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Transthyretin (TTR) Cardiac Amyloidosis

Frederick L. Ruberg, MD; John L. Berk, MD

The systemic amyloidoses are a family of diseases induced by misfolded or misassembled proteins. Extracellular deposition of these proteins as soluble or insoluble cross β -sheets disrupts vital organ function.¹ More than 27 different precursor proteins have the propensity to form amyloid fibrils.² The particular precursor protein that misfolds to form amyloid fibrils defines the amyloid type and predicts the patient's clinical course. Several types of amyloid can infiltrate the heart, resulting in progressive diastolic and systolic dysfunction, congestive heart failure, and death. Treatment of cardiac amyloidosis is dictated by the amyloid type and degree of involvement. Consequently, early recognition and accurate classification are essential.³

The diagnosis of amyloidosis requires histological identification of amyloid deposits. Congo Red staining renders amyloid deposits salmon pink by light microscopy, with a characteristic apple green birefringence under polarized light conditions (Figure 1). Additional immunohistochemical staining for precursor proteins identifies the type of amyloidosis (Figure 2).⁴ Ultimately, immunogold electron microscopy and mass spectrometry confer the greatest sensitivity and specificity for amyloid typing.^{5,6}

Two types of amyloid commonly infiltrate the heart: (1) Immunoglobulin light-chain (AL or primary systemic) amyloid and (2) transthyretin (TTR) amyloid. Transthyretin-related amyloidoses, in turn, encompass 2 forms of disease: Familial disease arising from misfolding of a mutated or variant TTR (familial amyloid cardiomyopathy or familial amyloidotic polyneuropathy [FAP]) and a sporadic, nongenetic disease caused by misaggregation of wild-type transthyretin (senile systemic amyloidosis [SSA]). Cardiac amyloidosis can also be caused by other precursor proteins such as apolipoprotein A1, but the prevalence of disease is low and beyond the scope of this review.³ In contrast, AL amyloidosis has an estimated incidence approaching \approx 2500 new cases annually,⁷ with cardiac involvement in \approx 50% of cases.⁸⁻¹⁰ Untreated, prognosis with AL disease is poor, with median survival after diagnosis of $<$ 1 year in the presence of heart failure symptoms.⁸ Unlike AL heart disease, transthyretin-related amyloid cardiomyopathy is slowly progressive and clinically well tolerated, often defying diagnosis until marked ventricular wall thickening, profound diastolic dysfunction,

and conduction disease have occurred. Untreated, survival with transthyretin-related cardiac amyloidosis is measured in years to decades. Secondary or AA amyloidosis results from misfolding of serum amyloid A, an acute phase reactant induced by chronic inflammation. Echocardiographic evaluations in 3 studies involving 48, 30, and 224 patients, respectively, with AA amyloidosis identified features of amyloid cardiomyopathy in only 1.3% of the aggregate cohort, and $<$ 1% of the population had recognized congestive heart failure.¹¹⁻¹³ Although 30% of Finnish rheumatoid arthritis patients have histological evidence of cardiac amyloid, heart failure is rarely reported in this population.¹⁴ These data indicate AA amyloid rarely infiltrates the myocardium or conduction system in a clinically meaningful fashion and that coronary infiltration is rarely reported.¹⁵

TTR, formerly known as prealbumin, is a 127-amino acid, 56-kDa transport protein primarily expressed by the liver. Under normal conditions, TTR circulates as a homotetramer, but because of genetic mutation or aging, tetramers can dissociate to monomers that misassemble into amyloid fibrils.¹⁶ Because 2 forms of TTR amyloid exist (age-related or senile amyloidosis [wild-type TTR] and familial transthyretin amyloidosis [variant TTR]), this review will compare the epidemiology, pathogenesis, diagnosis, and treatment of SSA and familial amyloid cardiomyopathy.

Epidemiology

Age-Related Amyloidosis or SSA

Soyka first described age-related cardiac amyloidosis in 1876,¹⁷ followed by numerous case reports and small case series published over the next 100 years. In 1965, Pomerance estimated the prevalence of senile cardiac amyloidosis to be \approx 10% in people $>$ 80 years of age and 50% in those $>$ 90 years of age.¹⁸ A study of 85 consecutive autopsies in patients \geq 80 years of age found amyloid deposits with prealbumin immunohistochemical staining (TTR) in the atria or left ventricle of 21 hearts, a 25% prevalence for age-related TTR amyloid in this elderly population.¹⁹ On closer review, however, only two thirds of the TTR amyloid-staining hearts had left ventricular involvement, described as "small and widely scattered" in $>$ 50% of cases. These data suggest histologically significant cardiac TTR amyloid occurs in 8%

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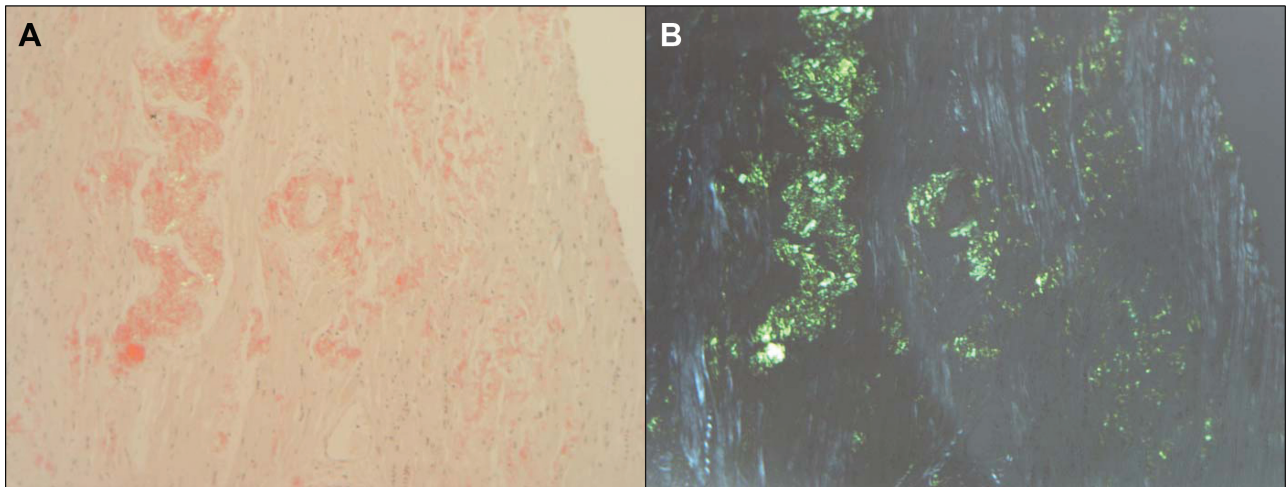


Figure 1. Congo Red staining of myocardial tissue from a patient with amyloid cardiomyopathy. **A**, Light microscopy; **B**, polarized light microscopy, 400× magnification.

to 16% of people >80 years of age. A prospective study of Finnish octogenarians (the Vantaa 85+ Study) also identified TTR amyloid deposits in 25% of hearts from 256 autopsies.²⁰ Once again, moderate or severe cardiac amyloid deposition occurred in only 5.5% of the total autopsy population.

Prevalence of SSA indisputably increases with advancing age, and virtually all patients are >60 years of age when diagnosed. SSA is a remarkably sex-specific disease, exhibiting ≈25 to 50:1 male:female expression.²¹ Although the Finnish autopsy study did not report a male predominance for amyloid deposition, male patients had more pronounced amyloid staining (greater amyloid burden) than did female SSA cases. By unclear aging mechanisms, aggregation and cardiac deposition of genetically normal TTR increase over time. Aggregate autopsy data suggest wild-type TTR, al-

though histologically present in the hearts of 25% to 30% of septuagenarians and octogenarians, drives cardiac dysfunction in a smaller but significant elderly cohort. Projections of octogenarian population growth over the next 20 years predict SSA will become the most common form of cardiac amyloidosis.

Familial Amyloid Cardiomyopathy

The TTR gene is located on chromosome 18q12.1 and spans 4 exons and 5 introns. There are >100 single nucleotide polymorphisms encoding variant TTR, with 80 confirmed pathogenic mutations.²² These mutations tend to cluster into geographic or ethnic groupings and exhibit an autosomal dominant pattern of inheritance. The clinical phenotype of variant TTR amyloidosis varies greatly by mutation, the age

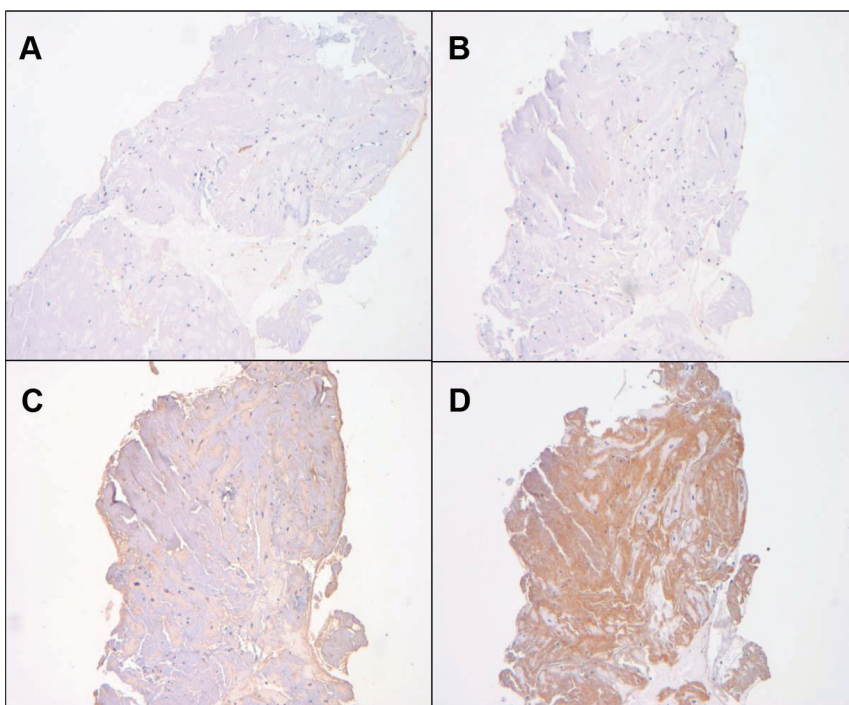


Figure 2. Transthyretin amyloid cardiomyopathy by immunohistochemical staining. Endomyocardial biopsy samples were stained with antibodies to **(A)** kappa light chain, **(B)** lambda light-chain, **(C)** serum amyloid A, and **(D)** transthyretin amyloid. Bright-light micrographs at 400× magnification.

Table 1. Characteristics of Wild-Type and Common Variant Transthyretin Cardiac Amyloidosis

| Mutation | Origin | Prevalence | Male:Female Ratio | Onset | Organs |
|----------|----------------------------------|----------------------|--------------------------|-------|----------------|
| SSA | Worldwide | 25% >85 y | 25:1 to 50:1 | >60 y | Heart, ST |
| V122I | United States, Caribbean, Africa | 4% Black | 1:1 Gene (+) 3:1 Disease | >65 y | Heart, PNS, ST |
| V30M | Portugal, Sweden, Japan | 1:1000 | 2:1 | >50 y | PN/ANS, heart |
| T60A | United Kingdom, Ireland | 1% Northwest Ireland | 2:1 | >45 y | Heart, PNS/ANS |

SSA indicates senile systemic amyloidosis, wild-type (no mutation); ST, soft tissue; PNS, peripheral nervous system; and ANS, autonomic nervous system.

of onset, disease penetrance, clinical course, and prognosis. Most FAP patients develop nervous system involvement with or without cardiac amyloidosis.²³ The mutations that most commonly induce variant TTR cardiac amyloidosis are summarized in Table 1. The most prevalent and best described mutation associated with amyloid neuropathy is V30M (Met 30), which predominantly affects patients originating from Japan, Portugal, or Sweden. People with the Met30 genotype have variable disease penetrance influenced by the place of origin, sex, parental gene transmission, and age affecting disease expression.²⁴ The T60A (Ala 60) TTR mutation is also a frequent cause of amyloid neuropathy and cardiomyopathy, affecting 1% of the population in northwest Ireland (County Donegal).²⁵ Although the UK National Amyloidosis Centre reports cardiac involvement in nearly 100% of T60A patients with familial amyloid cardiomyopathy referred with neuropathy,²⁶ the US experience is more heterogeneous.

Among the variant TTRs that predominantly target the heart, the valine-to-isoleucine substitution at position 122 (V122I or Ile122) affects the greatest world population.²⁷ First described in 1989 by Gorevic et al,²⁸ Jacobson et al^{29,30} reported V122I TTR genopositivity in roughly 3% to 4% of the US black population, with virtually undetectable prevalence in the white population.³¹ The V122I founder appears to have originated in West Africa, which explains clinical expression of V122I TTR in the Caribbean islands (Haiti, Jamaica, and Bermuda; D. Jacobson, personal communication). Although penetrance of this particular allele is unknown, there appears to be a strong association between carrier status, the development of heart failure (relative risk 2.6),³² and echocardiographic features of cardiac amyloidosis.³³ Examining stored samples from the Beta-Blocker Evaluation in Survival Trial (BEST), Buxbaum et al³⁴ demonstrated that ≈10% of black study participants with heart failure who were >60 years of age carried V122I, which suggests that unrecognized cardiac amyloidosis may be a contributor to the development of heart failure. This trial enrolled patients with systolic dysfunction (left ventricular ejection fraction <35%), a cardiac profile more frequently observed in V122I than in other TTR mutations.^{35,36} By recent US Census statistics, ≈1.5 million blacks carry the V122I mutation and are at risk for the development of ATTR cardiac amyloidosis.³⁷ More specifically, blacks >65 years of age carrying V122I constitute the population at immediate risk for clinical expression of TTR cardiomyopathy, and they number ≈99 600 to 132 800 according to the 2010 census figure (3%–4% of 3 320 000 blacks), rendering V122I a potentially important cause of heart failure in the elderly black community.

Pathogenesis

Transthyretin, a 127-amino acid protein, is encoded by 7 kb of DNA spanning exons 1 to 4 of a single gene on chromosome 18.³⁸ In its native state, transthyretin circulates as a tetramer with 2 C2 symmetrical funnel-shaped thyroxine-binding sites at its dimer-dimer interface.³⁹ Elegant thermodynamic studies demonstrate that tetramer dissociation is the rate-limiting step in misfolding of monomeric transthyretin to amyloid fibrils.¹⁶ In SSA, incompletely described age-related events such as posttranslational biochemical alterations in wild-type TTR or its chaperones appear to contribute to amyloid fibril formation. Data from transgenic mice overexpressing human SSA suggest that alterations in hepatic chaperone production or proteasome clearance of unfolded protein may determine which aging heart develops amyloidosis.⁴⁰ In FAP, 1 amino acid substitution in native TTR (variant TTR) destabilizes the tetramer, promoting disaggregation of monomeric protein. Ultimately, the amyloidogenicity of a particular variant transthyretin is determined by the capacity of a specific amino acid substitution to destabilize circulating TTR tetramers, releasing monomeric TTR to permit misfolding to occur. To date, >100 variant TTRs have been described. Many amino acid substitutions associate with patterns of organ involvement, predicting distinct clinical courses. Typically, variant transthyretin is induced by a single-nucleotide substitution; however, 2-nucleotide changes or complete codon deletion have been shown to produce 1 amino acid change in transthyretin.⁴¹

Affected organs invariably harbor extracellular amyloid deposits. Whether these deposits induce organ dysfunction or represent epiphenomena (disease markers) is debated. Review of sural nerve biopsy samples in 31 V30M ATTR patients described epineural amyloid deposits and nerve degeneration in half of the cohort. No correlation between the presence of amyloid deposits and histological nerve damage could be assigned,⁴² which suggests an alternative mechanism of injury. In the kidney, the degree of functional disruption is not predicted by the extent of amyloid deposits.^{43,44} Moreover, serial kidney biopsies in patients with AL amyloidosis before and after clinically successful treatments revealed unchanging amyloid burden despite significant improvement in proteinuria.^{45,46}

Increasingly, data support a central role for circulating or prefibrillar amyloidogenic proteins in the disruption of cardiac function in AL and TTR-mediated amyloid disease. Murine hearts perfused with clonal immunoglobulin light chain (AL-LC) isolated from patients with AL amyloid cardiomyopathy rapidly induced diastolic dysfunction. In contrast, light chain isolated from patients without AL amy-

loidosis did not alter *ex vivo* heart function.⁴⁷ *In vitro*, exposure of cultured cardiomyocytes to physiological levels of AL-LC stimulated production of reactive oxygen species and upregulated heme oxygenase-1, a redox-sensitive protein that identifies cell injury.⁴⁸ In addition to altering the cardiomyocyte intracellular redox state, AL-LC reduced intracellular calcium levels and cardiomyocyte contractility, in the absence of amyloid fibril formation.⁴⁸ Recent data indicate AL-LC alters cardiomyocyte ion fluxes, contractility, and programmed cell death through a noncanonical p38 α mitogen-activated protein kinase pathway.⁴⁹

Similar evidence of end-organ damage by prefibrillar TTR amyloid exists in FAP. Nerve biopsies from asymptomatic V30M ATTR carriers revealed prefibrillar/Congo Red stain-negative protein aggregates by anti-TTR immunohistochemistry and immunogold electron microscopy.⁵⁰ Upregulation of nuclear factor- κ B and proinflammatory cytokines in these nerve biopsy samples signaled cell toxicity affected by these nonfibrillar TTR aggregates, well before the onset of clinical disease. Exposure of neuronal cell cultures to L55P ATTR nonfibrillar aggregates induced caspase-3 generation and expression of programmed cell death. In contrast, mature L55P ATTR amyloid fibrils did not stimulate caspase-3 expression, which suggests that prefibrillar TTR aggregates are the neurotoxic mediator of disease. Additional data support a role for receptors of advanced glycosylation end products (RAGE) in mediating ATTR organ injury.⁵¹ Initial studies of V30M versus L55P ATTR transgenic mouse models of FAP did not detect differences in nerve toxicity mediated by the nonfibrillar forms of these variant TTR species. Taken together, data generated in AL and ATTR models of disease provide evidence that prefibrillar protein aggregates, and not mature amyloid fibrils, contribute to organ toxicity.

Diagnosis

Diagnosis of systemic amyloidosis requires histological identification of amyloid deposition by Congo red staining. Unlike light-chain (AL) amyloid disease, kidneys and tongue are rarely involved in clinically significant fashion, and thus, biopsy of these organs is usually not pursued.³⁶ Abdominal fat aspirate is a simple, office-based biopsy procedure that identifies amyloid deposits in $\approx 70\%$ of those with variant TTR such as V122I ATTR disease.³⁵ Cardiac biopsy remains the gold standard for amyloid cardiomyopathy and is not complicated by sampling artifact (yielding false-positive or -negative findings) as occurs with other infiltrative processes such as sarcoidosis. Once amyloid is identified by Congo red staining, immunohistochemical stains for κ and γ light chains, AA, and TTR can be performed to determine the precursor protein. Confirmation of histology and identification of the amyloidogenic protein may be aided by review by pathologists at international amyloidosis referral centers. Immunohistochemistry demonstrating TTR protein in amyloid deposits requires further analysis to distinguish variant from wild-type TTR. Isoelectric focusing electrophoresis in most cases permits separation of variant from wild-type TTR by charge (Figure 3). In our experience, isoelectric focusing reveals distinct electrophoretic mobility differences for

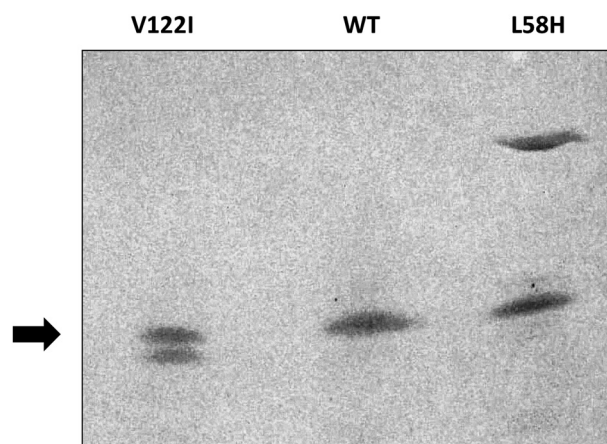


Figure 3. Isoelectric focusing gel electrophoresis. Sera from patients with V122I, wild-type (WT), and L58H transthyretin amyloidosis (ATTR). Arrow indicates wild-type transthyretin migration. Note presence of 2 distinct bands in V122I and L58H lanes.

$\approx 95\%$ of the variant TTRs tested. Polymerase chain reaction amplification and sequencing of TTR exons 1 to 4 validates the isoelectric focusing findings and definitively establishes variant from wild-type TTR genotype. Alternatively, biopsy tissue can be processed by laser dissection/liquid chromatography-tandem mass spectrometry to identify the precursor protein with 98% sensitivity.⁶ Occasionally, TTR cardiac amyloidosis can be diagnosed without cardiac biopsy; when variant TTR genopositivity is established, tissue biopsy from another site documents TTR amyloid deposits, and noninvasive data (see below) support cardiac involvement.

In many international amyloid centers, AL amyloidosis and monoclonal gammopathy of unknown significance dominate the clinic population, occasionally obscuring recognition of variant or wild-type TTR amyloidosis. Lachmann et al⁵² identified TTR mutations among 4% of referrals to a national amyloid center for evaluation and treatment of presumed AL disease. None of these TTR amyloid patients had family histories that suggested genetic disease. Interestingly, all of the ATTR patients presented with cardiomyopathy. Immunohistochemical staining of tissue sections ultimately identified TTR as the amyloid subunit protein.⁵² Similarly, Connors et al³⁵ reported biopsy-proven AL amyloidosis in 12% of V122I gene-positive patients. These data from large amyloid referral centers illustrate the importance of establishing the correct subunit protein that forms the amyloid tissue deposits, particularly in patients with ATTR genopositivity.

Noninvasive Testing

Diagnosis of cardiac amyloidosis can be based on invasive heart biopsies or a noninvasive approach, given the proper clinical context, supportive noninvasive testing, and identification of amyloid tissue deposits from a noncardiac source such as abdominal fat aspirate (Figure 4). A contemporary approach to noninvasive diagnosis of TTR cardiac amyloidosis includes echocardiography with strain imaging, cardiac magnetic resonance (CMR), electrocardiography (ECG), and serum biomarker testing, including B-type natriuretic peptide (BNP or N-terminal pro-BNP) and cardiac troponin (T or I).

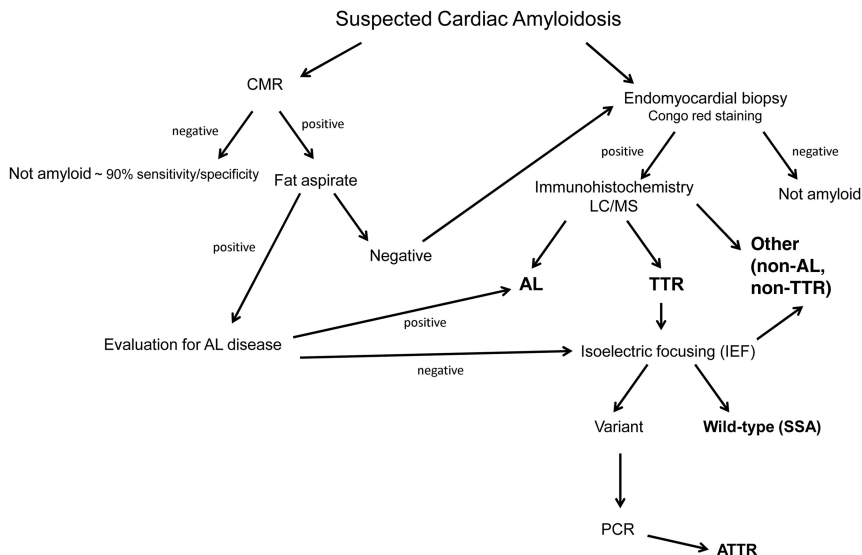


Figure 4. Diagnostic algorithm for diagnosis of transthyretin (TTR) cardiac amyloidosis. CMR indicates cardiac magnetic resonance imaging; AL, light-chain amyloidosis; LC/MS, laser dissection/liquid chromatography/tandem mass spectrometry; SSA, senile systemic amyloidosis; PCR, polymerase chain reaction; and ATTR, TTR amyloidosis.

Physical examination does not typically assist in differentiation of amyloid type, with the notable exceptions of macroglossia and periorbital ecchymoses, which herald AL amyloidosis.³⁶ Physical findings vary significantly depending on the severity of heart dysfunction, ranging from a relatively normal examination in early-stage disease to extensive signs of congestive heart failure, including pleural effusions, elevated jugular venous pressure, and peripheral edema. In many cases of TTR amyloid, isolated cardiac involvement or inconclusive biopsy samples from other sites (fat pad aspirate, gastric or rectal biopsies, extensor retinaculum sampling at carpal tunnel surgery, or salivary gland biopsies) warrant direct endomyocardial sampling.

Echocardiography

Echocardiography remains the most useful imaging modality for identifying and monitoring cardiac amyloid disease. Ease of image acquisition and interpretation, relatively low cost, unparalleled diastolic functional assessment, and capacity for serial studies despite technical differences in data acquisition or disease progression make echocardiography the universal instrument for cardiac amyloid assessment. Recent reports validate detection of subtle systolic dysfunction by tissue Doppler imaging and speckle tracking technology.⁵³

By classic echocardiographic teaching, the cardiac amyloid phenotype is a thick-walled ventricle with a speckling appearance of the myocardium, small left ventricular chamber volume, valve thickening, atrial enlargement, and signs of elevated filling pressures (pericardial effusion, pleural effusions, dilated vena cava) caused by restrictive diastolic filling (Figure 5; online-only Data Supplement Movie I).⁵⁴ Although the preponderance of data on which these echocardiographic features are based are derived from the AL population, similar findings have been reported in TTR cardiomyopathy.²¹ Wall-thickness increase remains the principal feature on which cardiac amyloidosis is diagnosed. According to an international consensus panel of experts in amyloid disease, interventricular septal thickness of >12 mm, in the absence of aortic valve disease or significant systemic hypertension, is the echocardiographic criterion that identifies cardiac in-

volvement in patients with AL systemic amyloidosis (there are no established criteria for TTR disease).⁵⁵ This single threshold fails to account for sex-specific differences in normal wall thickness⁵⁶ and confers a high degree of specificity but low sensitivity for identification of cardiac involvement. The continuum of cardiac involvement makes early disease recognition challenging when wall thickness and diastolic function are only mildly abnormal. The perceived rarity of amyloid disease compared with other, more common entities that produce ventricular thickening, such as hypertensive remodeling and hypertrophic cardiomyopathy, likely lowers cardiologists' recognition of new cases. Echocardiography alone is often unable to differentiate these very different processes, prompting multimodality assessment. However, the presence of prominent right ventricular wall thickening, interatrial septal thickening, and restrictive (grade 3) diastolic dysfunction are uncommon in hypertensive remodeling or hypertrophic cardiomyopathy and can suggest that TTR amyloidosis may be present.^{24,33}

The challenges of amyloid diagnosis coupled with mimicry of hypertensive and hypertrophic cardiomyopathy result in late recognition of TTR cardiac disease. Consequently, advanced remodeling changes are more often present on diagnosis of TTR cardiomyopathy than in AL heart disease. Patients with SSA cardiac amyloidosis tend to have the largest wall thickness and myocardial mass compared with AL²¹ and variant TTR disease.³⁶ Although systolic dysfunction is frequently a manifestation of more advanced disease in light-chain and variant TTR cardiac amyloidosis, it is fairly common in SSA disease, again likely because of delayed recognition. Among patients with ATTR, a lower left ventricular ejection fraction (<50%) is associated with reduced survival.⁵⁷

Longitudinal strain measurement by tissue Doppler and echocardiographic speckle tracking have emerged as useful clinical tools for the identification of cardiac involvement in AL disease⁵³ and can assist in differentiation of cardiac amyloidosis from other causes of wall thickening, including hypertension and hypertrophic cardiomyopathy.⁵⁸ TTR car-

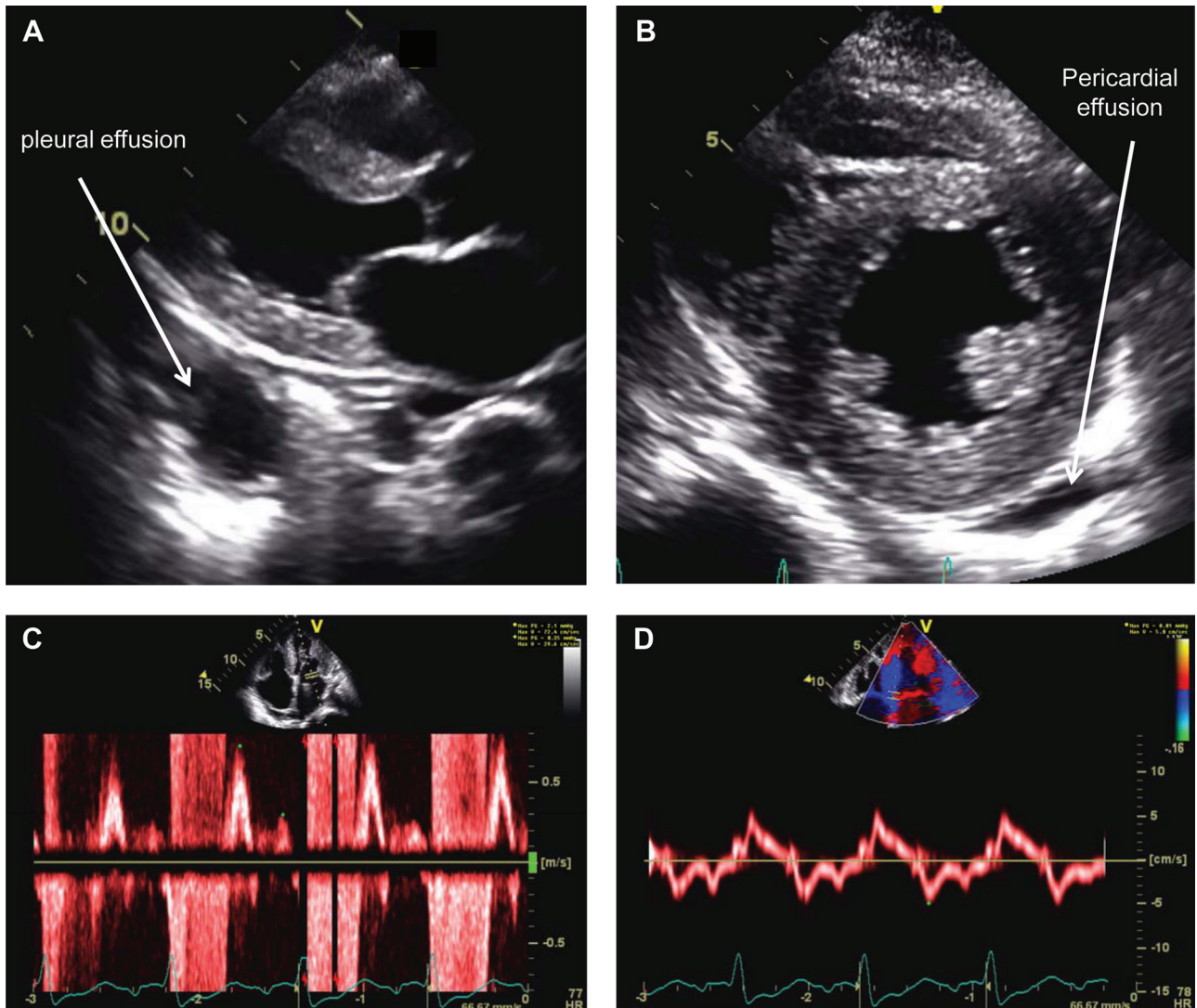


Figure 5. Echocardiographic appearance of V122I transthyretin cardiac amyloidosis (ATTR). Parasternal long-axis (A) and short-axis (B) views are illustrated, demonstrating increased ventricular wall thickness and pleural and pericardial effusions. C, Restrictive transmitral Doppler pattern. D, Tissue Doppler velocities consistent with reduced longitudinal systolic shortening (reduced S' velocity) and diastolic dysfunction (reduced e' velocity).

diac amyloidosis also results in reduction in longitudinal shortening, although its prognostic significance has not been established.

Cardiac Magnetic Resonance Imaging

Compared with echocardiography, CMR offers superior myocardial border delineation and a 3-dimensional approach to quantify ventricular volumes, wall thickness, and mass (online-only Data Supplement Movie II). It is more precise and reproducible than echocardiography but also more expensive, less widely available, and limited by the inability to image patients with pacemakers or implanted cardioverter-defibrillator devices. At present, echocardiography provides the best imaging technique for assessment of ventricular diastolic function, conferring better temporal resolution than CMR. However, the principal advantage of CMR over echocardiography is the capacity to directly identify amyloid infiltration by means of late gadolinium enhancement (LGE) imaging. Gadolinium is an extracellular contrast agent, and

under normal conditions, it is not retained in the myocardium after administration. Amyloid infiltration results in expansion of the extracellular space and abnormal myocardial gadolinium distribution kinetics, which result in contrast retained in the heart. Signal from normal myocardium is nulled or suppressed in LGE imaging, but because of diffusely retained contrast, this is difficult to achieve in cardiac amyloidosis. An important limitation of the application of contrast-enhanced CMR in TTR cardiac amyloidosis is coexistent chronic kidney disease and the risk of nephrogenic systemic fibrosis.⁵⁹ If the creatinine clearance is <30 mL/min, gadolinium contrast cannot be administered safely, and LGE imaging cannot be performed.

Maceira et al⁶⁰ first reported a CMR profile that identified cardiac amyloidosis, but with an imaging technique that required an unusually short delay after contrast administration to obtain optimal LGE images. Subsequent studies, in mixed AL and TTR cohorts, have determined that the

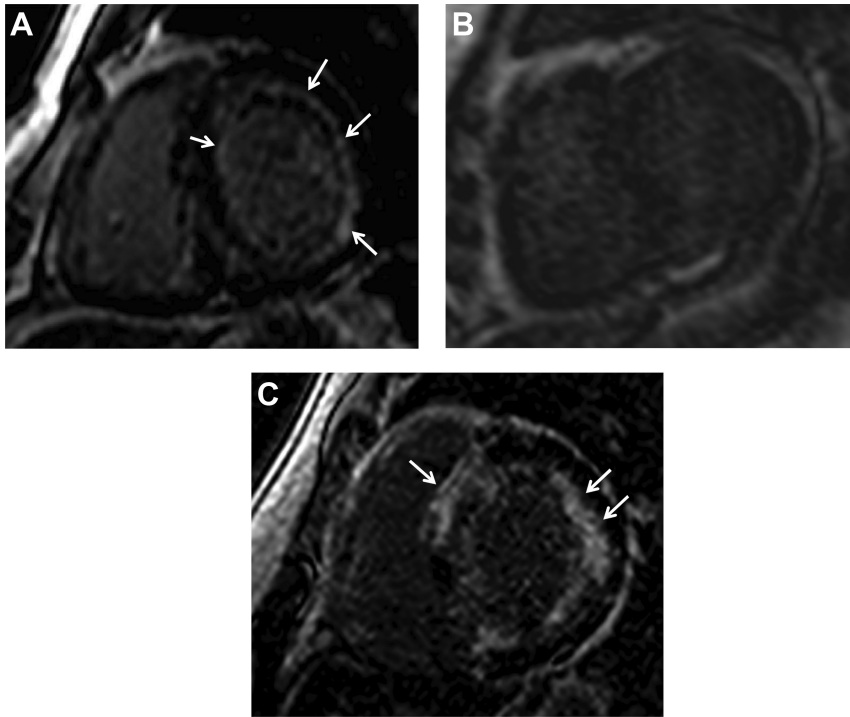


Figure 6. Cardiac magnetic resonance imaging of transthyretin amyloidosis. Late gadolinium enhancement (LGE) images from midventricular short-axis slices are depicted illustrating the different LGE patterns observed. **A**, LGE is evident (arrows) in a characteristic, diffuse subendocardial pattern (patient with wild-type transthyretin). **B**, Diffuse transmural low-intensity signal is seen with poor contrast between the blood pool and myocardium (patient with V122I variant transthyretin). **C**, High-signal-intensity, patchy LGE is evident involving the subendocardium in the lateral wall but transmurally involving the septum as well (arrows; patient with wild-type transthyretin).

sensitivity and specificity of CMR for the identification of cardiac amyloidosis compared with endomyocardial biopsy approaches 90%.^{61–63} Unlike LGE abnormalities associated with myocardial infarction, in which focal regions of high-signal-intensity LGE are seen, patterns in cardiac amyloidosis are variable, with global subendocardial, diffuse, and focal foci noted (Figure 6).⁶⁴ Furthermore, the retained gadolinium greatly shortens myocardial T1,⁶⁵ a fundamental magnetic resonance characteristic on which LGE contrast is founded. Because of retained contrast, myocardial T1 approaches that of the ventricular blood pool, rendering a distinct pattern of early myocardial signal suppression (coincident to the blood pool) representative of diffuse amyloid infiltration.

The majority of published reports of CMR in cardiac amyloidosis involve mixed cohorts of patients with relatively advanced AL and TTR disease, with the notable exception of the report by Di Bella et al,⁶⁶ wherein CMR and nuclear scintigraphy were used to identify cardiac involvement in patients with FAP. Although precursor proteins differ, the LGE findings in TTR and AL cardiac amyloidosis appear relatively similar. As reported in the echocardiographic literature, CMR-determined wall thickness and mass are greater in TTR cardiac amyloidosis than in light-chain disease.⁶⁷

Electrocardiography

Classically, cardiac amyloidosis is electrocardiographically typified by low QRS voltage and a pseudoinfarct pattern of Q-wave or T-wave changes on ECG. The presence of low QRS voltage and increased left ventricular wall thickness by echocardiography should prompt consideration of cardiac amyloidosis. Notably, certain ECG features support TTR cardiac disease more than AL heart disease. In different case series, low QRS voltage has been reproducibly identified in approximately 46% to 60% of AL patients but only 25% to

40% of those with TTR disease.^{36,68,69} However, a “pseudoinfarct” pattern is also equally seen in AL disease (47%–69%) and TTR disease (66%–69%).^{36,68,69} The presence of conduction system disease is more common in SSA disease, particularly left bundle-branch block. Finally, although most patients present with sinus rhythm, atrial fibrillation is more commonly observed in SSA disease ($\approx 30\%$) than in variant TTR ($<10\%$) and AL disease (typically $<20\%$).^{36,68}

Nuclear Scintigraphy

Nuclear pharmaceutical identification of cardiac amyloid deposition is a developing field. Three classes of nuclear tracers have application to cardiac amyloidosis: (1) Positron emission tomography agents, (2) bone-avid compounds, and (3) amyloid-directed molecules.

Positron Emission Tomography Agents

Design of the positron emission tomography agents is based on the structure of thioflavin T, a benzothiazole dye that fluoresces when bound to β -rich amyloid fibrils. The clinical limitations of the short-lived original ^{11}C compounds led to the development of ^{18}F -tagged agents, with recent US Food and Drug Administration market approval for ^{18}F -aV-45 [florbetapir] (Amyvid) as a tracer for β -amyloid in Alzheimer dementia. No published data are available on the use of positron emission tomography agents in amyloid cardiomyopathy.

Bone-Avid Compounds

Three different bone-complexing molecules have varying avidity for cardiac amyloid deposits, including the 99m-technetium-labeled agents pyrophosphate ($^{99\text{m}}\text{Tc}$ -PYP), methylene diphosphonate ($^{99\text{m}}\text{Tc}$ -MDP), and 3,3-diphosphono-1,2-propanodicarboxylic acid ($^{99\text{m}}\text{Tc}$ -DPD). Although the binding mechanism is debated, reports of bone scans with increased 99mTc-PYP uptake in the heart of

patients with amyloid infiltration date to the early 1980s. Testing in small cohorts of amyloid patients confirms ^{99m}Tc -PYP uptake in amyloid hearts; however, low-intensity signal and false-positive results in hypertensive, sarcoid, and dilated cardiomyopathies limit the use of this agent.^{70,71}

^{99m}Tc -MDP binds preferentially to cardiac amyloid, although with less avidity and sensitivity than ^{99m}Tc -PYP. Data comparing ^{99m}Tc -MDP to ^{99m}Tc -PYP in 7 patients with biopsy-proven cardiac amyloidosis reported 100% versus 56% sensitivity with the respective agents.⁷² Additionally, ^{99m}Tc -MDP uptake is less intense than ^{99m}Tc -PYP, which diminishes its potential as the tracer of choice for nuclear study of cardiac amyloidosis.

^{99m}Tc -DPD, in contrast to the other bone-avid molecules, displays preferential uptake in ATTR cardiomyopathy. In the first of 2 studies from Bologna, Italy, 15 patients with ATTR and 10 with AL cardiomyopathy underwent ^{99m}Tc -DPD scanning. Radiotracer uptake was reported in all ATTR and none of the AL hearts.⁷³ A second study involving 79 patients with amyloid cardiomyopathy (45 ATTR and 34 AL) and 15 control subjects identified mild ^{99m}Tc -DPD signal in AL hearts, which lessened the tracer selectivity for ATTR heart involvement.⁷⁴ Although not definitive, ^{99m}Tc -DPD uptake in the heart of a patient with systemic amyloidosis strongly suggests ATTR disease.⁷⁵ Further studies will be needed to fully characterize the utility of ^{99m}Tc -DPD scanning in patients with suspected cardiac amyloidosis.

Amyloid-Directed Scans

Serum amyloid P (SAP) is a stabilizing component of all amyloid deposits. Scans using ^{123}I -SAP can assess the amyloid burden affecting the liver, spleen, and kidneys (reticuloendothelial system) in patients with amyloidosis. In contrast, ^{123}I -SAP scans do not provide sufficient signal within affected hearts to permit its use as a diagnostic or serial measure of cardiac amyloidosis.⁷⁶

Aprotinin is a serine protease inhibitor and constituent of amyloid matrix ground substance, which has prompted the use of ^{99m}Tc -aprotinin as an amyloid marker. Two studies reported ^{99m}Tc -aprotinin uptake in 19 (40%) of 47 documented amyloid hearts.^{77,78} In addition to relative insensitivity in patients with documented amyloid cardiomyopathy, ^{99m}Tc -aprotinin scans have low signal-to-background ratios for heart uptake, which makes interpretation of studies challenging.

^{123}I -metaiodobenzylguanidine (MIBG) is a nuclear-labeled tracer of neurotransmitter uptake by sympathetic neurons used in amyloid patients to document cardiac denervation.⁷⁹ It has not proved as useful in disease detection as other modalities such as echocardiography or CMR.

Cardiac Biomarkers

Cardiac serum biomarkers, specifically BNP or its N-terminal form (NT-pro-BNP) and cardiac troponins, are elevated in AL cardiac amyloidosis and are associated with survival.^{80,81} The mechanism of BNP elevation involves increased cardiac filling pressures from amyloid infiltration, whereas troponin elevation results from myocyte cell death. In AL disease, BNP elevations may reflect direct toxicity of light chains by oxidant stress⁴⁸ versus protofilament-induced injury in TTR.

Suhr et al⁸² analyzed 2-dimensional, M-mode, Doppler, and strain echocardiography signs of amyloid cardiomyopathy versus troponin and BNP measures collected in 29 patients with ATTR. BNP elevations highly correlated with interventricular septum thickness and basal septal stain pattern in 76% of the cohort. Troponin I and T, however, did not segregate with echocardiographic abnormalities. Despite extensive reporting in light-chain disease, few data exist regarding the prognostic utility of BNP or troponin in TTR cardiac amyloidosis, although both appear to be elevated in advanced disease and may track with disease progression.⁸³ For diagnostic purposes, biomarker elevation can be viewed as supportive evidence of the presence of TTR amyloid disease.

Clinical Course

The clinical progression of TTR cardiac amyloidosis depends on fibril type (wild-type versus variant), specific mutation, age of onset, and potentially, fragmented versus full-length fibrils.⁸⁴ Untreated, TTR disease is associated with significantly longer median survival than AL amyloidosis but progresses to intractable heart failure and death from systolic heart failure or dysrhythmia. Rapezzi et al reported 2- and 5-year survivals of 98% and $\approx 75\%$ for variant TTR disease, respectively.³⁶ Notably, the study cohort did not include V122I ATTR, the most common mutation in the United States. In a retrospective analysis of V122I patients referred to our treatment center, median survival for V122I cardiac amyloidosis was only 27 months after diagnosis.³⁵ Furthermore, the Transthyretin Amyloidosis Cardiac Study (TRACS), a prospective, multicenter observational study comparing V122I ATTR and SSA, found that survival after diagnosis was lower for V122I patients (26 versus 46 months, respectively).⁸³ Survival in both the study by Connors et al³⁵ and TRACS⁸³ was likely biased by the relatively advanced stage of cardiac amyloidosis at the time of clinical referral. The clinical course of Ala60 appears somewhat slower, with median survival reported to be 6.6 years after onset of symptoms and 3.4 years from diagnosis.²⁶ Survival in wild-type TTR disease appears most favorable among the amyloid cardiomyopathies; reported median survival varies from 43 to 75 months.^{21,83}

There are limited data regarding the rate of disease progression in TTR cardiac amyloidosis. Benson et al,⁸⁵ using echocardiography and volumetric CMR (notably without LGE imaging reported) to study disease progression in ATTR disease, reported significant increases in myocardial mass (mean increase of 8% by CMR, 22% by echocardiography) after 1 year of observation. The multicenter TRACS study observed declines in left ventricular ejection fraction and 6-minute walk duration and coincident rises in NT-proBNP for both SSA and ATTR patients over 18 months.⁸³ Unlike AL disease, in which successful treatment stabilizes myocardial amyloid infiltration or induces regression in wall thickness,^{86,87} the effect of treatment on TTR amyloid heart disease is unknown.

Treatment

The treatment approach in TTR cardiac amyloidosis is 2-fold: First, directed toward alleviation of heart failure symptoms,

and second, to slow or stop progressive amyloid deposition. In contrast to large data sets for other forms of cardiomyopathy, there are few data in TTR cardiac amyloidosis on which to base treatment decisions.

Symptom-Directed Treatment of Heart Failure

Like AL cardiac amyloidosis, TTR disease results in progressive biventricular wall thickening, diastolic dysfunction resulting from loss of compliance, and symptoms of congestive heart failure from elevation in cardiac filling pressures. Because of the challenges of cardiac amyloid diagnosis, many patients are recognized later in the disease course, often presenting with heart failure symptoms.

Optimization of fluid status is the cardinal tenet in cardiac amyloidosis management. This is best accomplished by administration of loop diuretics and spironolactone. Frequently, acute or chronic renal failure complicates management of heart failure, and overdiuresis precipitates hypotension. Although Lobato et al⁴³ demonstrated amyloid deposition in the kidneys of V30M ATTR patients independent of clinical signs (proteinuria or decreased glomerular filtration rate), significant renal insufficiency occurs in only $\approx 3\%$ of this population.⁸⁸ In general, chronic kidney disease is not a hallmark of ATTR. Comorbidities such as diabetes mellitus, hypertension, or age-related reduction in nephron mass, on the other hand, can precipitate renal failure. Hemodynamic factors, including renal hypoperfusion caused by diastolic congestive heart failure, can contribute to reduced renal function. It is critical to reduce the dose or discontinue agents that impair inotropic or chronotropic compensatory mechanisms. Because TTR amyloid infiltration markedly impairs diastolic filling and reduces stroke volume, tachycardia is a critical compensatory mechanism that maintains cardiac output. Consequently, high doses of β -adrenergic receptor–blocking agents are often poorly tolerated as they blunt compensatory tachycardia drive and induce greater negative inotropic effects in amyloid-infiltrated hearts. Calcium channel blockers^{89,90} and digitalis⁹¹ are contraindicated in cardiac amyloid disease because of binding of amyloid fibrils and potentiation of drug toxicity. Low doses of angiotensin receptor antagonists or converting enzyme inhibitors may be beneficial as afterload-reducing agents, particularly in coexisting hypertension, to improve forward cardiac flow and renal perfusion.

Dysrhythmia Management

Atrial fibrillation is the most common dysrhythmia associated with wild-type TTR cardiac amyloidosis affecting $\approx 30\%$ of cases. Given the atrial dilation from increased ventricular end-diastolic pressures, as well as atrial amyloid infiltration, restoration of sinus rhythm is challenging and frequently unsuccessful in the long term. It is reasonable, however, to attempt sinus rhythm restoration with DC cardioversion, provided no atrial thrombus is present by transesophageal echocardiography. Atrial fibrillation recurs in most patients, and as such, a rate-control and anticoagulation strategy is warranted in most circumstances. Patients with AL disease are at extremely high risk of thromboembolism in atrial fibrillation, and it is generally assumed that the risk in TTR

disease is also elevated over and above that of non-amyloid-affected hearts, with warfarin reducing embolic risk.^{92,93} Low doses of β -adrenergic receptor antagonists are useful as rate-controlling agents; however, care should be taken to not exceed doses in which the negative chronotropic and inotropic effects of these agents overwhelm the benefit (typically 50–100 mg of metoprolol per day). Amiodarone can be useful as a rate-controlling agent and may organize atrial fibrillation into a slow atrial flutter, rendering rate control easier. Amiodarone is presumed safe in TTR cardiac amyloidosis, although naturally, patients must be monitored for the known toxicities, and the drug should be avoided in the presence of significant conduction disease (eg, left bundle-branch block) without pacemaker placement. Dronedarone is uncommonly used in TTR cardiac amyloidosis because it is contraindicated in advanced heart failure.

If rate control in atrial fibrillation is impossible to achieve without worsening heart failure or compromising cardiac output, then atrioventricular junctional ablation and placement of a permanent ventricular (or biventricular) pacemaker are required. It is also our practice to recommend prophylactic pacemaker placement for TTR cardiac amyloidosis patients with symptoms of presyncope and a high degree of heart block (for example, first-degree AV block with right bundle and left anterior fascicular block), to avert possible complete heart block from progression of amyloid infiltration, an approach that is consistent with the most recent American College of Cardiology/American Heart Association recommendations for device placement.⁹⁴ There are no data regarding the utility of radiofrequency ablation for either atrial fibrillation or atrial flutter in TTR cardiac amyloidosis; however, our experience is that typical flutter circuits, in particular, can be treated with this approach.

The role of cardioverter-defibrillator implantation for primary prevention of sudden cardiac death in TTR cardiac amyloidosis remains largely unexplored. In a small case series of high-risk AL patients, implantation of a cardioverter-defibrillator did not improve overall survival because the principal cause of arrhythmic death was pulseless electric activity.⁹⁵ Thus, implantable cardioverter-defibrillator therapy is reserved for secondary prevention indications, ie, those with aborted sudden cardiac death, or those in whom a documented rhythm disturbance, including sustained ventricular tachycardia, is noted.

Organ Transplantation

Transthyretin is predominantly produced by the liver, with minor secretion by the choroid plexus (central nervous system) and retinal pigment epithelium.⁹⁶ Orthotopic liver transplantation (OLT), a genetic experiment to replace the major organ synthesizing variant transthyretin with a producer of wild-type TTR, was undertaken by Sweden's Karolinska Institute in 1990 and by Deaconess Hospital, Boston, MA, in 1991.⁹⁷ Over the ensuing 20 years, transplant centers have voluntarily posted 1844 OLTs on the FAP World Transplant Registry (<http://www.fapwtrorg>), with 911 (49%) reported by Portugal, 235 (12.7%) by France, 137 (7.4%) by Sweden, 97 (5.3%) by Brazil, 83 (4.5%) by the United States, and 80 (4.3%) by the United Kingdom. The vast majority of OLTs have been performed in V30M ATTR patients

(94.3%), with non-V30M ATTR limited to 5.7% of the transplanted population. Cumulative data on 579 OLTs performed over the first 10 years of FAP World Transplant Registry listing indicated a 5-year survival rate of 77%,⁹⁸ with a high percentage of cardiac deaths (39%). The Karolinska Institute subsequently reported a single-center experience of 141 transplantations with 10- and 15-year survival rates of 83% and 60%, respectively, significantly better than a medically treated control cohort (62% and 19%, respectively).⁹⁹

Liver transplantation successfully eliminates circulating levels of variant transthyretin; however, reports of progressive cardiac infiltration after OLT began circulating 6 years after the first transplant in the United States, initially identifying those with non-V30M ATTR as the at-risk population.¹⁰⁰ Later, V30M ATTR patients were noted to experience similar progressive interventricular septal thickening,¹⁰¹ with preexisting amyloid cardiomyopathy the apparent predisposing condition in both V30M and non-V30M cohorts. Biochemical analysis of heart biopsy samples collected after OLT from patients with progressive cardiomyopathy revealed wild-type TTR fibrils deposited on variant ATTR-rich amyloid matrix,¹⁰² a phenomenon later described in patients with progressive neuropathy after OLT.¹⁰³

Progression of pre-existing amyloid cardiomyopathy following successful liver transplantation prompted the first orthotopic heart transplantation (OHT), eliminating the nidus for further amyloid deposition. To date, the FAP World Transplant Registry lists 26 patients undergoing combined orthotopic liver and heart procedures, including 16 simultaneous OLT/OHT procedures and 9 sequential organ transplants. Dubrey et al¹⁰⁰ reported the United Kingdom experience with OHT for amyloid cardiomyopathy. Among 24 OHTs, 17 were performed in AL amyloid patients, 3 in ATTR patients, 2 in SSA patients, and 2 in patients with other inherited amyloidoses. Five-year survival after OHT was 38% in AL patients, 67% at 2-year follow-up in ATTR patients, and 100% at 3 years in SSA recipients.¹⁰⁰ The literature includes 5 OHTs for SSA, the oldest a 77-year-old Korean man, with survival extending up to 4 years without biopsy evidence of amyloid recurrence in the transplanted heart.^{104,105}

The cumulative transplantation experience in FAP defines the following clinical parameters as optimal determinants of survival after OLT: (1) Age <50 years, (2) disease duration <7 years, (3) female sex, (4) modified body mass index > 600 (BMI [kg/m²] × serum albumin [g/dL]), (5) normal autonomic vasomotor regulation and bladder function, (6) absence of amyloid cardiomyopathy, and (7) V30M ATTR.⁹⁹ Notably, the V30M ATTR survival advantage is based on a small non-V30M ATTR cohort experience. In 2004, Herlenius et al⁹⁸ published data on survival after OLT in 449 V30M ATTR patients and 62 non-V30M ATTR patients, reporting 85% 5-year survival in V30M and 60% 5-year survival in non-V30M patients. At the latest posting (December 31, 2010), the FAP World Transplant Registry data set remains limited to 106 non-V30M ATTR patients, which includes 46 different ATTR genotypes.

The frequency of cardiac deaths in ATTR patients after OLT prompted consideration of prophylactic versus arrhyth-

mia event-driven pacemaker insertion. Limited experience confounds the analysis. A retrospective single-center (Karolinska Institute) experience involving 104 V30M ATTR patients documented 26 pacemaker insertions, with 7 placed preoperatively and 19 inserted a median 5 years after OLT.¹⁰⁶ OLT did not decrease the rate of arrhythmias, and pacemaker insertion did not improve survival.¹⁰⁶ A second center (Johannes Gutenberg University, Mainz, Germany) reported no difference in 5-year survival rates among ATTR patients undergoing OLT with (n=9) or without (n=7) a pacemaker.¹⁰⁷ A definitive position on the survival impact of pacemakers in OLT for ATTR disease awaits a prospective randomized study.

Small-Molecule Inhibitors

Discovery and study of a Portuguese family with V30M ATTR genopositivity without clinical disease manifestations identified them as compound heterozygotes, possessing both V30M ATTR and a disease-inhibiting second mutation (T119M ATTR).¹⁰⁸ Convinced that the intragenic transsuppressor effect of T119M on V30M ATTR represented a key to understanding protein misfolding, Kelly's laboratory at The Scripps Research Institute (La Jolla, CA) generated recombinant TTR tetramers that expressed the spectrum of V30M/T119M combinations.¹⁰⁹ The introduction of T119M TTR into V30M homotetramers inhibited tetramer dissociation under a variety of denaturing stresses.¹⁰⁹ Thermodynamic studies demonstrated that T119M expression raised the activation barrier of TTR tetramer dissociation, which slowed the rate-limiting step of TTR amyloid formation.^{109,110} Noting that thyroxine binding also stabilized the TTR tetramer, Kelly et al characterized the thyroxine-binding site, selected compounds with steric similarity to thyroxine, screened candidate molecules for a TTR tetramer-stabilizing effect, and identified 2 promising small ligands that inhibited ATTR amyloid fibril formation in vitro: Diflunisal, a US Food and Drug Administration-approved nonsteroidal anti-inflammatory drug, and a novel agent, now known as Tafamidis. By complexing the thyroxine-binding site at the dimer-dimer interface, both diflunisal and Tafamidis tighten TTR tetramer associations, inhibiting TTR monomer release and suppressing TTR amyloid fibril formation.^{39,110} Although Tafamidis binds the TTR tetramer more tightly, diflunisal overcomes weaker TTR binding coefficients with high serum drug concentrations. A proof-of-concept clinical trial involving Tafamidis in 126 V30M ATTR patients with early (stage I) peripheral neuropathy demonstrated significant slowing of disease progression in subjects completing the 18-month trial.¹¹⁰ The Diflunisal Trial (<http://www.clinicaltrials.gov>, unique identifier: NCT00294671) adopted more inclusive entry criteria, enrolling 130 subjects with a broad range of neurological disease and unrestricted variant TTR. Data collection over 24 months of study participation will conclude in December 2012. The European Medicines Agency has recently granted Tafamidis market approval to Pfizer Pharmaceuticals, Inc for sale of the drug in the European Union. The US Food and Drug Administration recently announced (February 2012) that it will accept Pfizer's new

Table 2. Small-Molecule Treatments for Transthyretin Amyloidosis

| Agent | Mechanism | Status | Route | Dosing |
|-----------------------|-----------------------|--|-------|--------------------------|
| Diflunisal | TTR stabilization | Phase 3 trial | Oral | 250 mg BID |
| Tafamidis | TTR stabilization | EMA approved; FDA request 2nd trial review | Oral | 20 mg QD |
| Doxycycline/ TUDCA | Amyloid disruption | Phase 2 | Oral | 100 mg BID 250 mg TID |
| Antisense ODN | TTR suppression | Phase 2/3 pending | IV/SQ | TBD |
| siRNA | TTR suppression | Phase 2/3 pending | IV/SQ | TBD |

TTR indicates transthyretin; BID, twice per day; EMA, European Medicines Agency; FDA, US Food and Drug Administration; QD, every day; TUDCA, tauroursodeoxycholic acid; TID, three times per day; ODN, oligodeoxynucleotides; IV, intravenous; SQ, subcutaneous; TBD, to be determined; and siRNA, small interfering RNA.

drug application for Tafamidis; however, at the present time, the agent is not available commercially in the United States.

Clinical trial evidence that small-molecule TTR stabilizers slow neurological disease progression in humans fueled speculation that the elimination of TTR expression might completely arrest ATTR disease (Table 2). As proof-of-concept, Benson et al¹¹¹ demonstrated that antisense oligonucleotides directed against human I84S ATTR suppressed TTR transcription and RNA translation by up to 80% in a transgenic mouse model. Isis Pharmaceutical (Carlsbad, CA) and Alnylam Pharmaceutical (Cambridge, MA) subsequently reported at international meetings that both antisense RNA and RNA interference techniques inhibit TTR mRNA and protein expression by 40% to 90% in transgenic mice models, nonhuman primates, and phase 1 clinical trials. Initial accrual to phase 2 and 3 clinical trials examining both RNA technologies in FAP is anticipated in late 2012 or early 2013.

Agents in Development

Epigallocatechin-3-gallate (EGCG), the predominant polyphenol in green tea, has been associated with decreasing interventricular septal thickness and left ventricular mass index, increasing left ventricular ejection fraction, and improved New York Heart Association classification in a cohort of patients with AL amyloid cardiomyopathy.¹¹² In FAP, EGCG stabilizes circulating TTR tetramers by binding a dimer-dimer site distinct from the thyroxine transport site through which small ligand stabilizers act.¹¹³ Unlike small-molecule stabilizers, EGCG also disrupts mature amyloid TTR fibrils *in vitro*.¹¹⁴ In a human V30M TTR transgenic mouse model of FAP, EGCG inhibited prefibrillary TTR deposition, by immunohistochemical measures, while suppressing biomarkers of endoplasmic reticulum oxidative stress (BiP, Fas, 3-nitrotyrosine) that signal toxicity of those early preamyloid intermediates.¹¹⁴ EGCG treatment of V30M TTR transgenic mice also decreased the mature amyloid fibril matrix, which confirms reports of amyloid fibril disruption in nerve cell culture.¹¹⁵

Resveratrol, or 3,5,4'-trihydroxystilbene, a polyphenol present primarily in grape skins, stabilizes tetrameric TTR by binding the thyroxine transport pocket.^{39,116} Studies in cul-

tured human cardiac cells demonstrate that resveratrol limits the toxic effects of prefibrillary TTR moieties by promoting tetramer formation from free monomers.¹¹⁶ The bioavailability of orally administered resveratrol is limited because of rapid conjugation in the intestine, with <5% of free drug ultimately circulating in blood plasma.¹¹⁷ Wine and grape ingestion are unlikely to clinically affect FAP (wine concentration <25 $\mu\text{mol/L}$).¹¹⁸ A phase 2 trial examining the effect of resveratrol (500 mg to 2 g daily) on biomarkers of Alzheimer disease has been initiated, which illustrates the magnitude of doses needed to influence the chemistry of neurodegenerative disease.

Doxycycline, a tetracycline antibiotic, inhibits amyloid fibril formation and disrupts deposited mature fibrils in FAP (human TTR-V30M/mouse TTR-KO)¹¹⁹ and AL transgenic mice (CMV- γ 6).¹²⁰ Notably, doxycycline administration in the AL transgenic mice experiments (15 mg/L) replicated serum concentrations achieved in humans with standard drug dosing (100 mg twice daily).¹²¹ Additionally, doxycycline inhibited matrix metalloproteinase-9, a mediator of amyloid-induced organ injury, in both mouse models.¹²² Despite *in vitro* data demonstrating clearance of mature deposited amyloid fibrils, doxycycline failed to resolve toxic prefibrillar TTR moieties.^{119,122} In contrast, tauroursodeoxycholic acid (TUDCA), a biliary acid with antioxidant and antiapoptotic activities, decreases toxic TTR prefibrillar aggregates in transgenic TTR mice without a significant effect on mature fibrils.¹²² When administered consecutively to TTR transgenic mice for 15 and 30 days, respectively, doxycycline and TUDCA induced significant reductions of fibrillar/Congo Red staining deposits and nonfibrillar oligomeric TTR.¹²² Measures of oxidant tissue injury (BiP, Fas, and 2-nitrotyrosine levels) all declined with treatment, which supports effective clearance of toxic TTR intermediates.¹²² To test these findings in an FAP cohort, a phase 2 open-label single-center study administering doxycycline (100 mg twice daily) and TUDCA (750 mg daily) for 12 months began in July 2010 at IRCCS Policlinico San Matteo, Pavia, Italy. The organizers anticipate final data collection in July 2012, with data analysis reported in December 2012 (<http://www.clinicaltrials.gov>).

Conclusions

Transthyretin cardiac amyloidosis is an underappreciated contributor to heart failure in elderly patients. Although diagnosis typically requires tissue biopsy and demonstration of amyloid by histological techniques, cardiac amyloidosis can also be identified noninvasively by echocardiography and CMR. Clinical management of TTR cardiac amyloidosis differs from other forms of heart failure, and thus, disease recognition is essential. Liver transplantation remains the established treatment for variant TTR-related amyloid neuropathy and cardiomyopathy, but small-molecule pharmaceuticals may prove effective alternatives to surgery. In addition, small molecules may provide much needed treatment options for SSA, because only heart transplantation averts disease progression at present. The role of new and developing medical treatments for ATTR gene carriers remains to be established.

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