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Prevalence and prognostic implication of arrhythmic burden in TTR amyloidosis

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1. Amyloidosis Definition

The term Amyloidosis refers to a rare group of heterogeneous pathologies, both localized or systemic, resulting from the extracellular deposition of amyloid substance; Amyloid is composed by a fibrillar material derived from alterations in the folding process of various protein precursors that assemble abnormally into insoluble aggregates. These deposits are formed by fibrils with antiparallel β sheet conformation (less frequently they can be found with parallel conformation), unbranched, with variable length and a diameter that range from 7.5 to 10 nanometers. The conformation of the amyloid substance is responsible for the characteristic and pathognomonic apple green birefringence when observed under a polarized light optical microscope after coloring with the Congo Red dye. Amyloid fibrils are for the major part composed by protein material, but there is also a polysaccharide component in the form of glycosaminoglycans and glycoproteins, of which the Serum Amyloid P Component (SAP) is the most represented substance.

Deposition of amyloid can occur in the presence of an abnormal protein (in the mutated Transthyretin (TTR) forms and in the AL light chain forms); o in the presence of a normal protein which for reasons not yet well known (wild type TTR previously known as senile systemic amyloidosis). Amyloid deposits could also be due to the presence of a normal protein which is found in circulation in greater quantities compared to normal status (AA reactive amyloidosis and dialysis-related b2 microglobulin) (1,2).

1.2 Amyloidosis Classification

From a biochemical point of view, the fibrillar structure differs according to the protein precursor, and each precursor differs in the amino acid sequence, while the polypeptide skeleton assumes the same spatial configuration as a folded β sheet, which makes the amyloid resistant to proteolysis processes (3).

By convention, the amyloidosis nomenclature provides for the use of two letters to indicate the types of amyloid fibril: the first letter designates the chemical nature of the protein and is always an A, which stands for amyloid, followed by one or more letters indicating the different protein precursor, for example L for light chain amyloidosis caused by deposition of monoclonal immunoglobulin light chains (AL) or TTR to indicate Transthyretin in the form determined by the deposition of the wild type or mutated Transthyretin protein.

To date, more than 30 proteins have been identified as amyloidogenic in humans, although recent mass spectroscopy analyzes suggest that there may be many more (4).

Figure 1 Amyloidosis Classification List

	Acquired or hereditary	Patients seen at UK-NAC (%; n=5100)	Underlying disorder	Precursor protein	Organ involvement					Treatment	Treatment target
					Heart	Kidneys	Liver	PN (AN)	Other		
AL	Acquired	4067 (68%)	Plasma cell dyscrasia	Monoclonal immunoglobulin light chain	+++	+++	++	+(+)	Soft tissue gastrointestinal	Chemotherapy or ASCT	dFLC <40 mg/L
AA	Acquired	633 (12%)	Inflammatory disorders (RA, JIA, IVDU, FPS)	SAA	-/+ (late)	+++	+(late)	-	Gastrointestinal (late)	Suppression of inflammation	SAA <4 mg/L
ATTR	Acquired	168 (3·2%)	..	Wild-type TTR	+++	-	-	-	Carpal tunnel syndrome	Supportive	Optimum control of heart failure
	Hereditary	339 (6·6%)	Mutations in TTR gene	Abnormal TTR	++	-	-	+++ (+++)	-	Liver transplant (younger patients with V30M-related ATTR), diflunisal, (doxycycline/ TUDCA) Supportive	Optimum control of congestive heart failure and symptoms of PN/AN
AFib	Hereditary	87 (1·7%)	Mutations in fibrinogen α-chain gene	Abnormal fibrinogen	-	+++	-/+	-	-	Supportive, organ transplant	Preserve renal function
ALect2	Acquired	16 (0·3%)	Uncertain	Lect2	-	+++	++	-	-	Supportive	Preserve renal function
AApoA1	Hereditary	40 (0·8%)	Mutations in apolipoprotein A1 gene	Abnormal ApoA1	+	++	++	+/−(-)	Testis	Supportive, organ transplant	Preserve renal function
ALys	Hereditary	17 (0·3%)	Mutations in lysozyme gene	Abnormal lysozyme	-	+	++	-	Gastrointestinal or skin	Supportive	..
AGel	Hereditary	4 (0·1%)	Mutations in gelsolin gene	Abnormal gelsolin	-	-/+	-	++(-) cranial	-	Supportive	..
Aβ2M	Acquired or hereditary	93 (1·8%)	Long-term dialysis	Aβ2M	-	-	-	-(+*)	Carpal tunnel syndrome, arthropathy	Supportive, renal transplant	..

Aggregation of misfolded proteins determines amyloid deposition, a process that can involve several organs and tissues giving rise to a systemic disease or on the contrary be a localized phenomenon. Among the forms of localized amyloidosis, some remain asymptomatic, as in the cases of autopsy findings of small amyloid deposits in the heart, brain, pancreas; while others, much more relevant, are located in the brain, such as Alzheimer's disease and some prion diseases. Most of the forms are acquired, but some are hereditary-familial diseases with clinical manifestations that often arise at a later age (5).

1.3 Pathogenesis

While amyloidosis are from the clinical point of view very heterogeneous pathologies, they are very homogeneous under the histological-molecular profile as they are characterized by the deposition in the tissues fibrils with an homogenous structure. All the amyloidogenic proteins, in fact, are capable of taking the Congo Red histological dye and refract polarized light in a similar way, with the characteristic apple green birefringence.

The deposition of these proteins in the form of fibrils plays a decisive role in the pathogenetic mechanism of the disease. The fact that amyloid deposits can potentially be found in almost all organs and tissues explains the considerable diversity of clinical presentation of this disease. In fact many of its manifestations are non-specific involving different organs and resulting in an increase difficult to achieve diagnosis. From a biochemical point of view, the stability of the amyloid fibril structure is determined by numerous hydrogen bonds between the juxtaposed polypeptide chains and by interactions between the side chains and the surrounding medium, forces that can accelerate or inhibit fibrillar aggregation.

The process that determines the transition from a circulating amyloidogenic protein to fibril deposited in the tissue is a dynamic and multi-step process that involves the so-called “folding” of proteins. Folding is a reversible process which consists in the ability of proteins to assume the native conformation, i.e. a stable three-dimensional structure in relation to its aminoacid structure, characterized by the lowest potential energy, and on which the biological function of the protein itself depends. The reverse process is called "unfolding"; and consists in the more or less complete destructuring of the protein conformation, with consequent progressive loss of its biological function. This process occurs by exposing, for example, a native protein to particular conditions of temperature, pH, ionic strength, to oxidative processes or to the action of denaturing agents or detergents.

Protein folding, although a thermodynamically spontaneous process, is often facilitated by certain proteins called molecular chaperones, which bind to the protein during its folding. In the extracellular area, proteins can undergo more or less extensive denaturation which determine the unwinding of the polypetidic chain and a rapid recovery of the native structure. These unfolding and refolding processes make hydrophobic areas of the polypeptide chain available to the solvent of the extracellular environment that are inaccessible in the native structure of the protein; in this way the protein is more exposed to the risk of self-aggregation or to proteolytic attacks by ubiquitous endoproteases capable of inducing cuts that can destabilize the protein, facilitating its denaturation.

The self-aggregation of fibrillar amyloid proteins occurs during the fluctuation between a partially denatured state and the native state and the mechanisms that induce the formation of misfolded proteins are of different types.

There are proteins that possess an intrinsic propensity to assume a pathological conformation as in the case of wild type Transthyretin which in elderly subjects can undergo tissue deposition. Other proteins, however, despite a partial tendency to denaturation and aggregation, form amyloid deposits only when serum concentrations remain persistently high; an example is β2-microglobulin

in patients subjected to hemodialysis for a long time, in which the dialysis membranes do not remove the protein causing an increase in its blood concentration by hundreds of times. In hereditary forms of amyloidosis, the tendency to aggregation and precipitation of the amyloidogenic precursor often derives from a gene mutation that determines an amino acid substitution in the primary sequence of the protein. Transthyretin and lysozyme represent the prototypes of this mechanism; over 100 different mutations affecting Transthyretin are reported in the literature, many of which are associated with amyloidosis (6,7).

It is not yet clear why the fibrillar aggregation and precipitation process occurs with a certain organ selectivity, which changes according to the amyloidogenic proteins and which can vary, even for the same protein, as a function of different types of point mutation.

It is important to emphasize that the amyloid fibrils extracted from the tissue do not consist exclusively of the amyloidogenic precursor but also of substances that are always present regardless of the type of protein from which the fibers are made by.

These molecules include glycosaminoglycans (GAGs), proteoglycans (PGs) and especially the serum amyloid protein (SAP). The latter is a glycoprotein with a specific binding site for all amyloid fibrils, which appears to be able to protect the amyloid deposit from protease-mediated proteolytic degradation (8).

It has been shown that proteoglycans, in particular, with their polysaccharide content represent an important component of the composition of amyloid deposits. The protein component of these structures, although not completely determined, seems to bind to constituent elements of the extracellular matrix thus anchoring the amyloid to the tissue. Proteoglycans could represent the initial molecular scaffold, in the role of molecular scaffold on which the first nuclei of aggregated amyloid protein could adhere and orient themselves, contributing to the nucleation and growth process of the deposit. Among the proteoglycans, heparan sulfate seems to be the one most involved in the amyloidogenic process also through its intrinsic cellular toxicity (9).

Animal models with genetically modified mice support this hypothesis. In fact, amyloidogenic peptides deriving from unfolded protein precursors tend to generate both insoluble fibrils and intermediate soluble fibrils which can then further aggregate in insoluble forms. Both of these peptide forms can damage the function of cells and tissues, creating pictures of amyloidosis. The tissue in which the deposition of amyloid fibers occurs undergoes cellular suffering through mechanisms that have not yet been fully explained (10).

The first anatomo-pathological studies on tissue samples from patients affected by amyloidosis showed that the cause of the tissue damage was substantially linked to the continuous deposition of proteinaceous, amorphous material in the perivascular site, without evidence of inflammatory manifestations. Faced with this evidence, it has been proposed that the cause of the tissue damage was due to a mechanical action caused by the infiltration in the interstitium resulting in chronic hypoxic-ischemic suffering, functional damage and cell death.

However, the infiltration and replacement of tissue by amyloid fibrils does not seem to be sufficient to explain all the pathogenic effect. Over the last few years, the increasingly important role of the cytotoxic action exerted by soluble prefibrillar aggregates has been elucidated, demonstrating that these pre amyloid form are be able to induce apoptosis of target tissue cells. The cytotoxic action of prefibrillar aggregates is widely documented in the case of the Aβ peptide and Transtyretin. It therefore seems that the most plausible hypothesis to date to explain the cell damage induced by the amyloid deposit is linked both to the presence of the fibrillar deposit itself and to the presence of prefibrillar oligomers that exert cytotoxic effects on the cell and on the affected tissue (11).

However, from a clinical point of view, it can be said that for cell damage to occur, the presence of both amyloid fibers and prefibrillar soluble aggregates is necessary. There are no signs of disease without deposit, while it is possible to eliminate signs and symptoms of disease if the cytotoxic prefibrillar soluble precursor is eliminated, even if the tissue deposit of amyloid remains substantially unchanged at least to the imaging analyzes available today (12).

These evidences emerge from some cases described in the past, but above all from a study conducted by the Pavia group on amyloidosis AL, in which it was shown that the serum concentration of the biohumoral marker of cardiac toxicity, the N-terminal portion of the natriuretic propeptide of type B (NT-proBNP), is directly correlated with the concentration of soluble free light chains and not with the amount of amyloid deposited in the myocardium. A possible explanation could derive from the recent observation that on the growth surface of the amyloid fiber there is a balance between molecules that associate and molecules that continuously dissociate from the fiber, according to a reversible reaction and which obviously presents an equilibrium shifted in the direction of growth. of the fiber. Regarding the deposition of fibrils, regardless of the etiology of amyloidosis, the role of "seeding" has also been demonstrated, suggesting the hypothesis that "amyloid generates amyloid", a phenomenon also called "nest effect" .

This could explain why the precipitation of proteins is concentrated in certain organs, sparing other parts of the organism (13).

2.1 ATTR amyloidosis etiopathogenesis

Transthyretin (TTR) is synthesized mainly by the liver and only minimally (<5%) by the chorioid plexuses of the brain and the epithelium of the pigmented retina.

It is present in plasma at concentrations of 20-40 mg / dl, in cerebrospinal fluid at concentrations of 10-40 mcg / ml, and was initially called pre-albumin as it showed a characteristic greater migration in the vicinity of the electrophoresis. anode with respect to albumin (14).

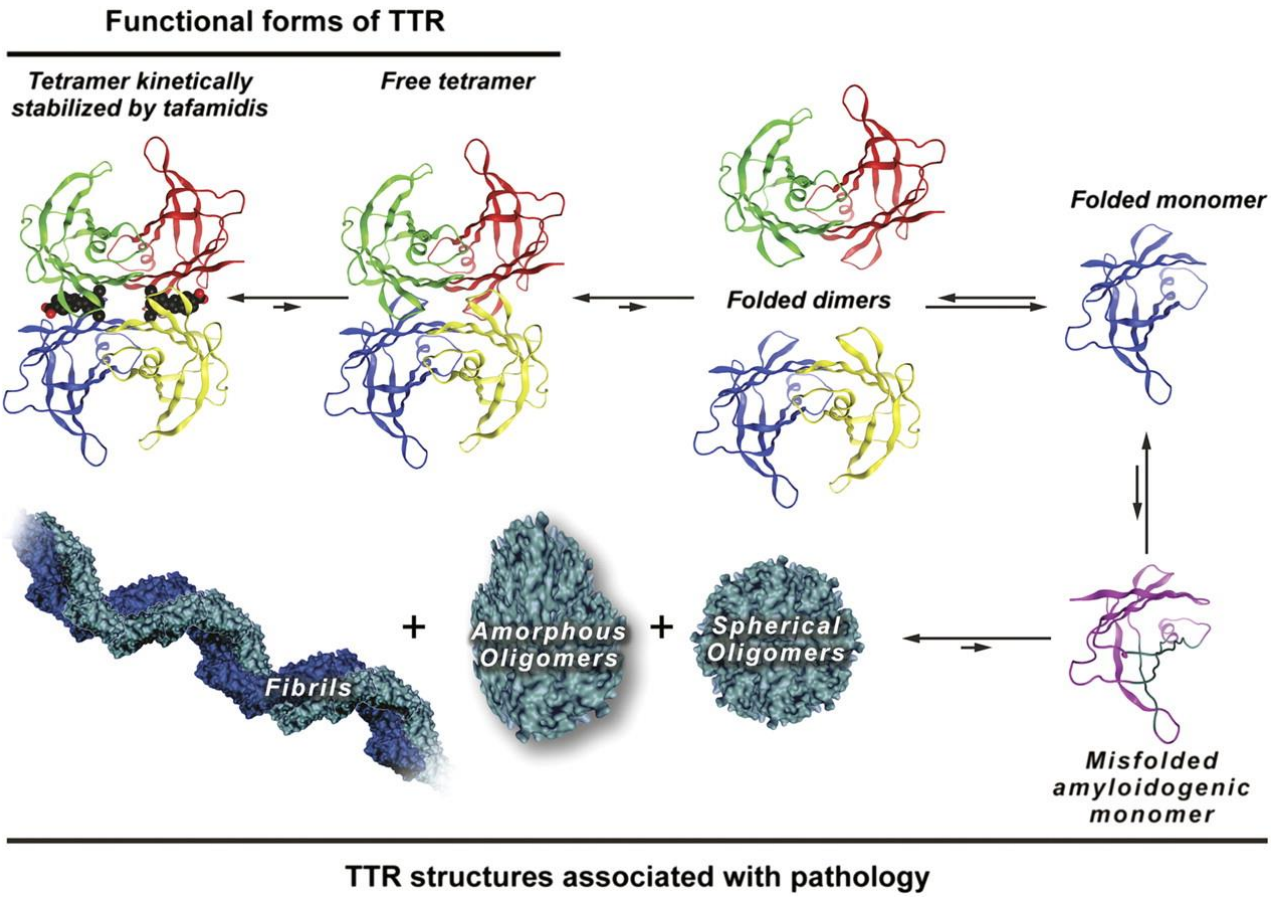
Transthyretin circulates as a homotetramer composed of 4 identical subunits linked together in a non-covalent way. Each monomer consists of 127 amino acids arranged in 8 antiparallel domains to beta-leaflet. In the circulation, TTR is linked both to thyroxine, for which it has an intermediate

affinity between TBG (thyroxine-binding globulin) and albumin, and to retinol / vitamin A as a trimeric compound with retinol-binding globulin.

In the cerebrospinal fluid it is the only protein capable of carrying thyroid hormones, as neither albumin nor TBG are present in appreciable quantities.

Transthyretin plays an important role in behavior, cognitive processes, regeneration of nerve fibers and axonal growth; it also tends to possess the innate ability to aggregate into insoluble amyloid fibrils that accumulate in oligomers composed of 6-10 monomers and cause toxicity and tissue damage.

Figure 2 TTR structure, misfolding and amyloid formation adapted from Bulawa C et al PNAS June 12, 2012 109 (24) 9629-9634



The gene that codes for TTR is located on chromosome 18 and consists of 4 exons and 5 introns. More than 80 mutations have been described in the literature from which as many protein variants derive (15).

Most of these mutations result from the replacement of a single nucleotide in the TTR gene, which results in a pathological protein with an increased propensity to form deposits of amyloid fibrils. The mutation most frequently detected worldwide is Val30Met with 3 main clusters in Portugal, Sweden and Japan. Transthyretin is associated with two distinct clinical forms of amyloidotic cardiomyopathy:

• ATTRwt previously known as Senile Systemic Amyloidosis (SSA), an acquired disease associated with unchanged Transthyretin deposits

• The familial transthyretin-related form, caused by mutations (usually punctiform) in the gene for Transthyretin (ATTRm).

The forms of TTRm are rare diseases with autosomal dominant transmission, in which only one mutated allele is sufficient to develop the disease and in which the majority of affected individuals are heterozygous for the pathogenic mutation. The penetrance of this pathology is also extremely variable and the same mutation can clinically present itself differently among the various members of the same family or not present at all (healthy carriers).

The prevalent neurological clinical manifestation of the disease related to the Val30Met mutation has meant that ATR has always been considered a disease of neurological relevance, so much so that it is commonly referred to as Familial Amyloid Polyneuropathy (FAP). In FAP the onset of the disease often occurs within the fourth decade of life, through the appearance of a progressive and rapidly disabling peripheral, sensory/motor and autonomic neuropathy, with a dysfunction of the peripheral nerve fibers of the lower limbs that extends to the extremities proximal and reaches the terminal stage in 10-15 years involving in this time also the upper limbs. The first symptoms accused are disturbances of thermal and nociceptive sensitivity, paraesthesia and dysesthesia in the

lower limbs. Motor function is generally preserved until the advanced stages of sensory impairment, but eventually manifests itself with significant disability. In association with sensory alterations, progressive dysautonomia also occurs early, due to both sympathetic and parasympathetic denervation, characterized by erectile dysfunction (which in young males can represent the initial symptom), gastrointestinal motility disturbances (alternating constipation with diarrhea with consequent loss of weight and severe malnutrition), urinary retention or incontinence and orthostatic hypotension, arrhythmias (16,17).

Carpal tunnel syndrome deserves a separate consideration, a common disease in the general population characterized by the entrapment and consequent dysfunction of the median nerve by the wrist tendons. Carpal tunnel syndrome for reasons still partly unknown is frequently associated with TTR amyloidosis, even 20 years before the cardiology clinic (18).

The main cardiological manifestations of patients with amyloidosis are due to the progressive replacement of the myocardium by the amyloid fibrils in the interstitial site that make the heart rigid and thickened, causing a progressive evolution towards a picture of infiltrative cardiomyopathy with a typical restrictive pattern. The clinical picture is characterized by dyspnoea especially from exertion, orthopnea, often atypical chest pain and peripheral edema up to a real anasarcatic state. The natural history of the disease leads to an evolution towards progressive heart failure, often refractory to conventional therapy.

Ocular involvement is manifested above all with vitreous opacities, and is confined to some specific TTR mutations, probably linked to the synthesis of amyloid by the pigment epithelium of the retina. Other manifestations are glaucoma, and pupillary changes due to amyloid deposits in the ciliary nerve that result in anisocyclia (19).

The involvement of the leptomenynx leads to a picture of cerebral angiopathy due to amyloid deposition in the medium and adventitious arteries of medium and small caliber, in the arterioles and occasionally in the cortical veins, probably due to the possibility that a small amount of

Transthyretin comes directly from the chorioid plexuses (20) . Clinical manifestations include hemorrhagic and ischemic stroke, ataxia, spastic paralysis, seizures and dementia.

Renal involvement in ATTR amyloidosis is much rarer than in AL and is characterized by proteinuria and renal dysfunction, which appear to be related to amyloid deposits in the glomeruli and arterioles, but not in the renal medulla (21).

2.2 AL amyloidosis etiopathogenesis

Light chain amyloidosis (AL) is a peculiar hematological form of systemic amyloidosis. In these patients, a neoplastic plasma cell clone is responsible for the production of fully assembled or fragmented monoclonal antibody elements. Among the immunoglobulin fragments, free light chains (FLC) are of considerable importance due to the high tendency to assemble together and precipitate when they are not associated with heavy chains. There are two types of free light chains, kappa type and lambda type. Normally in patients with AL amyloidosis there is a clear prevalence of only one type of light chain, which can be evidenced both in the serum and in the urine. Lambda chains are found three times more frequently than kappa chains.

The amyloidogenic plasma cell clone is typically small and indolent, however when the clone accounts for more than 10% of the bone marrow cellularity the prognosis is poor, similar to that of patients with AL amyloidosis associated with Multiple Myeloma. Monoclonal plasma cells in AL amyloidosis have phenotype and copy number variations profile similar to the plasma cells of Multiple Myeloma, but the transcriptome is similar to that of normal plasma cells. Using the Next Generation Sequencing technique to investigate the mutation landscape in a patient cohort with plasma cell dyscrasias, the pattern of mutations in AL amyloidosis was found to be intermediate between that of monoclonal gammopathies of uncertain significance (MGUS) and myeloma Multiple (22,23).

The diagnosis of AL amyloidosis is usually performed around the sixth decade. This form of systemic amyloidosis affects numerous vital organs and tissues simultaneously, with the exception of the CNS. Cardiac involvement is very common (82%) and is manifested by a restrictive cardiomyopathy associated with heart failure with preserved ejection fraction. The second more often involved organ is the kidney (68%) and often the suspicion of AL amyloidosis can be aroused precisely by the occasional laboratory finding of unexplained proteinuria with normal serum creatinine values (24). If glomerular proteinuria reaches the nephrotic range it causes hypoprotidemia with declining edema. Renal function can deteriorate severely over time until it reaches the stage of end uraemic renal failure. The manifestations affecting the soft tissues are less frequent but almost pathognomonic: in 17% of cases there are one or more objective findings including carpal tunnel syndrome, macroglossia, shoulder pad, nail dystrophy and periorbital purpura. The latter clinical sign is defined as the "raccoon sign" (raccoon eyes) and is caused by the deposit of amyloid substance in the periorbital capillaries resulting in brittleness and bruising. In the early stages of the disease, patients with AL amyloidosis may also experience peripheral or autonomic nerve dysfunction such as peripheral sensorimotor neuropathy, orthostatic hypotension, erectile dysfunction, constipation or diarrhea, and gastrointestinal changes with weight loss, anorexia, malabsorption and bleeding (25,26).

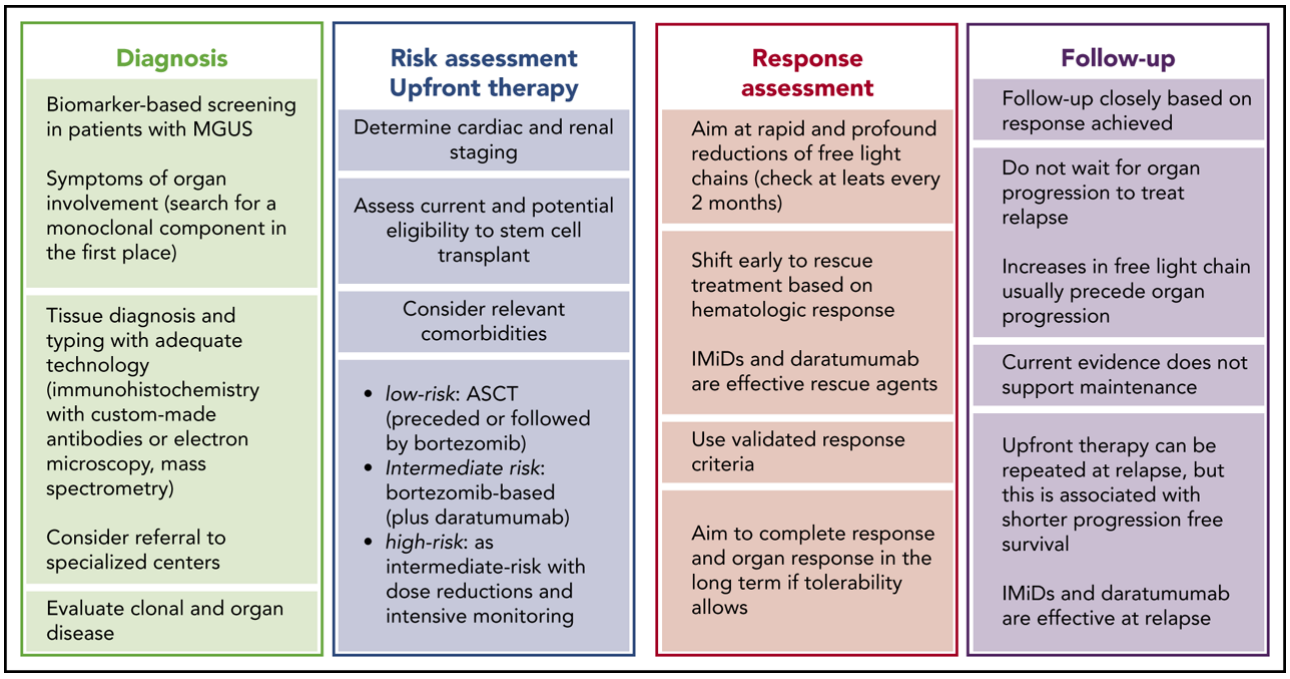
AL amyloidosis is a form of systemic amyloidosis characterized by a worse prognosis than the transthyretin forms (27). The goal of AL amyloidosis therapy is the abolition of fibrillar precursor, or the free circulating light chains, through the suppression of the plasma cell clone responsible for their production.

AL amyloidosis therapy involves the use of the same chemotherapy protocols used in the treatment of Multiple Myeloma. Traditionally, therapy has always been based on the combined administration of Melphalan and Prednisone for 12-24 months depending on the response. Prolongation of therapy is associated with a dramatic increase in the incidence of acute leukemia and myelodysplastic

syndrome. Treatment with high-dose Dexamethasone showed a lower toxicity profile, while the combination of Melphalan and Dexamethasone has high therapeutic efficacy with an average of 67% haematological response, representing the gold standard in AL amyloidosis therapy for non-patients. eligible for autologous stem cell transplantation.

Today's therapeutic landscape proposes further molecules with interesting therapeutic efficacy, such as proteasome inhibitors (Bortezomib) and immunomodulatory drugs (Thalidomide, Lenalidomide). For patients with low-risk AL amyloidosis the gold standard remains treatment with high doses of Melphalan followed by autologous stem cell transplantation (28).

Figure 3 Management of AL amyloidosis in 2020 Palladino G e al. Blood 2020



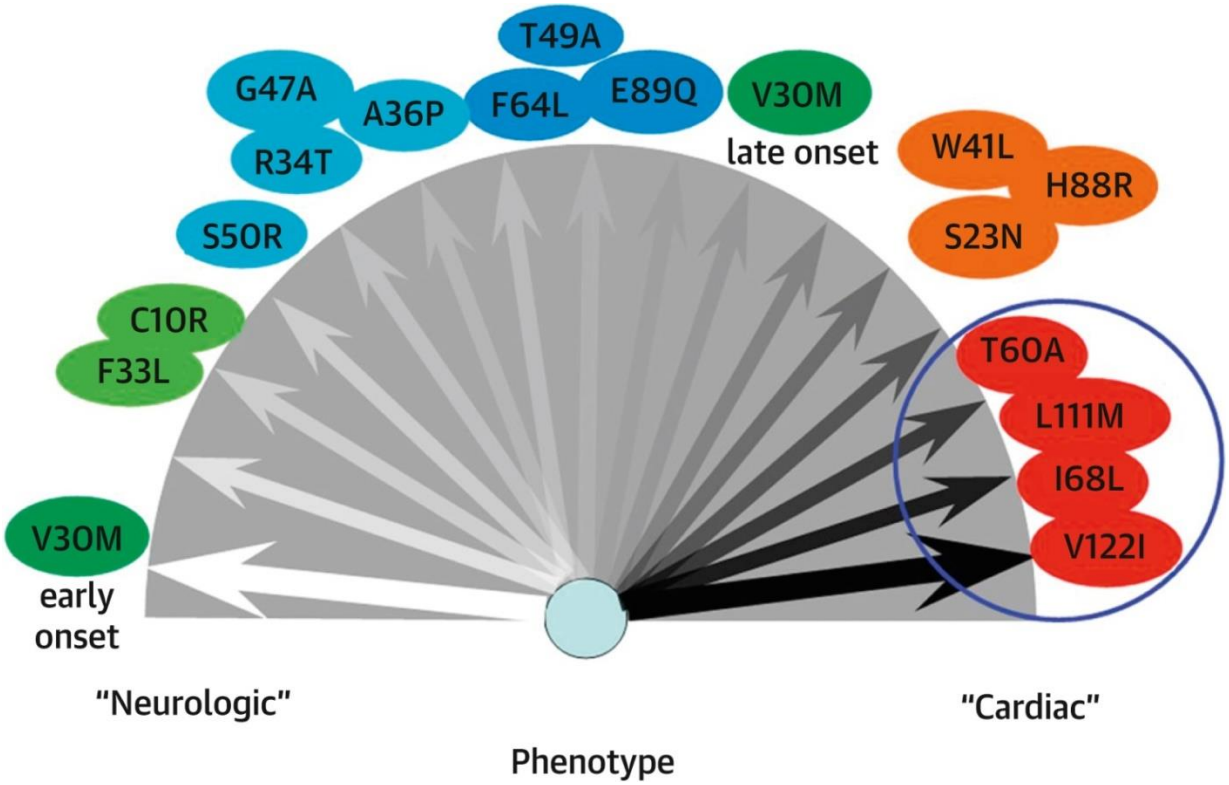
3.1 Cardiac involvement

The presence of cardiac involvement in amyloidosis and its prevalence significantly change according to different type of amyloid precursor.

In AL amyloidosis cardiac involvement is present in more than half of the patients, but subclinical involvement can be detected in almost all cases at endomyocardial biopsy and autopsy. It classically manifests itself with varying degrees of myocardial infiltration up to the evolution towards a restrictive cardiomyopathy picture (29,30).

Amyloidosis related to transthyretin, both mutated and wild type, has a very variable clinical spectrum from an exclusive neurological involvement to a predominant cardiac involvement. This heterogeneity is linked to various factors such as the specific genetic mutation of the TTR gene, parental transmission, geographical distribution and endemic / non-endemic aggregation (figure 4).

Figure 4 Different phenotypes distribution according different TTR mutation



For wild type TTR forms, cardiac involvement is predominant and occurs mainly in men over the age of 65 with a phenotype generally classifiable as heart failure with a preserved systolic function with an evolution towards a restrictive pattern. For the mutated TTR forms, on the other hand, a much more heterogeneous and complex picture is established.

Numerous studies have focused on the characterization of the Val30Met mutation with its almost exclusive neurological manifestation; little is known about cardiac expression mutations. Among the various mutations known to date at least 4 are responsible for a predominant cardiac phenotype: Val122Ile, Thr60Ala, Leu111Met, and Ile68Leu. In the case histories of Italian registries it has been shown that despite the neurological manifestations as a whole they are responsible for almost 80% of the symptoms, in about 15% of patients there was also an important cardiac involvement often misunderstood or classified as hypertrophic cardiomyopathy (31). Although the molecular basis of this diversity is still largely unknown today, the most probable hypotheses involve several factors such as protein stability, tissue-specific proteolytic remodeling and interactions with the extracellular matrix including also chaperone proteins. Regardless of the pathogenesis of the amyloidosis subtype, the progressive substitution of the myocardium by amyloid fibrils in the interstitial site makes the structure rigid and thickened, causing a progressive evolution towards a cardiomyopathy with a typical restrictive pattern. The clinical picture is characterized by dyspnoea, orthopnea, atypical chest pain and the formation of peripheral edema up to the onset of a true anasarcatic state. The natural evolution of the disease is a rapidly progressive heart failure often refractory to conventional therapy. The physical examination can be absolutely negative, especially in the early forms, or be dominated by signs of mainly right heart failure. Characteristic is the reduction of blood pressure values, which also occurs in previously hypertensive patients. Furthermore, due to the frequent involvement of the sympathetic nervous system, patients with amyloidosis quite often experience episodes of orthostatic hypotension (32,33).

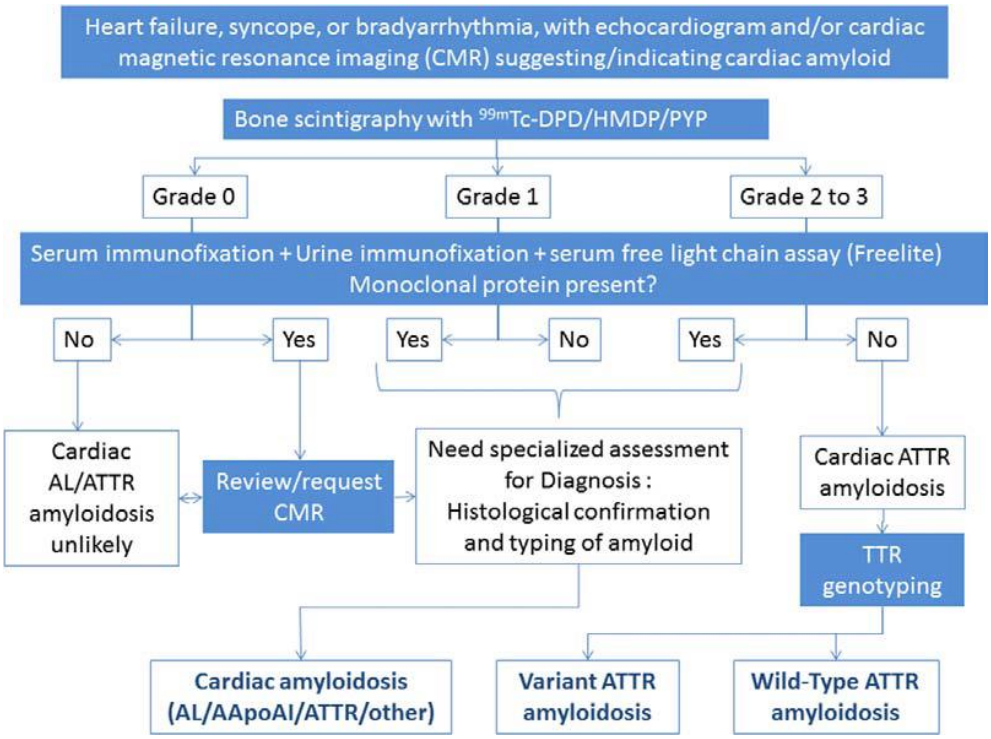
4.1 Diagnosis

Amyloidosis is a rare and extremely heterogeneous disease from a clinical point of view.

Due to these characteristics, clinical suspicion and diagnosis rise often when the disease is in an advanced stage. Very often, in fact, several months or even years can pass between the onset of amyloidosis related symptoms and diagnosis. This is because, especially in the Transthyretin forms, both mutated and wild type, the symptoms can be unclear up to the advanced stages of the disease. In consideration of the advanced age of the patients, other co-pathologies due to the advanced age can act as a confounding effect (coronary heart disease, aortic stenosis, arterial hypertension, diabetes mellitus, the absence of particular and specific striking extracardiac manifestations) (34).

Gillmore et al. have therefore recently proposed a diagnostic algorithm which, starting from the clinical suspicion, through the execution of simple laboratory and instrumental tests, allows to identify the 3 main classes of Amyloidosis (AL, ATTRm and ATTRwt figure 5) and then proceed to the most appropriate therapy and correct prognostic stratification (35).

Figure 5 Cardiac amyloidosis diagnostic algorithm



4.2 Echocardiography

Echocardiography is the central instrumental examination for the non-invasive diagnosis of cardiac amyloidosis. Cardiac amyloidosis is characterized by a thickening of the ventricular walls due to the deposition of amyloid substance in the extracellular space, in the context of the myocardium and valves.

The infiltrated myocardium has a particular echogenicity with a hyper-reflectance defined as “granular sparkling”. This aspect is observed with a widely distributed or localized pattern in some specific areas of the myocardium. The areas that most frequently show the granular sparkling appearance are the interventricular septum and the left ventricular posterolateral wall. This echocardiographic finding reflects the site-specific deposition of amyloid fibrils in the heart and also justifies the peculiar normal kinesis of the apex compared to remaining parts of ipokinetic infiltrated myocardium, in what is defined in terms of segmental motility "apical sparing".

The papillary muscles are initially spared from the deposition of amyloid, although in the most advanced stages of the disease they are also infiltrated and therefore thickened and hyper-reflective.

There is also widespread thickening of the atrioventricular valves and the presence of a pericardial effusion.

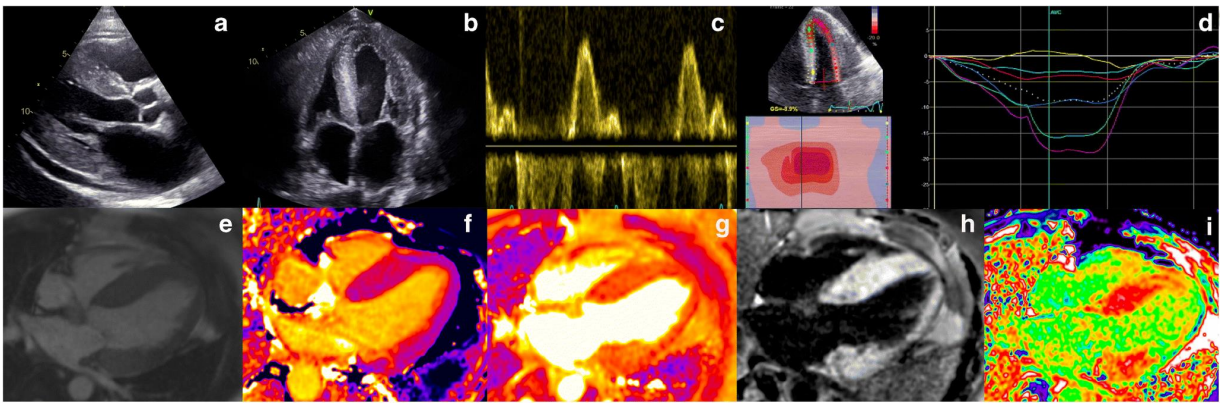
The infiltration of amyloid in the context of the ventricular myocardium occurs at the expense of the left ventricular cavity; the right ventricle presents a thickening of the free wall. The atrial septum is also frequently thickened and with an increase echogenicity, the atrial chambers are dilated due to diastolic dysfunction and increased atrial pressure. The deposition of amyloid in the context of the ventricular myocardium, in fact, determines alterations of the properties of its release leading to what is called "heart of stone". Diastolic dysfunction is one of the first alterations to appear and evolves towards a restrictive diastolic pattern with high filling pressures, while systolic dysfunction occurs only in the most advanced stages of the disease (36).

The myocardial velocity of the ventricular walls and both the degree of strain (“strain”) and the rate of strain over time (“strain rate”) of the ventricular myocardium are frequently depressed. Alterations in the longitudinal contractility of the left ventricle (detectable with tissue Doppler and speckle tracking images) are frequent and appear in the early stages of the disease, when the radial shortening function is still preserved. The longitudinal "strain" also shows a typical pattern that differentiates it from other cardiomyopathies: severe reduction of the deformation values at the base of the left ventricle and sparing of the apex (apical sparing) (37-39) .

The standard pattern of TDI includes an anterograde (positive) velocity during the isovolumetric contraction phase, a systolic wave (S ') during the ejection phase, and two retrograde (negative) velocities during the protodiastole (E') and during contraction atrial (A '). Wave E ’, which represents the speed peak at the mitral annulus during the initial phase of diastole, turned out to be a good index for the study of myocardial relaxation. Even more important was a combined index of traditional Doppler and TDI (the E / E 'ratio), which proved to correlate well with left ventricular filling pressure and therefore with left atrial pressure (figure 6).

The combined analysis of transmitral Doppler and tissue Doppler frequently suggests elevated end-diastolic pressure and / or a restrictive filling pattern.

Figure 6 Echocardiography findings in a patient with advanced cardiac amyloidosis.



4.3. Scintigraphy

Total body bone tracer scintigraphy has become the most common nuclear medicine method in evaluating cardiac amyloidosis. Numerous tracers (mainly bisphosphonates) have been used for the identification and evaluation of amyloidotic cardiomyopathy with very heterogeneous results. The main tracers used in Europe are 99mTc-3,3- diphosphono-1,2-propanodicarboxylic acid (99mTc-DPD) and 99mTc-hydroxymethylene diphosphonate (99mTc-HMDP) while in the United States the most common is 99Tc-PYP.

Perugini et al. (41) firstly demonstrated how the use of this methodology is of fundamental importance to discriminate between AL and TTR amyloidosis. From their study it emerged that the cardiac uptake of 99mTc-DPD, even if evaluated in a semi-quantitative manner and by visual analysis, occurs exclusively in patients with the Transthyretin-related amyloidosis form.

A semiquantitative visual score is thus outlined in 4 degrees where grade 0 indicates absence of cardiac uptake, grade 1 mild cardiac uptake (however less than that of bone), grade 2 moderate cardiac uptake equal to that of bone and Grade 3 with marked cardiac uptake and reduced bone uptake (Figure 6). The semi-quantitative method consists in comparing cardiac uptake with that of another region (ROI, Region Of Interest), which may consist of the contralateral mediastinum, kidney, bladder and skull. In a recent study it has been shown that 99mTc-HMDP scintigraphy is capable of highlighting a certain degree of pulmonary retention in almost 60% of subjects.

Furthermore, the incidence of pulmonary uptake appears to be significantly correlated with the degree of cardiac retention according to the Perugini visual score (Figure 7 and 8). This data contributes to the attenuation of the bone signal which is the marker of the highest cardiac uptake score 3. In other words, the extreme cardiac uptake of the tracer in relation to the bone is linked to a subtraction of the tracer from the bone not only by the cardiac TTR deposits but also by the extraosseous ones, much less intense but much more widespread. and therefore quantitatively

important. The significant difference in distribution of 99mTc-HMPD in the lungs in AL and TTR amyloidosis suggests an etiological tropism of fibril, comparable to the already well documented cardiac tropism related to transthyretin correlated in the first hypothesis to the presence of amyloid deposits in the lung tissue. The presence of pulmonary uptake could therefore drastically influence this semi-quantitative method, reducing the heart to lung ratio and therefore other quantitative methods such as the heart and whole body retention ratio (H/WBR) or the heart and heart ratio should be preferred. skull (H/S).

Figure 7

Examples illustrating the spectrum of 99mTc-HMDP uptake among patients using Perugini Visual Score. Perugini 0 absent cardiac uptake and normal bone uptake, Perugini 1 mildcardiac uptake, inferior to bone uptake, Perugini 2 moderate cardiac uptake associated withattenuated bone uptake, and Perugini 3 high cardiac uptake with decreased or absent bone uptake.

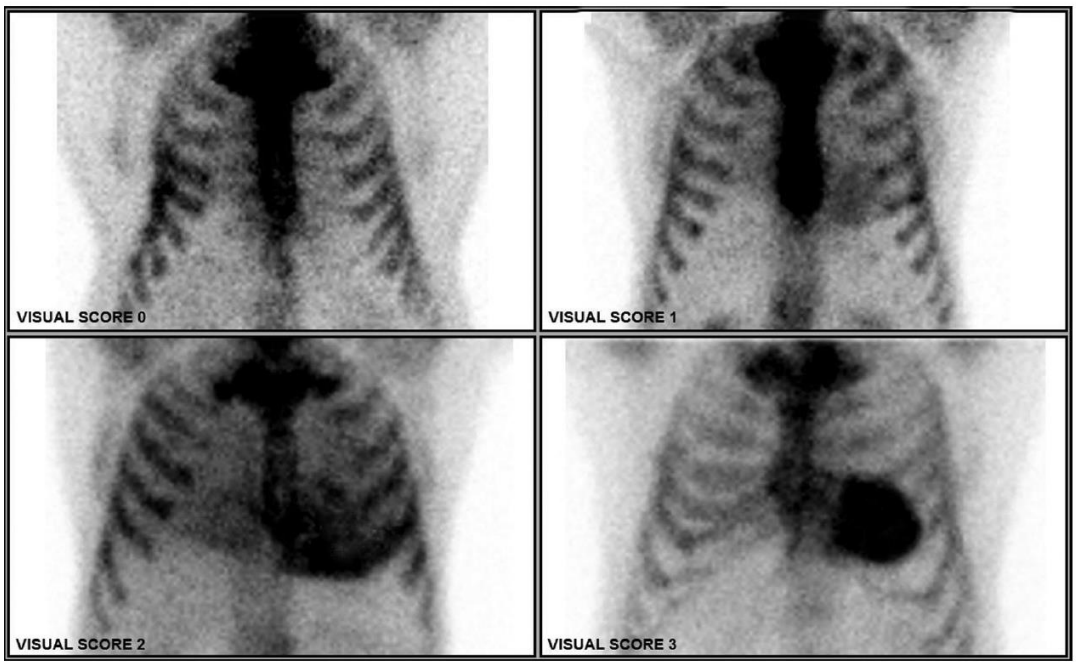
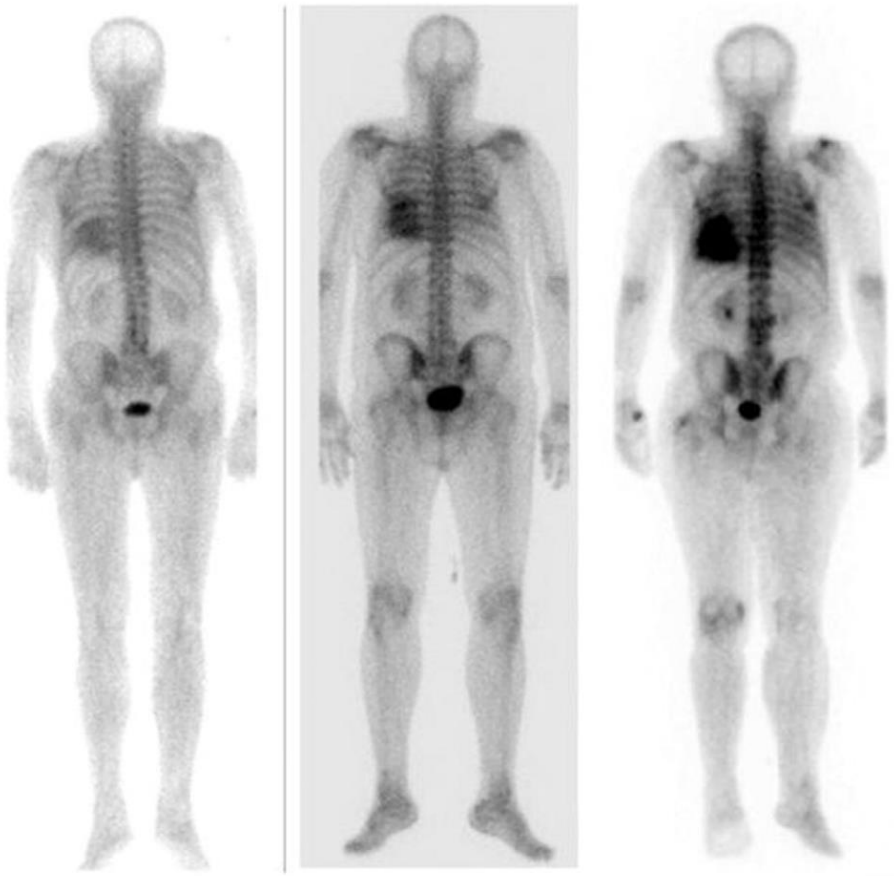


Figure 8

Examples illustrating the spectrum of 99mTc-HMDP lung uptake among patients using Janssen modified visual score. (Left) No lung uptake (grade 0) and Perugini visual scoreHeart Uptake 1, (centre) lung uptake less than ribs (grade 1) and Perugini visual score Heart Uptake 2, (right) lung uptake more than ribs (grade 2) and Perugini visual score Heart Uptake 2.



4.4 Cardiac Magnetic Resonance Imaging

Cardiac MRI (CMR), have gradually acquired an increasing importance in the early diagnosis and risk stratification of cardiac amyloidosis. Compared to echocardiography alone, CMR allows to obtain a better quantification of cardiac volumes and mass, the degree of hypertrophy, biventricular systolic function, as well as a more detailed and accurate characterization of myocardial tissue.

One of the parameters used for myocardial characterization is the T1 phase tissue study, both pre-contrast (native T1) and post-contrast, which quantitatively assesses the signal deriving from the myocardium. Each pixel image represents the absolute value of T1, which reflects the amount of extracellular tissue, encoded by an algorithm with a range of colors from red to blue and an absolute numerical value. The longitudinal relaxation time T1 indicates the time in which the protons that have been excited by a radiofrequency pulse, take to return to the equilibrium situation. We have two main types of sequences: MOLLI (modified look-locker inversion recovery) which allows T1 measurement values in a single apnea in 17 consecutive cardiac cycles and ShMOLLI (shortened modified look-locker inversion recovery) where the cycles needed are nine. Reduced T1 values indicate intracellular infiltration as occurs in lysosomal storage pathologies (Fabry's disease) and in the accumulation of iron; increased T1 values instead indicate an expansion in the extracellular component as for the presence of fibrosis, edema and amyloid.

In cardiac amyloidosis the native T1 signal appears to be increased in intensity, with higher values in the forms of cardiac AL amyloidosis compared to those of ATTR, and it appears to be an early marker of disease, since the increase in the signal is also found before the onset. of hypertrophy. The native T1 is therefore a useful parameter for obtaining an estimate of the amount of amyloid deposition, as well as for differentiating the left ventricular hypertrophy observed in patients with cardiac amyloidosis from that of patients with hypertrophic cardiomyopathy, as shown by recent studies (42) .

The main limitation of T1 is due to the fact that it measures a composite myocardial signal, coming both from the interstitium and from myocardiocytes; moreover, it does not allow to fully distinguish the underlying pathological process. Evaluation of the extracellular volume (ECV), which gives an estimate of the myocardial interstitial space and therefore indirectly of the amount of total amyloid deposition mostly overcome the limitation of native T1 mapping (43).

By measuring the ECV, it is also possible to obtain an estimate of the intracellular volume, with further improvement of tissue characterization. In cardiac amyloidosis, the ECV is increased, with higher values in the ATTR forms, both mutated and wild-type, compared to AL. Combined together, the evaluation of the ECV and native T1 yields a more accurate assessment of cardiac amyloidosis in relation to the degree of infiltration (ECV), edema (native T1) and myocardial mass (intracellular volume).

By administering a contrast medium such as Gd-DTPA (gadolinium-diethylenetriamine penta-acetic acid) it is possible to highlight the extracellular tissue contrast, obtaining a T1 signal which will be strongly reduced in case of widespread and massive amyloid infiltration.

In addition, in cardiac amyloidosis there is a characteristic pattern of Late Gadolinium Enhancement, LGE with global and diffuse subendocardial involvement, with non-coronary distribution in the early disease stage and then a trans mural diffuse patchy pattern.

LGE occurs in areas with increased extracellular space, due to a high regional concentration of the contrast agent and a slower distribution kinetics than normal myocardial tissue, due to the presence of amyloid.

Highly variable results emerge from studies that have focused on the prognostic value of LGE in patients with amyloidosis. In fact, in this type of disease, due to its extracellular infiltration characteristics, a very variable pattern is outlined after administration of contrast medium with gadolinium.

A continuous evolution is therefore configured that follows the course of the disease burden: from

the absence of LGE, to its presence in spots and subsequently to a diffuse subendocardial ring up to the more advanced stages where the pattern is clearly transmural. The latter stage correlates with a worse prognosis and is an important marker of cardiac mortality (44).

The main features of cardiac amyloidosis identified by cardiac MRI are:

• the morphological phenotype with major asymmetric hypertrophy of the septal segments and consequent inversion of the septal profile (reverse septal contour);

• typical pattern of Amyloidosis with LGE varying from a diffuse subendocardial hyperintensity to a transmural pattern;

• an increase in extracellular volume

4.5 Electrocardiogram

In cardiac amyloidosis patients, electrocardiographic tracing often shows characteristic pattern, with low voltages (<5 mm in all peripheral leads and <10 mm in the precordial leads). Low voltages found in patients with amyloidosis are a consequence of the thickening of the heart walls due to an amorphous, electrically isolating substrate. It act in an opposite direction as an increase wall thickness on a hypertrophic basis in which the increase ventriculat mass is mainly due to increasing contractile myocells. However, only one third of patients with TTR-related amyloidosis have a low potential pattern.

The detection of low voltages on the ECG must be completed with an echocardiographic examination showing thickened, infiltrated ventricular walls. The mismatch between echocardiographic and electrocardiographic findings makes cardiac amyloidosis different from any other form of hypertrophic cardiomyopathy, given that cardiac amyloidosis is a pseudohypertrophy.

Other characteristics of the trace during amyloidosis are: poor progression of the R wave in the

precordial leads and sometimes pseudonecrosis Q waves, defined as patological Q waves (1/4 of the amplitude of the R wave) or QS waves in two consecutive leads in the absence of previous myocardial ischaemia, or segmental dyskinesia at imaging evaluation. A frequent finding is the presence of atrial fibrillation, due to the deposition of amyloid substance in the context of the atrial wall, which prevents the physiological propagation of the impulse through the atrial chambers. Various degrees of conduction alterations can also occur for the same reasons, such as 1st degree atrioventricular blocks, nonspecific intraventricular conduction delays and 2nd and 3rd degree atrioventricular blocks (45-46).

4.6 Tendinopathies as amyloidosis red flag

4.6.1 Carpal tunnel syndrome

Carpal tunnel syndrome is the most studied and most common peripheral nerve entrapment disorder in the world. Carpal tunnel syndrome is due to compression of the median nerve passing through a very narrow osteofibrosal canal.

This channel, known as the carpal tunnel, is a passage located proximally on the palmar side of the wrist. The median nerve is a mixed sensory-motor nerve that arises in the axillary cavity from the union of the medial and lateral secondary trunks of the brachial plexus. Carpal tunnel syndrome is characterized by an initial numbness of the hand, especially during the night. Paresthesias are reported and, to a lesser extent, pain in the area of innervation of the median nerve which can reach up to the forearm and shoulder.

Women are affected more frequently than men. The gender difference in incidence explains how part of the pathogenesis is linked to hormonal factors since pregnancy, breastfeeding and menopause involve an increased risk of developing carpal tunnel syndrome (47).

In systemic amyloidosis etiopathogenesis of carpal tunnel syndrome is mainly due to the

characteristics of amyloid to be deposited in the extracellular space of various organs and tissues, including the various tenosynovial structures of the carpus. Deposits of amyloid substance were found around and inside the retinaculum of the flexor muscle tendons, in the synovial tissue, in the sheath of the flexor tendon, in the fascia, in the wall of the venous and arterial vessels. Endoneurial deposits of the median nerve have also been believed to be responsible for the pathogenesis of carpal tunnel syndrome.

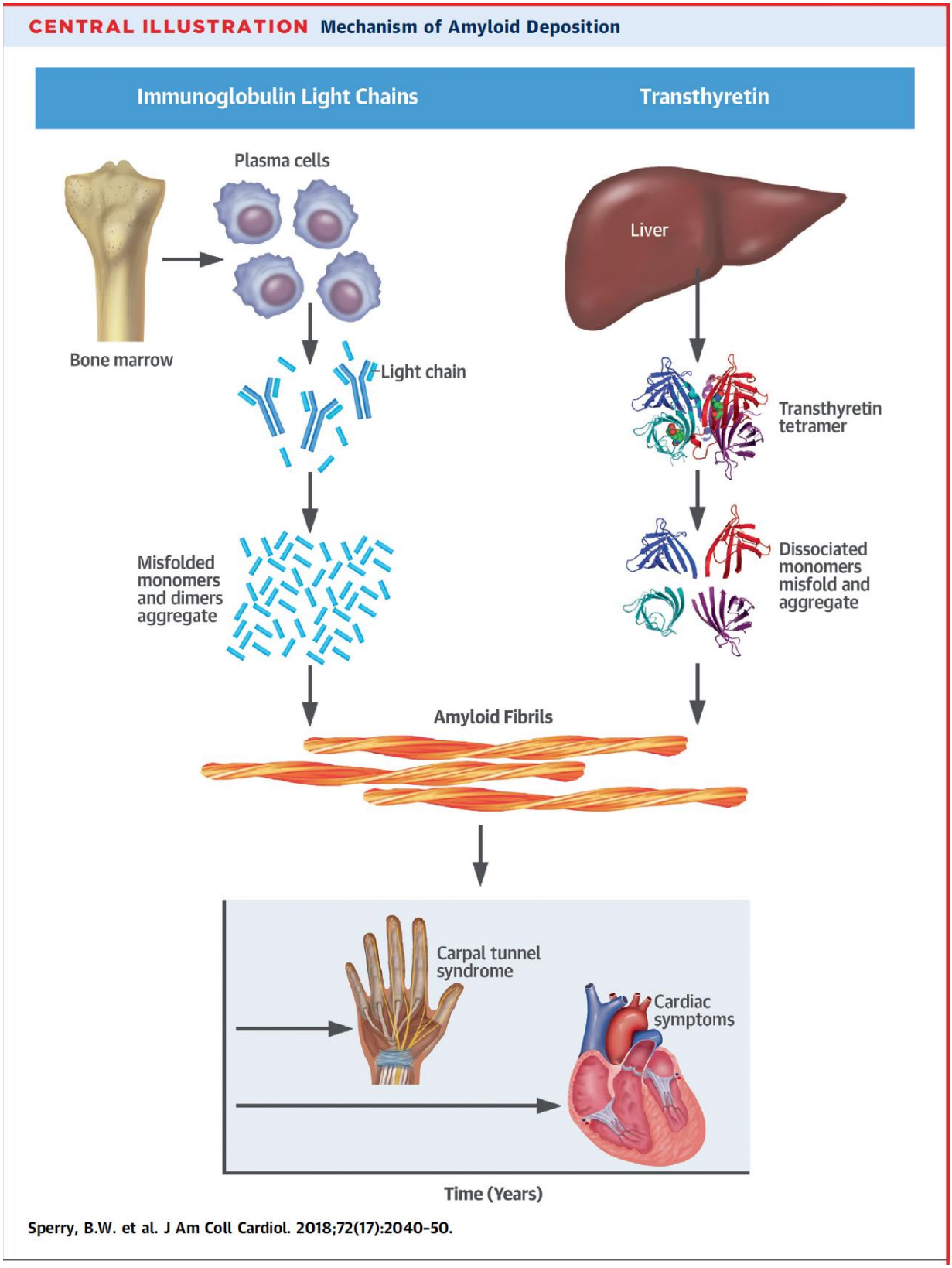
In patients with amyloidosis can often be reported by patients a medical history of carpal tunnel syndrome, often bilateral, with previous surgery, sometimes multiple median nerve decompression due to symptoms relapse (48).

In a recent retrospective study by Sperry et al. in a cohort of patients with a minimum age of 50 years and with a history of idiopathic carpal tunnel syndrome undergoing surgery, the presence of deposits of amyloid substance in the carpal ligament was found in 10.2% of cases. Interestingly, all patients with amyloid deposits presented symptoms attributable to carpal tunnel syndrome bilaterally (figure 9) (49).

In another American study involving a cohort of patients with cardiac amyloidosis, 40% of patients had a history of carpal tunnel syndrome and 16% of patients presented carpal tunnel syndrome as the first systemic sign of damage from deposition. of Amyloid substance (50).

Carpal tunnel syndrome seems to be less frequent in AL amyloidosis than in TTR amyloidosis and the time from carpal tunnel symptom onset and amyloid diagnosis seem to be shorter in AL (51).

Figure 9 Mechanism of amyloid deposition in carpal tunnel ligament (49)



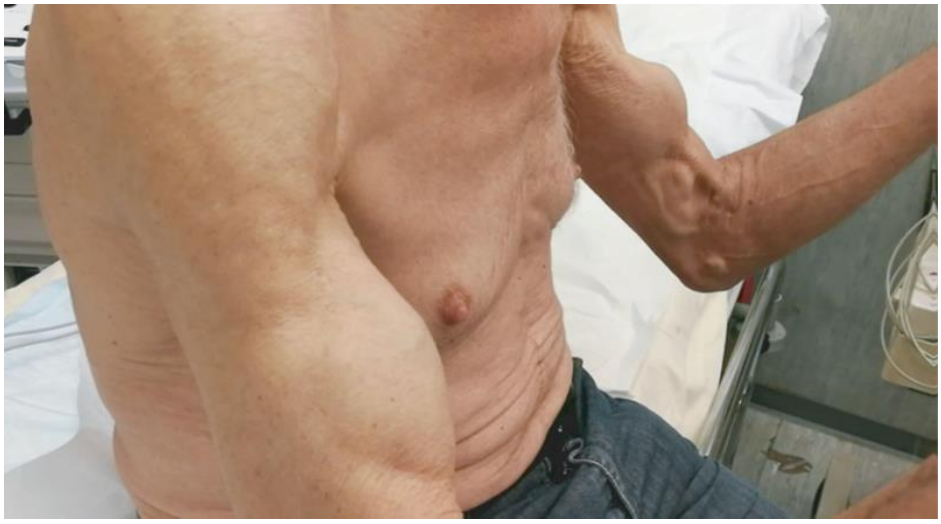
4.6.2 Brachial biceps tendon rupture

The brachial biceps muscle is part of the anterior arm muscle group. The long head of the biceps originates from the supraglenoid tubercle of the scapula with a long tendon which descends into contact with the head of the humerus, sliding inside the scapulo-humeral joint and then running into the intertubercular groove. The short head originates medial to the long head from the apex of the coracoid process of the scapula. The two ends join in the middle of the arm forming a voluminous belly which, descending towards the elbow, is inserted into the tuberosity of the radius. It is innervated by the musculocutaneous nerve.

Popeye's sign i.e the brachial biceps tendon rupture is reported in the literature to affect 0.53 per 100,000 people in five years, with a male: female ratio of 3:1, affecting the age group between 40-60 years. The limb of the dominant side is the one most frequently affected.

Patients generally trace the break to a traumatic event, described in most cases as due to a sudden load applied to the flexed and supinated forearm. Rupture of both the proximal tendon and the distal tendon is described, although the former is observed more frequently. Rupture frequently occurs at the level of the insertion of the long head tendon at the level of the supraglenoid tubercle in its musculotendinous junction (52,53).

Figure 10 Bilateral Brachial biceps tendon rupture with the typical Popeye sign



Advanced age, smoking exposure, taking statins and corticosteroids, prolonged work or sports-related stress are the most common risk factors. Additional situations that lead to tendon degeneration are metabolic disorders such as diabetes, hypercholesterolemia, hyperuricemia, hemochromatosis and obesity. To these already known risk factors, recent researches have demonstrated that also amyloidosis is linked to development of Popeye sign (54-56) (figure 10).

In patients with amyloidosis, often, the deposition of amyloid substance is slowly progressive over time and involves a gradual deterioration of the tendon up to the rupture that is described by patients as non-traumatic and therefore difficult to place over time.

The rupture of the biceps tendon does not involve a total functional limitation of the limb, patients generally maintain the complete flexion capacity of the elbow and partial pronation / supination force. A loss of strength in flexion in 8-16% of cases and of strength in pronation-supination in 11-21% of cases is reported in the literature.

On physical examination, the break can be seen by asking the patient to flex the forearm against resistance, a distal retraction of the muscle belly or a lack of contraction is appreciated.

Diagnosis of total or partial tendon rupture can also be instrumental using ultrasound or magnetic resonance imaging.

5.1 ECG patterns and arrhythmias in cardiac amyloidosis

Systemic Amyloidosis is a disease that frequently involves the heart. Amyloid deposition in myocardial tissue reduces ventricular compliance by initially altering the mechanism of diastolic relaxation and in later stages affecting contraction. Amyloid fibrils can also deposits in myocardial vessels reducing blood flow and then causing local ischemia. Amyloid deposition is associated with fibrosis of the cardiac conduction system, resulting in conduction abnormalities and arrhythmias (57).

In recent years, increased disease attention and improved screening have generated an increase in the prevalence of cardiac amyloidosis, with estimates reporting of 18 to 55 cases per 100,000 people per year, and are estimated to represent about 13% of hospitalizations for heart failure with preserved ejection fraction in over 65s (58).

Arrhythmias and ECG abnormalities are frequent in patients with cardiac amyloidosis and are an important cause of morbidity and mortality, although to date they represent an aspect that has not yet been adequately investigated (59).

According to the data reported in the literature, the most recurrent electrocardiographic anomalies in patients suffering from cardiac amyloidosis consist of low QRS voltages in the peripheral leads; pseudonecrosis in the anterior precordial leads and in the lower peripheral leads; changes in atrioventricular conduction in the form of atrioventricular blocks of various degrees; intraventricular conduction abnormalities such as fascicular blocks;

Atrial arrhythmias, in particular atrial fibrillation but also atrial flutter and atrial tachycardia are common in cardiac amyloidosis patients, leading to important clinical repercussion (60-62).

5.2 Bradiarrhythmia

Cardiac amyloidosis patents often show bradyarrhythmic patterns on ECG. Infiltration of the

electrical conduction system represents a valid causal element to which this phenomenon can be attributed.

In particular, atrioventricular conduction delay or AV blocks are more common than sinus node disease, in fact, despite the high frequency of atrial involvement in amyloidosis, sinus node disease is poorly represented and relevant episodes are limited to isolated events in patients with ATTRm amyloidosis probably more related to dysautonomia than direct infiltration (60). This is in agreement with the results obtained by Reisinger and colleagues in a study aimed at evaluating the activity of the sino-atrial node in 25 patients with AL cardiac amyloidosis. Authors demonstrated that at electrophysiological study a normal pacemaker function of sino atrial node was detected in 88% of the cases (61).

Conduction defects of the His-Purkinje tract are very common and are often associated with symptomatic atrioventricular blocks. First degree atrioventricular block (1^{st} Degree AV block) is often due to a delay in the His-Purkinje system with conduction preserved at the nodal level. Despite the anomalies of atrioventricular conduction have been identified in both patients with AL amyloidosis and with the ATTR form, symptomatic atrioventricular blocks have a higher incidence in the ATTR form. This could be related to a better prognosis with longer life expectancy of ATTR form and therefore longer time to develop symptomatic arrhythmias.

The ECG pattern may provide some differences between the AL and ATTR form: left bundle branch block has been documented in 40% of patients with wild-type ATTR but is rare in the AL form (4%), while the typical low QRS voltages were identified in 40% of wild-type ATTR patients versus 60% of AL patients (62).

Low QRS voltages are an important finding to investigate in the suspicion of cardiac amyloidosis, especially if the echocardiogram shows increased ventricular thickness and mass. In the literature, the prevalence of low QRS voltages in patients with cardiac amyloidosis ranges from 45% to 70%. The variability of this data is partly a consequence of the different degree of cardiac involvement

but partly reflects the lack of uniformity in low voltage definition. Some authors use the Sokolow-Lyon index and define low voltages as a value ≤15 mm (1.5 mv) obtained from the sum of the S wave in V1 and the R wave in V5 or V6. According to the most common definition, it is sufficient that the amplitude of the QRS complexes is ≤ 5 mm (0.5 mV) in all peripheral leads, although some authors add as a criterion the presence of QRS complexes with an amplitude ≤ 10 mm (1 mV) in all precordial derivations. Finally, other authors follow both of the above criteria in combination.

The sensitivity of low voltages still remains uncertain, but from a prognostic point of view in patients with AL amyloidosis the correlation between the presence of low voltages and a more severe cardiac involvement of the disease has been made (63).

In patients with ATTR amyloidosis, ECG findings show a higher frequency of pseudonecrotic pattern (21%) than low voltage (8%), although both are significantly less common than in patients with AL amyloidosis cardiac involvement (45% and 47% respectively). Putting these two aspects together, Cheng and colleagues demonstrated through a study carried out on 276 patients diagnosed with systemic amyloidosis that the combination of low voltages in the peripheral leads and pseudonecrotic pattern has high specificity and positive predictive value for the diagnosis of cardiac amyloidosis (64).

5.3 Atrial Fibrillation

Among the atrial tachyarrhythmias, atrial fibrillation is the most represented arrhythmia in patients with cardiac amyloidosis and appears to be frequently represented in ATTR amyloidosis patients, Although large scale direct comparison study are lacking it seems that the highest frequency of AF is demonstrated in TTRwt and less represented in AL Form. Data on ATTRM are lacking but seems that in this heterogeneous subtype AF frequency could be intermediate between ATTRwt and AL (65,66).

From a physiopathological point of view Henein and colleagues demonstrated that in patients with ATTR amyloidosis myocardial function is compromised regardless of the size of the atrial cavity and ventricular deformation (67). This result reinforces the concept of arrhythmogenicity conferred by amyloid substance deposits, underlining how the mechanism of onset of atrial fibrillation in patients with cardiac amyloidosis cannot be considered superimposable to the mode of onset of atrial fibrillation in those who are not affected by the disease. It has also been shown that in patients with atrial fibrillation undergoing ablation the rate of arrhythmia recurrence is significantly higher for patients with cardiac amyloidosis (83% at one year) than in people without the disease (25% at one year) (68) .

In cardiac amyloidosis atrial arrhythmias are often highly symptomatic and poorly tolerated, especially in case of high ventricular rate and the irregular ventricular response that compromises ventricular filling and contractility. In fact, from a clinical point of view, after atrial fibrillation onset, patients often present with an exacerbation of the symptoms of heart failure.

The importance of knowing the real incidence of atrial fibrillation in patients with cardiac amyloidosis is aimed at the need to apply effective therapeutic management aimed at controlling symptomatic-clinical discomfort, but above all at reducing the risk of thromboembolism and stroke that accompanies this type of arrhythmia. Furthermore, numerous studies have reported the presence of an elevated risk of stroke and systemic embolism that requires anti-coagulant treatment regardless of the CHADS2 and CHA2DS2-VASc scores (69).

5.4 Ventricular Arrhythmias

Regarding ventricular arrhythmias few data are available and results are not univocal. A study carried out on patients with an advanced form of AL amyloidosis reports the incidence of non-sustained ventricular tachycardia (VT) in 27% of patients, while in a more recent study events of non-sustained VT -sustained were observed in only 0.05% of the cohort. This discrepancy probably

reflects the different cardiac infiltration of disease between the two populations under study rather than a reduction in incidence. In the AL form there is a higher incidence of ventricular arrhythmias than in the ATTR type. The prognostic significance of non-sustained VT in patients with cardiac amyloidosis has not yet been defined, as the rate of sudden cardiac death in patients with cardiac type AL amyloidosis is similar among patients in whom non-sustained VT has been documented. and patients in whom it was not detected (70-71). A study by Dubrey and colleagues (72) showed in a cohort of 195 patients with cardiac amyloidosis AL who underwent 24 hours of continuous recording with ECG-Holter the presence of non-sustained VT in 27% of patients with form advanced disease. In a more recent study conducted by Sayed and colleagues in 2015, non-sustained VT was detected by loop recorder in only 1 in 20 patients with cardiac AL amyloidosis. In both studies, cases of sudden cardiac death were mainly associated with terminal bradycardia followed by pulseless electrical activity (73).

To date, due to the few studies available the correlation between the prevalence of ventricular arrhythmias and prognosis in ATTR patients is poorly understood.

On the other hand Paladini and colleagues highlighted a clear correlation between the presence of ventricular arrhythmias and a negative impact on prognosis in AL patients (74).

5.5 Pacemaker and Implantable Cardiac Defibrillator

To date in patient with cardiac amyloidosis there are no specific indication to suggest the implat of pacemaker or intra cardiac defibrillator device (ICD). In fact current ACC / AHA / HRS guidelines recommend pacemaker implantation in patients with cardiac amyloidosis in the presence of advanced conduction impairment (Class IIa indication, defined as Mobitz type II second-degree atrioventricular block, atrio-ventricular block degree ventricular, or third degree atrioventricular block (75). No clear evidence is present in literature to guide the timing of pacemaker implantation

in patients with cardiac amyloidosis, especially in those at risk of developing conduction abnormalities without conventional indications for pacing. Some studies have suggested that, despite the high prevalence of conduction system pathology, few patients develop clinically significant bradyarrhythmias requiring pacemaker implantation. On the other hand, in a small cohort of patients with AL and advanced cardiac involvement, routine loop recorder implantation demonstrated that all deaths are preceded by bradycardia, especially complete heart block (3^{rd} degree AV block). Further research is needed to define whether the preventive pacemaker implantation would improve the outcome in this scenario, in particular when death occurs secondary to electro-mechanical dissociation. In fact, the most frequently documented terminal event in cardiac amyloidosis is pulseless electrical activity (PEA) or agonal bradycardia, a variant of asystole and also in the case of sudden death, the mechanism commonly underlying is electro-mechanical dissociation. secondary to end stage heart failure, however PEA is not a shockable rhythm.

ICD implantation in primary prevention is associated with a high frequency of appropriate discharges for ventricular arrhythmias in patients with AL amyloidosis (32% in the first year), but does not translate into an improvement in survival. In fact, AL amyloidosis, with its rapid negative course after the onset of heart failure, has a higher risk of incidence of ventricular arrhythmias compared with the risk of the ATTR form. However, there are few systematic studies available to date regarding the prevalence of ventricular arrhythmias and despite clear evidence of arrhythmic risk in patients with cardiac amyloidosis, guidelines for the management of these patients are still limited (76-78).

6 Methods

6.1 Study population

A multicenter data base including cardiomyopathy dedicated unit committed to amyloid clinical management was settled. From six referral centres (Rome Sant'andrea Hospital, Genova Policlinico S. Martino, Trieste ASUGI, Firenze Azienda Ospedaliera Careggi, Messina Policlinico universitario, Padova Policlinico universitario), we retrospectively collected data of CA patients charts. Clinical data at first and last evaluation were collected, with a special focus on ECG characteristics and arrhythmia.

6.2 Diagnostic work-up

As per standard clinical practice diagnosis of AL amyloidosis was confirmed by biopsy of abdominal fat pad or of an involved organ. All positive biopsies demonstrated typical Congo Red birefringence under polarized light and staining by anti-k or anti-λ light-chain antibodies at immune histochemistry or electronical microscopy, combined with elevated serum or urine levels of the corresponding monoclonal light chain (79). Diagnosis of TTR amyloidosis was confirmed by tissue biopsy stained with anti-TTR antibodies (irrespectively of cardiac uptake at bone-tracer scintigraphy), or according to the non-invasive algorithm suggested by Gillmore et al.[80] whenever a positive tissue biopsy was not available. The non-invasive algorithm required a combination of a Perugini grade 2 or 3 cardiac uptake on bone-tracer scintigraphy with the absence of either an abnormal free light chain ratio or a detectable serum/urinary monoclonal protein by immunofixation, in a patient with echocardiogram and/or MRI suggestive of CA. The diagnosis of

cardiac involvement in AL amyloidosis was made according to the International Symposium on Amyloid and Amyloidosis criteria[81]. Cardiac involvement in TTR amyloidosis was defined by demonstration of amyloid deposits on the endomyocardial biopsy or by a positive 99mTc-hydroxymethylenediphosphonate (TcHMDP) or 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid (Tc-DPD) imaging. All patients with ATTR amyloidosis had undergone sequencing of the TTR gene and 30 subjects were diagnosed with ATTRv amyloidosis.

Clinical variables and parameters

Clinical data including weight, height, systolic and diastolic blood pressure obtained at the first visit to the Center, NYHA functional class clinically assessed at the time of the first visit to the Center, were considered for each patient. History of hypertension, coronary heart disease and atrial fibrillation were investigated.

It has been indicated whether the patient has a Pacemaker (PM) or Implantable Cardiac Defibrillator (ICD) in primary or secondary prevention. The date of the last contact with the patient has been specified or, if the patient has died, the date of death.

For each patient ECG clinical and biohumoral data were collected at the last visit available at the referring center. Among biohumoral data according to center clinical practice standard TN or High sensitive Troponin, NT-proBNP or BNP were collected.

In accordance with the guidelines of the ethics committee of each the Center, written informed consent was obtained for each patient to use clinical data for research purpose

6.3 Electrocardiographic variables and parameters

During each visit to the Center, all patients underwent 12-lead electrocardiography. For the purposes of the study, the ECG performed during the first visit to the Center (1^{ST} evaluation ECG) and the ECG performed at the most recent visit to the Center (2^{nd} evaluation ECG) were taken into

consideration. The presence of sinus rhythm (RS) or atrial fibrillation (AF) was defined and heart rate (HR) and mean ventricular rate were indicated, respectively. Atrioventricular conduction abnormalities, i.e. first, second or third degree atrioventricular blocks (BAV1; BAV2; BAV3) were identified and right bundle bundle block (RBBB) left bundle bundle block (LBBB) and left anterior fascicular block (EAS) were considered for intraventricular conduction abnormalities. Atrioventricolar delay was also measured as a continuous variable between the onset of the p wave and the onset of QRS wave. In the same way QRS duration and P wave duration in milliseconds (ms) was measured. Both peripheral and precordial low voltages and both anterior and inferior pseudonecrosis or the presence of T wave inversion were investigated. Ventricular repolarization T waves reversed in anterior, lateral, or inferior location were identified. Finally, it was indicated whether overall the ECG is normal or pathological.

Atrial Fibrillation was identified using the following elements:

• Absence of P waves;

• Irregular R-R intervals;

• Evidence of atrial activation f-waves characterized by low amplitude, frequency of 400-650 per minute and variable morphology.

Atrioventricular conduction alterations of the first degree atrioventricular block (1^{st} degree AV block) type were identified using the following elements:

• Each P wave is followed by the QRS complex and the frequency of the QRS complexes is regular between them.

• Duration of the P-R interval> 200 ms.

Atrioventricular conduction alterations of the second degree atrioventricular block (2^{nd} degree AV block) type were identified using the following elements:

• Progressive lengthening of the P-R interval until the sinus impulse is not conduced to the ventricles, with resumption at the next beat of the P-R interval of normal duration and a new

progressive lengthening (Mobitz 1);

• Rhythmic P waves and constant P-R interval, however in the presence of occasional P waves not conducted to the ventricles and therefore not followed by the QRS complex (Mobitz 2).

Atrioventricular conduction alterations of the third degree atrioventricular block (3^{rd} degree AV block) type were identified using the following criterion:

• Rhythmic P waves without their QRS complex. No sinus impulses reach the ventricles, which are activated by another pacemaker located distal to the block (atrioventricular dissociation).

Right bundle branch block (RBBB) changes in intraventricular conduction were identified using the following elements.

• QRS lasting> 120 ms;

• rSR 'complex in the right precordial muscles (V1-V2) with a wide R' wave (≥ 40 ms);

• qRS complex in left precordial muscles (V5-V6) and in D1 and aVL with wide S wave (≥ 40 ms);

Left bundle bundle branch block (LBBB) changes in intraventricular conduction were identified using the following:

• QRS lasting> 120 ms;

• QS or rS complex in the right precordial muscles (V1-V4);

• Complex R, RR 'or RsR' in the left precordial muscles (V5-V6) and in D1 and aVL.

Left anterior fascicular block (EAS) type intraventricular conduction changes were identified using the following:

• QRS of normal duration (<110 ms);

• qR complex in D1 and aVL;

• rS complex in D2, D3 and aVF;

• Electric axis between -30 ° and -90 °.

Low voltages were identified by the following elements:

• Low peripheral voltages: total QRS amplitude <5 mm in all peripheral leads (D1, D2, D3, aVR,

aVL, aVF);

• Low precordial voltages: total QRS amplitude <10 mm in all precordial leads (V1, V2, V3, V4, V5, V6).

Pseudonecrosis was identified using the following elements:

• Q wave duration> 40 ms;

• Q wave amplitude> 25% of the total QRS amplitude;

• Anterior pseudonecrosis: the aforementioned criteria found in leads V1-V4 in at least two contiguous leads;

• Lower pseudonecrosis: the aforementioned criteria found in at least two leads between D2, D3 and aVF;

• Negative medical history for ischemic heart disease.

6.4 Echocardiography

All patients underwent a comprehensive echocardiographic evaluation according to the American Society of Echocardiography recommendations[16]. Interventricular septum (IVS) thickness,. LV ejection fraction (LVEF) was assessed using biplane Simpson's equation method. Tricuspid annular plane systolic excursion (TAPSE) was measured to assess global right ventricular function. The E-to-e′ ratio was assessed as the ratio between early diastolic trans-mitral flow velocity (E) and the average of tissue Doppler derived early diastolic peak velocity (e') at lateral and medial mitral annulus.

6.5 ECG 24 hour Holter monitoring

During the follow-up at the Center it is common for patients, especially those in whom there are alterations to the 12-lead electrocardiogram or with symptoms of suspicion, undergo one or more in-depth studies using ECG Holter. Each exam has a duration of 24 hours of continuous recording. Average, minimum and maximum heart rates were assessed. The possible detection of atrial fibrillation was indicated even for only a part of the total recording time. Atrioventricular conduction alterations were evaluated. The presence of pauses greater than 2 seconds and pauses greater than 3 seconds has been indicated. The total number of supraventricular ectopic beats (BESV), the number of single ventricular ectopic beats (BEV) and the number of ventricular pairs (CPT) were reported. The presence of non-sustained ventricular tachycardia (TVNS) was evaluated, how many times it occurred, the maximum number of beats belonging to the longest run and the maximum speed reached in the fastest run. Finally, the presence of sustained ventricular tachycardia (SVT) was indicated.

6.6 Statistical analysis

Continuous and categorical variables were expressed as mean ± standard deviation and as frequencies or percentages and were compared with ANOVA followed by Bonferroni post Hoc analysis and with Pearson $\chi 2$ test, respectively. Time to all-cause mortality was the endpoint selected for survival analysis in AL group, ATTR group and overall population while a Log Rank (Mantel-Cox) test was used to test the equality of survival distribution according to electrocardiographical variables . Analyses were performed with SPSS 20 Version IBM Package, with values <0.05 considered statistically significant.

7 Results

7.1 Baseline characteristics

A total of 459 patients (129 with AL and 322 with ATTR-related amyloidosis) were enrolled in the study. In the ATTR subgroup, 266 patients were wild-type while 66 carried a TTR mutation (22 pure cardiac phenotype Ile68Leu, Val122Ile, and 44 mixed phenotype). At baseline ATTRWT were older, more often male (9 to 1 male to female ratio) and with higher BMI compared to other groups.

AL patients showed higher resting heart rate (HR) and were more often in NYHA functional class III than ATTR patients (**Table 1**). Troponin hs I serum levels were higher in AL patients and significantly lower in ATTRM patients while creatinin level were higher in AL subjects. BNP was available for 126 subjects, despite a gradient with lower values in ATTRm no significant difference was evidenced.

On the other hand NT-proBNP that was available in a larger population, i.e. 236 subjects, showed significantly lower values in ATTRm patients compared to AL and ATTRwt.

Compared to AL and ATTRm patients, those with ATTRwt related amyloidosis had larger Left Atrial (LA) chambers, thicker InterVentricular Septum (IVS) thicness, lower LV ejection fraction and higher Pulmonary Sistolic Artery Pressure (PASP). While the presence of a restrictive pattern was not statistically diferent between groups, E/E' ratio was significantly better in ATTRm subjects. No significant difference was showed in RV systolic function according to Tricuspid Annulus Plane Systolic Excursion (TAPSE).

7.2 Electrophysiological profile at presentation

At the time of diagnosis, the prevalence of at least one episode of AF greatly higher in ATTRwt patients (148/266 patients, 55%) (**Table 1, figure 11**). Despite not significant a clear trend in PM implant before CA diagnosis was present. In fact 6% and 9 % and 13% of AL, ATTRm and ATTRwt patients reported a PM implant before diagnosis. The reason of device implantation prior to first diagnosis were similar between groups and were, slow AF, extreme bradycardia/advanced AV block, ablate and pace for resistant high rate AF, and chronotropic incompetence.

ATTRwt showed higher degree of atrio ventricular and intraventricular conduction delay with significantly prolonged PR interval and QRS duration (**Figure 12**). On a morphological analysis ATTRwt

patients showed more often a Left Bundle Branch Block (LBBB) while Right Bundle Branch Block was similar between the three etiology. No advanced BAV were recorded during baseline ecg evaluation while the prevalence of first degree AV block was by far more common in ATTR wt patients compared to other groups.

Conversely, low voltage pattern was more common in AL patients, occurring in 50% (61/122) . T wave inversion and the presence of anterior pseudenocrosis was also more common in AL patients compared to other etiology.

Table 1 baseline clinical biohumoral echocardiographic and ecg characteristics at diagnosis

	AL (N=127)	ATTRm (N=66)	ATTRwt (N=266)	P=
BMI	24,3±3,5	24,8±4,0	25,6±3,2*	0,002
Age y	66,2±10,2	66,4±11,8	79,1±6,1 **	0,0001
Gender m/f	83/44	50/16	249/17	0,0001
NYHA class				
I	22	12	38	0,008
II	62	46	161	
III	38	8	65	
IV	5	0	2	
IVS mm	15,5±2,6	16,7±3,8§	17,8±3,2**	0,000
LA diameter mm	44,3±7,9	42,3±6,1	46,9±7,3**	0,000
LVEF %	55,9±9,3	55,7±9,5	52,2±10,8**	0,001
E/E'	17,9±8,6	14,1±6,5§	18,3±8,1	0,002
Restrittive pattern Y/N	44/83	21/45	173/93	0,189
TAPSEmm	18,6±4,5	18,5±4,5	18,3±4,5	0,870
PASP mm hg	33,7±11,2	32,7±10,9	38,9±12,5**	0,000
Troponin hs N=(169) pg/ml	164±224	40±38£	100±159	0,007
Creatinin mg/dl	1,5±1,1	0,94±0,28§	1,19±0,42**	0,0001
BNP n=126	668±595	345±518	504±449	0,065
NT-proBNP ng/l N=233	5808±7636	2408±2401£	4625±4828	0,0001
Heart rate BPM	78±14&	73±10	72±12	0,0001
AV 1st degree block y/n	24/81	17/36	79/72	0,0001
PR ms	176±35	184±38	205±47**	0,002
QRS ms	103±23	103±22	113±32**	0,001
QRS score mv	101±45	98±35	116±37 **	0,0001
LBBB Y/N	4/122	6/56	34/224	0,009
RBBB Y/N	16/110	11/52	48/210	0,343
Low voltages Y/N	61/61	20/42	76/178	0,001
T wave inv Y/N	45/82	9/54	43/214	0,000
Pseudonecrosis ant	47/79	16/47	61/196	0,019
Pseudonecrosis Inf	13/112	7/54	31/226	0,892
Hystory of AF Y/N	40/87	15/51	148/118	0,0001
PM implant at diagn. Y/N	8/119	6/60	34/232	0,135

*TTRwt vs AL, ** TTRwt vs other, § TTRm vs Other, £ TTRmvs AL,& al vs other

Figure 11 Different ECG rhythm (in percentage) at first evaluation according to amyloid subtype

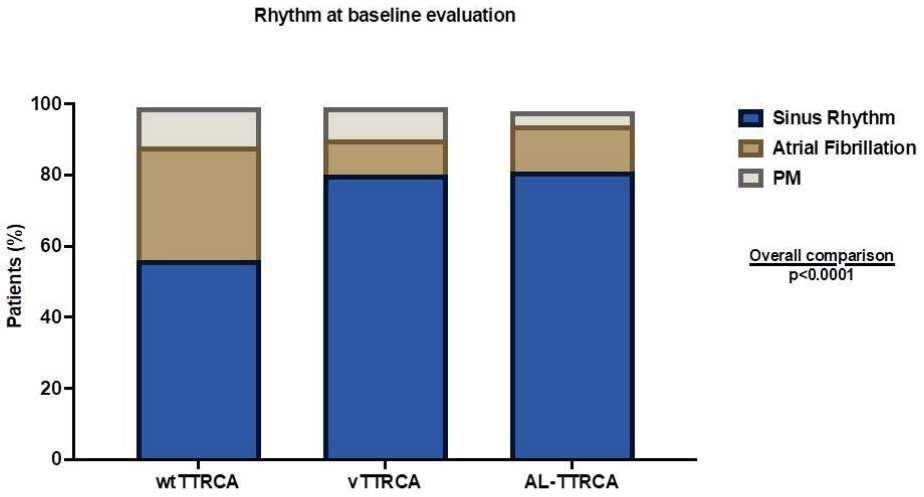
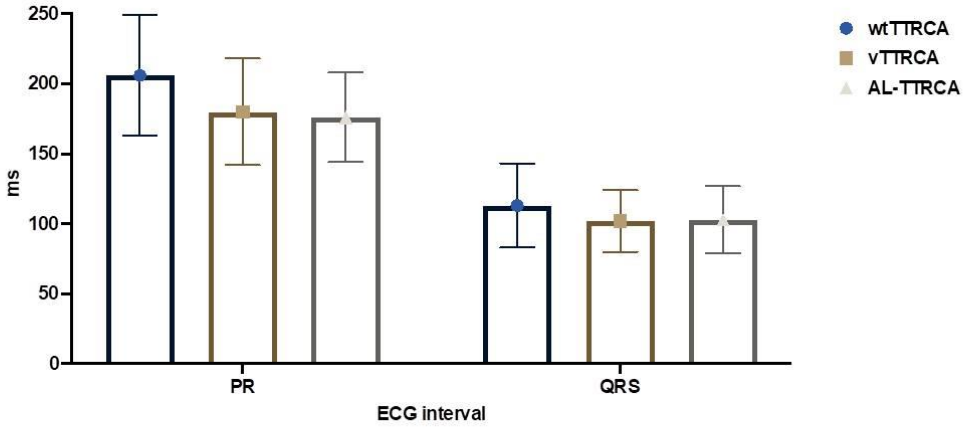


Figure 12

QRS and PR interval duration in ms according to amyloid subtype at first evaluation



7.3 ECG abnormalities and arrhythmias during follow-up

At the end of follow up a worsening in HF symptoms class according to NTHA classification was evidence in all the three CA subtypes (Table 2). No difference was evidenced in resting heart rate while significantly higher plasma NT-proBNP, creatinine and Troponin hs plasma values were evidenced in AL patients. Al patients on the other hand showed smaller IVS thickness and higher LV EF compared to ATTRwt and ATTRm patients, RV systolic function showed a reduction in ATTRwt patients with higher PASP values. LV diastolic function showed lower E/E' values in ATTRm patients compared to other etiologies while the frequency of restrictive pattern was not significantly differ between groups.

PM implant was required in almost 10% of subjects irrespectively to the CA etiology subtypes. On the other hand despite quite common in all subtypes new AF incidence was higher in ATTRwt. Moreover the incidence of AF in ATTRwt was significantly higher with more than 70% of patients presenting at least one episode of atrial arrhythmia in medical history.

TTRwt patients, as in the baseline evaluation, presented higher incidence of LBBB, prolonged PR interval and QRS duration. The prevalence of low voltage and T wave inversion at follow-up was greater in AL subjects, while the prevalence of a pseudonecrosis pattern was comparable between groups.

Major arrhythmic events were absent in ATTRm group and rare in AL and TTRwt subgroup respectively recorded in almost 5% and 3% of cases. In cardiac AL amyloidosis 2 patients presented an episode of ventricular fibrillation and 4 a sustained ventricular tachycardia. IN ATTRwt 6 patients presented at least one episode of sustained ventricular tachycardia.

Table 2 baseline clinical biohumoral echocardiographic and ecg characteristics at follow up

	AL (N=127)	ATTRm (N=66)	ATTRwt (N=266)	P=
Follow up days	709±658	741±431	585±422	0,017
NYHA class				
I	19	7	35	0,715
II	52	33	126	
III	45	22	88	
IV	10	3	12	
IVS mm	15,6±3,0&	17,4±3,7	18,4±3,4	0,0001
LA diameter mm	46,2±8,4	44,3±6,7	49,3±8,7**	0,0001
LVEF %	53,6±10,2	44,3±6,7£	49,3±8,7*	0,004
E/E'	18,0±8,6	14,8±6,3&	18,9±7,4	0,01
Restrittive pattern Y/N	49/73	25/39	110/130	0,450
TAPSE mm	17,9±5	17,4±4,7	15,8±4,6**	0,0001
PASP mm hg	33,9±11,4	32,7±9,3	38,4±11,5**	0,0001
Troponin hs N=169 pg/ml	161±353	59±79	104±180	0,098
Creatinin mg/dl	1,80±1,35&	1,16±0,65	1,37±0,49	0,0001
BNP n=109	770±966	271±255	598±644	0,102
NT-proBNP N=280 ng/l	6430±9821	2917±3201£	4463±4461	0,012
Heart rate BPM	78±14	75±15	77±15	0,480
PR ms	187±43	194±41	212±44**	0,0001
QRS ms	112±26	109±25***	120±31	0,009
QRS score mv	105±57	99±40	113±41	0,042
LBBB Y/N	9/119	10/53	41/207	0,050
RBBB Y/N	25/97	12/50	51/97	0,977
Low voltages Y/N	61/61	20/42	76/178	0,001
T wave inversion Y/N	42/81	9/53	48/200	0,001
Pseudonecrosis ant	46/76	19/43	64/184	0,062
Pseudonecrosis Inf	12/111	10/52	41/209	0,213
New onset AF Y/N	19/81	9/44	43/93	0,0001
History of AF	59/68	24/42	191/75	0,0001
PM implant during FU Y/N	11/111	4/56	27/208	0,108
Major arrhythmic events	6/121	0/66	6/266	0,119

*TTRwt vs AL, ** TTRwt vs other, *** TTRm vs TTRwt, § TTRm vs Other, £ TTRmvs AL,& AL vs other

7.4 Incidence and impact of ventricular arrhythmia at Holter monitoring

We retrospectively analysed data from 24-hours ECG monitoring of 181 patients (67% male, age 74.6 $\pm$ 9 years). In particular, 51 (28.2%) patients had AL amyloidosis, 105 (58%) patients wild-type transthyretin (ATTRwt) amyloidosis and 25 (13.8%) patients mutated transthyretin (ATTRm) amyloidosis. Median time from diagnosis to 24-hours ECG monitoring was 8 months (range 0-70 months), with 32% of patients completing the 24-hours ECG monitoring within 3 months from diagnosis. Median follow-up (defined as time from ECG monitoring to last evaluation or death) was 12 (1-93) months. A total of 53 patients (29.3%) experienced NSVT during the 24-hours ECG monitoring. The mean number of beats of NSVT was 7 (range 3-22) and mean rate of all the runs was 149 (range 120-218) beats per minute. Prevalence of NSVT was similar in the three subtypes of CA: 17/51 (33%) in AL, 31/105 (29.5%) in ATTRwt, and 5/25 (20%) in ATTRv amyloidosis (p=0.73). Patients with NSVT presented higher prevalence of NYHA functional class III-IV (p=0.03), lower left ventricular (LV) ejection fraction (p=0.02) and higher NTproBNP values (p=0.03) compared to those without NSVT at ECG Holter monitoring (Table 1). When analysing data according to CA subtypes, AL patients with NSVT had lower LV ejection fraction (p= 0.02) and higher troponin value (p=0.02), whereas ATTRwt patients with NSVT were mainly female (p=0.03), younger (p=0.04) and with slightly higher NT-proBNP values (p=0.08) compared to patients without NSVT. No significant clinical differences were noted between ATTRv patients with and without NSVT. NYHA functional class III-IV was the only independent predictor of occurrence of NSVT at 24-hours ECG monitoring (OR 2.00, 95% CI 1.03-4.10, p=0.03). During follow up, 14 (26%) of the 53 patients with NSVT and 30 (23%) of the 128 patients without NSVT died (p=0.80). Four patients died suddenly (1 had NSVT), 34 patients died due to advanced decompensated HF (12 had NSVT) and 6 patients died for non-cardiac causes (none had NSVT). Fifty-four CA patients underwent hospitalization for congestive HF, 14 (26%) with and 40 (31%) without NSVT (p=0.31) (Table 1). At survival analysis, presence of NSVT was not related to

outcome (combined end-point of death and HF hospitalization) in the total population and in each amyloidosis subtype (log rank, p=0.64).

Table 3 . Clinical characteristics of CA patients with and without NSVT at holter recording

	NSVT n=53	**No NSVT n=128**	**p**
Age, years	74 ± 8	75 ± 10	0.82
Male sex	33 (62)	89 (69)	0.21
Amyloidosis subtype			0.73
AL	17 (32)	34 (27)	
ATTRv	5 (9)	20 (16)	
ATTRwt	31 (58)	74 (57)	
NYHA functional class III/IV	22 (41)	33 (26)	0.03
Atrial fibrillation	7 (29)	13 (24)	0.43
Beta blockers	27 (51)	54 (42)	0.19
Antiarrhythmic drugs	2 (4)	8 (6)	0.72
NTproBNP, pg/ml	4147 (236-34782)	2515 (38-30198)	0.03
Troponin I, ng/ml	0.07 (0.01-1.9)	0.07 (0.005-1.1)	0.28
Maximal wall thickness, mm	18 ± 3	17 ± 3	0.17
Left atrium dimension			0.67
Normal	0 (0)	3 (2)	
Mild (<42 mm)	12 (23)	24 (19)	
Moderate (42-46 mm)	21 (40)	53 (42)	
Severe (> 46 mm)	20 (38)	47 (37)	
LV ejection fraction, %	51 ± 8	54 ± 10	0.02
TAPSE, mm	17 ± 5	18 ± 5	0.17
Follow up, months	17 ± 15	17 ± 16	0.95
Death, n (%)	14 (26)	30 (23)	0.40
HF Hospitalization	14 (26)	40 (31)	0.31
Combined endpoint	19 (36)	53 (41)	0.51

Categorical variables are presented as absolute frequencies with percentages and were compared using Chi-squared test or the Fisher exact test; continuous variables are presented as mean value ±

standard deviation or median (range) and were compared using the Kolmogorov-Smirnov test. Abbreviations: CA, cardiac amyloidosis; AL, light chain amyloidosis; ATTRv, mutated transthyretin amyloidosis; NSVT, non-sustained ventricular tachycardia; ATTRwt, wild-type transthyretin amyloidosis; HF, heart failure; LV, left ventricle; TAPSE, tricuspid annular plane systolic excursion

7.5 Impact of electrocardiographic abnormalities on mortality prediction in TTR cardiomyopathies

At the end of follow up in the ATTR cardiomyopathy group were recorded 5 heart transplantation, 3/66 in the ATTRm CA group and 2/266 in the ATTRwt CA group (p=0,05). In ATTRm CA were recorded 6 death while in ATTRwt subgroup 43 patient deceased (p=0,20).

Clinical and demographic characteristics are reassumed in table 4. Significant differences between survival and deceased or transplanted patients in LVEF, NYHA class and NT-proBNP plasma values. History of AF, or presence at first evaluation of permanent AF were not linked to a worse outcome at follow up. Either the presence of a PM or prolongation of atrioventricular conduction delay were not related to survival. On the contrary atrioventricular conduction delay according to QRS duration and also morphological conduction disturbances as LBBB and RBBB were significantly correlated to worse outcome. A Kaplan Meyer curves according to QRS duration above 120 ms were performed (**Figure 13**)

Figure 13 Kaplan Meier survival analysis according to QRS duration in ms (cut off 120 ms)

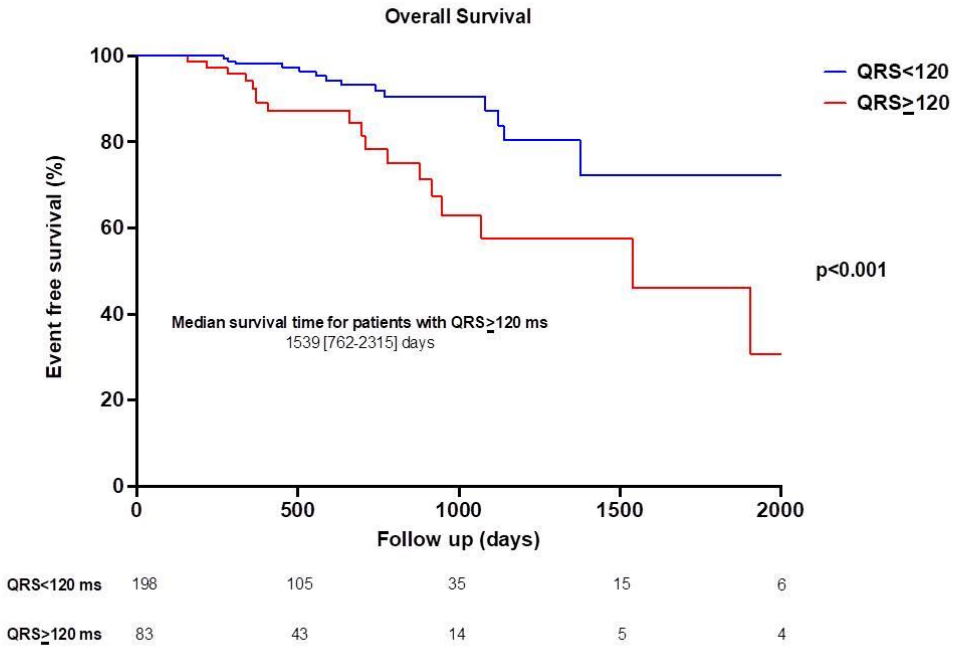


Table 4. Clinical characteristics of ATTRCA patients according to survival at end of follow up

	Alive n=284	Death or TXP n=48	p
Age, years	76 ± 9	76 ± 9	0.82
NYHA functional class			
I	49	1	0,001
II	181	23	
II	53	23	
IV	1	1	
AF medical Hystory	137/284	26/48	0.273
PM implant at diagnosis	32/253	8/48	0,201
LBBB	30/277	10/43	0,002
RBBB	46/277	13/43	0,015
Low voltages	85/279	12/45	0,151
Pseudonecrosis	65/276	12/44	0,145
Heart rate bpm	72±12	72±12	0,891
PR ms	201±46	194±41	0,433
QRS ms	108±29	125±37	0,001
QRS score mv	116±32	116±36	0,906
T wave inversion	45/276	7/44	0,166
Restrictive pattern	95/284	19/48	0,264
NT-proBNP pg/ml	3901±4231	6377±1461	0,027
IVS mm	17 ± 3	18 ± 3	0,399
LV ejection fraction, %	53 ± 10	49 ± 12	0,009
TAPSE, mm	18 ± 5	17 ± 4	0,210
E/E’	17±8	18±9	0,282

Categorical variables are presented as absolute frequencies and were compared using Chi-squared test or the Fisher exact test; continuous variables are presented as mean value ± standard deviation or median (range) and were compared using the Kolmogorov-Smirnov test. Abbreviations: CA,

cardiac amyloidosis; LV, left ventricle; TAPSE, tricuspid annular plane systolic excursion, RBBB right bundle branch block, LBBB left bundle branch block.

According to multivariable Cox regression analysis (**Table 5**), qrs duration was the only electrocardiographic predictor of a combinate end point of death or heart transplantation.

Table 5 Cox regression model

	B	**SE**	**Wald**	**Sig.**	**Exp(B)**	**IC 95,0% per**	
QRS ms	-,007	,003	7,695	,006	,993	,988	,998
Age y	,016	,007	4,475	,034	1,016	1,001	1,031
LVEF	-,014	,007	3,383	,066	,986	,972	1,001
NYHA	-,239	,123	3,745	,053	,787	,618	1,003

8 Discussion

8.1 ECG evaluation at baseline and follow up

Through our retrospective study we wanted to investigate the frequency of arrhythmic phenomena and ECG abnormalities in patients with amyloidosis, making as a final analysis the comparison of the final electrocardiographic pattern in the subcategory of patients with ATTRm and ATTRwt amyloidosis compared to the subgroup of patients with AL amyloidosis.

During first ECG evaluation, atrial fibrillation was, by far, the most common abnormality in ATTR patients . This result is in line, but with an increase prevalence compared to the data reported in the literature (82-84). This net prevalence of atrial fibrillation in patients with ATTR amyloidosis, is probably due to the high tropism of transthyretin for the heart. For example, Yamamoto and colleagues report a prevalence of atrial fibrillation of 11% for AL amyloidosis and 38-67% for ATTRwt amyloidosis (85). The study by Sanchis and colleagues (82) also demonstrates the higher frequency of atrial fibrillation in ATTRwt amyloidosis (71%; n = 123) compared to AL amyloidosis (26%; n = 115). However, in this same study, the authors highlighted that this arrhythmia has no impact on cardiovascular mortality in both categories of patients.

Intraventricular conduction delay was the most frequent baseline electrocardiographic abnormality for patients with ATTRwt amyloidosis while among the subgroup of patients with AL amyloidosis the most frequent finding on the first ECG were low voltages, with a surprisingly superimposable presence with ATTRm subgroup. Low voltage QRS in CA has been explained by amyloid fibril deposition leading to myocyte destruction and replacement with electrically silent material. The pathophysiological basis for the higher prevalence of low voltage in AL amyloidosis could be related to greater cardiomyocyte damage (despite lower grade of amyloid deposition) induced by light-chain toxicity. In this scenario we can hypothesized that also ATTRm precursor can induced a similar degree of cardiotoxicity that is less represented in ATTRwt subtype.

While is now a well-established aspect that this ECG finding is more frequent in the AL form and numerous studies confirm this (84,86), is not reported a so high prevalence in variant ATTR cardiomyopathy.

On the other hand, as regards intraventricular and atrioventricular conduction delay , the results of our analysis revealed the presence of a more pronounced delay in ATTRwt patients with, despite not statistically significant an higher degree of PM implant before CA diagnosis. A pseudonecrosis pattern was present at baseline in approximately one third of patients in all CA subgroups. At follow-up visit, the prevalence of pseudonecrosis had remained similar in the three groups consistent previous studies [87-88]

The analysis of ECG data referring to the last contact at the Centers (Table 2) highlighted and confirmed the presence of statistically significant differences between patients with ATTRwt, ATTRm and AL amyloidosis as regards the prevalence of atrial fibrillation, new onset atrial fibrillation during follow up, atrio-ventricular and intra ventricular conduction delays.

8.2 Ventricular arrhythmia at Holter monitoring

The occurrence of NSVT during 24-hours ECG monitoring is common in CA, being present in about a third of patients, regardless of the amyloidosis aetiology. The prevalence of NSVT in our cohort is in line with previous studies both on AL amyloidosis and other cardiomyopathies (89-92). CA patients with NSVT had more symptomatic HF with lower ejection fraction and higher biomarkers values. However, the presence of NSVT on 24-hours ECG monitoring did not confer a worse survival, suggesting that these arrhythmias may be considered a marker of disease severity rather than a predictor of arrhythmic mortality. Different circumstances may contribute to lessen the prognostic weight of NSVT in CA. First, in most cardiomyopathies ventricular arrhythmias are predictors of sudden death, but this dramatic complication seldom occurs in CA and its causes still

remain unclear (electromechanical dissociation, ventricular tachyarrhythmia or advanced AV block). Moreover, as evolving therapies are leading to a significant outcome improvement in CA, the influence of NSVT on prognosis may be mitigated or undervalued in a mid-term follow up, as that in our study.

The close association of NSVT with other clinical features of severe HF underscores the importance of 24-hours ECG monitoring for a complete assessment of the clinical profile of patients with CA. Longitudinal and longer ECG monitoring studies are warranted to provide additional characterization and prognostic information about ventricular arrhythmias in CA.

8.3 Survival prediction analysis in ATTR CA amyloidosis

According to survival analysis no difference were reported in ATTR CA patients with low voltages or reduced QRS scores. These results are in contrast to two other studies (93-94) that have demonstrated an increase mortality in AL patients with low voltages at first evaluation. Our result are not surprisingly due to the great pathophysiological difference between AL amyloidosis and TTR amyloidosis. Has previously hypothesized by Rapezzi et all. (95) low voltages could be more strictly related to the direct toxic effects of light chains and AL amyloidosis precursor more than the amount of amyloid already deposited in tissues. In ATTR CA amyloid precursors seems to have less toxic with cardiomyopathy more related to the amount of misfolded protein settled between myocites. On the other hand our research clearly confirmed that the delay in intraventricular conduction is related with a worse outcome, both according QRS duration or different pattern of bundle branch blocks. This correlation has been already suggested in other researches again mainly based on AL CA patients (63,96). Further study are needed to understand if , in ATTR CA the increase in intraventricular delay or the development of bundle branch block are related to the amount of amyloid settled in tissue or a related to direct damage of the intraventricular conduction system.

9. Conclusion

In conclusion, the three main types of CA show markedly different electrophysiological profile, both at baseline and during follow-up, dictating important aspects of clinical management. Overall, AF and atrio ventricular and intraventricular delays are more common in ATTR patients especially in the TTRwt subtype. Regarding to the Holter monitoring surveillance we can assume that the occurrence of NSVT is common in CA, being present in about a third of patients, regardless of the amyloidosis aetiology. CA patients with NSVT had more symptomatic HF with lower ejection fraction and higher biomarkers values but the presence of NSVT did not confer a worse survival, suggesting that these arrhythmias may be considered a marker of disease severity rather than a predictor of arrhythmic mortality. These findings have implications for the implementation of tailored arrhythmic surveillance strategies in CA cohorts underscoring the importance of serial 24-hours ECG monitoring during follow up and the evaluation of longer ECG monitoring studies are warranted to provide additional characterization and prognostic information about ventricular arrhythmias in CA.

10 Limitation

The study was retrospective and conducted among referral centres for the diagnosis and management of AL and ATTR-CA in Italy. Patients with CA are a heterogeneous cohort with different stages of disease severity. he heterogeneity of TTR mutations did not allow to perform subgroup analyses in specific cohorts on ATTRv-CA patients. The National Amyloidosis Centre stage could not be measured as NT-proBNP values were not available for the whole study cohort.

For Holter ECG monitoring analysis data were available only iin a minority of subjects and median time from diagnosis and Holter recording was 8 months.

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List of publication published in peer review journals during the PHD.

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