

THE PRESENT AND FUTURE

REVIEW TOPIC OF THE WEEK

Diagnosis, Prognosis, and Therapy of Transthyretin Amyloidosis



Morie A. Gertz, MD,* Merrill D. Benson, MD,† Peter J. Dyck, MD,‡ Martha Grogan, MD,§
Teresa Coelho, MD,|| Marcia Cruz, MD,¶ John L. Berk, MD,# Violaine Plante-Bordeneuve, MD,**
Hartmut H.J. Schmidt, MD,†† Giampaolo Merlini, MD‡‡

ABSTRACT

Transthyretin amyloidosis is a fatal disorder that is characterized primarily by progressive neuropathy and cardiomyopathy. It occurs in both a mutant form (with autosomal dominant inheritance) and a wild-type form (with predominant cardiac involvement). This article guides clinicians as to when the disease should be suspected, describes the appropriate diagnostic evaluation for those with known or suspected amyloidosis, and reviews the interventions currently available for affected patients. (J Am Coll Cardiol 2015;66:2451-66) © 2015 by the American College of Cardiology Foundation.

In 1858, Rudolf Virchow described the reaction of tissue deposits with iodine and sulfuric acid. This reaction was known as a marker for starch in plants; thus, Virchow referred to the deposits as starch-like or “amyloid.” Recognition that these deposits stained with Congo red occurred in 1922, and the apple-green birefringence was discovered in 1927 in the brain of a patient with Alzheimer’s disease (1). Deposits of transthyretin (TTR), a tetrameric protein rich in β strands that is highly conserved and present in all human serum, can cause amyloidosis. TTR’s physiological function includes transportation of thyroxine and retinol-binding protein; the name *transthyretin* was coined from *transports thyroxine and retinol*.

TTR is synthesized primarily by the liver, with <5% synthesized in the choroid plexus of the brain and the

retinal pigment epithelium. TTR has important roles in behavior, cognition, nerve regeneration, and axonal growth (2). TTR has an innate ability to aggregate into insoluble amyloid fibers. Transient accumulation of TTR oligomers, composed of 6 to 10 monomers, may cause cell toxicity or tissue damage. Single point mutations can increase the likelihood of TTR misfolding into an insoluble β -pleated sheet, which deposits in the heart, nerves, and other tissues, causing familial amyloid cardiomyopathy, familial amyloid polyneuropathy (FAP), and leptomeningeal amyloidosis (3,4). **Table 1** provides the most common mutations recently reported from a single U.S. center. More than 80 TTR mutations have been described, including the nonpathogenic G6S mutation found in 6% of the white population. V30M is the second most common mutation in the United States, but, to date, it

From the *Division of Hematology, Mayo Clinic, Rochester, Minnesota; †Indiana University School of Medicine, Indianapolis, Indiana; ‡Division of Peripheral Nerve, Mayo Clinic, Rochester, Minnesota; §Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota; ||Hospital de Santo Antonio, Porto, Portugal; ¶Federal University of Rio de Janeiro of Brazil, University Hospital, Rio de Janeiro, Brazil; #Amyloidosis Center, Boston University School of Medicine, Boston, Massachusetts; **University Hospital, Henri Mondor, Créteil, France; ††Universitätsklinikum Münster, Münster, Germany; and the ‡‡Department of Molecular Medicine, University of Pavia, Pavia, Lombardy, Italy. Dr. Gertz is an advisory board member of and has received honoraria from Isis Pharmaceuticals. Dr. Coelho has received financial support to attend scientific meetings from Isis Pharmaceuticals, Alnylam, and Pfizer; and has received honoraria from Pfizer for educational activities. Dr. Cruz has served as an advisor for Pfizer, Isis Pharmaceuticals, Alnylam Pharmaceuticals, and FoldRx Pharmaceuticals; has received support as a clinical investigator from FoldRx Pharmaceuticals, Isis Pharmaceuticals, and Alnylam Pharmaceuticals; and currently serves on the THAOS (a disease registry capturing natural history and effect of the therapies on TTR amyloidosis) scientific advisory board. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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**ABBREVIATIONS
AND ACRONYMS**

- AL** = immunoglobulin light chain
- ATTR** = transthyretin amyloidosis
- CMR** = cardiac magnetic resonance
- DPD** = diphosphono-propanodicarboxylic acid
- FAP** = familial amyloid polyneuropathy
- NT-proBNP** = N-terminal pro-B-type natriuretic peptide
- siRNA** = small interfering ribonucleic acid
- TTR** = transthyretin
- wt** = wild type

is the most frequent reported globally. Three major clusters in Portugal, Sweden, and Japan have been described. The Portuguese and Japanese appear to have a single founder in the 15th century (brought from Portugal to Japan by explorers) (5,6). The V30M mutation seems to have appeared later in Sweden than in Portugal and Japan (7). The largest populations of mutant TTR, V30M (p.V50M), are in endemic areas of Japan, Sweden, and Portugal, with large cohorts in Brazil (8). The T60A mutation originated in northwest Ireland and came to the United States, where it was termed *Appalachian amyloidosis*. The V122I founder likely originated in West Africa, as indicated by V122I TTR expression in the Caribbean islands.

Wild-type (wt) TTR can also misfold into the amyloid configuration. Previously termed *senile cardiac amyloidosis* and subsequently as *senile systemic amyloidosis*, wt TTR amyloidosis will be called wt transthyretin amyloidosis (ATTR) in this paper. Wt ATTR is sporadic, with no known biomarkers for its diagnosis. Deposition of the wt protein occurs almost exclusively (90%) in men >60 years of age.

**CLINICAL CHARACTERISTICS
OF CARDIAC AMYLOIDOSIS**

Amyloid infiltration results in poor diastolic relaxation (poor filling, with low end-diastolic volume). Doppler measures of inflow velocity can detect left ventricular diastolic filling abnormalities, and Doppler diastolic filling variables are prognostic in cardiac amyloidosis. Shortened deceleration time and an increased early diastolic filling velocity to atrial filling velocity ratio are stronger predictors of cardiac death (9). Amyloid cardiomyopathy should be suspected in any patient who presents with heart failure and preserved ejection fraction. Findings of

right-sided heart failure predominate, including lower-extremity edema, hepatomegaly, ascites, and elevated jugular pressure. Right ventricular dilation is linked to more severe cardiac involvement and short survival (median 4 months) (10). A study of 74 patients with biopsy-proven immunoglobulin light chain (AL) amyloidosis showed an association of right ventricular dysfunction with more severe involvement of the left ventricle, higher plasma levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP), and poor prognosis (11).

Using strain echocardiography, left atrial dysfunction was identified in 32% (lateral left atrial criteria) and 60% (septal left atrial criteria) of patients with amyloidosis (12). Severe atrial and ventricular infiltration by amyloid may result in mechanical atrial standstill, with resultant thrombus formation (13). These findings have been identified by cardiac magnetic resonance (CMR) imaging (14). CMR has also been used to estimate cardiac amyloid burden by quantification of myocardial extracellular volume fraction (15). In addition to heart failure, patients may present with atrial arrhythmias or conduction system disease. In patients who have ventricular thickening without a history of hypertension or valvular disease, an infiltrative cardiomyopathy should be considered (16). Deposition of amyloid into the myocardial wall causes diastolic dysfunction, restrictive physiology with late loss of systolic function, arrhythmias, and heart failure (17). The finding of increased wall thickness, small ventricular volume, and occasional dynamic left ventricular outflow tract obstruction can be confused with true hypertrophic conditions such as hypertrophic cardiomyopathy and hypertensive heart disease. Although the electrocardiogram classically shows low voltage in the QRS complex in amyloidosis and increased voltage in myocyte hypertrophy disorders, the overlap is great; voltage can be useful but is not reliable (18,19).

Clues to the presence of amyloid cardiomyopathy are seen in the widespread deposition of amyloid. In patients with wt ATTR, one-half have associated carpal tunnel syndrome caused by deposition of TTR amyloid into the carpal tenosynovial tissue, with hand symptoms typically preceding cardiac manifestations by 8 to 10 years. Of patients with idiopathic carpal tunnel syndrome, 34% will have amyloid deposition in tenosynovial tissue, possibly representing an early symptom of wt ATTR cardiomyopathy (20).

Clinical presentation of mutant ATTR is variable and driven by the specific mutation, of which ~110 have been described. Patients with mutant ATTR present on a spectrum from exclusive neuropathy to

TABLE 1 Most Common TTR Mutations Identified in Patients With Symptomatic Amyloidosis

TTR Mutation	Mayo Clinic	TTR Amyloidosis Outcomes Survey
T60A	26.0	1.7
V30M	16.0	73.3
V122I	11.0	4.4
S77T	6.0	1.6
L111M	0.0	1.9
E89Q	0.0	2.1
All others	41.0	16.7

Values are %. Data from Coelho et al. (21) and Swicicki et al. (22).
TTR = transthyretin.

cardiomyopathy-overlapping phenotypes. Among 611 symptomatic patients with hereditary TTR, multi-system involvement was seen with each mutation (21). The quality of life in patients with hereditary ATTR is severely impaired.

GENERAL CHARACTERISTICS OF TTR AMYLOIDOSIS

In a study of ATTR cardiomyopathy, 212 of 266 patients were men (80%) (22), and in the Transthyretin Amyloidosis Outcomes Survey data registry, 72% of patients with mutant ATTR were male and 99% with wt ATTR were male (21). The mean left ventricle wall thickness was higher in wt ATTR than in mutant ATTR, and mutant ATTR less often had low voltage in the QRS complex (only 25% of patients). Mutant and wt ATTR had a favorable survival rate compared with that of AL amyloidosis and other forms of amyloid cardiomyopathy (19).

A particularly important form of amyloid cardiomyopathy caused by mutant TTR in the United States is the V122I mutation, which has been identified in 124 of 3,856 (3.2%) of blacks (23) and should be considered in black men >50 years of age with heart failure symptoms and thickened ventricular walls with echocardiography. Of interest, mortality rates are not markedly different between V122I carriers and noncarriers (23). However, the V122I variant is associated with a 47% higher risk of incident heart failure, worse systolic and diastolic cardiac function, and significantly higher levels of NT-proBNP compared with noncarriers. V122I ATTR may be more benign than previously thought. Nevertheless, even when clinically apparent amyloidosis is absent, patients with the V122I mutation have a higher incidence of New York Heart Association functional class 3 and 4 heart failure (10%) (24).

Extracardiac symptoms provide an important clinical clue when mutant ATTR is the cause of an infiltrative cardiomyopathy. Among a cohort of patients with T60A (p.T80A) amyloidosis, 82% had cardiomyopathy, but 65% also had peripheral neuropathy (22). Among those with V30M amyloidosis (the most commonly recognized mutation globally to date), 43% have cardiomyopathy and 95% have peripheral neuropathy. Furthermore, even in V122I amyloidosis, which causes cardiomyopathy for all patients, we found that 30% also had peripheral nerve symptoms that were generally mild but part of the chief concern in 7%. When present, peripheral nerve symptoms in a patient with cardiomyopathy may suggest systemic amyloidosis (25). The finding of peripheral neuropathy is suggestive of amyloidosis in

a patient with: 1) heart failure with preserved ejection fraction; or 2) imaging findings consistent with infiltrative cardiomyopathy. Patients with some forms of mutant TTR amyloid also ultimately have renal dysfunction; in a cohort of patients with V30M ATTR, dialysis was initiated at an average of 10.2 years after the onset of neuropathy (26). Dialysis-dependent renal failure was preceded by nephrotic-range proteinuria. When renal replacement therapy was initiated, the 2-year treatment survival rate was only 38.4%.

The natural history of wt ATTR is much better than other forms of amyloid cardiomyopathy (19). In 1 of the first studies, the median survival was 60 months from presentation with heart failure symptoms, whereas it was 5.4 months for patients with cardiomyopathy from AL amyloidosis (27). Characteristics of wt ATTR include greater left ventricular wall thickness than that of patients with AL amyloidosis, less-severe heart failure, and a median survival of 75 versus 11 months, again suggesting a more protracted clinical course (28). Despite the long clinical course of heart failure, the disease is progressive with time. Echocardiography, 6-min walk, and NT-proBNP revealed significant declines in myocardial performance over a 6-month observation period (29). In a large series of more than 100 patients with wt ATTR, median survival from onset of symptoms was 6.07 years. Predictors of shorter survival included troponin elevation, the need for a pacemaker, and elevation of New York Heart Association functional class (30).

All patients with amyloid peripheral or autonomic neuropathy need screening for cardiac involvement. Amyloid neuropathy is classically a symmetric, ascending length-dependent, sensorimotor, axonal polyneuropathy. It is associated with a high incidence of carpal tunnel syndrome and autonomic neuropathy manifesting as orthostatic hypotension, sweat abnormalities, urinary incontinence, erectile dysfunction, alternating diarrhea and constipation, and orthostatic syncope. In a screening study of 81 patients with FAP due to V30M, 17% had severe thickening of the intraventricular septum (>15 mm) (31).

The following clinical features are characteristic of TTR FAP and are useful for distinguishing this polyneuropathy from inherited and acquired polyneuropathies. First, TTR FAP is a symmetric, distal polyneuropathy that typically begins in the lower limbs, progresses to the upper limbs, and then affects more proximal aspects of the limbs and even the trunk. Second, it is an axonal polyneuropathy affecting all functional classes of nerve fibers (i.e., motor, sensory [large and small], and autonomic fibers), which can distinguish it from other

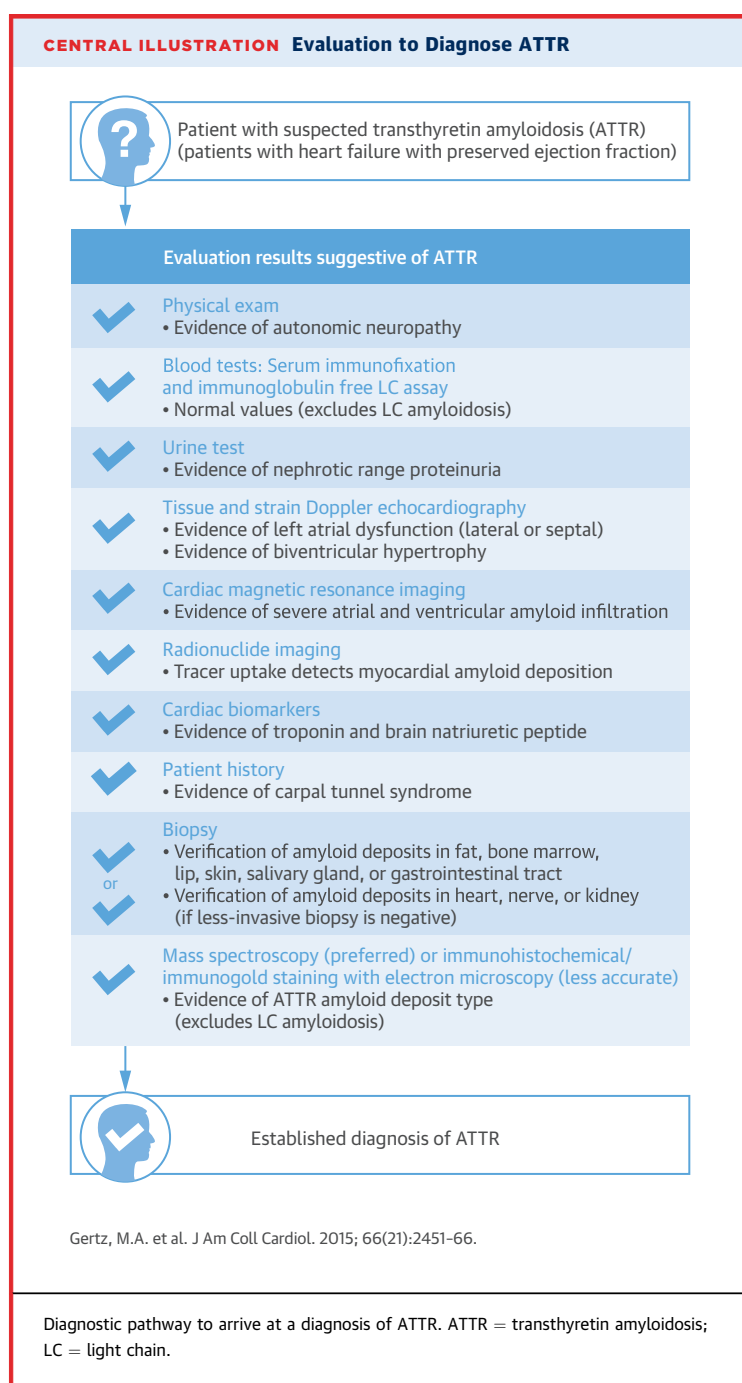
inherited neuropathies, especially those predominantly involving small sensory nerve fibers (hereditary, sensory, and autonomic neuropathies) or selectively involving large sensory fibers (spinocerebellar degenerations). Third, its onset typically begins at the end of the second decade of life or later and progresses substantially during the next 1 or 2 decades. Fourth, a family history of a similar polyneuropathy is usually present but often is not

recognized clinically because family histories are not rigorously obtained. Fifth, concurrent involvement of visceral organs (e.g., heart or kidneys) is common. A high index of suspicion is required because amyloid polyneuropathy is initially suspected in only 38% of patients presenting with peripheral nerve symptoms and no family history (32). The **Central Illustration** lists the recommended diagnostic evaluation for patients with suspected amyloidosis. The purpose of serum immunofixation and the immunoglobulin free light chain assay is to exclude AL amyloidosis. Although an echocardiogram or cardiac magnetic resonance (CMR) imaging may suggest infiltrative cardiomyopathy, these cardiac diagnostic tests cannot reliably predict amyloid type. Absence of a monoclonal light chain should not reduce the index of suspicion for amyloidosis because ATTR is not associated with the presence of a monoclonal protein.

PREVALENCE OF ATTR. Because of the wide geographic distribution of mutant ATTR and regional differences in mutation rate, accurate statistics on the prevalence of mutant ATTR are difficult to obtain. Relative frequencies, however, have been reported. Among 100 patients seen at an amyloid referral center who all had endomyocardial biopsy, 74 had AL amyloidosis, 22 had wt ATTR, and 4 had mutant ATTR (33). This study, conducted in the United States (where the incidence of AL amyloidosis is 8 per million/year) would translate to 0.4 per million/year for mutant ATTR.

However, a recent review suggests that wt ATTR may be under-recognized (34). Histologically, cardiac vascular involvement is seen in 88% of patients with AL cardiomyopathy and in only 26% with wt ATTR, but the biopsy finding of cardiac amyloid with no vascular involvement should heighten the suspicion of ATTR. A study of cord blood from 1,000 children born at a U.S. county hospital to mothers who self-identified as black showed a 3% frequency of the TTR mutation V122I (35), although its penetrance is probably low. On the basis of a prospective French study, the estimated prevalence gave an age-standardized incidence rate of amyloidosis at 14 cases per million person-years, constituting 66 patients in 3 years (36). Of these patients, 60% had wt ATTR and 20% had AL amyloidosis; no cases of mutant ATTR were reported. The nature of data collection makes the conclusions uncertain.

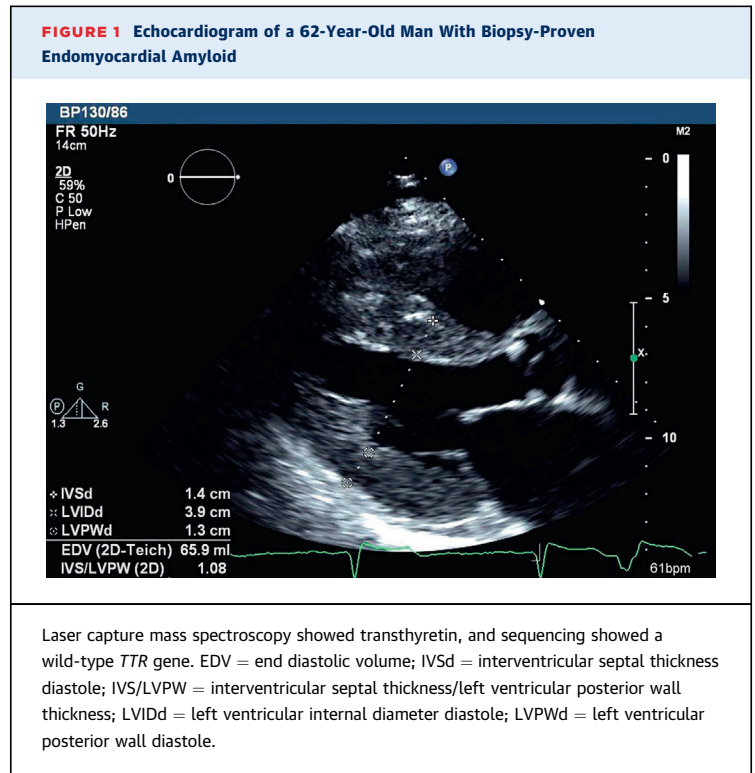
Autopsies suggest that the prevalence of wt ATTR is much greater than previously reported. In 1 autopsy study of people >85 years of age, wt ATTR was present in 25% (37). The fraction of autopsied patients with clinically significant symptoms is not known. In a consecutive series of surgical pathology specimens



with ATTR (n = 33), mutant TTR was found in 36% and wt TTR in 64%, emphasizing that wt TTR is more common (38). Endomyocardial biopsies from 101 patients with amyloid showed AL in 54 and ATTR in 42 (39). Overall, 5 of 42 patients with ATTR had mutant TTR.

Among patients presenting with peripheral neuropathy, 6 of 16 (38%) had cardiac amyloidosis, as evidenced by cardiac radiotracer uptake and by CMR, emphasizing the need for cardiac screening in all patients with peripheral neuropathy due to amyloid, even those without clinical signs of cardiac involvement (40). The true prevalence of wt ATTR is unknown because true population-based surveillance has never been performed. In an autopsy study of 109 patients with heart failure with preserved ejection fraction (but not suspected of having amyloidosis) and 131 control subjects, amyloid deposits were found in 18 patients with heart failure with preserved ejection fraction (17%) and 7 control subjects (5%) (32). The frequency increased with each decade of life (from 70 to >90 years of age). Wt ATTR is an increasingly recognized cause of heart failure with preserved ejection fraction, and amyloidosis should be considered in the differential diagnosis of this increasingly recognized syndrome.

ECHOCARDIOGRAPHY. Echocardiography is the modality by which virtually all patients with cardiac ATTR are recognized. The most common finding is thickening of both the left ventricle free wall and the septum (41) (Figure 1). This can be misdiagnosed as hypertrophic cardiomyopathy or hypertensive cardiomyopathy. Patients with an exclusively cardiac phenotype (i.e., lacking neuropathy or autonomic features) show more pronounced cardiac involvement on echocardiography and electrocardiogram. In a study of 13 patients, all with ATTR, echocardiography showed biventricular hypertrophy, left atrial enlargement, and normal to slightly reduced left ventricular ejection fraction (42). Tissue Doppler septal e was low, and median E/e was high. “E” and “e” represent waves seen on tissue Doppler and reflect early diastolic filling and mitral inflow; the ratio E/e reflects diastolic compliance. Right-sided catheterization showed the classic restrictive filling pattern, with a median pulmonary wedge pressure of 21 mm Hg. Electrocardiograms with low-voltage QRS complexes were seen in only 36%, pseudoinfarction pattern with poor R-wave progression in 65%, and atrial fibrillation in 36% of patients. In a study of 48 patients with ATTR (29 with wt TTR, 19 with mutant TTR), all patients met criteria for cardiac involvement with a left ventricle thickness >12 mm (43). Patients



with ATTR had increased left and right ventricular chamber volume, increased left and right ventricular wall thickness, reduced left ventricular ejection fraction, and fractional shortening. Despite this, these patients had lower NT-proBNP levels than patients with AL amyloidosis. ATTR also appears to have a slower progression than AL amyloidosis. When echocardiograms from patients with AL amyloidosis and ATTR (36 mutant, 56 wt) were compared, longitudinal strain was severely impaired for both but was worse in AL amyloidosis.

Compared with mutant ATTR, wt ATTR is characterized by a greater left ventricular wall thickness, greater depression of ejection fraction, and higher longitudinal strain. Worsening left ventricle function correlated with increasing wall thickness. Patients with wt ATTR had greater wall thickness but a lower mortality rate (19,44). Tissue and strain Doppler imaging are essential for detecting cardiac amyloidosis because these techniques can detect early amyloid myocardial involvement before it can be seen with traditional echocardiography (45). A total of 55 patients with cardiac amyloidosis were compared with 30 control patients with left ventricular hypertrophy. A relative apical longitudinal strain (LS) of 1.0 (defined using the equation: Relative apical LS = average apical LS/[average basal LS + average mid LS]) had 93% sensitivity and 82% specificity in

differentiating patients with amyloidosis from control patients. Relative apical longitudinal strain was the only parameter predictive of cardiac amyloidosis ($p = 0.004$) (46).

CMR IMAGING. CMR has enhanced the ability to recognize cardiac amyloidosis (Figure 2) because it shows a distinct pattern of late gadolinium enhancement over the entire subendocardial circumference (Figure 3). This pattern was found in 12 of 15 patients with positive endomyocardial biopsy (47). The sensitivity of CMR for cardiac amyloidosis was 80% and the specificity was 94%, with a positive predictive value of 92% and a negative predictive value of 85%. CMR is useful when distinguishing cardiac amyloidosis from hypertrophic cardiomyopathy and hypertensive heart disease. In 1 study, late gadolinium enhancement was observed in 6 of 6 patients with cardiac amyloid, and the number of enhancing segments was significantly greater in cardiac amyloidosis (seen in 8 of 9) than in hypertrophic cardiomyopathy or in hypertensive heart disease (seen in 6 of 11) (48). In a prospective study of 53 patients with mutant ATTR and 14 asymptomatic carriers, positive late gadolinium enhancement was detected in 60% of patients but not in carriers (49). A diffuse pattern of enhancement was seen exclusively in patients with cardiac symptoms. Cardiac amyloid on CMR was observed in 19% of patients with

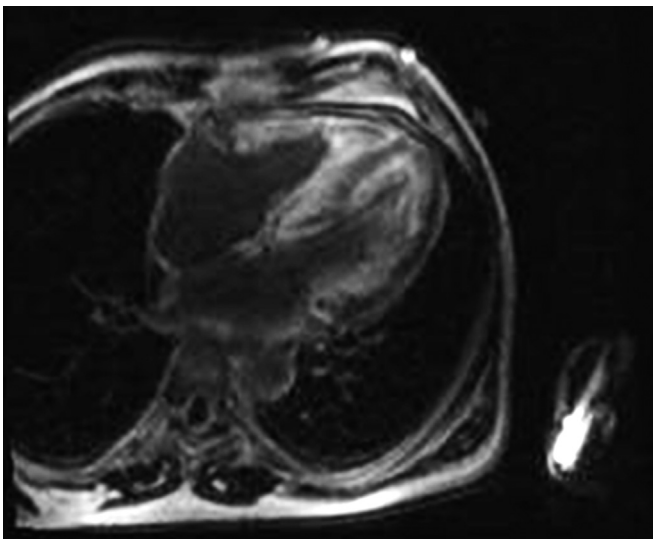
isolated mutant ATTR neuropathy and 20% of patients without wall thickening. An attempt was made to use CMR to differentiate AL from ATTR in patients with cardiac amyloidosis. With use of a late gadolinium enhancement scoring system in conjunction with age and wall thickness, ATTR was identified with greater sensitivity (87%) and specificity (96%) compared with AL (50). Moreover, CMR was able to differentiate AL from cardiac ATTR. A total of 90% of patients with ATTR demonstrated transmural late gadolinium enhancement, compared with 37% of patients with AL (50).

Native myocardial T1 mapping by CMR was studied in 85 patients with ATTR amyloid (50 with wt TTR) and 79 patients with AL amyloid. Native myocardial T1 mapping detected early cardiac ATTR amyloid and had similar performance characteristics for diagnosing and tracking disease in both ATTR and AL amyloidosis (51).

Magnetic resonance imaging is also capable of detecting lower-limb nerve injury and quantifying nerve injury in vivo on a microstructural level. The use of magnetic resonance imaging to assess FAP is ongoing (52).

RADIONUCLIDE IMAGING OF CARDIAC AMYLOIDOSIS. The utility of radionuclide imaging in recognizing cardiac amyloidosis dates back over 30 years (53). Ten years ago, in an attempt to differentiate AL amyloidosis from ATTR, the diagnostic accuracy of ^{99m}Tc -3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) was investigated (54). Using echocardiography as the reference standard for cardiac involvement, sensitivity and specificity were 100% for ATTR, and DPD was proposed as a useful diagnostic component for distinguishing between forms of cardiac amyloidosis (Figure 4). However, the same group later reported that although ^{99m}Tc -DPD scintigraphy could be useful for differentiating ATTR from AL amyloidosis-related cardiomyopathy, its diagnostic accuracy was lower than previously reported because of a mild degree of tracer uptake in about one-third of patients with AL amyloidosis (55). Another publication from the same group reported that ^{99m}Tc -DPD showed tracer uptake in all 63 patients with ATTR, 23 of whom did not have echocardiographic evidence of infiltrative cardiomyopathy, indicating the ability of this tracer to diagnose cardiac involvement early in the disease course, before the appearance of echocardiographic abnormalities (56). ^{99m}Tc -DPD has been compared with CMR for assessing cardiac involvement. Both techniques have similar capabilities for identifying myocardial amyloid deposition, but the infiltration burden can

FIGURE 2 Cardiac Magnetic Resonance Imaging Without Gadolinium Shows Dramatic Thickening of the Right Ventricle, Interventricular Septum, and Left Ventricular Free Wall



Amyloid infiltration causing wall thickening.

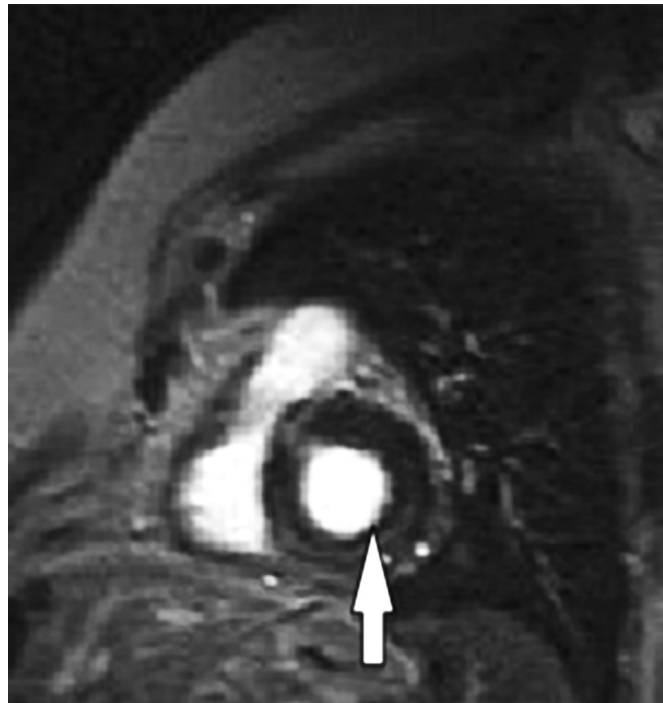
be markedly underestimated by visual analysis of CMR compared with ^{99m}Tc -DPD (57).

^{99m}Tc -pyrophosphate also has been used as a cardiac tracer and was reported to be equally efficacious in both mutant and wt ATTR (58). Further investigation of ^{99m}Tc -pyrophosphate showed 97% sensitivity and 100% specificity for identifying cardiac ATTR. In the United States, ^{99m}Tc -pyrophosphate is more easily obtained than the DPD tracer (59). In a review, the sensitivity and specificity of the ^{99m}Tc -DPD and ^{99m}Tc -pyrophosphate tracers appeared comparable (60). A new tracer, ^{99m}Tc -hydroxymethylene diphosphonate, can detect ATTR amyloid before echocardiographic evidence of cardiac involvement (61).

Subclinical cardiac involvement has also been detected with the tracer when CMR and echocardiography were nondiagnostic (62). Quantification of tracer retention of ^{99m}Tc -DPD can be used to characterize the severity of cardiac involvement by ATTR, although echocardiography remains the standard (63). Radionuclide scanning with Tc-pyrophosphate or ^{99m}Tc -DPD can heighten the suspicion of TTR cardiac amyloidosis. A negative result can exclude cardiac involvement or suggest that cardiac amyloid may not be of TTR origin.

CARDIAC BIOMARKERS. Both troponin and B-type natriuretic peptide are important cardiac biomarkers for assessing the severity of cardiac amyloidosis. Although initially used to study AL amyloidosis, they were recently applied to detect cardiomyopathy in ATTR. Among 29 patients who were evaluated, troponin T was detectable in only 3 and troponin I was detectable in 6, suggesting that TTR amyloid is less toxic to myocytes than AL amyloid (64). This fits with the finding that, although cardiac function declines as the ventricular wall thickens, patients with wt TTR tolerate much greater degrees of wall thickening and strain abnormality than patients with AL cardiac amyloidosis. This is consistent with a hypothesis of additional toxicity by soluble light chains in AL (44). B-type natriuretic peptide was elevated in 76% of patients and correlated significantly with septal thickness and basal septal strain; it appears to be a more sensitive marker for familial amyloid cardiomyopathy. In an evaluation of NT-proBNP in ATTR, the biomarker correlated with left ventricular mass (calculated by CMR) and with late gadolinium enhancement, suggesting the utility of this biomarker as a measure of cardiac amyloidosis severity (65). In a study of 36 carriers of mutant TTR (either asymptomatic or with only neuropathy symptoms), NT-proBNP had 92% sensitivity and 90% specificity for predicting echocardiographic left ventricle abnormalities (66). In

FIGURE 3 Late Gadolinium Enhancement on Cardiac Magnetic Resonance Imaging



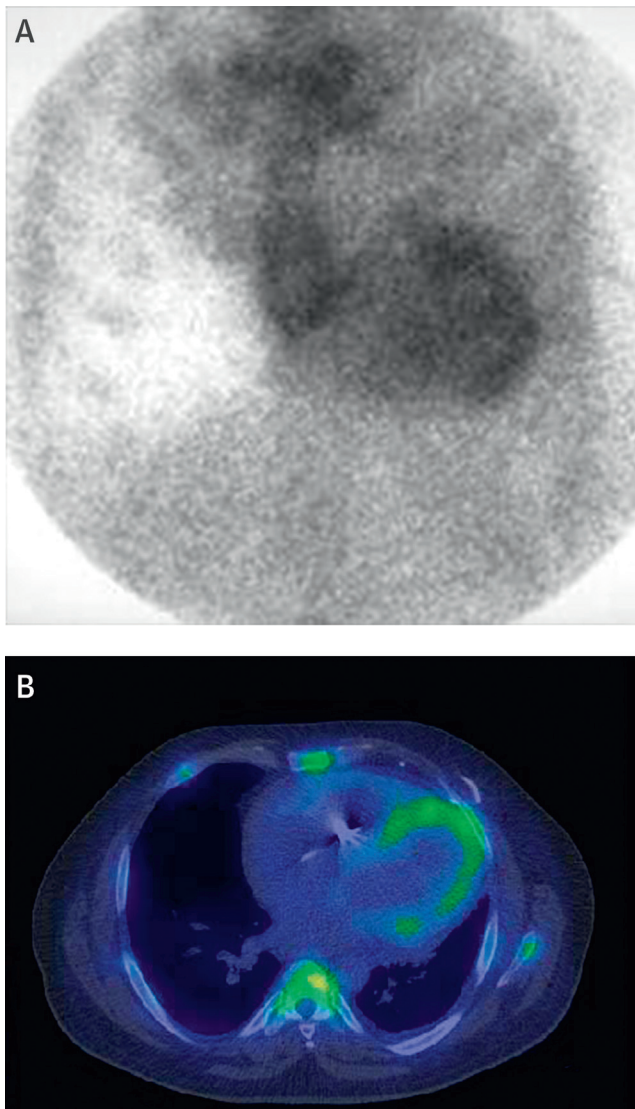
Arrow indicates myocardial nulling.

carriers without cardiac symptoms or in patients with only neurological symptoms, NT-proBNP can be used to identify the best time to begin echocardiographic assessment.

In another study, 13 patients with wt ATTR showed significant correlations between the log of B-type natriuretic peptide and left ventricle wall thickness (67). Although the data are limited, B-type natriuretic peptide level is likely a useful prognostic marker of cardiac function in wt ATTR.

DIAGNOSIS AND CLASSIFICATION

When treating a patient with evidence of infiltrative cardiomyopathy consistent with amyloidosis, biopsy verification is essential. However, endomyocardial biopsy (Figure 5) often is not necessary, particularly in the elderly or in patients debilitated by neuropathy. Noncardiac biopsies frequently will show amyloid deposits in patients with echocardiography or CMR findings consistent with amyloidosis, although positive noncardiac biopsies generally are less common in ATTR than in AL amyloidosis. Surgical skin biopsy can be performed safely at the bedside, with 1 study reporting 11 patients with wt ATTR undergoing

FIGURE 4 Bone Scan Demonstrating TTR Amyloid in the Heart

(A) ^{99m}Tc -3,3-diphosphono-1,2-propanodicarboxylic acid imaging of wild-type transthyretin amyloid. The 64-year-old man had a score 3 uptake of the tracer.
(B) ^{99m}Tc -pyrophosphate with single-photon emission computed tomography imaging.
 Courtesy of Geoffrey B. Johnson, MD, PhD, Mayo Clinic, Rochester, Minnesota (used with permission).

that a negative biopsy of an unaffected organ does not exclude a diagnosis of ATTR, and in wt TTR, most patients will require an endomyocardial biopsy to establish the diagnosis. Bone marrow biopsy was positive in 41% and 30% of patients with mutant and wt TTR, respectively. Rectal and sural nerve biopsies were positive in 81% and 83% of patients with mutant and wt ATTR, respectively. Only 4 rectal biopsies were performed among 100 patients with wt TTR; 2 were positive. Biopsy of minor salivary glands is used in many centers, with a reported sensitivity of 61% (70). Nerve biopsy should be reserved for patients with symptomatic peripheral neuropathy. In an autopsy study of 6 subjects with wt ATTR and 5 with mutant ATTR, 44% had positive amyloid deposits from the gastrointestinal tract and subcutaneous tissues (71). Noncardiac biopsy or fat aspiration could be considered an initial diagnostic test to confirm ATTR, but it is important to remember that abdominal fat is negative for most patients with wt TTR.

A diagnosis of wt TTR cardiac amyloidosis is plausible in a patient with classic echocardiographic features of amyloid cardiomyopathy, a technetium scan that shows strong uptake, and sequencing that shows no mutations in the *TTR* gene. Although these findings are considered consistent with wt ATTR, none of the actively enrolling trials currently accept patients without histological confirmation of amyloidosis for wt ATTR. Some will enroll patients with mutant ATTR if they have the classic presentation phenotype of cardiomyopathy or peripheral neuropathy and a proven *TTR* pathogenic mutation. In general, histological confirmation of the amyloid is strongly encouraged, even for patients with known *TTR* mutations.

After amyloid is detected in tissue, correct classification of the type of amyloid protein is critical. Historically, immunostaining with immunoperoxidase or immunogold was used to confirm ATTR, but false positives can occur. In a study of 24 endomyocardial biopsies for amyloid (72), 8 showed strong TTR staining of cardiac amyloid deposits. Mass spectroscopy, considered the criterion standard for classification, was performed in 5, with all showing light-chain amyloid protein. Among 15 patients with plasma cell dyscrasias, 7 biopsies showed strong staining for the corresponding monoclonal light chains, and 8 were equivocal. Strong false-positive immunostaining for TTR and cardiac amyloid is a significant pitfall, particularly when AL amyloid frequently fails to stain because of deletion or misfolding of the epitopes (which makes them unrecognizable by commercial antisera). In 142 consecutive biopsy specimens from 38 different

skin biopsy from the abdominal wall, with 8 having TTR amyloid deposits (sensitivity, 73%) (68). In a retrospective review of 186 patients with mutant ATTR and 100 patients with wt ATTR (69), 210 patients (73%) were positively identified after noncardiac tissue sampling, including 94% with mutant ATTR and 35% with wt ATTR. Subcutaneous fat was positive in 67% of patients with mutant ATTR (Figure 6) and only 14% with wt ATTR. This indicates

tissue types, positive immunohistochemistry was 100% concordant with mass spectroscopy findings. Mass spectroscopy improved the diagnostic accuracy from 76% to 94% (73). Amyloid type should be confirmed with mass spectroscopy.

In a study of 117 patients with amyloid (74), immunohistochemical analysis had a sensitivity of 96% and specificity of 100% for identifying inherited amyloidoses. Immunohistochemistry allowed definite classification of 92% of patients, and mass spectroscopy was used to classify inconclusive cases. In a large study of 423 cases with confirmed systemic amyloidosis (75), immunoelectron microscopy of abdominal fat showed 80% sensitivity and 100% specificity. However, the analysis of abdominal aspirate was informative in only 40% of patients with ATTR.

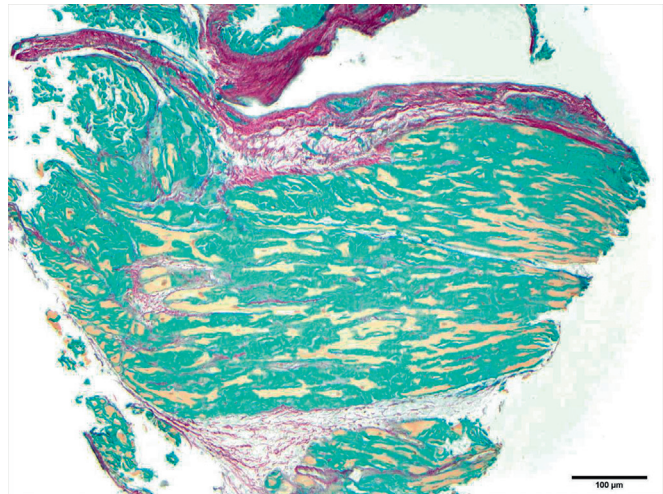
Mass spectroscopic proteome analysis is applicable to amyloid in a subcutaneous fat aspirate. Among 366 analyses, the sensitivity of the assay was 88% and the specificity was 96%; this analysis identified all forms of amyloid, including immunoglobulin light chain in 74%, TTR in 13%, and others in 3% (76). A universal amyloid proteome signature has been identified, and databases have been created to detect amyloidogenic peptides. Although not widely available globally, mass spectroscopic confirmation of the type of amyloid is strongly recommended. The **Central Illustration** presents the diagnostic evaluation for a patient with an established diagnosis of amyloidosis.

THERAPY

SUPPORTIVE CARE. Although diuretic agents are commonly prescribed for patients with heart failure, their use in amyloidosis is complicated. As a consequence of poor ventricular compliance, end-diastolic volumes are low. Patients often require a higher filling pressure to distend the stiffened heart, and diuretic therapy reduces preload, which can further reduce stroke volume and systolic blood pressure, with resultant cerebral hypoperfusion. Diuretic agents must therefore be used with care. Beta-blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers are poorly tolerated in cardiac amyloidosis and should be avoided (77). Digoxin binds to amyloid fibrils and can lead to locally high levels; it also must be used with caution (78,79).

CARDIAC DEVICES. Death in cardiac amyloidosis can result from progressive pump failure and is hemodynamically characterized by high filling pressures and low cardiac output. Right atrial pressure, pulmonary artery diastolic pressure, pulmonary capillary wedge pressure, and pulmonary artery saturation

FIGURE 5 Endomyocardial Biopsy Shows Amyloid



Sulfated Alcian blue; original magnification $\times 1,000$.

are predictors of death (80). Sudden death from bradyarrhythmia and electrical mechanical dissociation is a competing cause of cardiac death. Given the high incidence of sudden death in patients with TTR cardiac amyloidosis, considering prophylactic placement of an implantable cardioverter-defibrillator is rational. In 1 study, 19 patients with cardiac amyloidosis and a history of syncope or ventricular extrasystoles had an implantable cardioverter-defibrillator placed (81). Seven patients

FIGURE 6 Fat Aspirate Viewed Under Polarized Light



Congo red; original magnification $\times 1,000$.

died of electrical mechanical dissociation. Bradycardia, as recorded in pacing, was rare. Better predictors of arrhythmia-associated sudden cardiac death are required to truly understand the effect of an implantable cardioverter-defibrillator in cardiac amyloidosis. We reported the placement of implantable cardioverter-defibrillators in 9 patients with mutant ATTR and 10 with wt ATTR (82). The rate of appropriate implantable cardioverter-defibrillator shocks was 32% in the first year, observed almost exclusively in patients with AL amyloidosis. Only 2 with wt TTR and none with mutant ATTR received appropriate implantable cardioverter-defibrillator shocks. Device placement did not translate into an overall survival benefit. Early insertion of a pacemaker can be helpful because bradyarrhythmia, when it occurs, causes severe symptoms, and implantable cardioverter-defibrillators have increased rates of complications. An implantable device for primary prevention was reported in 4 patients with wt TTR and 3 with mutant TTR. Four of these were defibrillators, and in 1 patient, a shock was aborted because of spontaneous termination. This patient survived at least 23 months (83). One concern about failure of an implantable defibrillator to rescue amyloidosis patients is a higher defibrillation threshold. A subcutaneous array lead system can reduce this threshold and result in successful defibrillation (84).

Selection criteria were published for patients with familial amyloidosis who would benefit from prophylactic pacemaker placement. A His-ventricular interval ≥ 70 ms, His-ventricular interval >55 ms when fascicular block is present, or Wenckebach anterograde point ≤ 100 beats/min were considered for prophylactic implantation. Follow-up of these patients documented a high-degree atrioventricular block in 25% (85).

Left ventricular assist devices have been implanted in patients with TTR cardiac amyloidosis. Among 9 patients with the device, 2 died before hospital discharge, 3 died after discharge (median survival 13.7 months), and the remaining 4 survived with 16 to 24 months of follow-up (86). Gastrointestinal bleeding was seen in 3 of the 9. Implantation of a left ventricular assist device is feasible, but its exact role in ATTR currently is undefined. A study of left ventricular assist devices in 28 patients with restrictive cardiomyopathy included 10 patients with amyloidosis (87). The 1-year survival for patients who did not undergo heart transplantation was 64% and was not significantly affected by amyloidosis status. The most common complication was right ventricular failure. Smaller left ventricle dimensions may represent an

advanced stage of the cardiomyopathy process that is less responsive to assist device support. Alternatively, smaller left ventricle dimensions present technical challenges for left ventricular assist device inflow cannula implantation and may result in sub-optimal device placement and inadequate mechanical support.

LIVER TRANSPLANTATION. Liver transplantation has been considered the first-line therapeutic intervention for patients with mutant ATTR. Because the bulk of TTR is produced in the liver, replacing mutant TTR production with wt TTR was initially postulated to stop the progression of amyloidosis. However, limitations to the value of liver transplant have been identified. Wt TTR deposits in the heart, kidneys, peripheral nerves, and gastrointestinal tract have been identified in autopsied patients with mutant ATTR polyneuropathy who did not undergo liver transplantation. The proportion of wt versus mutant TTR in deposits and age at death are significantly correlated. The presence of wt and mutant TTR codeposits in the heart can be a major impediment to the long-term success of liver transplantation (88). Over a decade ago, patients with mutant TTR polyneuropathy were noted to have codeposits of mutant and wt TTR in nerves and in the heart (60% mutant, 40% wt) (89). Deposition of wt TTR on a mutant TTR substratum can result in progression of cardiac amyloidosis after liver transplantation. In an autopsy study (90), patients without a liver transplantation showed 60% mutant TTR and 40% wt TTR, but patients who underwent liver transplantation had a mutant to wt TTR ratio of 25:75, suggesting continued deposition of wt TTR in cardiac tissue after liver transplantation.

Ongoing deposition of wt TTR affects health-related quality of life after liver transplantation. Studies have shown that quality of life is stable in the first 4 years after transplantation, but physical well-being after 4 years was significantly lower compared with non-ATTR liver transplant recipients (91). Patients with the longest follow-up had deterioration in all health domains. Low health-related quality of life after liver transplantation is an ongoing issue in patients with mutant ATTR. Deposition of wt TTR after liver transplantation appears to be time-dependent, with a higher proportion of wt TTR at 1 year compared with 3 weeks; furthermore, the deposited protein is full-length TTR, not TTR fragments (92). Among 10 patients with mutant TTR receiving a liver transplant in Spain, 4 subsequently died (on post-transplant days 3, 510, 730, and 2,290) (93). Patients who underwent combined heart and liver transplant had better survival than those with heart transplantation alone. This

suggests that cardiac transplantation, when combined with liver transplantation, removes the nidus of mutant TTR (upon which wt TTR codeposits) and prolongs survival.

After liver transplantation, peripheral neuropathy can progress, just as cardiac amyloidosis progresses. However, current pathophysiological understanding of specific mechanisms is limited by clinical complexity (i.e., neuropathy can be caused by numerous factors, such as neurotoxic immunosuppression, post-transplant hyperinsulinemia, and other disease entities). In 1 study, a subject who died 5 years after liver transplantation had a nerve containing 75% wt TTR, which was associated with clinical symptoms of progressive neuropathy (94). Serial echocardiographic monitoring of patients with V30M ATTR after liver transplantation showed markedly increasing interventricular septal thickness with time, presumably due to cardiac deposition of wt TTR (95). Results after liver transplantation appear to be mutation-dependent, with the V30M mutation being associated with the most favorable outcomes. At many centers, patients with non-V30M ATTR are not commonly offered liver transplantation as an option (96). With long-term follow-up (>10 years) after liver transplantation, wt TTR deposits are seen in the heart, tongue, and spinal cord (97). Clinical involvement of the central nervous system appears to occur in V30M ATTR, regardless of liver transplantation; 31% of patients with V30M ATTR had clinical manifestations of focal neurological episodes (98).

Altogether, long-term outcomes of liver transplantation for mutant ATTR indicate that neuropathy and organ impairment are not usually reversed. Five-year survival rates in 1 study (99) were 100% and 59% for V30M and non-V30M ATTR, respectively, with death primarily caused by cardiac problems and sepsis. Lower survival is associated with malnutrition, disease duration, and older age at diagnosis (99). A recent analysis of the Familial Amyloidotic Polyneuropathy World Transplant Registry (n = 1,940) reported an overall 20-year survival rate after liver transplantation of 55.3% (100). Multivariate analysis showed modified body mass index, early onset of disease (age <50 years), disease duration before transplantation, and TTR V30M versus non-V30M TTR mutations as independent, significant factors affecting survival. In this large study, 5-year survival was 80%, and 10-year survival ranged from a low of 28% in men >50 years of age with V30M to 78% for men <50 years of age with V30M (100).

Combined cardiac and liver transplantation has been reported. In a series of 25 patients with ATTR,

18 of whom showed end-stage heart failure (101), 5 patients (age 43 to 57 years) had cardiac transplantation, with 1 having a subsequent liver transplant. A total of 3 were listed for liver transplantation, and 1 patient showed recurrent cardiac amyloid by electron microscopy only. In the short term, combined cardiac and liver transplantation is feasible. In 1 report, 7 patients received combined heart and liver transplantation for TTR amyloid cardiomyopathy (102). Two died within the first year. Five patients, all carrying an L111M (p.L131M) mutation, survived with normal ejection fraction and no recurrence of cardiac amyloid. These results are encouraging. Cardiac transplantation to restore normal function, followed by liver transplantation to prevent recurrent amyloid deposition, is feasible in younger patients with mutant TTR cardiac amyloidosis.

Isolated heart transplantation has also been performed. It may be particularly beneficial for the patients with the V122I TTR mutation, which has late onset and often does not deposit outside of the heart. In a case report, no systemic abnormalities were detected 5 years after heart transplantation (103). A second patient with V122I ATTR was well 3 years later, without systemic amyloid deposition (104). A case series of 10 patients (2 with wt TTR, 7 with mutant TTR, and 1 unknown) undergoing cardiac transplantation reported only 1 episode of amyloid in the cardiac graft, suggesting that in the short to intermediate term, isolated cardiac transplantation is feasible (105).

PHARMACOLOGICAL THERAPY OF ATTR. Considering the limitations of liver transplantation, the age at which many patients are diagnosed, and the lack of donor organs, pharmacological agents are needed (106). Techniques have been developed that serially assess patients with familial amyloid neuropathy to measure the rate of regression or progression of neuropathic symptoms (107). **Table 2** lists some agents currently being investigated in registered clinical trials (106).

In vitro studies suggest that epigallocatechin gallate, the most abundant catechin in green tea, inhibits amyloid fibril formation of several amyloidogenic proteins. One study described 19 patients with ATTR cardiomyopathy who were serially evaluated with echocardiography and CMR after consuming green tea or green tea extracts (108). After 12 months, no increase in left ventricular wall thickness or left ventricular mass was observed by echocardiography, suggesting that green tea or its extracts might inhibit progression of cardiac amyloid.

In 2006, orally administered diflunisal was reported to stabilize the TTR tetramer against association with amyloidogenic monomers (109). Diflunisal can bind

TABLE 2 Selected Trials for TTR Amyloidosis

Drug	Phase	TTR Type	Organs	Study Number
Doxycycline + tauroursodeoxycholic acid	II	Wild type and mutant	Cardiac, nerve	NCT01171859
Revusiran (ALN-TTRsc), Alnylam Pharmaceuticals Cambridge (Cambridge, Massachusetts)	III	Mutant	Cardiac	NCT02319005
Tafamidis	III	Wild type and mutant	Cardiac	NCT01994889
Diflunisal	II/III	Mutant	Nerve	NCT01432587
Patisiran (ALN-TTR02), Alnylam Pharmaceuticals Cambridge (Cambridge, Massachusetts)	III	Mutant	Nerve	NCT01960348
ISIS-TTR _{Rx} , Isis Pharmaceuticals (Carlsbad, California)	III	Mutant	Nerve	NCT01737398
SOM0226, SOM Biotech SL (Barcelona, Spain)	I-II	Mutant	Nerve	NCT02191826

TTR = transthyretin.

99% of unoccupied L-thyroxine binding sites in TTR, increasing the tetramer dissociation barrier and stabilizing the tetramer from forming monomers that misfold into the amyloid configuration (110). This phase I trial supported the safety of diflunisal administration, with kinetic studies showing binding to TTR and slowing of urea-mediated dissociation, setting the stage for future clinical trials (109).

Diflunisal was shown to be safe for patients with compensated cardiac ATTR (111). A randomized clinical trial reported in 2013 showed reduced progression of neurological impairment and preserved quality of life 2 years after randomization to therapy with diflunisal; however, no information was provided regarding the cardiac response (112). Diflunisal may also be effective for autonomic dysfunction and late-onset FAP caused by V30M ATTR (113). Because diflunisal is a nonsteroidal anti-inflammatory drug, potential adverse effects must be considered. Many patients with ATTR are taking diflunisal on the basis of these reports.

Tafamidis has been approved in the European Union and in Japan for treatment of mutant ATTR. It can produce kinetic stabilization to prevent TTR amyloidogenesis, preventing dissociation of the native TTR quaternary structure (114). Tafamidis binds to the thyroxine-binding sites of the TTR tetramer and inhibits its dissociation, thereby blocking the rate-limiting step of the TTR amyloid cascade. In a randomized, double-blind trial, tafamidis significantly reduced neurological decline in most variables examined, supporting the hypothesis that prevention of TTR dissociation can delay peripheral neurological impairment (115). Analysis of the open-label extension of this study showed that long-term treatment

with tafamidis was well tolerated, with the reduced rate of neurological deterioration sustained over 30 months (116). In a cohort of patients with non-V30M ATTR, patients treated with tafamidis (20 mg/day) did not show clinically relevant worsening of health-related quality of life, NT-proBNP, or echocardiographic parameters (117). In a post-approval trial by the French Network for FAP, neurological impairment scores worsened in 55% after 1 year of tafamidis treatment, suggesting that it could not stop disease progression (in terms of neural impairment) or disability in patients with advanced disease (118). A review of tafamidis (119) suggested that slowing deterioration of neurological function benefited health-related quality of life in long-term extension studies (up to 66 months). Tafamidis was capable of stabilizing TTR tetramers in patients with non-V30M ATTR and was well tolerated, with few patients discontinuing treatment. Currently, tafamidis is not approved for use in the United States, but trials are underway and are actively accruing patients (Table 2). Tafamidis also prevents biochemical and echocardiographic decline in TTR cardiomyopathy (120).

Ten years ago, studies showed that small interfering ribonucleic acid (siRNA) could selectively silence V30M TTR gene expression in cell culture systems (121). Advances in siRNA delivery and target selection provided the opportunity to initiate human trials using this technology (122). RNAi technology has been harnessed to knock down the disease-causing TTR protein, which is synthesized primarily in the liver. Data from a mouse model showed that the degree of TTR knockdown correlated with decreasing TTR tissue deposits. Patisiran (Alnylam Pharmaceuticals Cambridge, Cambridge, Massachusetts), an investigational drug, uses a TTR-targeting siRNA that knocks down both wt and all mutant forms of TTR. The drug is encapsulated in lipid nanoparticles, is administered through intravenous infusion, and targets the liver. It was studied in a single-dose, placebo-controlled, phase I trial in 32 ATTR patients and 17 healthy volunteers (123). At doses of 0.15 to 0.3 mg/kg, mean reductions in TTR ranged from 82.3% to 86.8%; at 28 days, reductions ranged from 56.6% to 67.1%. This siRNA suppressed mutant and wt TTR production, establishing proof-of-concept for currently accruing phase III trials (APOLLO). The trial is placebo controlled, and duration of therapy is 15 months. The primary endpoint is change from baseline of modified Neuropathy Impairment Score +7. Initial results from a pilot, phase 2 study of Revusiran (ALN-TTRsc) (Alnylam Pharmaceuticals Cambridge), an investigational RNAi therapeutic for cardiac ATTR, show that it was

generally tolerated in patients with clinically significant disease.

In addition to technologies investigating siRNA, antisense oligonucleotide drugs have been developed that inhibit hepatic expression of *TTR*. Antisense oligonucleotides are deoxyribonucleic acid- or ribonucleic acid-like molecules consisting of a short string of nucleotides. For *TTR*, an antisense oligonucleotide that is exactly complementary to the messenger ribonucleic acid (mRNA) molecule that encodes *TTR* is used. After the drug binds the mRNA, protein production may be inhibited by ribonuclease H-mediated destruction of the mRNA. Thus, the antisense drug can prevent or dramatically decrease *TTR* production. A transgenic mouse model with human *TTR* I84S suppressed *TTR* levels by as much as 80% (124). In phase I studies, the safety profile was favorable, and the agent produced dose-dependent reductions in circulating *TTR* levels. In healthy volunteers, ISIS-TTR_{rx} (Isis Pharmaceuticals, Carlsbad, California) produced rapid, dose-dependent reductions (approximately 75%) in plasma *TTR*, although some subjects had an approximate 90% reduction (125). Placebo-controlled phase III trials are underway for amyloid neuropathy (Table 2). Therapy is administered subcutaneously once a week. The primary endpoint is efficacy of ISIS-TTR_{rx}, as measured by change from baseline in the modified Neuropathy Impairment Score +7 and change from baseline in the Norfolk Quality of Life Diabetic Neuropathy questionnaire. The trial runs for 64 weeks.

Doxycycline can disrupt fibrils (126), and doxycycline treatment of a mouse model of amyloidosis resulted in amyloid disaggregation and improvement in some tissue markers associated with *TTR* deposition (127). Importantly, when doxycycline was administered in combination with the antiapoptotic agent tauroursodeoxycholic acid, a more pronounced, synergistic effect on removal of tissue *TTR* deposits was observed, paralleled by complete normalization of known FAP tissue markers. On the basis of these pre-clinical studies, a phase II, open-label study was designed to evaluate the efficacy, tolerability, safety,

and pharmacokinetics of doxycycline (100 mg, twice daily, orally) and tauroursodeoxycholic acid (250 mg, 3 times/day, orally) administered continuously for 12 months. Preliminary data support a beneficial effect with an acceptable toxicity profile (128).

Administration of antihuman antibodies against serum amyloid P component to mice with amyloid deposits containing human serum amyloid P component triggers a reaction that removes amyloid deposits. Antibody treatment is feasible because circulating human serum amyloid P component can be depleted in patients by the bis-d-proline compound CPHPC, thereby enabling injected antibodies to reach residual serum amyloid P component in the amyloid deposits (129).

SUMMARY

ATTR is easily confused with other forms of infiltrative and hypertrophic cardiomyopathy. The coexistence of systemic symptoms involving the peripheral or autonomic nervous system and cardiac dysfunction are important clues. Diagnosis of mutant *TTR* is often established without an endomyocardial biopsy. A positive noncardiac biopsy is much less likely in wt *TTR* compared with mutant *TTR*. If typical echocardiographic or CMR findings are present, a DPD or pyrophosphate nuclear scan of the heart can establish the diagnosis of ATTR, although current clinical trials require tissue confirmation. Establishing the diagnosis early is relevant because therapeutic interventions exist; treatments include liver transplantation and newly developed pharmacological agents that can stabilize the tetramer, prevent misfolding, or block hepatic synthesis of *TTR*. The standardized evaluation of patients with this disorder (Central Illustration) can be used to guide clinicians.

REPRINT REQUESTS AND CORRESPONDENCE: Dr. Morie A. Gertz, Division of Hematology, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905. E-mail: gertz.morie@mayo.edu.

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