Addressing Common Questions Encountered in the Diagnosis and Management of Cardiac Amyloidosis

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Abstract

Advances in cardiac imaging have resulted in greater recognition of cardiac amyloidosis (CA) in everyday clinical practice, but the diagnosis continues to be made in patients with late stage disease, suggesting that more needs to be done to improve awareness of its clinical manifestations and the potential of therapeutic intervention to improve prognosis. Light chain CA (AL-CA) in particular, if recognized early and treated with targeted plasma cell therapy, can be managed very effectively. For patients with transthyretin amyloidosis, there are numerous therapies that are currently in late phase clinical trials. In this review we address common questions encountered in clinical practice regarding etiology, clinical presentation, diagnosis and management of cardiac amyloidosis, focusing on recent important developments in cardiac imaging and biochemical diagnosis. The aim is to show how a systematic approach to the evaluation of suspected CA can impact the prognosis of patients in the modern era.

Introduction

What is amyloidosis?: Amyloidosis is a localized or systemic deposition disease in which proteins with unstable tertiary structures misfold, aggregate and form amyloid fibrils that deposit with a range of chaperone proteins in the heart, kidneys, liver, gastrointestinal tract, lungs and soft tissues[1]. Individual amyloid fibrils are non-branching, 7 to 10 nm in width and of variable length. They are insoluble and notably resistant to proteolysis[2]. The term "lardaceous disease" was first used to describe such deposits at autopsy but was replaced by the botanical term "amyloid" because of their starch-like affinity for iodine[3]. In polarized light under the microscope, the deposits, when stained with the dye Congo red, display apple-green birefringence, changing from a hyaline pink appearance in normal light[4]. Under electron microscopy amyloid fibrils appear as needle-like structures with multiple filaments that have a unique highly organized cross β-pleated-sheet configuration[5].. Among the signature chaperone proteins found in all amyloid deposits are the pentraxin serum amyloid P component (SAP), clusterin, vimentin, vitronectin, glycosaminoglycans and apolipoproteins[6].

What forms of amyloidosis affect the heart? There are more than thirty proteins that can form amyloid fibrils, five of which frequently infiltrate the heart and cause CA: immunoglobulin light chain (AL or primary amyloidosis), immunoglobulin heavy chain (AH), transthyretin (ATTR), serum amyloid A (AA), and apolipoprotein A I (AApoA1). The overwhelming majority of patients with CA are affected by either AL or ATTR CA. While the general mechanism of extracellular deposition of fibrillary proteins leading to tissue and organ dysfunction is common to all forms of CA, there are three major

precursor proteins causing CA, each with differing natural histories and therapies including AL and ATTR due to a mutation (ATTRm) or wild type (ATTRwt).[7]

Epidemiology and Delays in Diagnosis

Is cardiac amyloidosis rare? AL-CA is a rare condition with an estimated prevalence of 8 to 12 per million[8-10]. There are ~3,000 newly diagnosed cases of AL amyloid per year in the United States (US) of which 30-50% of which have symptomatic cardiac involvement and 10-15% occur in association with multiple myeloma[11]. There are currently ~10,000 individuals affected with AL in the US, consistent with the definition of an orphan disease as one that affects less than 200,000 persons. The population prevalence of ATTR-CA is less certain, but recent data suggest that it is overlooked as a cause of common cardiovascular conditions in older people including heart failure with preserved ejection fraction (HFpEF), low flow aortic stenosis and atrial fibrillation[12, 13]. Autopsy data has demonstrated that among adults more than 80 years of age, 25% have transthyretin amyloid deposits in the myocardium[14]. Among patients with HFpEF, autopsy data reveal amyloid deposits in 32% of those over 75 years of age compared with 8% of those aged <75 years (8%)[15, 16]. Emerging data using nuclear scintigraphy has suggested that 13% (95% CI 7.2-19.5%) of patients hospitalized with HFpEF may have ATTRwt-CA[17]. Some patients, however, have disease caused by mutations in the TTR gene and although these are relatively rare, some genetic forms are endemic in particular geographic regions. The Val122Ile mutation, which almost exclusively affects individuals of African or Afro-Caribbean descent, has a population prevalence of 3-4%[18] and while clinical penetrance of this variant is incomplete in subjects up to 80 years of

age, it is almost certainly under-recognized as a cause of heart failure[19]. Emerging data suggest that ATTR-CA, especially due to ATTRwt, is not uncommon and will become the most commonly diagnosed form of CA.

How often is the diagnosis missed or delayed? CA is often misdiagnosed and the delayed diagnosis has significant consequences for patients. In a survey of more than 500 patients with AL (37% of whom had cardiac involvement) the average time from initial symptoms to diagnosis was 2 years. A substantial proportion of patients (31.8%) reported seeing at least 5 physicians before receiving a diagnosis of amyloidosis. Cardiologists were consulted more often than hematologists, oncologists or nephrologists, but were responsible for making the diagnosis in only 18.7% of cases. Similar data for ATTR-CA, albeit in much smaller populations, reveals that less than half of subjects were diagnosed within 6 months, mainly by cardiologists.[20]

What are the impediments to early and accurate diagnosis? CA is a "great pretender", often misdiagnosed as another condition or delayed in its recognition as a result of both physician and disease related factors. Optimum diagnosis and care requires multidisciplinary management (cardiology, neurology, nephrology and hematology) but amyloidosis teams are relatively few. Additionally, the absence of disease modifying therapies for ATTR and the late presentation of AL-CA patients has contributed to a nihilistic attitude about the condition.

The main disease-related factor that hinders a correct and timely diagnosis is heterogeneity with respect to the cardiac phenotype as well as systemic involvement.

The need for a histological demonstration of target organ amyloid infiltration has also delayed the diagnosis as the technique is restricted to referral centers with expertise in the performance of endomyocardial biopsy and requires skilled pathologic analysis of obtained samples. Phenotypic variability of genetic forms of ATTR is also influenced by genetic heterogeneity, geography, endemic vs. non-endemic aggregation, age, sex of the patient and transmitting parent, and probably amyloid fibril composition[21, 22 23]. Patients also rarely report a family history because of the late presentation and incomplete penetrance.

In whom should one consider cardiac amyloid? The diagnosis of CA requires a high index of clinical suspicion. In the past, the presence of multi-organ involvement was over-emphasized, resulting in consideration of the diagnosis only in the presence of extreme extra-cardiac findings such as macroglossia and periorbital purpura that while specific, are present only in a minority of AL cases and absent in ATTR-CA. A number of diagnostic red flags (Table 1) in a patient with otherwise unexplained left ventricular hypertrophy or restrictive cardiomyopathy can foster the correct suspicion. A history of carpal tunnel syndrome in ATTR (particularly if bilateral in a male), atraumatic rupture of biceps tendon, unexplained neuropathic pain, orthostatic hypotension and a diagnosis of "hypertrophic cardiomyopathy" after the 6th decade of life are useful clues.

Key point*: Increased awareness of cardiac amyloidosis is essential to reduce under-diagnosis and misdiagnosis*, ***which is critical because prognosis worsens rapidly with advancing organ dysfunction.***

Pathophysiology and Natural History

Why is the heart affected in some cases of amyloidosis and not others? The heterogeneity of systemic amyloidosis remains puzzling. Patients with the same type of amyloidosis, whether AL or ATTR, can have different patterns of organ involvement and different amounts of amyloid in the involved organs. Patients with systemic AL amyloidosis without heart involvement at diagnosis are likely to develop cardiac involvement if their culprit light-chain proteins are not eliminated and if they live long enough[30]. The basis for the organ tropism of AL is not known. Studies of the immunoglobulin light-chain genetic clones underlying AL indicate that the repertoire of light-chain germline genes used in AL is restricted; four light-chain variable region germline genes (*IGKV1-16*, *IGLV6-57*, *IGLV2-14*, *IGLV3-1*) account for two thirds of cases, and there is tropism for the heart with *IGLV2-14* and *IGLV3-1*[31-35] although the mechanism is unknown[36]. The explanation for the differential involvement of the heart and peripheral nervous system in patients with ATTR is also unexplained.

What is the natural history of ATTR cardiac amyloidosis? ATTR-CA may be due to specific mutations that have a predominant involvement of the heart (Val122Ile, Leu111Met, Ile68Leu) or ATTRwt (Table 2). Ile68Leu and Leu111Met are mutations reported almost exclusively in Italy and Denmark, respectively, causing a severe cardiomyopathy at an early age with a malignant course[22, 37, 38]. In the US, ATTRwt followed by Val122Ile and Thr60Ala mutation forms are most common[39]. Original reports[7, 40, 41] suggested that ATTRwt-CA had a median survival of >5 years. However, recent reports[42-44] have demonstrated that outcomes are worse with median survivals of ~3.5 years after initial evaluation[45]. These discrepancies are in part related to difficulty

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in defining when the disease begins. Initial manifestations of TTR deposits often

include bilateral carpal tunnel syndrome[46], which is found in ~70% of individuals with

ATTRwt-CA on average 5-7 years prior to the cardiac manifestations. Other

manifestations include atrial arrhythmias and a progressive decline in effort tolerance,

which are difficult to definitively attribute to amyloidosis as they occur commonly with

advancing age. However, it is clear that ATTRwt-CA can manifest as early as the fifth

decade of life. Cohort studies among subjects with ATTR-CA are predominately single

center reports[42, 44, 47]. One, multicenter, prospective cohort study (Transthyretin Amyloid

Cohort Study – TRACS)[43], demonstrated in a small (n=29) population that mortality was

high (29%) and differed between ATTRwt (22%) and Val122Ile (79%). Larger single

center studies have not confirmed this difference in survival and multivariate analyses of

TRACS and the THAOS registry data suggests that cardiac function and not the

presence of a mutation was independently associated with mortality[39, 47, 48]. Progressive

left ventricular dysfunction in ATTR-CA is mediated by physiologic derangements that

include reduction in chamber capacitance, declines in chamber contractility and

increases in arterial elastance.[48]. Patients with ATTRwt-CA have impairments in peak

VO2 and in a large series from the UK National Amyloidosis Center, a positive troponin

T, presence of a pacemaker, and NYHA class IV symptoms were associated at baseline

with a worse outcome[44]. Clinically, as ATTR-CA progresses, blood pressure falls due to

reduction in cardiac output and heart rate increases to compensate for a reduced stroke

volume. Every 6 months, the mean 6-minute walk distance declined by 25.8 meters,

NT-pro-BNP increased by 1,816 pg/mL, and left ventricular ejection fraction fell by

3.2%[43]. Cardiac cachexia is particularly common with ATTR-CA, potentially mediated in

part by right heart failure, liver congestion, and possibly alterations in bowel flora.

Key point: ATTR has been considered a neurologic disease but data from worldwide registries suggest that the heart is the main affected organ in at least half of the cases. ATTR cardiac amyloidosis is a progressive disorder associated with significantly morbidity and mortality.

Diagnosing Cardiac Amyloidosis

Is the ECG useful for identifying CA? Classically, CA is characterized by low QRS voltage in spite of an increase LV wall thickness observed with non-invasive imaging. In contemporary series, however, the prevalence of low voltage varies with etiology, ranging from 60% in AL to 20% in ATTR[61, 62]. The absence of low QRS voltages does not, therefore, preclude CA, especially in patients with ATTRwt in whom left ventricular hypertrophy or left bundle branch block is present in up to 30% of patients. The hallmark of CA is the disproportion between left ventricular wall thickness and QRS voltages. While this relationship can be expressed in a relatively complex way[63], it can be assessed more simply using left ventricular wall thickness/total QRS voltage ratio[64]. Pseudo-infarction patterns, present in up to 70% of cases, can lead to an initial misdiagnosis of coronary heart disease. A significant number of patients develop conduction disease and the presence of AV block in an older patient with left ventricular hypertrophy should always prompt consideration of CA.

Is there a lab test to diagnose CA? As yet, no blood test can diagnose ATTR-CA. In

contrast, in AL, serum and urine immunofixation and quantification of serum free light chains in combination have a 99% sensitivity for identifying the underlying substrate for AL-CA. Importantly, an abnormal free light chain ratio alone is not specific for AL amyloidosis as up to 5% of the population over 65 years of age have a monoclonal gammopathy of undetermined significance (MGUS)[54]. This can lead to a misdiagnosis of AL-CA in elderly patients who actually have TTR-related CA and MGUS (up to 10% of misdiagnosis even in referral centers)[55]. In addition, free light chains are filtered by the glomeruli, with renal dysfunction resulting in increased serum concentrations. Renal dysfunction affects kappa and lambda free chains differently and a wider reference range for FLC κ/λ ratio has been proposed for use in patients with advanced renal failure[65]. The broader K/L ratio normal ranges in patients with low GFR makes the diagnosis of AL-CA more challenging in patients with renal failure. While many patients with renal failure have increased left ventricular wall thickness and serum β2-microglobulin can be associated with increased wall thickness[66], beta-2 microglobulin amyloid deposition rarely causes meaningful cardiac dysfunction[67]. Natriuretic peptides tend to be particularly high in CA, often out of proportion from the hemodynamic burden[56]. Circulating light chains exert a direct toxic effect by modulating p38 mitogen-activated protein kinase, which can directly promote NT-pro-BNP expression.[29, 57, 58] Thus, for the same range of hemodynamic abnormalities, plasma levels of NT-pro-BNP are higher in AL compared to ATTRwt and ATTRm[56]. An apoptotic effect can cause an elevation of troponins, sometimes leading to false diagnoses of acute coronary syndrome in patients with CA hospitalized for heart failure. Accordingly, the use of new blood tests such as the free light chain assay, NT-pro-BNP and troponin in patients with

new onset dyspnea and no obvious etiology may enable the earlier diagnosis of AL-CA. This approach is particularly relevant to patients with pre-existing clonal plasma cell diseases such as MGUS or smoldering myeloma or in patients with unexplained increased wall thickness on echocardiography.

Key point*:*

While there is no test diagnostic for CA, there are blood based tests (sequencing of transthyrein for ATTR and free light chains/immunofixation for AL) that can identify the substrates for this illness??(Just an idea – edit as you see fit)

Should everyone with TTR cardiac amyloidosis have gene sequencing? TTR gene sequencing is recommended in all cases of TTR-related amyloidosis as hereditary types of amyloidosis cannot be distinguished from acquired types on clinical grounds alone and a family history indicating autosomal dominant inheritance is often absent due to incomplete and late disease penetrance. Thus sequencing the transthyretin gene in conjunction with genetic counseling is recommended in cases in which hereditary TTR is proven by mass spectroscopy or suspected even with a negative profile on mass spectroscopy.

Do laboratory tests predict prognosis in CA? There are well established prognostic models in AL amyloidosis. Current staging systems for AL-CA are based on serum levels of N-terminal pro-brain natriuretic peptide (NT-pro-BNP), cardiac troponin T, and the concentration of circulating free light chains. The combination of the three

biomarkers constitutes the most powerful prognostic tool available in AL-CA and is the basis for the staging systems elaborated by the Mayo Clinic[68] (Figure 1). The scoring system assigns one point for each of the following: NT-pro-BNP ≥ 1800 pg/mL, Troponin T ≥0.025 ng/mL, and difference between the kappa and lambda free light chains ≥ 18 mg/dL. Median survival of stage III patients was only 3.5-4.1 months. Within stage III, NT-pro-BNP > 8500 pg/ml combined with a systolic blood pressure less than 100 mmHg identifies patients with particularly high mortality (Stage III B). However, even patients with advanced cardiac amyloid can achieve meaningful improvements in survival if the light chains are reduced and accompanied by a cardiac response reflected by a decline in NT-pro-BNP response, defined as a decrease in NT-pro-BNP of >30% and >300 ng/L assuming a baseline value were ≥650 ng/L. Such responses predict clinical outcome and survival independent of therapy type, treatment class, or regimen[69]. Emerging data, suggest cardiac biomarkers are also a useful prognostic marker of cardiac function in ATTR[70]

<mark>If you like, you can pull the old key point down here from the diagnosis section</mark>

What is the role of imaging modalities in diagnosing CA? The diagnosis of cardiac amyloidosis should be systematic and directed towards the cause as well as the presence of amyloid protein in the myocardium. Laboratory tests, bone-tracer scintigraphy, genetic analysis and tissue biopsy all have a place in achieving these goals. In clinical practice, cardiac imaging is relevant to CA in three scenarios: (1) differential diagnosis of hypertensive hypertrophic and restrictive cardiomyopathy (most common); (2) the evaluation of patients known or suspected to have systemic AL or

familial TTR amyloidosis; and (3) in the presence of definite CA, to stage the disease and monitor the response to therapy. Cardiac imaging must be interpreted alongside other clinical findings and different imaging techniques should be considered complementary. The question is not what the "best" examination is, but which is most useful and appropriate for each step of the diagnostic and therapeutic pathway (Table 3).

What are the findings on cardiac ultrasound that raise suspicion of CA? The echocardiographic findings of CA have been extensively reviewed elsewhere and are summarised in Figures 2 and 3[71-73]. Nearly all are non-specific, but in context they can be highly suggestive of CA. The major morphological abnormalities in patients with advanced disease are symmetrical thickening of the left ventricle, thickening of the free wall of the right ventricle and a small pericardial effusion. Classical signs also include thickening of the atrioventricular valves and inter-atrial septum and abnormal myocardial "texture" characterized as a speckled appearance, although the latter is less reliable when using harmonic imaging. Amyloidosis is the archetype for diastolic LV dysfunction, with findings dependent on the stage of disease[74]. Early cardiac involvement is often associated with impairment of relaxation which progresses to typical restrictive LV pathophysiology in advanced symptomatic disease. Analogous changes in RV diastolic function occur in the RV inflow, superior vena cava, and hepatic vein flow velocities[75]. CA is often listed as a cause of HFpEF, but this underplays its impact on ventricular systolic performance [7, 44, 76-79]. While LVEF may be normal until the advanced stage of disease, reduction in peak systolic wall motion

velocities, especially in the longitudinal axis, are present even in early disease[80]. In general, CA alters strain parameters to a much greater degree than other causes of LV hypertrophy and is characterized by a base-to-apex strain gradient (Figure 3, upper right panel)[78, 79, 81]. This is readily appreciated in parametric polar maps that show relative apical sparing of longitudinal strain, representing an important diagnostic clue differentiating CA from other cardiomyopathies[78]. In general, differentiating AL from ATTR-CA with echocardiography is not possible but on average patients with AL-CA tend to be more restrictive while those with ATTR-CA tend to have greater LV wall thickness[7, 44, 64].

What is the role of cardiac magnetic resonance imaging in diagnosis? Much of the structural information derived from CMR is similar to that obtained using echocardiography, but the ability to interrogate tissue composition with gadolinium based contrast agents has led to an increase in the frequency of diagnosis of CA[82-87]. Gadolinium is a purely extracellular agent and does not enter intact cardiomyocytes[82]. In some cases of CA, there is a virtually pathognomonic appearance of global sub-endocardial late gadolinium enhancement in a non-coronary artery territory distribution with a dark blood pool. However, enhancement can also be diffuse and transmural or more localized and patchy[82, 84, 88]. False negative studies can also arise for technical reasons when scans are nulled using abnormal myocardium [83]. This latter problem is reduced by using phase-sensitive inversion recovery (PSIR) sequence which reduces the need for an optimal null point setting and makes scans less operator dependent[82, 89].T1 mapping is a new magnetic resonance technique in which a quantitative signal

from the myocardium is measured, before (native T1) or after contrast administration[82, 85, 86]. CA substantially increases native T1 and in AL and in ATTR-CA (Figure 3, lower right panel). T1 values are higher in AL-CA compared with ATTR-CA, while the extracellular volume is higher in ATTR-CA than in AL-CA[87]. As native T1 requires no contrast administration, it can be used in patients with renal impairment and may be abnormal before the left ventricular wall thickens. Combined, native T1 mapping and measures of extracellular volume (ECV) post contrast can delineate three aspects of CA including amyloid burden or infiltration via measure of ECV, edema via measure of native T1 and myocyte response via measure of intracellular volume. Such an approach can provide a richer understanding of the pathophysiological processes that may be used to monitor disease progression and response to therapy. Current limitations of the T1 imaging are the effect of confounding pathologies such as myocardial edema and platform dependent variation in normal ranges for T1.

What is the role of myocardial scintigraphy in making a specific diagnosis? The 99mTc-phosphate derivatives, originally developed for bone imaging, were observed to accumulate in the early healing phase after acute myocardial infarction. 99mTc-PYP imaging was first described as a potential diagnostic test for CA in the early 1980s following reports describing increased cardiac uptake in patients with amyloid heart disease [90-92]. More recently in Europe, studies have evaluated the role of the tracer 99mTc-3,3-diphosphono-1,2-propanodicarboxylic (DPD) in diagnosing CA (Figure 3, lower left panel) [93-95]. Collectively, the data show that ATTR-CA is particularly avid for bone tracers, whereas uptake in AL-CA is either absent or only minimal. The

explanation for this differential uptake is unknown, but it has been suggested that the preferential binding to ATTR may be a result of higher calcium content. Bone tracers seem to be useful for early identification of ATTR-CA and may more closely correlate with amyloid load than estimates from CMR [96] Additionally, there is also uptake in skeletal muscle, which may appear to suppress bone activity due to masking by extensive soft-tissue uptake. In selected patients the specificity of "bone tracer scintigraphy for ATTR CA is so high that this method can be considered a "diagnostic standard" (see below).

Can PET be used to image and quantify amyloid? 18F-florbetapir is approved for imaging beta amyloid protein in the brain. Recent studies have shown that it is also taken up in the heart in patients with ATTR-CA and AL-CA (Figure 3, lower right panel). Similar findings have been reported for another beta amyloid imaging agent 11C-PiB [97]. These agents hold promise for absolute quantification of amyloid burden.

How can one integrate the various imaging modalities into a diagnostic algorithm? A consensus on the role of bone scintigraphy for non-invasive diagnosis of CA has emerged (Figure 4). If CA is suspected, blood and urine should be analysed for evidence of a plasma cell dyscrasia and imaging with bone tracer considered if ATTR-CA is a possibility. If these tests are negative, then current evidence suggests that CA is very unlikely[98]. CMR scanning may be used prior to nuclear scintigraphy as an aid to diagnosis, but a 10-20% false negative (particularly in-CA) and false-positive rate[87] (possibly more so in people of Afro-Caribbean ancestry) for conventional contrast

enhanced scans means that it does not substitute for other tests. Until very recently, a tissue diagnosis was considered essential in all cases of suspected CA. However, in the setting of a positive 99mTc-phosphate scan without evidence for plasma clone on blood and urine testing, a diagnosis of ATTR-CA can be made without a biopsy. For those patients with evidence for a plasma cell dyscrasia, a histologic diagnosis is still required as the presence of uptake on a 99mTc-phosphate scan is not 100% specific for ATTR-CA.

When and how should tissue biopsy be undertaken? The diagnostic accuracy of an extra-cardiac biopsy depends on the type of amyloidosis and on the examined tissue. In general, the yield of an extra-cardiac biopsy (abdominal fat pad, gingiva, skin, salivary gland, or gastrointestinal tract) is higher in AL than in ATTRm; the yield is low in ATTRwt. In a series of 131 patients with CA and a positive endomyocardial biopsy (EMB), only 73% showed amyloid infiltration on non-cardiac tissue sampling [99]. In ATTR, peri-umbilical fat biopsy, especially in patients with ATTRwt has a sensitivity of <20%[99]. Thus, while a fat pad biopsy is a preferred initial site, a negative result is insufficient to exclude the diagnosis and an endomyocardial biopsy should be performed.

How does one confirm the precursor protein causing amyloidosis? The ultimate goal of a biopsy, in addition to documenting amyloid infiltration, is to provide a definite etiological classification. Immunohistochemistry remains the most widely available method for fibril typing. Its diagnostic value is very high in AA amyloidosis and in most

cases of ATTR amyloidosis, but the results are not always definitive in patients with AL amyloidosis. The most frequent pitfall is the coexistence of positivity for more than one type of antisera, typically those for TTR and lambda or kappa-chains, which are the result of the antibody binding to circulating proteins present in the pathological specimen. In a recent series, 8/15 patients with monoclonal gammopathy, showed strong TTR staining in the histological samples whereas mass spectrometry demonstrated light chain amyloid in 5 of these 8 patients[100]. Mass spectrometric analysis of amyloid is the new gold standard for fibril typing[6]. This method involves laser micro-dissection and laser capture of amyloid using a microscope followed by tryptic digestion and tandem mass spectrometry. Computer algorithms match the peptides to a reference database, where amyloid fibril subtype can be identified. Frequently, mass spectroscopy can identify a mutant TTR genotype, though when a mutation is not identified, gene sequencing may be necessary.

***Key point**: Tissue diagnosis is required for AL amyloid and endomyocardial biopsy should be pursued if index of suspicion is high despite a negative fat pad. Nuclear scintigraphy agents combined with assessment for monoclonal proteins are reducing the need for tissue confirmation in ATTR; CMR characterization of myocardial tissue can be used to monitor disease progression and response to therapy.*

Standard and Emerging Therapies for Cardiac Amyloidosis
Are there specific treatments for CA? Diagnosis and clinical care have been held

back by the erroneous belief that there are limited effective treatments for AL-CA and by the absence of disease modifying therapy for ATTR-CA. It is now recognized that a reduction in the precursor proteins that forms amyloid fibrils is the key to improving prognosis in CA. Combination chemotherapy is effective in lowering free light chains in AL-CA and orthotropic liver transplantation to remove the hepatic source of genetically variant amyloid-forming proteins either alone or in combination with cardiac transplant has a role in selected patients with ATTRm-CA. TTR stabilizers and silencers hold promise for managing all forms of ATTR-CA and newer anti-fibrillar therapies aimed at reversing cardiac dysfunction are in clinical trials. The key determinant of the efficacy of therapy is the severity of disease at diagnosis. In advanced disease, therapy is poorly tolerated and often ineffective[24]. Multidisciplinary team management and supportive care is critical in controlling symptoms, optimizing nutritional status, maintaining cardiac function and managing the toxicities of treatments. Collectively, therapeutic advances have begun to shift CA from a universally fatal disease to a manageable chronic condition.

AL Cardiac Amyloidosis

What are the goals of therapy for AL cardiac amyloidosis? The toxic amyloid-forming light chains that cause AL are produced in the bone marrow and the clonal plasma cells in AL possess many of the same genetic abnormalities found in other plasma cell dyscrasias [102-105]. The major difference between AL and myeloma is that in AL organ damage occurs because of the monoclonal light chains while in myeloma the organ damage is due largely to cell mass[106]. Anti-plasma cell treatments for AL have

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evolved from those used in myeloma, including steroids, high-dose melphalan, proteasome inhibitors and immunomodulatory agents [107]. The goals of therapy in AL-CA are to eliminate the clonal plasma cells and provide optimal supportive care to address and control side effects of therapy. Patients with advanced cardiac involvement cannot tolerate high-dose therapy with autologous stem cell transplantation (SCT) and therefore patient selection for SCT has become restrictive[49, 108]. Despite excellent hematologic response rates, patients with elevations of cardiac biomarkers who are poor candidates for SCT are also at significant risk even with standard bortezomib-based therapy with 40% dead within 2 years of diagnosis and only 20% of responders experiencing cardiac improvement by biomarker criteria[109]. However, when a response is achieved survival can be meaningfully improved.

Is halting deposition of amyloid the only treatment or can removal of amyloid and reversal of organ dysfunction be achieved? Hematologic response and degree of cardiac involvement are the deciding factors regarding overall survival in systemic AL amyloidosis[4]. The categories of hematologic response with initial therapy in newly diagnosed patients are complete (CR, normal FLC ratio and negative serum and urine immunofixation), very good partial response (VGPR, difference between involved and uninvolved free light chains < 40 mg/L or <4 mg/dl), partial response (PR, difference between uninvolved and involved free light chains decreased > 50%), and no response (NR)[25]. The frequency of cardiac responses defined as a decline in NT-pro-BNP to <30% of baseline and of at least 300 pg/ml in 374 newly diagnosed treated AL-CA patients were 57% with a CR, 37% with a VGPR, 18% with a PR and 4% with NR[25]

(Figure 1). At 6 months after starting anti-plasma cell chemotherapy, cardiac responses

occurred in 26% and progression in 45% of patients, with 85% of responders and 30%

of progressors still alive after 2 years[25]. Such data indicate that anti-plasma cell

chemotherapy, including the use of high-dose chemotherapy with SCT, does not enable

reliable cardiac improvement for all patients especially those with advanced cardiac

involvement. Many patients with hematologic responses continue to have persistent

cardiac dysfunction despite improved overall survival. After initial therapy, the median

hematologic progression-free survival (PFS) is 1 to 4 years, depending on the type of

initial therapy. The PFS for the 25% of newly diagnosed patients who were candidates

for SCT appears to be higher than that of patients receiving standard therapy with

bortezomib-based combinations. Indeed, about 80% of the long-term post-SCT

survivors with CR have organ responses.[49]. At hematologic relapse, the majority of

patients have stage 2 or 3 chronic renal disease and stage 2 cardiac disease (see

Figure 1)[30].

The unmet need for patients is therapy that reverses organ damage and

dysfunction[36]. Several approaches are in clinical trials at this time, including three

monoclonal antibody based therapies that may enhance phagocytic clearance of

amyloid deposits. The anti-amyloid 11-1F4 monoclonal was used in a phase I trial

(NCT02245867) in AL patients with relapsed refractory disease, and cardiac and GI

organ responses were reported in 3 of the first 6 patients treated[50]. Employing a

different approach in their phase I trial (NCT01777243), based on the fact that amyloid

fibril deposits always contain the non-fibrillar normal plasma protein, serum amyloid P

component (SAP), investigators from the National Amyloidosis Center in the UK used a

combination of two agents. The drug, R-1-[6-[R-2-carboxy-pyrrolidin-1-yl]-6-oxo-

hexanoyl] pyrrolidine-2-carboxylic acid (CPHPC), efficiently depletes SAP from the

plasma but leaves some SAP in amyloid deposits that can be specifically targeted by

therapeutic IgG anti-SAP antibodies, inciting an inflammatory multinucleated giant cell

response[51]. In this phase I trial, whole-body scans using iodinated SAP demonstrated

depletion of hepatic amyloid in 50% of patients with liver involvement. There were also

reductions in in C3 and spikes in CRP, indicating ongoing inflammation. Patients with

cardiac involvement were excluded from the trial[52]. The third, and furthest along in

clinical development, is the anti-amyloid monoclonal NEOD001 that binds to an epitope

in amyloid fibrils. In a phase 1/2 trial (NCT01707264), previously treated AL patients

who were in hematologic remission with biomarker evidence of on-going organ damage,

received NEOD001 monthly[53]. There were no toxicities and organ responses were seen

in majorities of both cardiac (57%) and renal (60%) patients. NEOD001 is now in a

phase 3 trial for newly diagnosed patients with cardiac involvement (NCT02312206), in

which patients receive bortezomib-based therapy and are randomized to receive either

NEOD001 or saline monthly.

What are the mechanisms of therapeutic efficacy (reduction in precursor

protein/amyloid production versus amyloid clearance)? Therapeutic efficacy in AL

has two dimensions, survival and improvement in organ function. Hematologic response

to therapy is measured in terms of the reduction or elimination of the clonal plasma cells

that produce the culprit light-chain protein. Patients who achieve a hematologic CR or

VGPR live longer than those who do not[25]. Improvement in organ function is measured

with cardiac and renal biomarkers and, for patients with other organs involved, with a

mix of exam and laboratory findings such as normalization of orthostatic symptoms and vital signs for patients with autonomic neuropathy or of liver span and alkaline phosphatase for those with hepatic involvement[110]. Patients who achieve cardiac biomarker responses live longer than those who do not [25, 60, 111].

Hematologic responses based on changes in the serum free light chain assay are validated surrogates for the impact of therapy on the clonal marrow cells. With elimination or near elimination of the culprit light-chain protein, patients usually experience improvement in organ function and in their QOL. However, while hematologic response is necessary for organ response, it is not sufficient[36]. There is general consensus that both early cardiac death and survival with persistent organ damage are critical unmet clinical challenges.

What factors influence treatment decisions (rapidity of response, toxicity and physiologic reserve)? The initial evaluation of newly diagnosed patients with AL is focused on the extent of both cardiac and extra-cardiac organ involvement, physiologic reserve and the character of the clonal marrow plasma cells[26]. In a cohort of over 800 patients from 6 countries, 26% were NYHA class 2 or 3, 25% CKD Stage 3, and 15% CKD Stage 4 or 5; 50% were Mayo cardiac stage II with either Troponin T or NTpro-BNP above the threshold and 34% were cardiac stage III with both elevated; 19% had liver and 16% peripheral nervous system involvement[25].

Who is eligible for SCT? Eligibility criteria for high-dose therapy with SCT include age less than 65, Karnofsky performance status of 80% or higher, adequate cardiac function

(systolic blood pressure > 90 mm Hg, ejection fraction >45%, troponin T <0.06 ng/ml, NT-proBNP < 8,500 pg/ml or pulmonary artery saturation of >55%) and compensated or controlled orthostatic changes in blood pressure[113]. Patients who are Mayo cardiac stage III or CKD 4 are rarely eligible; young patients on hemodialysis, however, who are cardiac stage I or II are often eligible for SCT. Most patients are not eligible for SCT and receive bortezomib-based therapy with doses adjusted for frailty, impaired liver function and NYHA class[114, 115]. Bortezomib may not be the first choice of proteasome inhibitors for patients with peripheral neuropathy because of the risk of worsened neuropathy due to the drug; alternatives exist[116]. After SCT, consolidation is recommended for AL patients who do not achieve a CR (in myeloma post-SCT consolidation is recommended for all)[4, 117]. For patients treated with bortezomib-based therapy, the lack of a hematologic response after 2 cycles is usually a signal to change therapy and, in most cases, bortezomib and dexamethasone will be retained but other agents such as melphalan or immune-modulatory drugs are added[26].

What is the data on efficacy of therapies? The mix of therapies used to treat newly diagnosed patients with AL and the reported outcomes are recounted in Supplementary Table 1. High-dose therapy with SCT and bortezomib-based combination chemotherapy are the two options used most frequently in the USA[108]. In the UK and EU, however, SCT is less often employed and other regimens such as oral melphalan and dexamethasone or oral cyclophosphamide, thalidomide and dexamethasone are commonly used[107, 118, 119]. Doses of agents in all of these regimens are often adjusted for age, frailty, renal and hepatic function, and NYHA class.

ATTR Cardiac Amyloidosis

What are the targets for therapy in TTR? Current treatment for ATTR-CA is focused on various stages in the TTR amyloid cascade including silencing of TTR production, TTR stabilization and amyloid clearance from tissues (Figure 5)[120-122].Because 95% of TTR protein is produced by the liver, orthotopic liver transplantation (OLT) has been employed in ATTRm to replace amyloid forming mutant with wild type TTR and theoretically arrest amyloid formation. The majority of OLTs for ATTRm amyloid are performed in patients with a primary neuropathic phenotype, most commonly the Val30Met variant[123]. OLT is considered a preventative measure to forestall the development of the sensorimotor neuropathy or multiple organ involvement. Survival after liver transplantation in Val30Met patients is > 50% at 20 years[123]. Since the liver function of TTR liver transplant recipients is otherwise normal, their livers have been transplanted into high risk recipients ("domino liver transplant"), causing TTR amyloidosis in recipients several years later[124]. While survival is prolonged by liver transplantation[123], there is slowing but not arrest of cardiac dysfunction. This is attributed to deposition of wild type TTR amyloid in the heart from the transplanted liver causing progressive cardiac dysfunction. Patients with fragmented ATTR fibrils (type A) developed HF after OLT while those who had intact ATTR fibrils (type B) did not deteriorate to the same degree[127]. The scarcity of organ availability, the need for lifelong

immunosuppression and the relatively older age of affected subjects make transplantation an a suboptimal option for treating ATTR-CA.

Both RNA interference (RNAi) and antisense oligonucleotides (ASOs) have been used to inhibit hepatic expression of amyloid forming TTR. These therapies capitalize on endogenous cellular mechanism for controlling gene expression in which siRNAs or ASOs, mediates the destruction of target messenger RNA (mRNA) essential for TTR protein production. These agents cause a robust and durable reduction in genetic expression (knockdown) of TTR and retinol binding protein[128-130]. Both methods of silencing have completed recruitment for phase III trials in ATTR peripheral neuropathy with results expected in 2017 but a phase III trial in ATTR cardiomyopathy was recently suspended with unexpected adverse impact on those receiving active treatment (Supplementary Table 2).

TTR stabilizers have demonstrated efficacy in FAP[131, 132] but their role in ATTR-CA is unknown. Diflunisal, a nonsteroidal anti-inflammatory drug, binds and stabilizes common familial TTR variants against acid-mediated fibril formation in vitro and has been tested in human clinical trials[131]. Use of diflunisal is controversial given the known consequences of chronic inhibition of cyclooxygenase enzymes including gastrointestinal bleeding, renal dysfunction, fluid retention, and hypertension that may precipitate HF in vulnerable individuals. Tafamidis is a novel compound that binds to the thyroxine-binding sites of the TTR tetramer, inhibiting its dissociation into monomers[133] and blocking the rate-limiting step in the TTR amyloid forming cascade (Figure 5).

Doxycycline disrupts fibril formation[134] and when combined with the bile salt, tauroursodeoxycholic acid, demonstrated a synergistic effect on removal of tissue TTR

deposits in an animal model, leading to open label trials in humans[135]. Epigallocatechin gallate (EGCG), the most abundant flavonoid in green tea, inhibits amyloid fibril formation in vitro, leading to open label trials in ATTR-CA[136, 137].

What are the results of recent trials in TTR? A majority of the trials completed to date have been small (<100 subjects), phase II, open label, intermediate duration (1-2 years) with primary endpoints being the NIS-LL and QOL for ATTR peripheral neuropathy and echocardiographic, biomarkers and six minute hall walk for ATTR-CA (Supplementary Table 2). Trials for neuropathy are further along than those for cardiomyopathy. Trials to date have demonstrated safety of the compounds, in the short term, with efficacy limited to either a surrogate maker (TTR stabilization) and/or the absence or slowing of disease progression, albeit with no control group.

The only phase III trials completed to date involved administration of diflunisal[131] and tafamidis[132, 138], both in FAP. In the diflunisal trial, echocardiography demonstrated a prevalence of presumptive CA in >50% of these FAP patients but analysis of echocardiographic variables including strain did not show significant differences ? after treatment, though statistical power was low[139]. Tafamidis, in patients with early-stage ATTRm due to Val30Met[132], slowed progression of neurological symptoms and early treatment was associated with greater preservation of neurologic function[138], which was not confirmed in late phase FAP[140]. Thus, early administration of tafamidis seems to be important for its efficacy. Additionally, all previous trials employed a dose of 20 mg but an 80 mg dose is being tested in an ongoing phase III trial, with biochemical data suggesting that a higher dose may be required for TTR stabilization[141]. Five year safety

data in a small cohort has been reassuring in that tafamidis was generally well tolerated at 20 mg, though efficacy data showed few patients with ATTR-CA were clinically stabilized at 3.5 years.

What therapies are in late phase development? Currently there is 1 clinical trial for ATTR-CA in Phase III involving tafamidis. A second trial with revusiran, a siRNA, was recently stopped and the development of the drug suspended. Both of these trials were placebo controlled with allocation to active therapy of ~2:1 and a fixed study duration that encourages participation by subjects who have a progressive, life threatening condition. Differences in study populations include a focus on ATTRm in the case of revusiran while the other (ATTR-ACT) enrolled both ATTRm and ATTRwt. The ATTR-ACT trial of tafamidis is expected to conclude in 2018.

<u>Key point:</u> The biologic process underlying ATTR-CA has led to the development of numerous therapies that are currently in late phase clinical trials. A recent trial of a siRNA for transthyretin in CA was stopped prematurely due to an unexpected worsening of subject's clinical status.

Cardiac Replacement therapy – Transplant and VAD

Is AL amyloidosis a contraindication to cardiac transplantation? CA from AL amyloid was previously considered a contraindication to orthotopic heart transplant (OHT) because of concerns regarding worse long term outcomes due to the amyloid involvement of other organs or the risk of recurrent amyloid in the graft[142]. However, the development of multiple therapies targeted at the monoclonal protein including high

dose chemotherapy with SCT has made long term control of the plasma cell dyscrasia possible. Accordingly, series in selected patients for whom heart transplant was performed at experienced centers with established multi-disciplinary collaborations followed by either stem cell transplant or ongoing chemotherapy have reported outcomes comparable to other subjects with restrictive cardiomyopathies.[143-146] Additionally, an analysis of UNOS data shows improved post-OHT survival in patients with CA, in part due to improved patient selection including an increase in those with ATTR-CA.[146]. The one year survival post OHT in UNOS for CA (? Is this specific to AL or also includes TTR?) from 2010 to 2012 was 81.6%. Accordingly, current guidelines endorse consideration of selected patients with either AL and ATTR-CA[147].

What additional testing is required for transplant evaluation? The degree of organs involved is an important prognostic variable influencing the outcomes of patients with AL undergoing SCT. Accordingly, multiple other organ systems must be thoroughly assessed for the functional effect of amyloid and if necessary for the anatomic/histologic degree of amyloid infiltration. Specific guidelines by the ISHLT have been published (see Table 4).[147]

Do outcomes differ in subjects with AL vs. TTR cardiac amyloidosis undergoing OHT? Data from Columbia University Medical Center, has shown better survival for ATTR-CA subjects (n=10) than AL-CA (n=16) undergoing OHT with transplants performed mainly from 1999-2008. However, a more contemporary series from Stanford has shown better survival in AL (n=10) than TTR (n=9) CA[145]. Multicenter data

obtained as part of the International Consortium in Cardiac Amyloid Transplantation (iCCAT) has demonstrated that outcomes of patients with CA do not differ statistically from those with AL compared to TTR[148].

How should the plasma clone be managed in patients with AL cardiac amyloid undergoing OHT? Control of the underlying plasma cell dyscrasia is critical to successful outcomes in AL cardiac amyloid. Data from iCCAT demonstrates that survival without recurrent amyloid post OHT is higher in those who receive chemotherapy prior to OHT than those who did not receive chemotherapy[149]. Accordingly, upfront plasma cell therapy to achieve a complete or very good hematologic response while waiting for OHT is increasingly being advised. While high or dose adjusted melphalan with SCT remains the most cytotoxic therapy for AL amyloid, combination therapy using proteasome inhibitors has been shown to be effective in AL-CA patients post OHT[145].

How can I bridge my patient with advanced cardiac amyloid to an OHT? The options for mechanical circulatory support as a bridge to OHT for CA patients is confounded by the small ventricular chamber size and increased wall thickness that increase the risk for suction events, right heart failure and overall worse outcomes in very small series reported to date.[150] Survival with a VAD is strongly associated with left ventricular end diastolic diameter[151]. Other forms of circulatory support including intra-aortic balloon pump, BiVAD or total artificial heart (the latter employed given the need for biventricular support) have been employed though survival at one year is reduced if

circulatory support is required[152]. A theoretical analysis using a cardiovascular

simulation of a novel micro-VAD for CA has shown that sourcing of blood from the left

atrium was not associated with suction, as assessed by declines in left ventricular end

systolic volumes, but did lower left atrial pressure and increase cardiac output and blood

pressure in subjects[154]. Whether such an approach can be effective in-vivo in CA

requires additional study.

Key point: *__Key point:__* *While the overall goal is to eliminate the need for OHT in CA, advances in patient selection and available therapies have made intermediate term outcomes of OHT in CA comparable to non-amyloid patients.*

Figure Legends

Figure 1: Mayo staging system for risk stratifying subjects with AL-CA in which one point is assigned for each of the following: NT-pro-BNP ≥ 1800 pg/mL, Troponin T ≥0.025 ng/mL, and difference in serum free light chains ≥ 18 mg/dL. Those with highest score have the worst prognosis[68] (Panel A).

Survival from the 3-month landmark of 300 patients with AL amyloidosis based on hematologic response. The proportion of stage III patients was not significantly different among the four hematologic response groups. CR, complete response; NR, no response; PR, partial response; VGPR, very good partial response and py, person-year; [25] (Panel B).

Prognostic relevance of cardiac response and progression criteria showing survival from the 6-month landmark of 377 patients with immunoglobulin light chain (AL) amyloidosis and baseline N-terminal natriuretic peptide type B (NT-proBNP)>650 ng/L according to NT-proBNP response and progression[25] (Panel C).

Survival according to NT-proBNP response in an ongoing phase 3 trial comparing melphalan-dexamethasone with melphalan-bortezomib-dexamethasone (NCT01277016)[101] (Panel D).

Figure 2: Sequence of still images showing typical echocardiographic features of cardiac amyloidosis. A: 2-D Parasternal long axis showing concentric left ventricular hypertrophy, bright myocardium and left atrial dilatation. B: 2-D Parasternal short axis showing concentric left ventricular hypertrophy and bright myocardium. C: 2-D Apical

four chamber view showing concentric left ventricular hypertrophy and biatrial dilatation D: Pulsed wave Doppler of mitral inflow showing an increase in E/A ratio, normal E wave deceleration time but a marked reduction in transmitral A wave velocity. E: Pulsed wave Doppler of pulmonary vein inflow showing marked diastolic prominence and increased duration and peak velocity of atrial reversal compared to the transmitral signal. F: Pulsed tissue Doppler of the lateral mitral annulus showing marked reduction in apical systolic and diastolic velocities (normal velocities being: ??, respectively).

Images courtesy of Dr Leon Menezes, University College London, UK

Figure 3: Upper left panel: Subcostal 2D echocardiogram showing some the typical findings in advanced cardiac amyloidosis. A: Biatrial enlargement, B: Thickened interatrial septum (and valves), C: Thickening of RV free wall and D: Concentric LVH and hyperechoic myocardial texture; Upper right panel: Apical 4-chamber peak systolic strain image illustrating relatively well-preserved apical strain with significant basal impairment. This is seen in the series of curves and the bull's-eye color-coded strain image*,* Lower left panel: Patient with atrial fibrillation and moderate to severely impaired LV function. Echocardiogram showed increased myocardial density and concentric LVH with moderate aortic stenosis. Cardiac MRI showed biventricular hypertrophy, moderate aortic stenosis, and diffuse fibrosis, in excess of that expected for LVH and aortic valve disease. A) Early whole body planar DPD image. B) 3h Delayed whole body planar image showing cardiac retention of racer and reduced bony uptake; Perugini Grade 2. C), D), and E) 2 chamber, short axis, and 4 chamber PSIR LGE cardiac MR images

showing diffuse fibrosis. **Images courtesy of Dr Leon Menezes, University College London, UK,**

Lower right panel: Fused Florbetapir PET/MR imaging. A) Native T1 map shows high (>1400) values pre-contrast. B) ECV map, the ECV/interstitial space expansion is as high as blood. C) LGE image- There is differential fibrosis, between the 'core' of the interventricular septum and the epi- and endocardial borders (white vs red arrows) and also in the lateral wall, which implies a non-uniform amyloid distribution. D) Fused Florbetapir PET/MR uptake with a LV basal septal predominance. **Images courtesy of Dr Leon Menezes, University College London, UK**

Figure 4: Diagnostic algorithm for patients with suspected amyloid cardiomyopathy. The grading system for bone scintigraphy is Grade 0 – absent cardiac uptake; Grade 1 – mild uptake less than bone; Grade 2 - moderate uptake equal to bone; Grade 3 - high uptake greater than bone. AApoA1 indicates apolipoprotein A-I; DPD, 3,3-diphosphono-1,2-propanodicarboxylic acid; HDMP, hydroxymethylene diphosphonate; and PYP, pyrophosphate (from Gilmore, Circulation 2016[98])

Figure 5: Disease modifying targets in TTR cardiac amyloidosis

Table 1: Red Flags and caveats in cardiac amyloidosis

- A high index of suspicion is mandatory for the recognition of CA (i.e. if you don't think of it you won't diagnose it)

- Cardiac amyloid should be suspected in any patient with heart failure, unexplained increased LV wall thickness and a non-dilated LV

- In a patient with a suspicion for HCM, look for the infiltrative features that suggest amyloid such as ….(AV block)

- A distinctive sign of CA is the abnormal ratio between LV thickness and QRS voltages rather than low QRS voltages alone. The absence of low QRS voltages doesn't rule out a CA and up to 20% of subjects with CA can have electrocardiographic evidence of left ventricular hypertrophy.

- In an elderly man with unexplained symmetric left ventricular hypertrophy, especially in the absence of hypertension, always consider the possibility of ATTRwt-CA

- CA in an elderly patient with a monoclonal gammopathy is not necessarily due to AL: consider the possibility of ATTRwt and MGUS

- Longitudinal LV function can be severely depressed despite a normal LVEF and the myocardial contraction fraction (MCF) is often low suggesting reduced global myocardial shortening.

- Myocardial deformation is reduced in cardiac amyloidosis but the apex is generally spared

- On cardiac MRI both T1 signal abnormalities and marked extracellular volume expansion in patients with LV hypertrophy are strongly suggestive of CA. LGE

distribution is heterogeneous and sub-endocardial enhancement is not the only pattern.

- A history of bilateral carpal tunnel syndrome in a man with HCM-like phenotype on echo is highly suggestive of ATTRwt-CA

Table 2: Phenotypic findings in AL-CA and in the most frequent types of ATTR-CA

	AL 44, 64	ATTRwt 39, 42, 44	Val122Ile 39	Ile 68Leu 37	Thr60Ala 155
Median age at diagnosis (yr)	62	76	70	70	62
Males (%)	66%	95%	75%	75%	70%
Common ethnicity	Variable	Caucasian	Afro-American Caribbean	Caucasian (Italy)	Caucasian (USA Ireland)
Cardiac referral route (%)	65	>80	>80	>80	30
IVS/PW (median values)	15/14	18/17	17/17	17/16	17/17
LVEF -%	56	50	50	50	53
Low QRS voltages (%)	45	33	45	30	16
Peripheral sensory-motor neuropathy (%)	10-20	<10	15	25%	54
History of carpal tunnel syndrome (%)	<10	30-45	30	37	Unknown
Autonomic symptoms (%)	24	12-20	10	<10	75
Median survival (yr)	Depends on stage	3.5	2-3	4-5	3.5
NTproBNP pg/L (median)	↑↑↑	↑↑	↑	↑	↑

Table 3: Diagnostic Tests and Their Role in the Various Phases of Evaluation and Management of Cardiac Amyloidosis

Imaging and biomarkers of Cardiac Amyloidosis

Phase of work-up	ECHO	MRI*	"Bone tracers" scintigraphy	BNP
Suspicion	+++	++	+/- (TTR)	++
Definite diagnosis	+/-	++	+++ (TTR)	-
Etiologic diagnosis	+/-	+/-	+++	+/-
Early diagnosis	+/-	+/-	++ (TTR)	+/-
Functional evaluation	+++	++	+ (MIBG)	+/-
Prognostic stratification	++	+	+	+++
Amyloid burden	-	++	+/-	-
Response to therapy	+/	+/-	+/-	+++ (AL)

*T1 native, LGE ECV, TTR – useful for TTR amyloid, MIBG - iodine-131-meta-iodobenzylguanidine,

+++ = very useful, recommended, ++= useful, to be considered, += possibly useful

+/- = role uncertain, - = not useful.

Table 4: Recommendation for Evaluation of Extra-cardiac Organs in AL Amyloid Prior to OHT according to ISHLT Guidelines

Organ System	Screening Tests
Pulmonary	Pulmonary function testing including arterial oximetry, diffusion capacity CXR and CT to assess for interstitial disease, effusions Thoracentesis may be necessary to differentiate amyloidosis from heart failure
Gastrointestinal	Nutritional assessment including pre-albumin, albumin. Assessment for bleeding by esophagogastroduodenoscopy, colonoscopy Assessment of amyloid deposition on random biopsy Assessment of intestinal motility with gastric-emptying studies
Hepatic	Serum alkaline phosphatase and bilirubin. An alkaline phosphatase >1.5 above upper limit of normal in the absence of congestion should prompt liver biopsy to assess for portal and parenchymal amyloid deposition.
Renal	Measured creatinine clearance or eGFR and 24-hour urinary protein excretion. An eGFR or measured creatinine clearance <50 ml/min/1.73 m^2 in the absence of decompensated heart failure or urinary protein excretion >0.5 g/24 hours should prompt renal biopsy to assess the renal amyloid burden.
Coagulation	Factor X and thrombin time Patients with a severe(<25%) factor X functional deficiency have o 50% survival after ASCT

Adopted from J Heart Lung Transplant. 2016 Jan; 35(1):1-23).

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