Transthyretin amyloidosis: an over review

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Amyloidosis is the extracellular deposition of fibrils, these consist of low molecular weight subunits of a variety of serum proteins. The most common familial amyloidosis is caused by the transthyretin protein. People who are born with inherited mutations in the transthyretin gene produce abnormal, (“variant”) transthyretin throughout their liver. The clinical manifestations vary, depending upon the particular substitution, but result either in neuropathy, cardiomyopathy, or both. One of the most common hereditary transthyretin amyloid cardiomyopathies is caused by the Val122Ile mutation. Progressive amyloid deposition in the myocardium and/or in the electrical conduction system is responsible for restrictive cardiomyopathy and unpredictable episodes of arrhythmias and/or severe conduction disorders. The vast majority of transthyretin-familial amyloidotic polyneuropathy cases are associated with Val30Met mutation. Progressive sensorimotor and autonomic neuropathy is the main neuropathic feature of transthyretin amyloidosis. The first step in evaluating a patient with transthyretin amyloidosis consists in establishing the diagnosis and then evaluating the extent of disease. Deposition of amyloid via tissue can be established by Congo red staining of biopsy specimens. In all cases, amyloid typing has to be completed by transthyretin gene sequencing and by immunofixation electrophoresis of serum and urine. Current treatment options for patients with transthyretin amyloidosis are limited. Patients with transthyretin- primary familial amyloidosis with mild or moderate disease, a diagnosis confirmed by genetic testing and biopsy, liver transplant is the current treatment standard.

Keywords: transthyretin; amyloid; gene therapy; Val122Ile; Val30Met


Introduction

Amyloidosis is the extracellular deposition of fibrils, these consist of low molecular weight subunits (5 to 25 kD) of a variety of serum proteins [1, 2, 3, 4]. The most common familial amyloidosis is caused by the transthyretin (TTR) protein. TTR amyloidosis (ATTR) is a group of autosomal dominant diseases of variable penetrance which are caused by mutated TTR that facilitates dissociation of TTR tetramers in amyloidogenic misfolded TTR and consecutive assembly into TTR amyloid which deposits in various organs [5]. ATTR is a rare but fatal disease, with an estimated incidence of less than 1 per 100,000 [4].

Apolipoproteins A1 and AII, Lysozyme, cystatin C, fibrinogen Aα - chain and gelsolin are other proteins associated with forms of hereditary amyloidosis [6].

What is transthyretin (TTR)?
Transthyretin (TTR) is a normal blood protein, present in everybody. Most TTR in the body is made in the liver and a small amount is made in the eye and the brain.\textsuperscript{[1, 4, 7]}

People with inherited mutations in the TTR gene produce abnormal, (“variant”) TTR throughout their liver. Over the course of several decades, usually after the age of 30 and often much later, they may develop symptoms of disease caused by the build-up of amyloid deposits.\textsuperscript{[8]}

**How frequent is hereditary TTR amyloidosis (ATTR)?**

ATTR is a rare disorder; it has an unequal distribution worldwide. Certain clusters have been described, especially in Portugal, Japan and Northern Sweden. In Europe, the incidence is estimated as 0.3 new cases per year per 1 million inhabitants, with a prevalence estimated of 5.2 cases per 1 million inhabitants. In the endemic area of Northern Sweden, the frequency of the gene is 1.5%. In USA the size of the patient population is estimated to not exceed 6,400 patients.\textsuperscript{[9, 10, 11]}

**Genetics**

The transthyretin (TTR or prealbumin) gene is located on chromosome 18q12.1, and more than 120 TTR mutations have been described, including single mutations, compound heterozygotes, and deletions. Nearly all mutant TTR gene products are amyloidogenic. The most common mutation is Val30Met (valine in position 30 is replaced by methionine). The vast majority of TTR-FAP (transthyretin-familial amyloidotic polyneuropathy) cases (85%) are associated with this mutation.\textsuperscript{[5, 12]}

Koike et al. studied and compared the pathologic findings of patients from 2 FAP-endemic regions in Japan to that of patients with later-onset who were not from the 2 FAP-endemic regions. All studied patients carried the common Val30Met mutation in the TTR gene.\textsuperscript{[13]} Overall, 14 TTR mutations manifest as cardiac-predominant familial disease, eight with an exclusively cardiac phenotype, and some have also associated with late -onset expression. Five TTR mutations (Gly30, Arg53, Ser64, His69, Cys114) have been associated with clinically significant central nervous system involvement, manifesting as leptomeningeal involvement, dementia, cerebellar dysfunction with ataxia, or cerebral hemorrhage.\textsuperscript{[6]}

**Clinical aspects of ATTR**

The affected anatomical structures are in particular the peripheral autonomic nerves, connective tissue of the carpal ligament, heart, kidneys, gastrointestinal tract and eyes.\textsuperscript{[5]}

The clinical manifestations vary, depending upon the particular substitution, but mostly result either in neuropathy, cardiomyopathy, or both.

**Cardiomyopathy**

Cardiomyopathy is the dominant clinical manifestation in patients with Val122Ile mutation or Thr60Ala, Ile68Leu, Leu111Met and Ser77Tyr mutations. One of the most common hereditary TTR amyloid cardiomyopathies is caused by the Val122Ile mutation. This mutation is associated with a late-onset amyloid cardiomyopathy characterized by progressive severe heart failure.\textsuperscript{[14, 15]}

Progressive amyloid deposition in the myocardium increases the thickness of both left and right ventricular walls and also the interventricular septum.\textsuperscript{[16, 17, 18]}

Bilateral atrial dilation, thickened atrial septum, infiltrated tricuspid and mitral valve leaflets are frequent in amyloidosis.\textsuperscript{[19]}

Progressive amyloid deposition in the electrical conduction system is responsible unpredictable arrhythmia episodes and/or severe conduction disorders. These include sinoatrial or high degree atrioventricular block with faintness, syncope, or even sudden death. Arrhythmia is often supraventricular (atrial flutter or atrial fibrillation) or less frequently ventricular.\textsuperscript{[20]}

Physical examination may reveal a variety of findings in patients with cardiac amyloidosis: the jugular venous pressure is frequently markedly elevated; hypertension is very uncommon, hypotension may be present; a right sided third heart sound is occasionally heard when the right ventricle is severely affected; a murmur of tricuspid or mitral regurgitation is occasionally heard; hepatomegaly due to congestion, and ascites may be present if heart failure is severe; the eyelids should be examined carefully for purpura.

Electrocardiogram (ECG) - although low voltage in the limb leads is common, electrocardiogram abnormalities in cardiac amyloidosis occur in approximately 25% of cases.\textsuperscript{[21]}

Echocardiography is the initial noninvasive test of choice to diagnose cardiac amyloidosis. Increased left ventricular wall thickness with evidence of diastolic dysfunction is one of the earliest echocardiographic found abnormality, and right ventricular diastolic dysfunction can also occur (figure 1).

Cardiac MRI is able to provide important information by evidencing restrictive cardiomyopathy and wall hyperechogenicity (bright and granite-like appearance).
secondary to amyloid deposits \cite{16,17}. Cardiac MRI could be useful in detecting preclinical cardiac amyloidosis. Deux JF et al evaluated 53 patients with hereditary ATTR amyloidosis and 14 asymptomatic carriers by cardiac MRI. A positive late gadolinium enhancement (LGE) suggesting cardiac amyloidosis was detected in 60% of patients. The pattern of LGE was diffuse, focal and circumferential in 32, 26 and 2% of patients, respectively. Diffuse pattern was exclusively encountered in patients with cardiac symptoms \cite{22}.

**Polyneuropathy**

The most widespread phenotypic presentation of the TTR amyloidosis is transthyretin familial amyloid polyneuropathy (TTR-FAP). The hallmark of TTR-FAP is neurodegeneration of sensory, motor and autonomic nerve fibers \cite{5}.

A length-dependent peripheral neuropathy is induced by TTR amyloidosis. The lower limbs are initially affected; symptoms generally include toe discomfort (numbness, spontaneous pain). At this disease stage amyloid typically first affects small nerve fibers (alters pain and temperature sensation). Clinical examination may detect impaired thermal sensitivity in the feet, with decreased pinprick sensation \cite{5,23,24}. Distal lower limbs motor deficit appears, also impairment of light touch and deep sensations. This indicates that larger sensory and motor nerve fibers are involved. As time passes (months and years), sensory deficit extends to the thighs, then the upper limbs, forearms, and fingers as the anterior trunk is involved \cite{5,7}.

A number of 19 unrelated Taiwanese patients with FAP and A97S mutation was reported by Yang et al. Within 5 years severe disease progression was observed and symptom onset ranged from 48 to 68 years. All patients presented sensory, motor and autonomic symptoms with loss of proprioception and loss of sensation to thermal stimuli \cite{25}.

An early but nonspecific manifestation of TTR-FAP is the carpal tunnel syndrome. A large number of patients with TTR-FAP are erroneously diagnosed with simple carpal tunnel syndrome; progressive symptoms or no improvement after release surgery for carpal tunnel frequently leads to the correct diagnosis \cite{5,23}.

**Ocular manifestations**

The TTR gene is expressed in the retinal pigment epithelium of the eye, about 20% of amyloidogenic TTR mutations are related with vitreous opacities derived from amyloid, this may lead to visual impairment \cite{26}.

**Gastrointestinal features**

Autonomic nervous system involvement causes disturbances of gastrointestinal motility. Gastrointestinal (GI) complications are frequent in a large number of cases, vary considerably in severity and type and may present before the onset of peripheral polyneuropathy \cite{27,28}. The most commonly GI complications are: diarrhea alternating with constipation, also constipation, diarrhea, vomiting and nausea \cite{27,29}. In early-stage disease weight loss and nausea is common, in late-stage disease severe chronic diarrhea and
cachexia are frequent. Patients with late-stage disease often have severe malnutrition and opportunistic infections; this may lead to death [30]. Endoscopic GI mucosa biopsy has a diagnosed sensitivity of approximately 85% [31].

Depletion of GI neuroendocrine cells was hypothesized to generate gastrointestinal disturbances, but after liver transplant no improvement of GI function was detected, even when the neuroendocrine cell count had been normalized. Loss of interstitial pacemaker cells of Cajal as a potential contributing factor involved in TTR-FAP GI complications is under investigation [32-36].

Recently, on a retrospective study of 266 patients with ATTR, the loss of weight was associated with a high risk of death (RR 1.81, p<0.002) [37].

In a cross-sectional observational study, a mean mBMI of 1,199 kg/m² g/l was found in healthy volunteers. This was statistically significantly higher than those of Portuguese Val30Met TTR-FAP patients having stage 1 disease (independent ambulation, 1,031.9 kg/m² g/l; n=29), stage 2 disease (assistance required to walk, 886.4 kg/m² g/l; n=16) and stage 3 disease (wheelchair bound/bedridden, 759.7 kg/m² g/l; n=16) [38].

It was discovered (prospective and observational studies) that patients with an mBMI (modified BMI=BMI X albumin level) <700 kg/m² g/l have lower survival rates than patients with a higher mBMI after liver transplant [39].

Other system involvement

Autonomic nervous system involvement includes sexual impotence, anhidrosis, orthostatic hypotension, neurogenic bladder and disturbances of gastrointestinal motility. In ATTR central nervous system symptoms are found on rare occasions except in very rare forms of familial leptomeningeal amyloidosis (cerebral hemorrhage with stroke like symptoms) [5, 8, 40].

Diagnosis

The first step in evaluating a patient with ATTR consists in establishing the diagnosis and then evaluating the extent of disease [8]. In order to confirm amyloidosis a tissue biopsy is mandatory, this has to demonstrate the presence of amyloid deposits. Deposition of amyloid via tissue can be determined by using Congo red staining on the biopsy specimens. When using Congo red staining the amyloid deposits present a distinctive green birefringence under polarized light. Suitable tissues for biopsy include rectal mucosa, gastric mucosa, skin, kidney and the abdominal wall (subcutaneous fatty tissue); other tissue obtained by carpal tunnel surgery as retinaculum, peritendinous fat and sural nerve tissue [10, 21, 41] (Figure 2 and 3).

In all cases, amyloid typing has to be completed by TTR gene sequencing and by immunofixation electrophoresis of serum and urine along with the measurement of circulating serum free light chains by immunonephelometry.

Amorphous hyaline deposits of the myocardium are predominantly seen in the extracellular space by using microscopic examination [9, 25]. On electron microscopy, these are seen to be composed of non-branching fibrils. The fibrils bind Congo red, this leads to green birefringence under polarized light, thioflavine T produces an intense yellow-green fluorescence and sulfated Alcian blue produces
a green color. Straight and unbranching fibrils are seen in electron microscopic examination.

Different investigations are necessary in order to assess the extent and stage of ATTR. Cardiac assessment is essential. At least, it requires an ECG, an echocardiogram, and a 24 hour ECG. The latter is recommended systematically given the paroxysmal and unpredictable nature of arrhythmias and/or conduction disturbances. Indications for invasive procedures such as endocavitary ECG recordings should be discussed on an individual basis [7].

In rare cases with central nervous system, MRI imaging can show meningeal enhancement in the brain and spinal cord.

The extent of amyloid deposition can be determined using scintigraphic studies with iodine 123 labelled SAP (serum amyloid P component), as SAP is present in amyloid deposits.

**Differential diagnosis and pitfalls**

Diagnostic difficulties arise when a patient presents with a sensory neuropathy mainly or purely involving small fibers in the absence of family history. The main differential for this type of neuropathy is diabetes mellitus or glucose intolerance. Other diagnoses that might be considered depending on the context are leprosy, HIV infection, drug toxicity, inflammatory diseases, Sjogren syndrome and occasionally vasculities [14, 42]. The absence of family history does not exclude the diagnosis of TTR FAP given the high frequency of “sporadic” cases particularly among late onset patients.

When amyloidosis is confirmed histologically, the differentiation between the two types of amyloid neuropathies (TTR-FAP and AL amyloidosis) can be challenging. Full sequencing of the TTR gene is essential for the diagnosis in any doubtful situation.

**Therapeutic strategies for ameliorating TTR amyloidosis**

Limited treatment options are currently available for patients with TTR amyloidosis. The current treatment standard is liver transplant for patients with TTR-FAP having mild or moderate disease (diagnosis confirmed by biopsy and genetic testing) [43]. Nonetheless a priority is the symptomatic treatment which must be able to provide an immediate relief [7].

Liver transplantation replaces the variant TTR gene by the wild type gene in the liver. As reported by data in the Familial Amyloidotic Polyneuropathy World Transplant Registry, approximately 2000 liver transplantations have been performed to date in 19 countries. After operation variant TTR serum concentration decreases fast, reaching almost zero. After liver transplantation the rate of survival at 5 years is 59% for non Val30Met patients and 82% for Val30Met patients [44]. Progression of cardiac amyloid depositions is more significance in non Val30Met transplant recipients and is associated with less favorable long term survival rates [45, 46].

Liver transplant eligible patients should have genetic proof of TTR-FAP, biopsy proof of amyloid variants, age, nutritional status, severity of both neuropathy and cardiac amyloid involvement. Major adverse events in patients undergoing orthotopic liver transplant for TTR-FAP are cardiac risks and complications. Cardiovascular complications following liver transplant are common. Furthermore, even after successful liver transplant cardiac disease may progress, particularly in patients with mutations non-Val30Met. This is as a result of the deposition of wild-type TTR fibrils on preexisting amyloid matrix. Combined heart and liver transplant is proposed in highly selected patients. Severe heart failure (as a result of amyloidotic cardiomyopathy) is the main indication for this procedure in patients without advanced neurologic involvement [47-52].

**Stabilizers of TTR tetramers: tafamidis and diflunisal**

Tetrameric TTR stabilizing seems to be an encouraging method in preventing amyloid formation and this is sustained by numerous studies. Tafamidis and diflunisal are two drugs that are undergoing clinical development worldwide. Tafamidis is a novel TTR stabilizer, diflunisal is a nonsteroidal anti-inflammatory drug that can stabilize TTR tetramers and was developed in 1971. Data show that tafamidis has beneficial effect and is well tolerated by stage 1 disease patients, although gastrointestinal side effects, vaginal and urinary tract infections may appear. In a randomized, placebo-controlled, double-blind trial in 125 patients with TTR-FAP (Val30Met) was assessed the ability of tafamidis to stabilize the TTR tetramer and evaluated its effect on clinical progression over 18 months. Treatment failed to achieve the prespecified statistical significance. Nevertheless, was demonstrated a significant reduction in neurologic deterioration, preservation of nerve fiber function, improved nutritional status, maintenance of quality of life (QOL), and TTR stabilization [53].
Data that support the treatment of patients stage 2 or 3 TTR-FAP or those who have familial amyloid cardiomyopathy is limited. Suhr et al conducted a post hoc analysis which evaluated the nutritional status of TTR-FAP patients that were treated with tafamidis while enrolled in clinical trials (double-blind, randomized, 18-month, placebo controlled trial and who continued into its open-label, 12-month extension). The authors observed, at month 18, that mBMI improved in the tafamidis group (n=38) and worsened in the placebo group (n=33). Moreover, by month 30 (completion of the open-label extension), placebo patients with 12 months of tafamidis treatment and tafamidis-treated patients with 30 months of treatment both tended to increase mBMI (28 ± 19 kg/m² g/l and 16 ± 18 kg/m² g/l, respectively). They concluded that mBMI is appropriate to monitor disease progression in patients with TTR-FAP and respectively). They concluded that mBMI is appropriate to monitor disease progression in patients with TTR-FAP and treatment with tafamidis can maintain or improve nutritional status [54]. In a small study on 21 patients with non-Val30Met and non-Val122Ile TTR amyloidosis, tafamidis showed to cause no harm in this population. Most of the patients had electrocardiographic or echocardiographic abnormalities at baseline and there was little progression of amyloid cardiomyopathy during the year-long course of treatment. The authors found only four patients that had mild increases in left ventricle wall thickness, the majority of participants did not have an important increase in NT-proBNP over time. This suggests that for more than 12 months the cardiac disease might have been stable under tafamidis treatment [55].

Low doses of diflunisal are able to stabilize TTR tetramers without toxicities, this was demonstrated in a phase 1 study. In a randomized controlled trial, diflunisal was able to inhibit polyneuropathy progression and preserve QOL. Recently it was demonstrated that in hereditary ATTR amyloidosis long-term diflunisal administration is tolerated and effective [56]. Adverse events which might appear in a low number of cases are GI bleeding, altered renal function, or fluid retention. Adverse events surveillance and patient selection are important elements of treatment [24, 43].

Gene therapy

Gene therapy for TTR amyloidosis has focused on inhibition of variant TTR mRNA expression by ribozymes, small interfering RNA (antisense oligonucleotides) and the mutated TTR gene by chimeric RNA/DNA oligonucleotides [45, 46].

Antisense oligonucleotides are short synthetic single strands or nucleotides that are designed to prevent expression of a target protein. This is achieved by selectively binding to the RNA encoding the target protein. Antisense drugs are currently researched to treat diseases such as diabetes, cancers, amyotrophic lateral sclerosis and Duchene muscular dystrophy.

Small interfering RNA (SiRNAs) is a class of double-stranded RNA molecules, they have 21-23 base pairs in length. SiRNAs have many roles, the most notable are in the RNA interference pathway. Here they interfere with the expression of specific genes with complementary nucleotide sequence [46, 48].

RNAi against transthyretin results were reported by Coelho et al in 2 phase 1 clinical trials. Two distinct formulations of first and second-generation were evaluated. The first in 32 patients with ATTR and the second in 17 healthy volunteers. In both trials a rapid dose-dependent durable lowering of transthyretin levels was achieved. Mutant and nonmutant transthyretin production was suppressed by both compounds, this established proof of concept for RNAi therapy targeting mRNA transcribed from a disease-causing gene [57].

Major candidates for TTR amyloidosis treatment are also immunotherapies. Gustavsson et al used various antigenic mapping methods to discover whether major antigenic sites differed for normal TTR, ATTR in situ amyloid fibrils. A major component of amyloid deposits in all types of amyloidosis is SAP [45, 58].

Evolution, prognostic and genetic advice

All forms of ATTR are progressive and have a variable rate of progression. This progression might be dependent on mutation and clinical phenotype. Patients with the ATTR Val30Met mutation generally have a mean life expectancy of 9 to 11 years from symptom onset. Cachexia, malnutrition, renal failure, cardiac disease or sudden death are the main causes of decease [45].

The increasing identification of genetic abnormalities responsible for hereditary diseases brings an essential question whether to test asymptomatic individuals with a positive hereditary family history. This kind of test might have a profound impact on all family members, not only for the tested individual [45].

Conclusions

TTR amyloidosis is a progressive, rare and fatal disease that is more and more diagnosed around the world. The affected anatomical structures are in particular the peripheral autonomic nerves, connective tissue of the carpal ligament, heart, kidneys, gastrointestinal tract and eyes. Genetic testing followed by tissue biopsy is the most reliable diagnostic
The current pioneering approach is liver transplantation in order to treat the familial TTR amyloidosis. In the future other less invasive therapies and general procedures such as small molecule TTR tetramer stabilizers and probably gene therapy approaches might be used.

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Conflict of Interest

Authors declare no conflict of interest.

List of abbreviations

TTR: transthyretin; ATTR: transthyretin amyloidosis; TTR-FAP: transthyretin- familial amyloidotic polyneuropathy; ECG: Electrocardiogram; MRI: Magnetic resonance imaging; LGE: late gadolinium enhancement; GI: gastrointestinal; SAP: serum amyloid P component; QOL: quality of life; SiRNAs: small interfering RNA.

Authors contributions

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