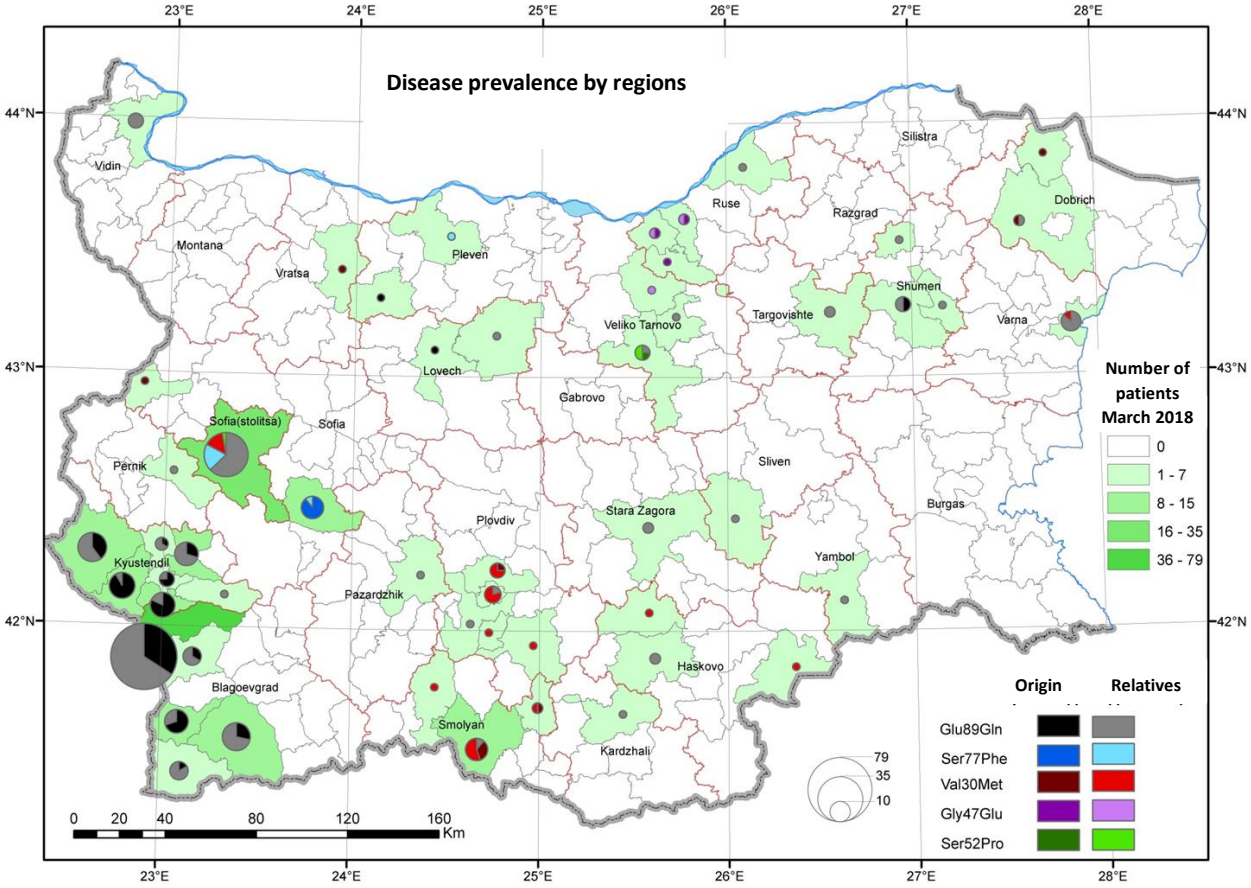


Figure 1. Geographic distribution of TTR gene pathological variant carriers in the territory of Bulgaria

By now, the efforts of the interdisciplinary team have resulted in the identification of a total of 370 TTR gene pathological variant carriers, including patients and their relatives (some of them with no clinical manifestations of the disease at present). Figure 2 shows the distribution of the different pathological variants of the gene. The genetic approach to diagnosing the disease in Bulgaria starts with testing for the most common mutations by geographic region. Where the target screening test is negative, a comprehensive testing of the coding sequence of the TTR gene follows. Based on the results, the mutant cases of wild-type TTR are differentiated.

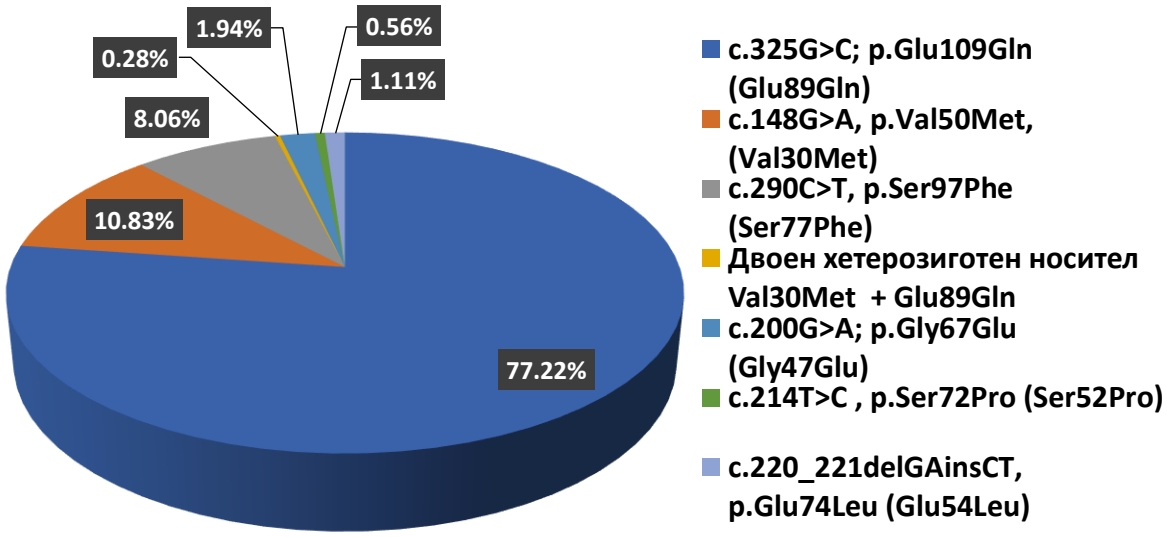


Figure 2. Distribution of pathogenic mutations in the Bulgarian population.

III. Genetic aspects.

1. Genetic aspects of hereditary transthyretin amyloidosis.

Hereditary TTRA is traditionally considered a monogenic autosomal dominant disease but nowadays it is known that the disease is much more complex. Globally, data points on the p.Val30Met variant are the most numerous but more detailed studies on other variants, for instance p.Glu89Gln, now known as the Bulgarian mutation, have recently become available, too. The following characteristics have been noted:

- Variations in the age of onset of the first symptoms: the usual age for the onset of the disease among p.Val30Met carriers in Portugal, Brazil, and Japan is the third or fourth decade of life. Cases of late onset are known (e.g., in Sweden), where the onset of the disease is in the fifth up to the sixth decade of life. In Bulgaria, the carriers of this pathological variant are characterized with a late onset.
- Differences in gene penetrance rates: in Portugal and Japan, more than 90% of the p.Val30Met carriers are middle-aged at the time of the onset of symptoms.

However, the disease penetrance rates in Sweden is only 2%, and some p.Val30Met homozygous individuals remain permanently asymptomatic.

- Certain atypical Portuguese and Japanese relatives follow the Swedish pattern of late onset with low penetrance.
- Some patients with no family history of amyloidosis have relatives who are asymptomatic carriers of the p.Val30Met variant.
- The onset of the disease is earlier in men than in women. For the Bulgarian p.Glu89Gln mutation, the difference of the age of onset between the two sexes is about 4.5 years, with earlier onset in males, too.
- In some cases, the onset age when the first symptoms manifest decreases in descendant generations. This feature is known as anticipation. The cause of anticipation is unknown and other factors, for instance epigenetic factors, distinct from the genetic causes may be responsible.

Anticipation, incomplete penetrance, and sporadic cases among relatives of unaffected pathological allele carriers have been reported for other pathological variants of the TTR gene as well.

2. Transthyretin-associated amyloidosis with normal (wild-type) sequence

Unlike the mutant TTRA type, the senile type which is free of pathological genetic variants (wild type), is associated with the processes of aging, usually in the seventh or eighth decade of life. The cases may be asymptomatic or with negligible clinical impact. On the other hand, other senile patients with TTRA with normal sequence have developed symptomatic and even fatal cardiac TTRA. The factors leading to wild-type TTRA are unknown. Involvement of other genetic and epigenetic factors is likely. The clinical presentations of severe SCA are like those observed in familial TTRA and in cardiac amyloidosis of the immunoglobulin light chain deposits (AL) type.

IV. Epidemiology of hereditary transthyretin amyloidosis.

1. Global epidemiology of hereditary transthyretin amyloidosis.

The first description of hereditary transthyretin amyloidosis came in 1952 from North Portugal in the Póvoa de Varzim districts by Portuguese neurologist Mário Corino da Costa Andrade (Andrade, C., 1952) and it was later identified in many other countries as well (Planté-Bordeneuve V. et al., 2007). At present, it is known to occur in 36 countries. Before the gene was sequenced in 1983, (Dwulet F., Benson M., 1983; Sasaki H. и et al., 1985) it was known as a Portuguese disease. Later, the Val30Met genetic mutation causing the disease was identified (Saraiva, M. et al., 1984). In the 1960s and the 1970s, two other large endemic regions were discovered in Japan (1968) and in Sweden (1976) and these featured the same mutation (Ikeda, S. et al., 2002; Saraiva M. et al., 1984).

Transthyretin amyloid polyneuropathy is a rare disease with a global burden of 1/10,186. The morbidity is highest 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 within the endemic regions in Portugal is 1/1,108 (Sousa A. et al., 1995).

The prevalence in the USA (Benson M. et al., 2000) is 1/100,000 but only among Caucasians. A diversity of mutations has been reported in the USA, due to the multi-ethnic origin of the population. According to the THAOS (Transthyretin Amyloid Outcome Survey) register, a total of 34 different mutations have been identified, the most common being Val122Ile (45.3%), Thr60Ala (20.4%), Val30Met (6.0%). Another mutation, Ser77Tyr, has also been reported (Maurer M. et al., 2016). The mean age of disease onset is about 63 years. It is considered that Val122Ile, which is characteristic for African Americans and can lead to familial amyloid cardiomyopathy, originated in West Africa (Pinto M. et al., 2017).

The disease prevalence in Japan (Kato-Motozaki Y. et al., 2008) ranges from 0.87 to 1.1/ 1,000,000 per the population. The highest prevalence has been reported for Nagano – 12/1,000,000, Kumamoto 10.2/1,000,000 and Ishikawa at 3.8/1,000,000.

Val30Met is also found in the Balearic Islands (a Transthyretin Amyloidosis Centre has been established in the city of Majorca). These islands are Spanish territory. The prevalence in Majorca is 5/100,000, and 1/100,000 in Minorca, another of the Balearic Islands. The prevalence in Cyprus is 43.34/1,000,000, and the morbidity rate is 0.69/100,000.

The French patient population is also reported (Adams D. et al., *2012)* to feature a great heterogenicity. A total of 29 different mutations have been identified. The most common one *is the* Val30Met (62%), followed by the Ser77Tyr (11.8%), and the Phe77 (6.2%).

Diverse mutations have been identified in Italy (Rapezzi C. et al., 2006). Interestingly, the Glu89Gln originally found in Sicily is the most common mutation in Bulgaria (Mazzeo A. et al., 2015).

In Turkey, seven mutations have been identified: Val30Met, Glu89Gln, Gly47Glu, Gly53Glu, Glu54Gly, Thr49Ser, and Glu54Lys. The first three occur in Bulgarian patients, too (Bekircan-Kurt CE et al., 2015; Durmuş-Tekçe H. et al., 2016).

In Israel, five different mutation types have been identified: Ser77Tyr, Phe33Leu, Gly6Ser, Val32Ala, Val30Met, and Ser77Tyr, Val30Met.

In Slovenia, the mutations discovered include Val122Ala, Val30Ala, Ile107Phe, Asp38Asn. In Romania, the Glu54Gln has been identified.

In December 2007, the international THAOS register was established. By 1 July 2018, more than 4,000 patients with TTRA have been recorded by 18 centres from all over the world (https://www.ttr-fapconnection.com/treatment-center-finder), including Bulgaria. According to data from the Register, some of the common mutations include Val30Met (73.3%), Val122Ile (4.4%), Glu89Gln (2.1%), Leu111Met (1.9%), Thr60Ala (1.7%), Ser77Tyr (1.6%), Phe64Leu (1.5%). Most patients (82.6%) were Caucasian (Coelho T**.**, 2013).

2. Epidemiology of hereditary transthyretin amyloidosis for Bulgaria

Bulgaria is among the countries with a high number of reported cases of TTPA. The most common TTP pathogenetic variant among Bulgarian patients is Glu89Gln, but other, less common variants have been observed: Val30Met, Ser77Phe, Gly47Glu, Ser52Pro, Glu74Leu.

As a result of a ten-year selective screening program among a high-risk population of patients and relatives of genetically verified affected people, out of a total of 1040 people tested, 370 carriers of TTP mutations (both affected and asymptomatic)

belonging to 134 pedigrees have been identified in Bulgaria, which are distributed in the last way:

- Glu89Gln – 100 families
- Val30Met – 16 families
- Ser77Phe – 13 families
- Gly47Glu – 2 families
- Ser52Pro – 1 family
- Glu54Leu – 2 families

All affected showed a mixed phenotype with variable age at occurrence and rate of progression depending on mutation. A regular follow-up program for asymptomatic carriers in the last 4 years has allowed to detect the transition of 47 (85) to symptomatic patients and to start timely treatment.

For Glu89Gln the effect of the precursor with approximate age of occurrence of the mutation was established 850 generations ago. Families with the different mutations have a certain region of origin as follows:

- **Glu89Gln epidemiology**

Glu89Gln is the most common TTP mutation in Bulgaria (78.53%).

The clinical phenotype in the Glu89Gln patient group was mixed, with peripheral nerve, heart and gastrointestinal system involvement.

Patients with this mutation originate exclusively from the southwest region of Bulgaria, from the town of Dupnitsa to the town of Petrich. The focal spot has a length of 200 km and is located along the state border with the Republic of North Macedonia. The affected areas are mostly within the Kyustendil and the Blagoevgrad administrative districts. Due internal migration, patients with this mutation are found also in the Northeast of Bulgaria, in the administrative districts of Varna, Shumen, Targovishte, Dobrich, Veliko Tarnovo and Ruse. They are less numerous in Central Bulgaria, in the administrative districts of Lovech, Pleven, Gabrovo, and in the Southeast and South of Bulgaria in the administrative districts of Haskovo, Kardzhali, Stara Zagora, Sliven, Pazardzhik, Plovdiv and Smolyan.

Within the Kyustendil administrative district, patients' places of origin are mostly the following municipalities: Kyustendil, Bobov dol, Boboshevo, Dupnitsa, Kocherinovo,

Nevestino. Within the Blagoevgrad administrative district, patients' places of origin are mostly the following municipalities: Blagoevgrad, Sandanski, Simitli, Strumyani, Petrich.

- **Val30Met epidemiology**

Patients hosting this mutation have been found in the administrative districts of Blagoevgrad, Varna, Plovdiv, Smolyan, and Sofia Region. In general, the distribution of patients with this mutation across the country displays a more scattered pattern. One of the families originates from the Xanthi region in Greece. Members of the Val30Met affected families live in the Plovdiv and Asenovgrad areas.

This mutation has a markedly endemic pattern, too. The district of Smolyan constitutes the smaller focus area. The larger focus area is probably spread over the whole of South Bulgaria, between the towns of Smolyan, Haskovo, Svilengrad, Stara Zagora, Plovdiv, Blagoevgrad. It may not be excluded that the endemic foci of the Glu89Gln and the Val30Met are adjacent along the Struma river course, suggesting the possibility of more than one double heterozygote for the two mutations.

Bulgarian nationals who belong to the affected families that display symptoms of the disease and who are currently undergoing tafaminis treatment, as well as asymptomatic carriers, also live in other countries such as the UK, France, Germany, Italy, Spain, Portugal, Canada, the USA, and the UAE.

- **Ser77Phe epidemiology**

All patients with this mutation originate from the village of Vakarel but from different parts of the village. One of the patients with this mutation is from the village of Venkovets situated 32 km from the village of Vakarel. A patient with the same mutation is from the village of Karapoltsi in the Elin Pelin Municipality. The distance between the two villages and the village of Vakarel allows for migration.

This mutation is most widespread in the French population (the Southwest of France, in the region of Toulouse). Historically, the mutation may have been transferred via the French crusaders during the Middle Ages but that is yet to be confirmed by haplotype analysis. According to another hypothesis, the mutation was carried to France by the Bogomils after they were driven out of Bulgaria during the Middle Ages.

- **Ser52Pro epidemiology**

The family hosting the Ser52Pro originates from the municipality of Elena. One carrier of the mutation lives in the city of Sofia.

- **Gly47Glu epidemiology**

Families with the Gly47Glu originate from the districts of Russe and Veliko Tarnovo and are from the Roma ethnic group, specifically the Muslim Roma group known as millet. The first recorded family is from the village of Tsenovo and the second family comes from the town of Polski Trambesh. The same mutation was reported for the first time in Italy in a large family of four generations and among Muslims from Xanthi, Greece.

- **Glu54Leu epidemiology**

The first family in three generations was found in Targovishte, but they are immigrants from the Blagoevgrad region. The second family is from Blagoevgrad.

Distribution and incidence of hereditary transthyretin amyloidosis worldwide:

The “Incidence” parameter indicates the number of newly diagnosed cases a year per the total population (the risk population), and the “Prevalence” parameter value indicates the total number of genetically confirmed cases per the total population.

To calculate the aforesaid two parameters, data from the National Statistical Institute on population density disaggregated by geographic origin - municipalities and administrative districts - has been collected (www.nsi.bg):

- **Glu89Gln**

The incidence of new cases in the Blagoevgrad district is 1/7,300 of the general population and in the Kyustendil district this parameter stands at 1/8,500. The combined incidence for the two districts amounts to 1/7,636.

Distribution in the Blagoevgrad district is 1/4,200 and in the Kyustendil district the morbidity rate stands at 1/3,900. The combined prevalence for the two districts is 1/4,150 of the general population.

- **Val30Met**

The combined incidence for the two districts amounts to 1/9,000 per the population.

Prevalence in Cyprus is 1/3,200 and the morbidity rate stands at 0.69/100,000. Patients and asymptomatic carriers of the Val30Met mutation who do not come from municipalities within the Smolyan district were excluded the calculations.

- **Ser77Phe**

The incidence (new cases) for the Municipality of Ihtiman amounted to 1/1,600 people and the prevalence were 1/850 people.

The resulting data show that there are endemic regions of different areas in the Bulgaria for each of the different mutations, Glu89Gln, Ser77Phe and Val30Met. The incidence and the prevalence values for the three mutations disaggregated by patients' place of origin serve as a criterion for endemic region identification (https://www.recognizingttr-fap.com/epidemiology) – ranging from 1/1,000 to 1/10,000.

For Bulgaria, with a population of 6.839 million (31 December 2022, National Statistical Institute of the Republic of Bulgaria), the overall mean incidence (new cases) of all the mutations combined and the clinically diagnosed patients over the 10-year survey period stood at approximately 1/54,500. The average disease prevalence in the country was 1/28,700.

These data are extremely dynamic and change each month as a result of newly diagnosed cases and asymptomatic carriers. This results in changes of both parameters with an incremental trend.

The number of identified carriers for the last 7 years amounts to 122.

V. Neurological impairment in hereditary transthyretin amyloidosis

Peripheral nerve impairment is one of the main clinical signs of the disease. It develops as:

- *Progressive distal sensorimotor polyneuropathic syndrome* (Plante-Bordeneuve V., Gerard S., 2011). It features hypo/areflexia in all four extremities, more marked in the lower extremities, impairment of all types of somatic sensations (touch, pain, temperature, muscle-articular sensitivity, vibrations, two-dimensional sensations) without a strictly defined onset order in our patients. In most cases, the first signs may be sensory disorders but changes in reflexes may also be the presenting sign of the disease. Sensory impairments determine the postural

instability during Romberg test to a score of marked ataxia, locomotion ataxia included. Onset is in the lower extremities with subjective hypesthesia, paraesthesia, and dysesthesia. Often hypesthesia may occur concomitantly with pain resistant to common analgesics used for the affected areas. Symptoms in the arms occur somewhat later (within the span of a few months) and rarely in more than a year's time. Parallel symptom development in the arms and legs is significantly less common. In the course of disease progression, distal muscle group hypotrophy develops, later followed by proximal muscle group hypotrophy combined with peripheral palsies/paralysis. Visible fasciculations may be present. Cranial nerve impairment is an uncommon clinical manifestation. Impairment, mostly of the bulbar and cranial nerves, may occur and result in vocal cord paresis (Plante-Bordeneuve V., 2007). Bulbar palsy is a rare finding and progresses with pharyngeal areflexia, dysarthria, dysphagia, tongue atrophy, and fasciculations, tongue and limb denervation, reflecting amyloid deposits in peripheral nerve structures in Val30Met cases (Ikeda K. et al., 1998). Cranial nerve impairment and mostly dysarthria have been reported in some of our patients with the Glu89Gln.

- *Carpal tunnel syndrome* is a common presentation of the disease (Sekijima Y., 2015; Kana T. et al., 2010). It is associated with amyloid deposits in the carpal ligament and direct compression of the medianus nerve due to deposited amyloid (Suhr O. et al., 2003). The finding is usually bilateral and more pronounced one side. The clinical picture is typical, with painful hypesthesia with paraesthesia, hyperesthesia with dysesthesia in the wrists and the fingers, resolving rapidly upon agitation of the hands. It is clinically indistinguishable from other causes of the syndrome. In the evenings, symptoms become more pronounced. Symptoms progress with time. These develop into a classical polyneuropathic syndrome over different time intervals, ranging from half a year to more than 20 years, and remain the only manifestation of the disease (Kana T. et al., 2010). Among our patients, the interval is 6 years on average. Those cases often (uni- or bilateral) undergo surgery but recurrent episodes are common.

- *Autonomous nervous system impairment.* It was suggested (Plante-Bordeneuve V. and P. Kerschen, 2013) that the signs of impairment include diarrhoea resulting from damage and count reduction of Cajal cells, regarded as the intestinal “pace maker,” urinary disorders (for instance urine retention and incontinence), erectile dysfunction in males (often reported as an early sign), sexual disorders in women, sweating disorders manifested with profuse sweating or as dry skin in advanced disease, arterial hypotension, with collapse episodes in severe cases. Impaired secretion of tears in the eyes, usually decreased and rarely increased in the early stages of the disease, and reduced resistance to tearing of the lacrimal film may be considered symptoms, too.

Classical transthyretin neuropathy affects the axons and depends on nerve length, the so-called length-dependent small fibre neuropathy described by Andrade in an early onset Val30Met case (Andrade C., 1952). Initially, the non-myelinated and the small, myelinated nerve fibres are affected, which results in impaired temperature and pain perception starting in the lower limbs (Conceição *I.* et al., 2015), while the tactile and the muscle-articular perceptions remain unaffected. Findings include paraesthesia, dysesthesia, allodynia, hyperalgesia, or spontaneous burning pain in the lower limbs. These are usually reported as the so-called sensory dissociation. Pain may not be present in all cases (Plante-Bordeneuve V., Gerard S., 2011). During this stage, reflexes are usually unaffected. Axon degeneration progresses in the proximal direction and reaches the arms in a period of four to five years on average. Within a period of several years, larger myelinated sensory and motor fibres structures may be affected, which leads to impaired perception of touch, muscle-articular sensations, two-dimensional space and vibration sensory perceptions. A motor deficit may occur, which will impair the gait. Trophic plantar lesions may occur, which resemble perforations as well as neurogenic arthropathies.

The clinical phenotype of our patients failed to strictly comply with this pattern of sensory dissociation. It was mixed, regardless of which of the five mutation variants (Glu89Gln, Ser77Phe, Val30Met, Gly47Glu, Ser52Pro) had been identified: peripheral nervous system and cardiac impairment was present, with gastrointestinal tract

disorders following in more advanced cases. The Glu89Gln had a markedly mixed phenotype (Coelho T. et al., 2013) with a mean onset age of 52 years, slightly earlier in males; Ser77Phe also exhibited a mixed clinical phenotype but had a mean onset age of 58 years; the mean onset age was the highest for Val30Met - 68 years, the phenotype was of the mixed type; however, the polyneuropathy prevailed over the cardiomyopathy. The average patient survival time was 8 years. Renal impairment was found in Ser52Pro and in Gly47Glu patients. Gly47Glu had an average onset age of 30 years among males and 35 among females; onset presentation involved gastrointestinal impairment and mild polyneuropathy. The median survival time was 5 years for Gly47Glu.

Worldwide, other mutations have been reported with other predilections: exclusive cardiac phenotype in Val122Ile without neuropathy, 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 associated with the Asp18Gly, Ala25Thr, Tyr114Cys mutations is related to cerebral amyloid angiopathy. It is characterized by amyloid deposits in the media and adventitia layers in medium and small-sized arteries, arterioles, and rarely in cortical veins. Clinical manifestations include cerebral infarction, brain haemorrhage, hydrocephaly, diverse stages of cerebral dysautonomia, ataxia, spastic palsy, epileptic seizures, dementia. Leptomeningeal amyloidosis is found in cases hosting the Val30Met and in post-transplant patients - the choroidal plexus continues to produce mutant transthyretin despite the liver transplantation (Sekijima Y., 2015).

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

Stage 1	Sensory and motor neuropathy limited to the lower extremities. Severe impairment of temperature and pain sensation with relatively unaffected proprioceptive perception (sensory dissociation). Mild motor disorders. Retained ability for unassisted walking without aids.
Stage 2	Walking with assistance or aids. The neuropathy progresses with

	impairment of the upper limbs and the body. Amyotrophy of the upper and lower limbs. Moderate neurological deficit.
Stage 3	Terminal stage. Confinement to a wheelchair or bed. Severe sensory, motor, and autonomous neuropathy in all four extremities.

A newer, modified neuropathy scoring scale has been developed for practical purposes, which scores the level of patients' disability (Ando Y. et al., 2013):

I. Sensory disorders but retained ability to walk
II. Walks with difficulties but without the need to use mobility aids when walking
III A. Using a single walking stick or a single crutch when walking
III B. Requiring the use of two walking sticks or two crutches when walking
IV. The patient is confined to a wheelchair or is bedridden

Nerve conduction examination

The basic diagnostic method in neuropathy, providing information about the large, myelinated fibres but non-sensitive for small, myelinated fibres impairment testing.

The findings of examinations of nerve conduction in patients with mixed type clinical phenotype with non-Val30Met mutations of non-endemic districts indicated different changes in the majority of parameters for the nerves examined: prolonged distal latencies of the medianus, ulnaris and the tibialis nerves of different percentage (Koike H. et al., 2008; Koike H. et al., 2011; Escolano-Lozano F. et al., 2018), decreased motor response amplitude from the medianus and ulnaris nerves, decreased conduction velocities along the sensory fibres of the medianus and ulnaris nerves, often, lack of sensory response from the suralis nerve (Koike H. 2009). At the time of diagnosis, the decreased motor and sensory response amplitude of lower limb nerves may be more pronounced (Koike H. et al., 2012).

Nerve conduction of the tibialis, medianus and the suralis nerves examination findings are characterized by axon neuropathy but with demyelination components due to the prolonged distal latency (Koike H. et al., 2008; Louise-Laure M. et al., 2015). Emphasis is laid on the combination of prolonged distal motor latency of the medianus

nerve plus axon polyneuropathy signs (Luigetti M. et al., 2013). A characteristic finding in our patients was the prolonged distal latency time along the motor fibres for both medianus nerves, often in combination with a lack of acquisition of sensory response conduction from those same nerves. A possible explanation may be myelin sheath damage caused by the amyloid associated with segment demyelination (Plante-Bordeneuve V. et al., 2007).

The electrophysiological parameters correlate with the clinical stage of the disease and the duration of polyneuropathy. The sensory potential is lost earlier than the motor one (as per examinations of ulnaris and tibialis nerves). A linear correlation was established between the duration of the disease and the aggravation of the parameters for the nerves examined (motor response from the m. abductor digiti minimi, velocity along the motor and the sensory fibres of the ulnaris nerve, distal latency from the ulnaris and tibialis nerves) (Kodaira M. et al., 2011). The most sensitive parameter for monitoring disease progression in transthyretin amyloid polyneuropathy is assumed to be the motor potential. The mutation does not affect examination findings for nerve conduction, but certain parameters may deteriorate with age (the amplitude and the conduction velocity along the sensory fibres of the medianus nerve, the amplitude of the sensory response along the peroneus and the suralis nerves (Lefaucheur J. et al., 2013).

Cerebrospinal fluid testing

Changes in cerebrospinal fluid are important for the differential diagnosis in order to avoid misdiagnosis of a chronic inflammatory demyelinating polyneuropathy. Patients with transthyretin amyloid polyneuropathy, regardless of the underlying mutation, may exhibit changes in their protein profile patterns. Most commonly, they are associated with the total protein in cerebrospinal fluid. It may be increased, although not as much as in chronic inflammatory demyelinating polyneuropathy (Conceigao I. et al., 2016). The majority of authors have reported mild to moderate hyperproteinorachia [increased CSF proteins] with decreased cell counts, i.e., cell vs. protein content dissociation (Cappellari M. et al., 2011; Koike H. et al., 2012). In isolated cases, high levels, 7.23 g/l (C Douglass, 2007), and extremely high levels of 2.20 g/dl have been found (Pelo E. et al.,

2002). In the most recent case reported, an increase in the albumin index was found as well (albumin levels in CSF vs. serum albumin levels).

Changes in cerebrospinal fluid do not consist only in the total protein increase but include impairment of the blood-brain barrier, too. Patients with leptomeningeal transthyretin amyloidosis were found to have increased albumin concentration quotient in their CSF/serum ratio (Nakamura M. et al., 2005). Histopathological findings in such patients include marked amyloid deposits in the leptomeninges, the vasculature and the parenchyma of the spinal cord and the brain. According to the authors, the finding is the cause for the blood-brain and blood-CSF barriers impairment.

VI. Cardiac impairment in hereditary transthyretin amyloidosis

Cardiac impairment features the development of infiltrative cardiomyopathy, manifesting with heart failure and rhythm and conduction disorders (Rapezzi C, 2010, Rodney H. Falk 2005, 2010, Frederick L. Ruberg, 2012).

The symptoms are unspecific and often the initial complaints reported consist in pericardial heaviness and palpitations. Many cases of patients with previous arterial hypertension or normotensive subjects report low arterial blood pressure, and the causes are complex, e.g., autonomous dysfunction, decreased stroke volume, hypovolemia due to chronic diarrhoea. In some of the patients, cardiac damage may be wrongly interpreted as a hypertensive heart even in the absence of history for arterial hypertension in some, while patients with pronounced left ventricular hypertrophy may be misdiagnosed with hypertrophic cardiomyopathy. The signs of heart failure are preceded by an asymptomatic period of varying length during which progressive infiltration of the myocardium with amyloid is taking place. Apart from the left and right ventricular myocardium, amyloid deposits may be found in the atria, the valves, around small blood vessels, and the conduction system may be involved, too. Heart failure signs may be masked by the decreased patient mobility associated with peripheral neuropathy. Common findings include rhythm and conduction disorders: atrial flutter, atrioventricular and sinoatrial block. Some patients may require a permanent pacemaker implant or cardioverter-defibrillator. Atrial flutter increases the risk of embolic stroke. The most common causes of death in patients with hereditary ATTR are progressive heart

failure and sudden cardiac death, usually due to electromechanical dissociation and asystole (Kristen, A.V. 2008).

VII. Gastro-intestinal tract (GIT) disorders in transthyretin familial amyloidosis

Digestive system impairment in hereditary TTRA clinically presents with gastrointestinal tract (GIT) symptoms and those is a common finding.

Over half of the patients with confirmed mutation (63%) have at least one GIT symptom. Symptoms may be from the upper part of the GIT (oesophagus, stomach, duodenum), from the lower GIT (the small intestines or the bowel) or both from the upper and the lower GIT concomitantly. Therefore, gastrointestinal symptoms in hereditary TTRA are very diverse. They may include difficulties in swallowing, or food stuck in the oesophagus (dysphagia); heaviness or bloating of the upper abdomen or of the whole abdomen, early (fast) satiation during feeding (after intake of small amounts of food); nausea, vomiting; stomach rumble; abdominal gas (meteorism or flatulence); constipation; diarrhoea; alternating diarrhoea and constipation; faecal incontinence (spontaneous, uncontrollable bowel movement/faecal discharge).

The most common symptoms include early satiation and changes in the rhythm of defecation – alternating from constipation to diarrhoea, followed again by constipation, diarrhoea, nausea, and vomiting. Concomitant weight loss may occur. A less common symptom is faecal incontinence and belching is the least commonly reported symptom; gastro-oesophageal reflux or abdominal pain related symptoms.

Gastrointestinal symptoms are more common among Val30Met carriers, as well as in early age disease onset - earlier than 50 years of age (Wixner J. et al., 2014).

Usually, those symptoms occur after the nerve system and/or heart changes have already presented but they may be the first presentation of the disease, too, i.e., preceding the onset of peripheral polyneuropathy (Wixner J. at al., 2014; Conceição I., 2016). Although diarrhoea is characteristic of the late stages of the disease, it may alternatively be the first and earliest sign. Once manifested, it persists and commonly co-occurs with an urge to defecate, faecal incontinence and severe malnutrition. The frequency and the severity of gastrointestinal symptoms increases with the duration of

the disease. During late stages, symptoms become frequent or daily and grow severe. New symptoms appear and existing ones worsen, resulting in malnutrition and progressive loss of body weight.

The presence of gastrointestinal symptoms has been linked with more severe stages of the disease, decreased quality of life and a reduced survival rate. Therefore, it is important that those symptoms be carefully and thoroughly evaluated, monitored, and managed (Wixner J. et al., 2014; Conceição I., 2016; Sekijima Y., 2015).

Physical examination of the abdomen may fail to detect specific changes and is useful for detecting only bloating or flatulence, mildly enhanced or slowed peristalsis. Pain upon palpation is frequently absent. Changes may be undetectable despite the severe symptoms. In advanced stages of the disease, the most obvious signs are those of malnutrition.

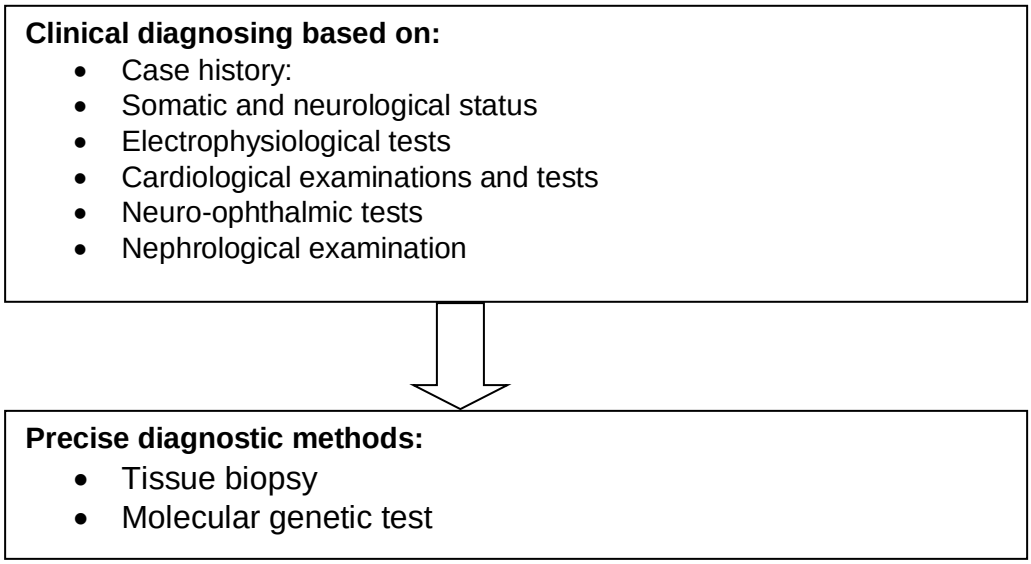
Changes in the GIT are associated with amyloid deposits in the gastrointestinal mucosa with impairment of the functions and the regulation of the autonomous and enteric nervous systems, decrease in the number of gastrointestinal neuroendocrine cells, and of the Cajal interstitial cells (Yoshimatsu S. et al., 1998; Ikeda S. et al., 2009; Wixner J. et al., 2013). This results in multiple generalized motor function impairments affecting the oesophagus, the stomach (impaired accommodation and slow emptying), changes in defecation (slow or faster intestinal passage), small intestine bacterial overgrowth, malabsorption of bile acids of basic nutrients (fats, proteins), lipid-soluble and water-soluble vitamins, electrolytes, microelements, etc., protein loss via the gastric and/or intestinal mucosa (Yoshimatsu S. et al., 1998; Ikeda S. et al., 2009; Wixner J. et al., 2013).

VIII. Diagnosing hereditary transthyretin amyloidosis

The early and precise diagnosing of hereditary TTRA is still a challenge for physicians due to the clinical heterogeneity of the disease (Adams D. et al., 2016; Conceiao I. et al., 2016).

The diagnostic process for hereditary TTRA consists of two main stages:

- Clinical diagnosis with additional confirmative tests
- Precise diagnostic methods: tissue biopsy and molecular genetic assays



It is important to distinguish between the diagnostic approaches in individuals with known familial predisposition originating from the so-called endemic regions and in patients with no family history (Adams D. et al., 2016; Conceiao I. et al., 2016).

In patients with evidence of familial predisposition/or pre-symptomatic evidence of genetic defects, any symptoms within the profile of peripheral neuropathy, autonomous dysfunction, rhythm, and conduction disorders need to be further examined to determine the clinical onset of the disease (see the additional tests).

The main **clinical findings** which necessitate testing for hereditary TTRA in patients without known family history, include:

Progressive symmetrical sensorimotor polyneuropathy	At least 1 of the following:	• Familial predisposition
		• Early autonomous dysfunction (erectile dysfunction, orthostatic hypotension)
		• Gastrointestinal manifestations (constipation, diarrhoea, or a combination of the two)
		• Weight loss
		• Cardiomyopathy, rhythm and conduction disorders
		• Bilateral carpal tunnel syndrome (especially in cases of familial predisposition)
		• Renal impairment (microalbuminuria, or mild azotaemia)
		• Amyloid deposits in the vitreous body

Additional manifestations:

- Rapid progression
- Lack of response to other therapies

Figure 3. Potential symptoms suggestive of a hereditary TTRA diagnosis

Referral for **additional tests/examinations** is important in patients with clinical signs suggestive of hereditary TTRA:

1. **Neurological examinations:**
    - The PND (polyneuropathy disability) scoring is presented in Table 1.

Table 1. PND scoring for the severity of peripheral nerve impairment in hereditary TTRA

0	Asymptomatic
I	Sensory disorders, no gait changes
II	Changed gait, walking possible without using a mobility aid
III A	Walking possible with only a single a mobility aid
III B	Walking possible with two mobility aids, or a roller walker
IV	Walking independently is impossible, patient confined to a wheelchair, or bedridden

- Staging in hereditary TTRA - is important for considering the option of using pathogenetic treatments (Table 2)

Table 2. Staging in hereditary TTRA (Coutinho P. et al., 1980)

I	The gait is not affected; mild sensory, motor, or autonomous impairment in the lower extremities
II	Walking independently is possible only with a mobility aid, moderate impairment of the lower and upper extremities and of the body
III	Walking independently is impossible, patient confined to a wheelchair, or bedridden

- Neuropathy impairment score (NIS) - see Appendix 1

PND scoring, staging of hereditary TTRA, and NIS scoring should be performed prior to the start of treatment and in order to assess the effect of treatment in patients with confirmed diagnoses.

- **Electroneurography** (ENG) and electromyography (EMG) with evidence of axon sensorimotor polyneuropathy with more severe impact on peripheral nerves of the lower extremities, and of sensory vs. motor fibres. The following combinations are possible:
  - Decreased amplitude (A) of the SNAP and SMAP at normal conduction velocity (CV) and distal latencies (DL)
  - Normally A and DL at reduced CV
  - Decreased A and CV at normal DL
  - DL are rarely prolonged, except for the DL of the medianus nerve, which may be up to twice higher than the upper limit range even with no clinical signs of the carpal tunnel syndrome.

During the early stages of the disease, in view of the predominant impact on small non-myelinated nerve fibres, the ENG test may give normal results, which will necessitate the performance of the following additional tests:

- **The sympathetic skin response** reflects sweat gland activation in response to electric impulse challenge or loud noise. It is tested at the palms and the soles of feet. Amplitude is evaluated, which is a measure of sudomotor nerve fibre integrity. Patients with hereditary TTRA show a decreased amplitude or lack of response (Conceição I. et al., 2014; Escolano-Lozano F. et al., 2017)
- **The sudomotor Sudoscan** test uses direct low intensity (≤ 4V) challenge and reverse ionophoresis for measuring the topical conductivity between the chlorides in perspiration secreted and the nickel electrodes of the device. Electrochemical conduction (EChC) of the skin, conferred by the non-myelinated C-fibres, is calculated in microSiemens (μS). Normal

EChC results are over 67μS. Where the skin EChC of the feet is less than 66 μS, the sensitivity of this method for confirming impaired autonomous regulation is 76%, and its specificity is 85% (Castro J. et al., 2016).

- **Quantitative sudomotor axon test** (QSART) evaluates sudomotor function but, because of the long duration of this test, it is not widely used in routine practice.
- **Heart rate variability** (HRV) is evaluated during deep breathing sessions and by the Valsalva technique but serves to assess mainly parasympathetic function. In cannot be used with patients with a permanent pacemaker (Escolano-Lozano F. et al., 2017).
- **The quantitative sensory test** (QST) evaluates vibration perception, the threshold of temperature perception for cold and the threshold of hot-pain perception. As with patients with hereditary TTRA, an increase in the threshold value for all the three modalities is assessed within the 95 percentile (Escolano-Lozano F. et al., 2017).
- **Laser evoked potentials (LEP)** use a CO_2-laser challenge on the back of the foot (wavelength of 10.6 mm; intensity of 15–20 W, duration of 15 ms). The inter-challenge intervals are varied between 10 and 20 s. N2–P2 LEP complex is acquired using a subcutaneous needle electrode from the vertex (Cz) and a frontal reference (Fpz). The peak latency and the N2–P2 complex amplitude are measured. In symptomatic subjects prolonged N2 latency is detected.

2. Cardiological examinations and tests

The diagnosis of hereditary transthyretin amyloid cardiomyopathy is based on: family history, ECG, imaging methods, histology examination (where needed) and confirmation of the diagnosis requires obligatory molecular genetic analysis. Light chain and wild-type transthyretin amyloidosis must be excluded. The other two most common types of amyloid cardiomyopathy, which require different management and treatment have different prognosis (Rodney H. Falk, 2010, González-López E. 2015, 2017, Rapezzi, C. et al 2009).

- **ECG** changes most commonly present as a pathological Q-dent (a pseudo infarction pattern), LAHB, low voltage in peripheral leads, and grade 1 A-V block. Left and right bundle branch blocks, atrial fibrillation, stimulated ventricular complex with a previously implanted pacemaker or resynchronization therapy system are less commonly reported. A specific but non-obligatory sign is the low ECG voltage on the background of LV hypertrophy from the imaging examination (O'Donnell E, 2013, Dungu, J. et al. 2010).
- **Transthoracic echocardiography** is an essential diagnostic imaging method (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 increased LV thickness greater than 12 mm and less than 7 mm for the right ventricle, with increased myocardial echogenicity
    - Normal or decreased LV sizes
    - Diastolic dysfunction of various severity - from impaired relaxation, through pseudo normal image, to restrictive LV filling patterns
    - Retained (>50%) or, in advanced cases, decreased EF
    - Early impairment of the longitudinal systolic function
    - Base-to-apex longitudinal strain gradient with relatively preserved cardiac apex deformity in STE
    - Left atrial dilatation
    - Right atrial and inferior vena cava dilatation
    - Thickening of valve flaps, usually with mild to moderate regurgitation
    - Thickened interventricular septum
    - Small pericardial effusion (in about 50% of patients)

- **Magnetic resonance imaging (MRI)**

  This method provides information on the structure and functioning of the heart and on tissue characteristics in the myocardium. Late phase contrast enhancement with gadolinium gives a typical image pattern 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 changes are characteristic of amyloid cardiomiopaty:

- Contrast amplification with gadolinium: gadolinium has an interstitial distribution and persists longer in segments with amyloid accumulation, resulting in late contrast amplification. It may be diffuse or spotted, more often subendocardial, but may be intra- or transmural
- T1 mapping is a quantitative method for measuring myocardial signals. With native T1 mapping (no contrast), significantly prolonged T1 relaxation time is detected, unlike other cardiomyopathies with a similar phenotype, such as hypertrophic BMD and Fabry's 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 LV hypertrophy occurs, and this may be used for earlier diagnosis of the disease.
- The MRI allows calculation of the extracellular volume which is increased in amyloidosis. This allows for early diagnosis, monitoring of amyloid accumulation and monitoring the effect of treatment.

3. Nuclear medicine

It has been established that ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD), 99mTc-pyrophosphate (^{99m}Tc-PYP) and 99mTc–hydroxy methylene diphosphonate (^{99m}Tc-HMDP) isotopes used for bone scintigraphy will accumulate in the myocardium of patients with transthyretin cardiac amyloidosis, both with hereditary and with wild-type variants. A visual assessment scale has been adopted for the evaluation of isotope distribution in the myocardium (0 - lack of myocardial accumulation, normal bone accumulation; 1 – mild myocardial accumulation and normal bone accumulation; 2 – moderate myocardial accumulation and decreased bone accumulation; 3 – severe myocardial accumulation with minimal or no bone accumulation) (Perugini, E. et al 2005). A finding of grade 2 to 3 isotope accumulation in the myocardium is of extremely high sensitivity and specificity, equal to almost 100% for transthyretin amyloid

cardiomyopathy. In disease carriers, the disease has been diagnosed based on the accumulation of the isotope in the myocardium before the onset of echography changes in the myocardium. To distinguish wild type from hereditary transthyretin amyloidosis, it is mandatory to perform genetic analysis. A negative result will exclude transthyretin cardiomyopathy, thus suggesting a different type of CMP, or a different type of cardiac amyloidosis. A small percentage of patients with light chain amyloidosis show isotope accumulation but in significantly lesser amounts – Perugini stages 1-2 – which will require obligatory exclusion of this rapidly progressing disease with extremely poor prognosis and different treatment and management requirements. These data have promoted the widespread use of the method because said method allows for diagnosing transthyretin amyloid cardiomyopathy without endomyocardial biopsy and ensures early diagnosis in mutant gene carriers (Figure 4). (Gillmore, JD et al. 2016, Glaudemans, AW et al 2009, 2014, Rapezzi, C. et al. 2011, Bokhari, S. et al. 2013, 2014).

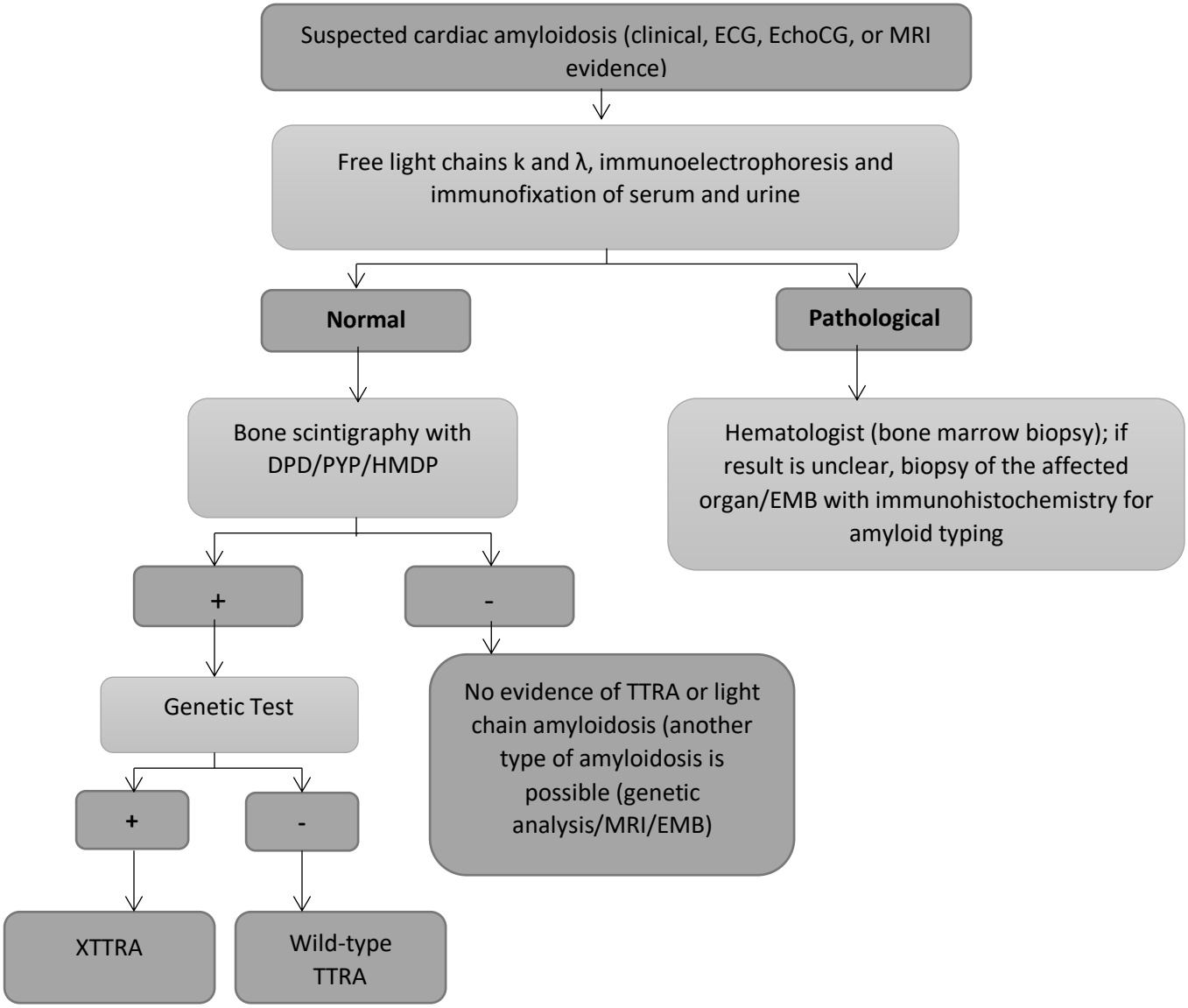


Figure 4. Diagnostic algorithm in patients with suspected cardiac amyloidosis
ECG – Electrocardiogram; EchoCG – Echocardiography; MRI - Magnetic resonance imaging; EMB - Endomyocardial biopsy; DPD - 3,3-diphosphono-1,2-propanodicarboxylic acid; HDMP - hydroxy methylene diphosphonate; PYP - pyrophosphate; HTTRA – hereditary transthyretin amyloidosis; TTRA – transthyretin amyloidosis.

- **Cardiac biomarkers**

  BNP, NT-proBNP and troponin are cardiac biomarkers which become positive in heart failure and in myocardial necrosis, respectively. The mechanism behind BNP and NT-proBNP increase involves increased pressure during left ventricle filling, while troponin increase results from cell death. These biomarkers provide information on the severity of cardiac impairment, have a prognostic value and may be used for the process of staging the disease (Panagiota Kyriakou 2018, González-López E, 2017, Krinsten 2014)

- **Holter ECG monitor**

  A 24 to 48 hours cardiac rhythm records are needed in case of any relevant complaints or periodically to check for asymptomatic atrial fibrillations or significant conduction disorders.

- **Early diagnosis and monitoring of carriers**

  Cardiac sings may present after an asymptomatic period of variable duration, with an already significant infiltration of the heart by amyloid. Generally, amyloid cardiomyopathy is suspected following echocardiography findings in patients with other non-specific complaints, or where disease onset is already suspected based on symptoms from the peripheral or the autonomous nervous system. Detecting transthyretin gene mutation carriers among affected families using genetic screening and monitoring of said families allows for identifying the earliest signs of the disease. The initial age and the regularity of the monitoring of carriers to detect any cardiac impact is done according to the generally adopted guidelines. The following cardiological examinations and tests are recommended: ECG, Echocardiography, NT-proBNP, MRI as needed (unsatisfactory echographic image), bone scintigraphy at the first examination where the age of the patient suggests possible disease progression, as well as in cases of suspicious or unconvincing results from the echocardiography or from the MRI during monitoring (Conceição I. et al., 2019).

Table 3. The tests required for diagnosing and monitoring of symptomatic patients and asymptomatic carriers of ATTR associated gene mutations

		ECG	EchoCG	MRI	Bone scintigraphy	NT proBNP	Troponin	Holter ECG monitor	Blood/ urine
Patients	Diagnosing	+	+	+/-	+/-	+	+	+	+
	Follow-up	+	+	-	-	+	+	+	+
Carriers	Diagnosing	+	+	+/-	+/-	+	-	-	-
	Follow-up	+	+	+/-	+	+	-	+/-	-

+ recommended; - not recommended; +/- at the professional's discretion in certain patients

ECG – electrocardiogram, EchoCG – echocardiography, MRI – magnetic resonance imaging; NT-proBNP, N-terminal pro-brain natriuretic peptide

ATTR-CM progression criteria.

One criterion from each group is the required minimum 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
More HF hospitalizations	Increase with 30% of NT-proBNP (300 pg/ml cut-off)	Increase of LV thickness with 2 mm

Increase of NYHA class	30% увеличение на тропонина	Increase in the degree of diastolic dysfunction
Decrease QoL with 5-10 points KCCQ, EQ-5D c 10%	Deterioration on the NAC scale	Change in systolic function (≥5% decrease of EF, ≥ 5 ml decrease of SV, ≥ 1% GLS increase
Decrease with 30-40 m in 6 MWT each 6 min		Newly occurring conduction disorders

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, SV – stroke volume, GLS - global longitudinal strain.

4. Gastroenterological examinations

To identify the cause of gastrointestinal symptoms and to exclude other diseases, upper or lower endoscopy with biopsy collection, computed tomography, or magnetic resonance enterography and enteroclysis may be conducted. Endoscopic and histological changes are mild and non-specific. Evidence of amyloid deposits in gastrointestinal tract mucosa by polarisation microscope histological examination and Congo red staining, or by immunohistochemical typing is important for diagnosing (Yoshimatsu S. et al., 1998; Ikeda S. et al., 2009). This is especially true of the early diagnosis of cases with gastrointestinal symptoms as a predominant, early presentation of the disease. Hereditary TTRA is characterized by the presence of small amounts of amyloid in the submucosa with marked deposits around nerve fibres, while primary and secondary amyloidosis is characterized by massive deposits located predominantly around the vasculature (Yoshimatsu S. et al., 1998; Ikeda S. et al., 2009).

Final confirmation of the diagnosis requires genetic and/or histopathological examination.

5. Histological examination.

It is the gold standard for the diagnosis of amyloidosis. The highest diagnostic value has a biopsy of an affected organ. When the heart is engaged, the limiting factor is the invasive

nature of the endomyocardial biopsy. In hereditary ATTR, the presence of typical changes in imaging tests, as well as a positive genetic test, reduce the need for a cardiac biopsy. About 5% of patients over 65 have monoclonal gammopathy of unknown significance (MGUS), and in these cases, endomyocardial biopsy with amyloid typing is mandatory. Amyloid typing is carried out by immunohistochemical testing using a panel of antibodies against known amyloidogenic proteins. With immunohistochemical examination, the presence of transthyretin can be detected without being able to differentiate a wild-type mutant. For this reason, DNA testing is mandatory. The most reliable method is considered mass spectrometry, but it is not widespread (Benson, M. et al., 2009).

6. Genetic assay

It aims to clarify the specific mutation in the TTR gene. In this way, the two forms of transthyretin amyloidosis - hereditary and wild type - can be distinguished. Amyloidogenic pathological variants in the TTR gene are most often single nucleotide substitutions in the protein-coding DNA sequence that result in a change in the encoded amino acid. The study of such genetic defects is extremely successfully carried out by PCR and direct sequencing by the Sanger method. Identification of the genetic defect and genetic counseling in cases of hereditary transthyretin amyloidosis is of utmost importance for follow-up 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 timely initiation of specific therapy.

7. Histopathological examination

The purpose is to identify amyloid deposits in different tissues, such as salivary glands, subcutaneous fatty tissue, gastrointestinal mucosa, peripheral nerves, or other affected organs (e.g., the heart, and the kidneys). Biopsies are usually collected from the abdominal subcutaneous fatty tissue, or from the salivary glands, and less commonly form the suralis nerve and the rectal mucosa. The tissue biopsy is stained with Congo red and is examined by means of a polarisation microscope to visualize extracellular amyloid deposits, which show in a bright green colour. After diagnosis has been confirmed by the Congo red stain, the specific type of amyloid needs to be

identified by immunohistochemical examination using anti-TTR serum. Control antisera against other types of amyloid precursors, including light immunoglobulin chains and amyloid A must be used to confirm the specific substance.

Histopathologic examination should be performed in pre-symptomatically diagnosed TTR gene mutation carriers who present later during their dynamic monitoring with subjective symptoms of peripheral nervous system damage and who had unclear, unconvincing results suggesting PNS impairment according to the results of the other relevant tests, i.e., ENG, sympathetic skin response, quantitative sensitivity test (QST), laser evoked potentials, quantitative sudomotor axon test, Sudoscan sudomotor test.

To evaluate whether an asymptomatic TTR genetic mutation carrier will transform into a symptomatic one, the person must have presented with symptoms attributable to the disease (polyneuropathy, autonomous dysfunction, consumptive syndrome, arrhythmia, rhythm, and conduction disorders), and with twofold or greater deviations in instrumental or conventional tests, and/or amyloid deposits found during tissue biopsies (Figure 5). A negative biopsy result is not an exclusion criterion where clinical symptoms are present in combination with abnormal electrophysiological and autonomous tests findings.

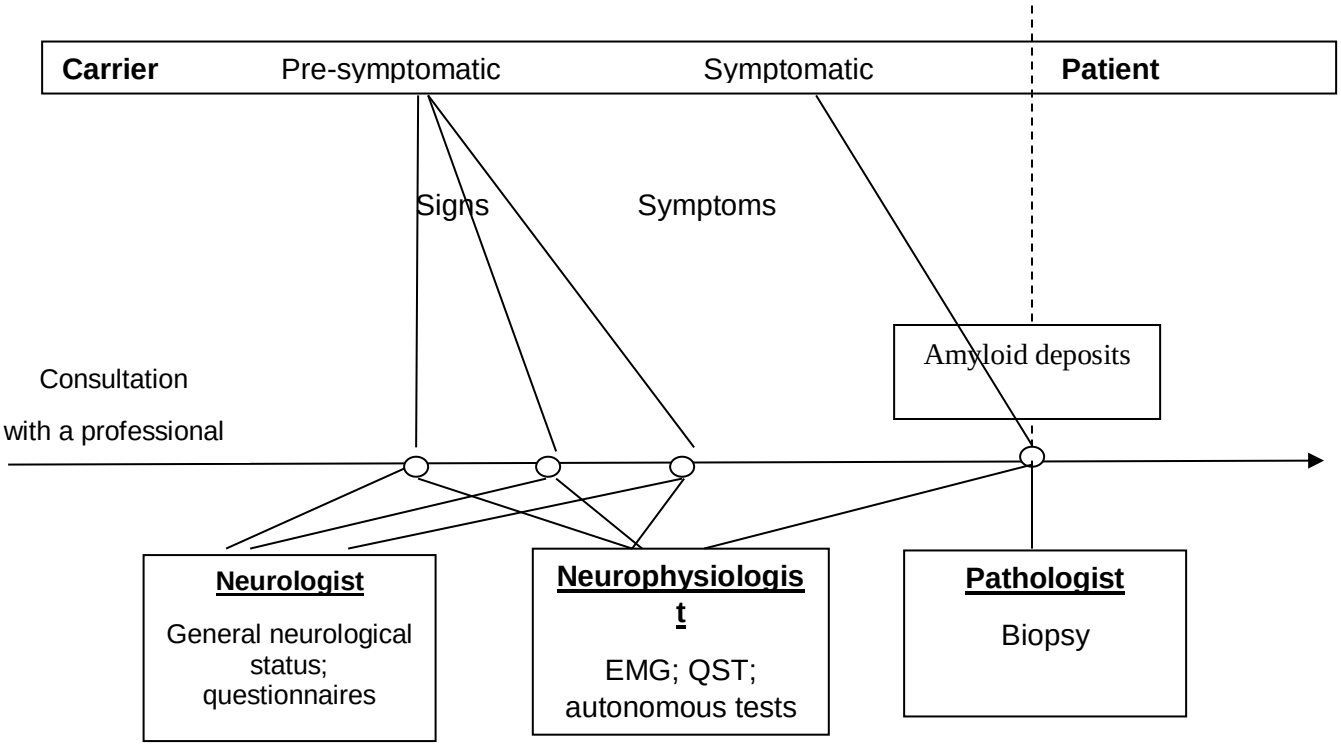


Figure 5. Main diagnostic components pertinent to assessing when an asymptomatic carrier will transform into a patient that requires the initiation of treatment

Diagnostic methods for monitoring patients with hereditary TTRA and treatment efficacy evaluation. Treatment should be initiated only after a baseline evaluation of the patient has been conducted. Thereafter, monitoring of patient's condition should take place once every six months and the effects of treatment should be monitored using the following tests and assessments:

- **Neurological:**
  - General neurological status:
  - Functional evaluations (gait, PND, staging of hereditary TTRA, NIS)
  - Quantitative sensitivity test (QST)

- Autonomous manifestations (urinary disorders, erectile dysfunction, orthostatic hypotension)
- Sympathetic skin test
- The Sudoscan sudomotor test
- Electroneurography

- **Cardiological:**
  - ECG
  - Echocardiography
  - Cardiac biomarkers
- **mBMI (modified body mass index)**
- **Consultation by a gastroenterologist**
- **Neuro-ophthalmological assessment**
- **Renal function and microalbuminuria evaluation**

IX. Differential Diagnosis

1. Peripheral impact pattern in TTRA should be distinguished versus (Ando Y. et al., 2013):

- Chronic inflammatory demyelinating polyneuropathy
- Idiopathic axon neuropathy
- Diabetic polyneuropathy
- Toxic polyneuropathies (most associated with alcohol abuse)
- Deficiency-related polyneuropathies (preceded by diarrhoea)
- Vitamin B_{12} deficiency
- Charcot–Marie–Tooth disease
- Hereditary sensory and autonomous polyneuropathies
- Fabry disease
- Other types of hereditary amyloidosis (gelsolin, apolipoprotein A1)
- AL amyloidosis in monoclonal gammopathies

Table 4 presents the major differences between TTRA, diabetic sensorimotor polyneuropathy, and AL amyloidosis

2. Hypertrophic cardiomyopathy

3. The gastroenterological signs should be differentiated from signs in:

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

Table 4. Major differences between TTRA, diabetic sensorimotor polyneuropathy, and AL amyloidosis

	Diabetic polyneuropathy	**AL amyloidosis**	**TTRA**
Clinical presentations	Polyneuropathy with small nerve fibre impairment, and subsequent large fibre impairment, sensory loss, neuropathic pain, tendon areflexia	Symmetrical predominantly sensory PNP (pain and temperature) Carpal tunnel syndrome - 25% Late motor impairment	Polyneuropathy with small nerve fibre impairment, and subsequent large fibre impairment Distal to proximal progressive weakness Carpal tunnel syndrome Tendon areflexia
Systemic presentations	Nephropathy, retinopathy	Macroglossia Purpura Nephrotic syndrome Multiple myeloma, paraproteinemia	Cardiomyopathy Rhythm and conduction disorders Nephropathy Eye impairment
Autonomous signs	+/-	+/-	++
Precursor protein	-	AL amyloid	TTR amyloid

Progression	Slow progression over the course of 5 to 20 years	Rapid progression	Progression over the course of 1 to 10 years
Familial predisposition	-	-	+/-
Cerebrospinal fluid protein	Normal	Normal	Normal/Elevated
Cranial nerve impairment	+/-	-	-
ENG	Axon sensorimotor	Axon sensorimotor	Axon sensorimotor

X. Pathogenetic treatment for hereditary transthyretin amyloidosis

1. Orthotopic liver transplantation (OLT)

Because TTR is predominantly produced in the liver, in 1990 orthotopic liver transplantation was adopted as a rational treatment for hereditary TTR amyloidosis preventing the production of mutant transthyretin. Until 2011, this used to be the only option for the treatment of TTR amyloidosis. Liver transplantation appeared to be most effective in patients with the Val30Met mutation with early onset of polyneuropathy and with no heart impairment.

Long-term follow-up of transplanted patients showed regression of amyloid deposits and a 77% five-year survival rate. In addition, data became available that neuropathy and/or cardiomyopathy progression could continue after the OLT in some patients, resulting from wild type transthyretin deposits. An international study in 362 subjects with hereditary TTR amyloidosis reported that about 33% of the patients had progressed in 3-5 years (PND score deteriorated) of OLT. OLT did not change TTR production in the eyes and the brain and therefore the central nervous and ocular signs failed to improve. As is the case with other transplantations, OLT is of limited use due to insufficient organ availability and risks from hepatic artery thrombosis, drug toxicity and malignancies associated with long-term immunosuppression.

Because most mutations identified in the Bulgarian population have mixed clinical phenotypes (neurological and cardiological), liver transplantation is not considered an

effective therapy for Bulgarian patients. The only Val30Met mutation which results in a predominantly neurological TTR amyloidosis phenotype has a late onset in our patients and therefore they are not suitable for OLT.

In November 2011, the European Medicines Agency delivered a favourable opinion on the first medicinal product, tafamidis, for the treatment of transthyretin amyloidosis in adult patients with symptomatic polyneuropathy (ATTR-PN) stage 1 for postponement of peripheral neurological impairment.

1. Tafamidis is a selective stabilizer of TTR. Tafamidis binds to TTR at thyroxine binding sites, stabilizing the tetramer, and delaying dissociation to monomers, the rate-limiting step in the amyloidogenic process. Tafamidis is associated with negative cooperativity with the two thyroxine-binding sites of the native tetrameric form of transthyrettin, thus preventing disintegration to monomers**. Inhibition of TTR tetramer dissociation is the basis for the use of tafamidis to slow disease progression in patients with ATTR-PN stage 1.**

1.1. Each capsule contains 20 mg micronized tafamidis meglumine, equivalent to 12.2 mg tafamidis. It is taken orally, once a day.

2. **The pivotal study** of tafamidis meglumine in patients with stage 1 ATTR-PN was an 18-month, multicentre, randomised, double-blind, placebo-controlled study that evaluated the safety and efficacy of 20 mg tafamidis meglumine once daily in 128 patients with ATTR-PN with Val30Met mutation, major stage 1 of the disease; 126 of the 128 patients do not need routine help walking. Eighty-six of the 91 patients who completed the 18-month treatment period were then enrolled in an open-label extension study where all patients received 20 mg of tafamidis meglumine once daily for another 12 months.

2.1. After 18 months of treatment, most patients treated with tafamidis meglumine responded to therapy according to the NIS-LL score (change of less than 2 NIS-LL points). Secondary endpoints showed that treatment with tafamidis meglumine resulted in fewer neurological function impairments and improvements in nutritional status (mBMI) compared to placebo. In the open-label extension study, the rate of change in NIS-LL

over the 12 months of treatment was like that observed in patients who had been randomised and treated with tafamidis in the previous 18-month double-blind period.

2.2. 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 a post marketing observational study in 39 patients. Based on the results of these studies the mechanism of action of tafamidis and the results for stabilization of TTR, tafamidis meglumine is expected to have a positive effect in patients in ATTR PN stage 1 due to other than Val30Met mutations.

2.3. The clinical program with tafamidis included 13 controlled and uncontrolled studies, as well as two non-interventional observational studies. Therapy with the medicinal product stopped 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 administration of tafamidis over 6 years is well tolerated (Barroso F., 2017). No life-threatening adverse reactions or death were observed.

2.4. According to the 2022 Consensus Recommendations for the treatment and follow-up of hereditary ATTR, an assessment of long-term tafamidis treatment (up to 6 years) in Portuguese stage 1 patients with Val30Met mutation showed that patients with early disease (NIS 10) and female patients had the best response to therapy, with prolonged and complete stabilisation of the disease. In all studies with Tafamidis 20 mg there were no observable serious safety signals.

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

3.1. Between 2013 and 2022, 162 patients with stage 1 ATTR-PN started treatment with tafamidis. 68 of them discontinued treatment: 20 - died, 45 - progression of the disease, 3 - refused treatment. All deceased patients

had advanced restrictive cardiomyopathy and heart failure. Some of them died suddenly from heart complications and others from stroke. 30% of patients have not progressed and continue to be in the first stage of polyneuropathy after 60 months of treatment. 62% continued to be in stage 1 of polyneuropathy after 48 months of treatment. A more persistent effect of tafamidis treatment was observed in patients with the Val30Met mutation, while in patients with Glu89Gln, despite a delay in the course of the disease, progression requiring discontinuation of treatment was more common. The weakest effect is the treatment and the fastest is the progression in the Glu47Gln mutation. Early initiation of treatment is critical to the outcome and effect of therapy. Most patients in whom disease progression has been detected are diagnosed more than 4 years after the onset of the disease.

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

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

4. Tafamidis is associated with negative cooperativity with the two thyroxine-binding sites of the native tetrameric form of transthyrettin, thus preventing disintegration to monomers. **Inhibition of TTR tetramer dissociation is the basis for the use of tafamidis in patients with ATTR-CM.**

4.1. Each capsule contains 61 mg of micronized tafamidis. Taken orally, once daily.

5. In patients with ATTR-CM, the effectiveness of tafamidis has been studied in two completed **clinical studies** (one controlled phase III and one open-label phase II study) as well as in two corresponding extensions of clinical studies. A total of 530 patients with ATTR-CM were followed, representing a large number for a clinical rare disease program. The efficacy of tafamidis was demonstrated in a

multicentre, international, double-blind, placebo-controlled, randomised 3-arm study (ATTR-ACT) in 441 patients with ATTRv-CM or ATTRwt-CM.

5.1. The results showed that patients treated with tafamidis had a significantly better combined indicator of overall mortality and incidence of cardiovascular hospitalisations compared to placebo after a 30-month treatment period (p=0.0006). There were also better outcomes in terms of overall mortality by 30% (p=0.026) and cardiovascular hospitalization rates by 32% (p<0.0001). Functional capacity was improved at six months compared to placebo and persisted until 30 months. Tafamidis reduced the deterioration of parameters when conducting a 6-minute test (p<0.0001) and general condition based on a questionnaire used in patients with cardiomyopathies (p<0.0001) (Kansas City Cardiomyopathy Questionnaire).

5.2. The results are better in patients with NYHA class I or II, which supports the conclusion that tafamidis should be started as early as possible during the course of the disease when the clinical benefit regarding disease progression is greatest (Maurer MS, 2018). A 64.4% reduction in overall mortality compared to placebo was observed in NYHA class I patients, 39.6% in class II NYHA and 16.2% in NYHA class III.

For the convenience of the patient, the tafamidis 61 mg free acid capsule was developed, comparable to a dose of tafamidis meglumine 80 mg (4 caps x 20 mg) with the same mechanism of action and biological effect. (Lockwood PA, Le VH, O'Gorman MT, Patterson TA, Sultan MB, Tankisheva E, Wang Q, Riley S.). The Bioequivalence of Tafamidis 61-mg Free Acid Capsules and Tafamidis Meglumine 4 × 20-mg Capsules in Healthy Volunteers. Clin Pharmacol Drug Dev. 2020 Oct;9(7):849-854. doi: 10.1002/cpdd.789. Epub 2020 Mar 20. PMID: 32196976; PMCID: PMC7754314.)

6. The clinical trial with **tafamidis with two different dosages, 20 mg and 80 mg**, was conducted globally in patients with ATTRv-CM and ATTRwt-CM for a

duration of 30 months. The primary efficacy end goal showed significant differences between 264 patients who received tafamidis and 177 patients who received placebo ($p<0.001$). Tafamidis was associated with lower overall mortality compared to placebo 78 (264) or 29.5% vs. 76 (177) or 42.9% and a lower rate of cardiovascular disease-related hospitalizations with a relative risk ratio of 0.68 (0.48 vs. 0.70 per year). At 30 months, tafamidis treatment was associated with a lower percentage of distance reduction from the 6-min walking test (6-MWT) ($p<0.001$) and a lower rate of decline in KCCQ-OS score ($p<0.001$).

6.1. 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 of the main study and LTE in terms of mortality were in favor of a higher dose – a 30% relative reduction in risk compared to a lower dose at 51 months. Tafamidis at both dosages had a safety profile comparable to placebo. (Damy T, Garcia-Pavia P, Hanna M, et al. Efficacy and safety of tafamidis doses in the Tafamidis in Transthyretin Cardiomyopathy Clinical Trial (ATTR-ACT) and long-term extension study. Eur J Heart Fail. 2021;23(2):277-285. doi:10.1002/ejhf.2027).

6.2. When comparing patients from different NYHA classes, NYHA class III patients experienced lower mortality than placebo patients. NYHA class III patients showed an increased risk of hospitalization. This is probably due to increased survival because these patients need more frequent hospitalizations.

6.3. When comparing the two doses 20 mg versus 80 mg in terms of efficacy, a higher dose showed a greater effect on mortality. No safety signals were found at 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 is a medicinal product with proven efficacy in clinical studies, with a favourable safety profile, easy to use for both patients and healthcare professionals. This is the

only approved treatment for ATTRv-CM and ATTRwt-CM. Tafamidis is a recommended treatment option for patients with ATTR-PN at an early stage of the disease.

- **Patisiran**

Patisiran is a small interferent RNA molecule which binds to the coding areas of the TTR matrix RNA and specifically suppresses the synthesis of transthyretin in the liver. A research study conducted in 44 sites in 19 countries with 225 patients (148 on intravenous patisiran (0.3 mg/kg body weight) and 77 on placebo) over 18 months showed that patisiran improved numerous clinical presentations of hereditary transthyretin amyloidosis (D. Adams et al., 2018). After 18 months of treatment, 56% of the patients on patisiran showed improvement in the mNIS+7 score vs. 4% in the placebo group. In addition, patisiran influenced walking speed and the modified BMI. Analysis of the APOLLO clinical study showed that patisiran reduced left ventricular wall thickness, global longitudinal strain, the NT-ProBNP cardiac biomarker and adverse cardiovascular events. These results suggest that patisiran can stop the progression of cardiac presentations and even provide add-on improvement (Adams D. et al., 2018; Solomon S. et al., 2018; Kristen A. et al., 2019).

The patisiran treatment group had an 81% decrease in transthyretin serum levels independent of age, sex, and genotype.

The clinical study confirmed that lowering transthyretin levels may be an effective therapeutic approach. Patisiran was found to produce a significant improvement in sensorimotor and autonomous signs of polyneuropathy in patients with hereditary transthyretin amyloidosis. In addition, the patisiran treatment significantly improved quality of life, walking, nutritional status, and everyday activities. Transition from aided to unaided walking was reported in 8% of patients in the patisiran group.

In the placebo group, the disease had a rapid, progressive course, with deterioration of symptoms and decreased performance status.

In terms of drug safety, mild to moderate adverse effects were reported in 20% of patients taking patisiran and their frequency deceased with time vs. 10% in the placebo group. The most common adverse reactions included peripheral oedema, diarrhoea,

nausea and infusion-related reactions. Mortality rates were similar across the two groups.

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

At present, there are no clinical trials directly comparing the effect of patisiran vs. tafamidis. A recent study indirectly comparing the treatment efficacy of patisiran vs. tafamidis applied Bucher's standard pairs method to the endpoints of the APOLLO and the tafamidis Fx-005 studies: the change from baseline in the Neuropathy Impairment Score-lower limbs (NIS-LL), the Norforlk QoL-Diabetic Neuropathy (QoL-DN) questionnaire, NIS-LL response and the mBMI vs. placebo. Analysis of the data concerning stage 1 FAP patients on patisiran suggested improved therapeutic outcomes when these were compared with data concerning pafamidis-treated patients across all endpoints, with a significant improvement in the median change in NIS-LL and QoL-DN at 18 months (Plante-Bordeneuve V. et al., 2019).

- **Inotersen - a treatment based on transthyretin decrease**

Inotersen is an antisense nucleotide therapy which suppresses the expression of wild-type transthyretin and of mutant transthyretin. The drug was authorized by the European Medicines Agency and by the FDA as a treatment for stage 1 and stage 2 polyneuropathy. This approval is based on the results from the NEURO-TTR clinical study in patients with hereditary TTR amyloidosis. Patients on inotersen treatment showed a significant improvement compared to placebo patients according to evaluations based on the mNIS+7 and the Norfolk QoL-DN. No improvement in cardiac parameters was found among patients in the subpopulation that had cardiac impact. In terms of safety, inotersen was associated with an increased risk for thrombocytopenia and glomerulonephritis, requiring careful monitoring to ensure early detection of those side effects (Kristen A. et al., 2019)

- **Diflunizal**

Diflunizal (Dolobid) is a generic non-steroidal anti-inflammatory agent used as a tetramer stabilizer. Although it is not authorized for hereditary TTRA, it is used in some

countries. As with tafamidis, a clinical trial has reported that, when used for 24 months, diflunizal can slow down neuropathy progression (measured by the NIS+7) compared to the rate in observed in the placebo group. Only 48% of subjects completed the study, and discontinuations were mostly due to disease progression with a median change in NIS+7 +8.2 over two years. Diflunizal in patients with cardiac impairment failed to show significant reduction in left ventricular wall thickness or left ventricular longitudinal strain vs. the placebo. The reported adverse events included impaired renal function, thrombocytopenia. This treatment is contraindicated in patients with heart failure and renal insufficiency. Since cardiac impairment was common among patient with hereditary TTRA, the use of diflunizal was limited (Kristen A. et al., 2019).

XI. Summary and perspectives in the treatment of ATTR according to the new recommendations (2022)

Guidelines and new recommendations in the therapy and monitoring of ATTRv amyloidosis (Amyloid The Journal of Protein Folding Disorders; https://doi.org/10.1080/13506129.2022.2052838)

The possibilities for influencing ATTR amyloidosis are embedded in its pathophysiology. The three therapeutic pillars are: TTR knockout mechanism, TTR stabilization and amyloid removal. The first two pillars include four disease-modifying therapies from clinical practice, liver transplantation, tafamidis, inotersen, and patisiran for ATTRv polyneuropathy and only one – tafamidis and ATTR cardiomyopathy (recommendations of the International Society for Amyloidosis from 2022).

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

Ideally, ATTR amyloidosis should be treated as soon as disease onset 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 choose the best therapy.

Patients with pure neuropathy should be treated with either tafamidis (early stage 1 of the disease) or patisiran, or inotersen gene silencers (stage 1 and 2), according to the doctor's discretion and local health authority criteria.

Level of evidence II, level of recommendation A

Patients with mixed phenotype, neuropathy, and cardiomyopathy (determined according to evidence of cardiac involvement by echocardiography with an interventricular septum wall thickness at the end of the diastole > 12 mm) may be treated with tafamidis as this is the only drug approved for the treatment of cardiomyopathy.

Level of evidence II, level of recommendation A

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

The use of diflunisal outside the indication (off-label) may be considered if no other options are available.

Level of evidence II, level of recommendation A

Patients with isolated cardiomyopathy should be treated with tafamidis

Level of evidence II, level of recommendation A

Multidisciplinary maintenance therapy is essential

Level of evidence IV, degree of recommendation D

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

Several phase III trials are close to completion and new approvals of medicinal products with new indications are expected soon. This will change the therapeutic options and recommendations.

Considering internationally approved guidelines for the treatment of hereditary transthyretin amyloidosis, we recommend:

Patients with pure neuropathy should be treated with either tafamidis (early stage 1 of the disease) or patisiran, or inotersen gene silencers (stage 1 and 2), according to the doctor's discretion and local health authority criteria.

Patients with mixed phenotype, neuropathy, and cardiomyopathy (determined according to evidence of cardiac involvement by echocardiography with an interventricular septal wall thickness at the end of the diastole > 12 mm) can be treated with tafamidis as this is the only drug approved for the treatment of cardiomyopathy, with a predominant cardiac phenotype the recommended dose being 61 mg.

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

Patients with isolated cardiomyopathy should be treated with tafamidis with the recommended dose of 61 mg.

XII. Symptomatic Treatment

1. Symptomatic treatment for neurological impairment

The symptomatic treatment for neurological impairment is targeted at managing neuropathic pain found in some patients, as well as paraesthesia, which are more common. Anticonvulsants, such as gabapentin are used (at doses of 1,200 mg to 2,400 mg), and pregabalin (at doses of 150 mg to 300 mg), or antidepressants, e.g., duloxetine (at doses of 30 mg to 90 mg). The daily dose may vary depending on symptom severity and individual tolerance to the agent. The agents should be titrated slowly and used with extreme caution due arterial hypotension, a risk of collapses and existing diarrhoea.

Symptomatic treatment for neuropathy symptoms is targeted at improving the quality of life of the patients affected.

2. Symptomatic treatment for cardiac impairment

At this stage, the treatment of transthyretin amyloid cardiomyopathy is symptomatic.

Diuretics are the first-choice agents for treating heart failure, but they should be used with caution in patients with cardiac amyloidosis the severe hypotension that often accompanies cardiac amyloidosis and results from low cardiac output combined with autonomous nerve system dysfunction and hypovolemia. The most effective treatment is a combination of a loop diuretic and an aldosterone antagonist. Angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) are poorly tolerated by patients since said patients are prone to hypotension. Beta blockers are also not indicated for patients with heart failure because a slower heart rate may lead to decreased cardiac output per minute. For patients with restrictive cardiomyopathy, tachycardia is a compensatory mechanism to make up for reduced cardiac output due to poor left ventricular filling. Cases of high frequency atrial fibrillations are exceptional in and beta-blockers may be used with caution to control heart rate in those. Even in cases where systolic dysfunction may be present, there is no evidence that beta-blockers, ACE inhibitors, and ARBs improve the prognosis of cardiac amyloidosis; quite on the contrary, these agents may lead to insufficient cardiac output related conditions. Calcium channel blockers are contraindicated because they can bind to amyloid fibrils and may cause extreme hypotension. Digoxin is normally avoided in patients with cardiac amyloidosis, because of the increased toxicity risk, but in certain cases of poorly controlled atrial fibrillations, it may be used with caution. Amiodaron is the drug of choice for maintaining the sinus rhythm in atrial fibrillations but its side effects in the lungs and the thyroid should not be neglected. In cases of confirmed atrial fibrillations, patients are indicated for anticoagulant therapy for embolic stroke prevention. Impairment of the conduction system of the heart may make it necessary to implant a permanent pacemaker. Because of the higher frequency of electromechanical dissociation as a cause of sudden cardiac death, cardioverter-defibrillator implantation as a means of primary prevention is considered to be of no significant benefit. According to recent guidelines, cardioverter-defibrillator implantation should be considered in patients with hemodynamically significant ventricular tachyarrhythmias after discussing the benefits and risks of this treatment with the patient. Orthostatic hypertension, associated with autonomous nervous system impairment, is difficult to manage. Midodrine, as well as external compression, may provide some benefits. Fludrocortisone

may also be used but only if no heart failure is present because of its fluid retention effects (González-López E 2017, Kristen, A.V. et al. 2008, Joseph P. Donnelly 2017, Maurer, MS et al. 2017, Rubinow, A., 1981, Bouhour, JB 1986, Lin, G. et al. 2013, Kojima, T et al 2012).

3. Diet and symptomatic treatment for GIT symptoms

Slow and relaxed feeding with properly chewed down bites is advised for any symptoms concerning the digestive system, with food offered as regimens of three main meals and two or more snacks. It is recommended to avoid spicy food and food with a lot of seasoning, fried or very harsh and hard foods (e.g., quinces), as well as any other food which may lead to symptoms.

In the case of upper GIT symptoms, frequent feeding times with small sized portions of food, preferably soups and purees, are recommended. Fatty food and meats, as well as alcohol, soft drinks, coffee, and tea should be avoided. Light and readily absorbable foods are recommended, e.g., rice, light broths, milk and dairy products (only where there is no lactose intolerance), particularly skimmed yogurt, fish, and cereals (whole grain bread). Water and other drinks should be drunk frequently and in small sips. It should be established whether the patient tolerates hot or cold drinks better and their intake should be increased. In the event of nausea or vomiting, toast, and baked potatoes, as well as ginger or chamomile tea, may provide some relief.

In the event of diarrhoea, it is advised to avoid dairy products and fatty foods rich in fibres and very sugary products, too. Bananas, salty foods, and fluids may be consumed, e.g., boiled rice, boiled potatoes, toast, carrots, roasted chicken (with the skin removed), rice infused water with salt, chicken soup containing salt, vinegar.

During episodes of constipation, increased fluid and fibre intake is recommended to ensure the necessary amount of faeces – 8 to 10 glasses of water; a varied diet with increased intake of foods and juices rich in fibres, e.g., fresh vegetables and fruit, plums, spinach, whole grain cereals, which should be used according to the tolerance threshold because of potential flatulence, abdominal bloating, and pain. Fats and low fibre foods should be limited, e.g., cheese, meats, and cakes, as well as drug that can cause constipation.

The management of gastrointestinal symptoms in hereditary TTRA is symptomatic and often unsatisfactory. It targets the major symptoms to provide control of the symptoms and improvement of quality of life.

In episodes of upper GIT symptoms, medicinal agents to improve /accelerate motor activity (prokinetics) should be prescribed, as well as agents to relieve vomiting (antiemetics), e.g., sodium citrate, dopamine (D_2) antagonists, e.g., metoclopramide, domperidone, itopride hydrochloride (3 to 4 times a day at standard doses). They have a mild effect on gastric emptying but frequently provide good symptomatic outcomes. Motilin agonists (e.g., erythromycin) favourably affect gastric emptying but would have a less pronounced effect on symptoms. It should be noted that some of those drugs can prolong the Q-T interval. They are used as agents reducing or binding gastric acids – antisecretors (proton pump inhibitors, e.g., omeprazole, pantoprazole, esomeprazole - 20 to 40 mg/day, or lansoprazole 30 mg/day); H_2-blockers, e.g., ranitidine 300 mg/day or famotidine 20 to 40 mg/day, alginates, and anti-acid agents (3 to 4 times a day in their standard doses).

All types of products for managing diarrhoea can be used; loperamide is the most emphatically recommended although it can often be ineffective. In certain cases, codeine may be considered. Severe cases my require somatostatin treatment. Milder cases may benefit from hydrasec and tanakan used in standard doses, simethicone and dimethicone against gas formation, absorbents: medical activated charcoal with clay admixtures (fine clay, kaolin). Anti-secretory agents, e.g., buscolysin, papaverine, duspathaline, opioid agents at standard doses, bismuth preparations, e.g., bismuth sub salicylate - up to 8 times and up to 30 ml or colloidal bismuth 4 times daily in doses of 120 mg per dose. Probiotics are recommended to both prevent and treat diarrhoea: e.g. two tablets of enterol two times a day. In moderate or severe diarrhoea, empirical antibacterial treatment is provided with ciprofloxacin or norfloxacin, metronidazole or tinidazole, as well as non-absorbable agents such as rifaximin in standard doses, with fluids and salts.

During constipation episodes, different laxatives may be used, to increase the volume of faeces (fibres, psyllium), to soften the faeces (surfactants, e.g., sodium or potassium docusate, phosphosoda enema), to stimulate peristalsis (contact agents -

sena, bisacodyl, olive oil, dried plums, rose; Ispaghula, husk, cascara), osmotic laxatives (lactulose, mannitol, glycerol, Polyethylene glycol), probiotics, etc. or enemas featuring ready-made solutions. Regular lactulose uses in the usual doses of 15-30 ml to 60 ml once a day in the morning, psyllium and polyethylene glycol may be very effective. Osmotically active agents, such as polyethylene glycol, which was found more effective and with fewer side effects than lactulose according to a meta-analysis. Sodium picosulfate sodium may be used, too. Except for lactulose, laxatives should be used with caution due to the risk of increased fluid and electrolyte loss or impaired absorption of nutrients.

Malabsorption control includes replacement treatment with pancreatic enzymes (Kreon 20-40,000 U daily during meals), and lipid-soluble vitamins (A, E. D, and K), particularly vitamin D, calcium, water-soluble vitamins from the B group, particularly vitamin B_{12}, electrolytes, magnesium, high-calorie foods rich in protein, enteral foods, infusions of human albumin in cases of severe hypoalbuminemia. Enteral nutrition, including via a nasogastric tube and a gastrostoma, should be preferred to parenteral nutrition.

Due to increased risk of osteoporosis and bone fractures, usually resulting from malabsorption, intravenous administration of bisphosphonates once a year is recommended to complement the vitamin D and calcium supplement. Oral bisphosphonate treatment should be prescribed with caution due to risk of oesophageal mucosa damage resulting from impaired motility.

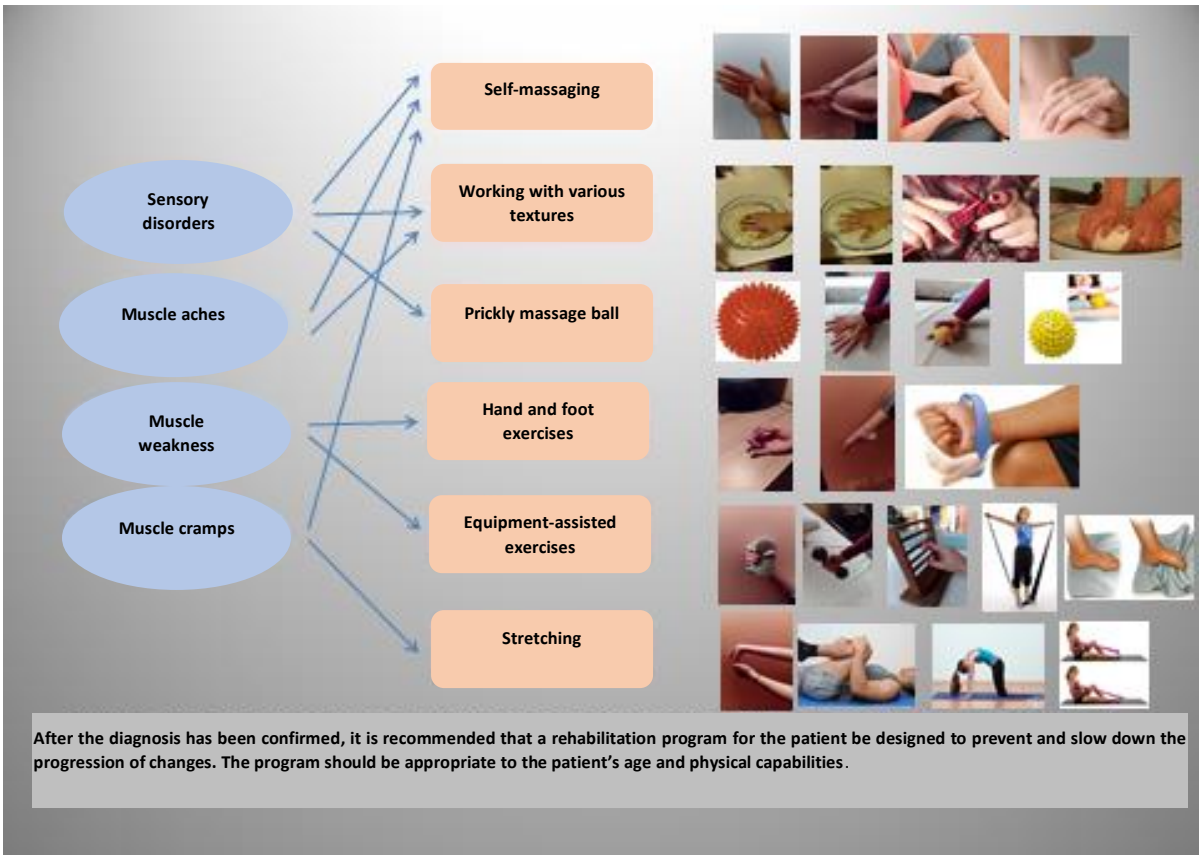
In the event of small intestinal overgrowth of bacteria, antibiotics are recommended: short-term doxycycline or metronidazole courses, probiotics. For longer-term treatment, non-absorbable antibacterial agents are preferred (Rifaximin).

The malabsorption of bile acids requires bile acid sequestrant (e.g., cholestyramine) treatment, with or without decreased nutritional lipid intake.

Patients with hereditary TTRA may have adrenal insufficiency which leads to nausea, vomiting, weakness, and orthostatic hypotension. Usually, a good response may be expected to hydrocortisone replacement therapy.

XIII. Rehabilitation of patients with transthyretin familial amyloidosis

One of the most important recommendations for improving general physical health is regular moderate walking. Slow regular walking in a calm and quiet area with fresh air (in a park or outdoors) for 5 to 10 minutes, 3 to 5 times a week is recommended. Walking time should be increased gradually by 1 to 2 minutes a week.



The main rehabilitation activities that patients may perform at home unassisted include:

- Self-massaging
- Working with various textures - may improve sensitivity and does not require a lot of energy
- Prickly massage ball
- Hand and foot exercises - may improve trophics and enhances sensitivity
- Equipment-assisted exercises - provide significant increase in strength and increase the number of muscle fibres in use

- Stretching – provide a more comprehensive muscle fibre use and improved recovery

XIV. Selective screening program for TTR gene mutation carriers among high-risk families to ensure their identification, dynamic monitoring, and the early initiation of treatment of symptomatic carriers

The target group for the screening program are the direct descendants of diagnosed patients coming from 100 affected families: brothers, sisters, children, and cousins who are at risk of developing the disease. During the screening program, genetic counselling is provided to families and all members of said families are offered information about the disease, namely: detailed explanation about the nature of the disease, why it occurs, how it can be inherited, what genetic testing is, what being a carrier means, how often a carrier's monitoring should take place, when treatment should be started.

Each person tested is counselled individually, their results are reported verbally and in writing and the significance of the results is explained, keeping all results strictly confidential.

Between 2014 and 2019, a total of 660 persons were screened and 175 patients with hereditary TTRA were identified among the 100 affected families. 100 of these patients are still alive and 75 have passed away. Over 111 asymptomatic carriers have been identified.

XV. Monitoring of asymptomatic carriers

Tests and examinations used for to monitor TTR mutation carriers should be specified based on the anticipated phenotypic presentations for each specific mutation. Different mutations have different clinical presentations, so it is important to use the most appropriate assay types for the carriers identified.

Monitoring should start 10 years prior to the estimated age of disease onset. Monitoring should be more frequent when it comes to mutations associated with a rapid course of progression. Carriers should be trained to recognize the early clinical signs associated with the relevant mutations. Monitoring of asymptomatic carriers should be

provided by a multidisciplinary team (neurologist., cardiologist, gastroenterologist, ophthalmologist, nephrologist) in an Expert Disease Centre.

1. **Identifying symptoms and relating these to the diagnosis of hereditary TTR amyloidosis** (Conceicao I. et al., 2019)

Neuropathic symptoms	**Neuropathic signs**
Positive sensory symptoms	*Small fibre damage*
Spontaneous pain	Loss of pain sensitivity
Burning pain	Loss of temperature sensitivity
Acute pain	Allodynia
Proximal pain	Hyperalgesia
Evoked pain	
Allodynia	*Large fibre damage*
Paraesthesia	Loss of the sensitivity to touch and of musculo-articular sensitivity and the perception of vibrations
Restless legs syndrome	lack of tendon reflexes
Negative sensory symptoms	muscle weakness
Sensory deficits	bilateral carpal tunnel syndrome

Autonomous symptoms	**ocular signs and symptoms**
Sexual dysfunction	Darkening of the vitreous body with gradual reduction in vision
Bladder dysfunction	Glaucoma
Alternating constipation and diarrhoea	
Orthostatic hypotension	
Dizziness and syncope	
Unexplained weight loss	
Early satiation	

Cardiac symptoms	Cardiac signs
Dyspnoea at exertion and in the resting state	Signs of right ventricular
Orthopnoea	failure - ankle oedema
Lung oedema	increased central venous pressure
Syncope	Signs of left ventricular failure:
Palpitations	dyspnoea, orthopnoea, lung oedema
	Atrial fibrillations, sinoatrial block, atrioventricular block
	Increased left ventricle wall thickness
	Decreased global strain with preserved cardiac apex deformation
	Cardiac deposits visualized on bone scintigraphy images

Potentially related symptoms	Definitely related symptoms
Paraesthesia	Spontaneous and evoked pain
Restless legs syndrome	Sensory deficits
Early satiation	Alternating constipation and diarrhoea
Dizziness	Orthostatic hypotension
Palpitations	Unexplained weight loss
Episodes of diarrhoea	Dyspnoea at exertion and in the resting state
Ankle oedema	Orthopnoea and syncope

Clinical monitoring includes neurological examination, including scoring as per the Neuropathy Impairment Score (NIS), electroneurography, sympathetic skin response and quantitative sensory testing. Tests for evaluating autonomous functions include postural blood pressure monitoring, a sudomotor test and heart rate variability.

The relevant cardiac tests include electrocardiography for rhythm evaluation, echocardiography, cardiac biomarker testing for functional assessment, magnetic resonance imaging of the heart and bone scintigraphy.

Amyloid deposit identification in biopsies is also helpful in diagnosing symptomatic TTRA. A tissue biopsy may be collected from salivary glands, abdominal fatty tissue or clinically affected organs.

2. Minimal criteria for establishing the onset of the disease

The diagnosis of symptomatic hereditary TTRA should be confirmed if at least one objective symptom or sign that is definitively related to the onset of symptomatic TTRA is present, or if at least one potentially related symptom plus one abnormal result from the confirmative investigations is present, or if two abnormal results from the confirmative investigations in the absence of clinical symptoms are in evidence (Conceicao I. et al., 2019).

The minimum criteria for confirming a symptomatic disease may be used for monitoring carriers from known affected families and ensure earliest diagnosing and an early initiation of treatment. Once the mutation responsible for the disease has been identified by the genetic assay, the age of onset of the disease may be predicted based on the type of mutation, the typical onset age of the clinical phenotype and the onset age in the other family members affected (Conceicao I. et al., 2019).

Patient monitoring and asymptomatic carrier monitoring are provided at the Expert Centre on TTRA Diagnostics, Treatment, Rehabilitation and Prevention at the Alexandrovska University General Hospital.

Appendix 1. Neuropathy Impairment Score (NIS)

1. Muscle weakness scoring

0= normal	3.25 - movement against gravity
1= 25% weak	3.50 - movement with gravity eliminated
2= 50% weak	3.75 - muscle flicker
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**
n. oculomotorius																
n. abducens																
Facial weakness																
Palatal weakness																
Tongue weakness																
Muscle weakness																
Respiratory																
Neck flexion																
Shoulder abduction																
Elbow flexion																
m. brachioradialis																
Elbow extension																
Wrist flexion																
Wrist extension																
Finger flexion																
Finger extension																
Thumb																

abduction																
Hip flexion																
Hip extension																
Knee flexion																
Knee extension																
Ankle flexion																
Ankle extension																
Great toe flexion																
Great toe extension																

2. Tendon reflexes scoring

0= normal; 1= weak; 2= absent

	Right			**Left**		
biceps	0	1	2	0	1	2
triceps						
stylo-radial						
knee						
Achilles						

3. Sensitivity scoring

0= normal; 1= hypesthesia; 2= anaesthesia

	Right			Left		
Thumb	0	1	2	0	1	2
Touch						
Pain						
Perception of vibrations						
Muscle-articular sensitivity						
Great toe						
Touch						
Pain						
Perception of vibrations						
Muscle-articular sensitivity						