Oxidative damage in immunoglobulin light chain and transthyretin cardiac amyloidosis - a closer look

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Abstract

Heart failure is a progressive disease, representing a growing cause of morbidity, hospitalization and mortality. An increasingly common type of heart failure with preserved ejection fraction (HFpEF) is the immunoglobulin light chain and transthyretin cardiac amyloidosis, in the pathophysiology of which oxidative damage appears to exert a strong impact. Reactive oxygen and nitrogen species have physiological signaling functions, but their overaccumulation, as in cardiac amyloidosis, leads to cardiomyocyte damage and apoptosis, and to cardiac hypertrophy and fibrosis. Moreover, such pathological processes worsen the redox damage with the perpetuation of an inflammatory state, in a vicious cycle. Here reviewed are the role of oxidative damage in the transthyretin and immunoglobulin light chain cardiac amyloidosis, the underlying pathogenic mechanisms, the therapeutic implications and possible future strategies.
Disclosure of interest

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Abbreviations and acronyms:

α-MHC  Alpha-myosin heavy chain
6MWT  Six-minute walking test
AL  Light chain immunoglobulin amyloidosis
ASCT  Autologous stem cell transplantation
ATTR  Transthyretin amyloidosis
ATTR-CA  Transthyretin cardiac amyloidosis
BNP  B-type natriuretic peptide
CA  Cardiac Amyloidosis
CamKII  ROS-activated calmodulin-dependent protein kinase II
CCM  Cardiac contraction modulation therapy
cGMP  Cyclic guanosine monophosphate
CTGF  Connective tissue growth factor
DCFDA  2',7'-dichlorodihydrofluorescein diacetate
DUOX  Dual oxidase
EMRE  Electromagnetic response elements
EGCG  Epigallocatechin gallate
FAP  Familial amyloid polyneuropathy
FLC  Amyloidogenic free light chains
GSH  Glutathione
HF  Heart failure
HFmrEF  Heart failure with mildly reduced ejection fraction
HFpEF  Heart failure with preserved ejection fraction
HFrEF  Heart failure with reduced ejection fraction
hTTR  Hereditary transthyretin amyloidosis
IL-6  Interleukin-6
LC  Light chain immunoglobulin
LVEF  Left ventricular ejection fraction
MnTMPyP  Manganese (III)tetrakis(1-methyl-4-pyridyl) porphyrin
NAC  N-acetylcysteine
NYHA  New York Heart Association
NO  Nitric oxide
NOXs  NADPH oxidases
NT-proBNP  N-terminal pro-B-type natriuretic peptide
OSAS  Obstructive sleep apnea syndrome
PBT2  5,7-dichloro-2-[(dimethylamino)methyl]-8-hydroxyquinoline
PICP  Procollagen type I
PIIINP  N-terminal propeptide of procollagen type III
PVC  Premature ventricular contraction
p38 MAPK  p38 mitogen-activated protein kinase
RAAS  Renin-angiotensin-aldosterone system
ROS  Reactive oxygen species
RyR2  Ryanodine receptor 2
SAP  Serum amyloid P
SERCA  Sarcoplasmic/Endoplasmic Reticulum Ca2+-ATPase
siRNA  Small-interfering RNA
sGC  Soluble guanylyl cyclase
1. Introduction

Among the wide variety of processes caused by or associated with oxidative damage, heart failure (HF) can be addressed with a particular significance. Although reactive oxygen and nitrogen species have physiological signaling functions in the heart, a dysregulated presence of these molecules seems to promote a redox imbalance leading to cardiomyocyte damage (Finkel, 1998; Lambeth and Neis, 2014; Sundaresan et al., 1995). Indeed, cardiomyocyte stress disrupts intracellular redox homeostasis by increasing the ROS/RNS steady-state concentration (Finkel, 1998; J. David Lambeth and Andrew S. Neis, 2014). This leads to further reactive species production responsible for the damage of subcellular components. Mitochondrial dysfunction has often been found in heart failure, as it plays a key role in ROS and RNS production (Aimo et al., 2020).

Heart failure (HF) is a complex, progressive disease, characterized by the impairment of ventricular filling or systolic function leading to symptoms such as dyspnea, fatigue, peripheral or pulmonary oedema (McDonagh et al., 2021). It represents a growing cause of morbidity and mortality and altogether a common cause of hospitalization. The prognosis of patients with chronic HF is about 50% within 5 years of initial diagnosis (McDonagh et al., 2021). The overall prevalence in the European adult population is estimated to be 2%, growing exponentially with age.

Within the group of diseases leading to HF, in particular HF with preserved ejection fraction (HFrEF), cardiac amyloidosis is highlighted by the likely important role that oxidative stress has on its pathogenesis and disease progression. Its main feature is the accumulation of amyloid fibrils in the myocardium, most frequently formed from misfolded immunoglobulin light chains or transthyretin, both altering the cardiomyocyte redox balance.

This review aims to elucidate the actual impact of oxidative stress in the pathogenesis of light chain immunoglobulin and transthyretin cardiac amyloidosis, AL and ATTR respectively, with at the end a glance at the therapeutic implications.

2. Heart failure

Heart failure is classified into three phenotypes based on the left ventricular systolic function measured with the left ventricular ejection fraction (LVEF):

1) Heart failure with reduced LVEF (HFrEF) is defined by an LVEF ≤ 40%;
2) Heart failure with mildly reduced LVEF (HFmrEF) is defined by an LVEF between 41% and 49%;
3) Heart failure with preserved LVEF (HFrEF) includes patients with an LVEF ≥ 50% and symptoms and signs of HF, with evidence of structural and/or functional cardiac abnormalities and/or raised natriuretic peptides (NPs).

HFrEF has long been considered the most frequent form, its pathology is well known and often characterized by an inflammatory response and oxidative damage resulting from a
133 primary cardiac insult, peripheral tissue hypoperfusion or pressure and volume overload 
134 (McDonagh et al., 2021).

Interestingly, HFpEF can derive from a combination of risk factors and comorbidities, 
135 like aging, female gender, obesity, systemic arterial hypertension, atrial fibrillation, diabetes 
136 mellitus, chronic kidney disease, anemia, iron overload, obstructive sleep apnea syndrome 
137 (OSAS) and chronic obstructive pulmonary disease (COPD) (McDonagh et al., 2021; Pieske et 
138 al., 2019). Notably, the large majority of these comorbidities is actually associated with 
139 oxidative stress and inflammation, two conditions indeed steadily associated with HFpEF as 
140 well. Furthermore, HFpEF can originate from infiltrative cardiomyopathies, such as 
141 amyloidosis, sarcoidosis, hemocromatosis, Fabry’s disease and glycogen storage disorders 
142 (McDonagh et al., 2021).

3. Light chain and transthyretin cardiac amyloidosis

In the context of HFpEF, cardiac amyloidosis seems to be a disease characterized by a 
dysregulated inflammatory response and ROS imbalance.

Amyloidosis is a group of multi-organ disorders caused by the extracellular deposition 
of proteolysis-resistant misfolded and insoluble proteins. Different precursor proteins can 
turn into amyloid fibrils, altering tissue structures and leading to various clinical phenotypes 
related to the specific protein infiltration (Lachmann and Hawkins, 2006).

Light chain amyloidosis (AL) and transthyretin amyloidosis (ATTR) are the two forms 
of amyloidosis with greater cardiac involvement, which are often the main cause of morbidity 
and mortality, regardless of the underlying cause of amyloid accumulation (Martinez-Naharro 
et al., 2018). In Table 1 we summarized their main features. Although ATTR amyloidosis was 
considered a rare disease in the past, advanced imaging techniques and non-invasive 
diagnostic methods are leading to increasingly frequent diagnosis of this entity.

The prevalence of cardiac amyloidosis (CA) was studied in asymptomatic and 
symptomatic subjects and in autopsy series (Aimo et al., 2022). 0.2%-0.5% of patients 
undergoing bone scintigraphy for non-cardiac reasons were suspected of ATTR CA (Bianco et 
al., 2021; Cuscaden et al., 2021; Longhi et al., 2015; Mohamed-Salem et al., 2018). 
Endomyocardial biopsies performed in patients with HFpEF of unclear etiology showed an 
overall CA prevalence of 14% (Hahn et al., 2020). Autopsies performed in unselected elderly 
individuals > 85 years of age demonstrated the presence of amyloid in approximately 25% of 
hearts, despite the absence of established cardiomyopathy. ATTR-CA was the most frequently 
diagnosed form (Aimo et al., 2022; Mohammed et al., 2014; Paraskevaidis et al., 2023). The 
prevalence of CA increases with age, while female sex seems to be a protective factor (Aimo 
et al., 2022).

AL amyloidosis is caused by a plasma-cell dyscrasia with misfolded immunoglobulin 
light chains deposited in multiple organs, including the heart in half of the affected patients. 
Extracardiac tissue infiltration leads to proteinuria and nephrotic syndrome, peripheral 
neuropathy or autonomic dysfunction, gastrointestinal motility disorders, hepatomegaly, 
periorbital purpura, and macroglossia.

ATTR amyloidosis is caused by the accumulation of misfolded transthyretin, a protein 
produced mainly by the liver that participates in transporting thyroid hormone and retinol. 
No relationship between thyroid disorders and amyloidosis was found (Giertz et al., 2015a; 
Zanotti and Berni, 2004). The structure of transthyretin is due to DNA mutations, in the case 
of the hereditary form (hATTR), while, in the wild type ATTR (wtATTR), the ATTR tetramer
misfolding is favoured by the normal aging process (Hellman et al., 2008; Plante-Bordeneuve, 2003).

Non-hereditary wild type TTR amyloidosis is often a senile disease with a strong male predominance (Connors et al., 2016) and a relatively late diagnosis because of its gradual progression. The heart is the most affected organ, while systemic involvement is rare except for some musculoskeletal disorders such as bilateral carpal tunnel, lumbar spinal stenosis, and biceps tendon rupture (Nakagawa et al., 2016).

Hereditary TTR amyloidosis (hTTR) is caused by mutations in the TTR gene, often following the autosomal dominant pattern of inheritance. More than 100 missense mutations can predispose greater instability of transthyretin and its misfolding, which leads to an earlier onset of the disease (Gopal et al., 2019). Specific mutations, together with epigenetic factors, determine the different onset timing, the geographical distribution, the prognosis and the predominant cardiac rather than neurological phenotype, with peripheral neuropathy and autonomic dysfunctions being the most common clinical manifestations besides cardiac involvement (Gertz et al., 2015a). Specific hATTR genotypes, such as Val122Ile, are associated with a poorer prognosis (Singh et al., 2017).

4. Oxidative stress and Inflammation in the myocardium: a vicious cycle that could lead to heart failure

Under physiological conditions, a significant bulk of reactive chemical species are generated within eukaryotic cells, including leukocytes and endothelial cells, as key intermediates in a variety of reactions of biochemical (e.g. mitochondrial respiration pathways) and biological (e.g. phagocytosis) importance. The principal reactive oxygen species include superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radical (HO’), and nitric oxide (NO). The generation of reactive oxygen and nitrogen species (ROS and RNS) results from oxidation-reduction reactions driven by transitional metal catalytic interactions or by specific enzymes such as NADPH oxidases (NOXs), dual oxidase (DUOX), xanthine oxidase, and nitric oxide synthase (Figure 1) (Halliwell and Gutteridge, 2015).

The critical point is maintaining an optimal, namely physiological, quantity of ROS/RNS within cells and tissues, through a delicate and dynamic balance between their production and degradation rates. Light chain immunoglobulin has indeed developed a pleiotropic and ubiquitous defense system in eukaryotes to preserve intracellular homeostasis by avoiding an excessive production and/or a diminished removal of ROS/RNS, namely a condition termed oxidative stress (Figure 2) (Halliwell and Gutteridge, 2015; Losada-Barreiro et al., 2022).

An oxidative stress condition can deregulate the inflammatory response and stimulate apoptosis through both extrinsic and intrinsic pathways, as well as induce fibrosis in many tissues and organs including the myocardium (Park et al., 2001). Fibrosis can occur through the action of growth factors, such as transforming growth factor-$\beta$ (TGF-$\beta$) and connective tissue growth factor (CTGF), and through the influence that the renin-angiotensin-aldosterone system (RAAS) has on fibroblast differentiation (Fujii et al., 2005; Liu and Desai, 2015). A sustained increase of ROS levels, as consequence of a chronic inflammation, could heavily affect the heart physiology in different ways. Oxidative stress could interfere with intracellular calcium homeostasis mainly through the ryanodine receptor 2 (RyR2) and ROS-activated calmodulin-dependent protein kinase II (CaMKII) (Johnston et al., 2015; Zima and Blatter, 2006). In addition, ROS disproportion leads to ion imbalance, promoting arrhythmias (Jeong et al., 2012). Overexpression of NOX, an important enzyme in physiological
vasculogenesis, seems to play a major role in the cardiac hypertrophy mechanism (Kuroda et al., 2010; Zhang et al., 2010).

Moreover, heart failure features such as neurohormonal activation, pressure-volume overload, and altered cardiomyocyte metabolism come out to have a fundamental role in the perpetuation of an inflammatory state, leading to a vicious cycle difficult to stop. This is revealed by the high levels of inflammatory mediators found in patients suffering from heart failure, such as Tumor Necrosis Factor alfa (TNF-α) (Higuchi et al., 2002) and interleukin 6 (IL-6) (Alogna A, Koepp KE, Sabbah M, et al., 2023; Ridker and Rane, 2021). Moreover, comorbidities such as diabetes mellitus, overweight, and obstructive sleep apnea promote systemic inflammation, exacerbating the myocardial ROS imbalance (Franssen et al., 2016; Kalogeropoulos et al., 2010; Paulus and Tschöpe, 2013).

ROS binding to NO decreases its circulating levels, leading to lower guanylate cyclase activation with consequent lower cyclic guanosine monophosphate (cGMP) production and cGMP-protein kinase G (PKG) downregulation. cGMP-PKG regulates cardiomyocyte contractility through cellular calcium homeostasis, suppresses hypertrophic changes, and favors ventricular diastolic relaxation by phosphorylation of troponin I and titin. Notably, the pattern of lower levels of NO and myocardial PKG hypoactivity is more frequent in HFpEF than in HFrEF (Francis et al., 2010; Mongirdienė et al., 2022). Besides involving NO, ROS also directly activates the TGF-β/Smad3 pathway, promoting myocardial fibrosis (Michels da Silva et al., 2019).

5. Oxidative stress and damage at the cardiac level in AL and ATTR amyloidosis

Amyloid fibril infiltrations in the heart lead to disruption of tissue structure, biventricular wall thickening and stiffness with consequent remodeling, elevated diastolic filling pressures with occurrence of HFpEF, atrial dilation, and supraventricular arrhythmias arising from either atrial fibrillation or atrial flutter (Merlini and Bellotti, 2003). Microvascular amyloid infiltration was shown to reduce myocardial perfusion. The conduction system is also involved, with atrioventricular conduction delays.

The simple mechanical displacement of normal parenchymal tissue cannot sufficiently explain the cardiac dysfunction observed both in AL and ATTR amyloidosis. Misfolded light chain immunoglobulin, altered/misfolded transthyretin and amyloid fibrils are believed to play the primary and crucial role in tissue toxicity (Quarta et al., 2014).

Both immunoglobulin light-chain and transthyretin-related amyloidosis are steadily associated with oxidative stress, and such an unbalanced redox status appears heavily involved in the pathogenesis of these disease processes (Gertz et al., 2015b). In fact, several experimental data strongly support a significant role of these two types of misfolded proteins in inducing an excessive accumulation of ROS in cardiomyocytes (Sharma et al., 2019).

With regard to the pro-oxidant property of abnormal deposition of misfolded immunoglobulin light-chain in the myocardium, a marked increase of ROS, as detected by using the 2',7'-dichlorodihydrofluorescein-diacetate reagent (DCFDA), and a net overexpression of the heme oxygenase 1 enzyme (HO-1), reliable marker of adaptation to cell oxidative challenge, were observed in rat ventricular cardiomyocytes incubated 24 hours in the presence of light chain immunoglobulin isolated from patients with cardiac light chain immunoglobulin amyloidosis, while an identical cell treatment with light chain immunoglobulin deriving from non-amyloid myeloma patients did not lead to a rise of the two oxidative stress markers (Brenner et al., 2004). The unbalanced redox status towards
oxidation was accompanied by cardiomyocyte damage, dysfunction and apoptotic death, changes that were prevented by suitable cell preincubation with the antioxidant superoxide dismutase/catalase mimetic manganese(III)tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP) (Brenner et al., 2004).

A source of intracellular overproduction of ROS in AL amyloidosis appears to be the membrane NADPH oxidase enzyme (NOX), the isoforms of which NOX2 and NOX4 are those mainly represented in cardiomyocytes. In fact, the NOX-triggered cell signaling driven by p38 MAPK pathway (Santillo et al., 2015) was demonstrated to be upregulated in the cardiomyocytes challenged with light chain immunoglobulin deriving from patients with cardiac AL (Shi et al., 2010). In parallel, the changes observed in the so treated cardiomyocytes were significantly quenched by pretreatment with the antioxidant MnTMPyP or the selective p38 inhibitor SB203580, thus indicating that oxidative stress may act as a primary cause of cardiac damage and malfunction (Shi et al., 2010).

Of note, p38 MAPK simultaneously plays a role in the transcription of brain natriuretic peptide (BNP). This confirms the association of cardiotoxic light chain action and MAPK signaling activation with elevated BNP levels (Shi et al., 2010). The level of abnormally circulating light chains might be useful in formulating the clinical prognosis in patients with AL, as it also corresponds with cardiac biomarker elevations (Kumar et al., 2012). Moreover, specific chemotherapy with the aim of reducing the underlying clone, thus the quantity of circulating amyloidogenic free light chains (FLC) was also seen to reduce BNP levels, regardless of the amount of amyloid deposition in the myocardium (A. D. Wechalekar et al., 2022).

A second source of ROS that is most likely up-regulated in AL are the cardiomyocyte mitochondria. In the Caenorhabditis elegans treated with light chain immunoglobulins from patients with cardiac amyloidosis the impairment of the contraction-relaxation rhythm of the pharyngeal muscle, appeared dependent upon a marked mitochondrial oxidant burst as detected by the MitoSOX Red staining, since not inhibited by the pretreatment with antioxidants like N-acetyl cysteine, ascorbic acid and epigallocatechin gallate. The mitochondrial burst was not observed when the C elegans pharyngeal pumping was treated with light chain immunoglobulins from nonamyloidogenic multiple myeloma patients (Diomede et al., 2014). The same group then demonstrated that the mitochondrial ROS burden observed in C. Elegans was dependent from the presence of metal ions, in particular copper (Diomede et al., 2017).

As regards the transthyretin-related amyloidosis, the tetrameric protein TTR accumulates in the extracellular spaces of various organs including the heart, then dissociates into monomers that misfold and aggregate to generate oligomers and amyloid fibrils (Wu and Chen, 2024). Both entities are provided with remarkable cytotoxicity, because of pro-apoptotic properties and overproduction of ROS and RNS (Sharma et al. 2019). As for the pro-oxidant mechanisms involved in the cardiotoxic effects of misfolded light chain immunoglobulin, also in the case of misfolded/aggregated TTR, the two main sources of ROS accumulation in cardiomyocytes were identified in up-regulated NOX and altered mitochondrial bioenergetics. In the murine cardiac muscle cell line HL-1 overloaded with TTR aggregates, the binding of the aggregates to the cardiomyocyte surface and the following internalization of oligomers was demonstrated. Consequently, an overproduction of ROS occurred, detected by the 2',7'-dichlorofluorescein diacetate (DCFDA) fluorescent probe method, due to a marked derangement of mitochondrial membrane potential (Sartiani et al.,
Such oxidative stress state condition was accompanied by a net change in intracellular calcium homeostasis with ensuing cardiac muscle cell dysfunction (Sartiani et al., 2016). The involvement of NADPH enzyme, as another main source of ROS, was demonstrated in human neutrophils showing an oxidative burst following the treatment with amyloid fibrils derived from aggregated TTR. The cell preincubation with the NADPH selective inhibitor diphenyleneiodonium (DPI) prevented the induction of ROS production, as detected by using the probe dihydrorhodamine 123 (Azevedo et al., 2012). The enhanced formation of ROS by aggregated TTR was shown to be paralleled by an overproduction of RNS, as measured in terms of nitrite and nitrate levels, in two human cell lines, namely the epidermoid A431 and the Schwannoma sNF943 cell lines (Fong and Vieira, 2013). The same authors later reported an increased concentration of hydrogen peroxide paralleled by a partial inactivation of catalase and a diminished level of reduced glutathione (GSH) in both epidermoid and Schwannoma cell lines when treated with aggregated TTR (Fong et al., 2017).

RNS, mainly generated by the endothelial nitric oxide synthase (eNOS), might contribute to the oxidative damage observed in cardiac amyloidosis, as some amyloidogenic variants were proven to become pathogenic after undergoing S-nitrosylation. The nitrosylation of the TTR oligomers seems to enhance the ability to form amyloid fibrils with consequent increased oxidative damage, leading to a vicious cycle (Saito et al., 2005). In addition to the “consumption” of antioxidant defense elements like catalase and reduced glutathione as a consequence of the sustained oxidative stress condition determined by deposition in the heart, as in other organs, of aggregated TTR, the misfolded protein was shown able to otherwise affect the antioxidant system. The TTR tetrameric protein, that usually binds two molecules of thyroxine and two retinol-retinol binding protein complexes, in condition of diminished availability of retinol (vitamin A), like sustained oxidative stress, exhibits its cryptic protease function and cleaves Apolipoprotein A1, by this way hampering the antioxidant activity of high density lipoproteins (Liz et al., 2004). Further, GSH could bind to -thiol residues of TTR, that is this protein becomes glutathionylated, by this way some of the antioxidant tripeptide becomes sequestered (Sharma et al., 2019). Figure 3 shows the main sources of ROS and RNS, as well as relevant causes of antioxidant depletion in AL and ATTR amyloidosis.

In the case of ATTR amyloidosis, it is noticeable that tissue dysfunction occurs even before TTR fibril deposition in tissue. This indicates a non-intermediary toxic action of the prefibrillar protein. In laboratory trials, small TTR intermediates (monomers and oligomers smaller than 100 kDa) cause cytotoxicity through interactions with membrane proteins and cholesterol. The consequential activation of apoptotic mechanisms through cleavage of caspase 3/7 and the rise of ROS by means of superoxide formation can justify, at least partially, cardiac dysfunction (Manral and Reixach, 2015; Reixach et al., 2004). Moreover, in individuals with ATTR amyloidosis, comorbidities such as diabetes, hypertension, chronic kidney disease, and atrial fibrillation contribute independently to impaired ventricular relaxation and promote a pro-oxidative state, accelerating amyloid deposition. This could be an explanation of the heterogeneous clinical manifestations and different progression timing in mutant TTR carriers.

In Figure 4 the possible consequences of the oxidative damage occurring in HFpEF are depicted.

6. AL and ATTR amyloidosis: from pathophysiology to therapeutic targets
At present, treatment of cardiac amyloidosis mainly consists of therapies based on general guidelines for the management of heart failure and arrhythmias.

**Treatment of the hematological disease underlying AL**

Specific therapy for AL amyloidosis aims at suppressing FLCs by treating the underlying hematological disease, therefore reducing the number of light chain-producing clones. Low-risk patients are eligible for high-dose melphalan administrations followed by autologous stem cell transplantation (ASCT) (Dispenzieri et al., 2004; Sher et al., 2016). Hematological response after treatment was shown to reduce organ damage and improve prognosis in 65-80% of patients.

Patients with organ damage [i.e. NYHA (New York Heart Association) class III-IV, increased Troponin T, arterial hypotension, renal failure] are not eligible for ASCT. In this setting, treatment regimens are BMDex (melphalan + dexamethasone + bortezomib) (Kastritis et al., 2020), CyBorD (cyclophosphamide + bortezomib + dexamethasone). In this setting, Dara-CyBorD, (CyBorD + daratumumab, a human CD38-targeting antibody) recently proved to be effective (Kastritis et al., 2021).

Regarding ATTR amyloidosis, orthotopic liver transplantation had been the only treatment option for decades. Although it was proven successful in patients with familial amyloid polyneuropathy (FAP), it was not effective in reducing the organ damage caused by the amyloid fibrils already deposited in tissues, nor the misfolding of circulating proteins caused by them. Therefore, several disease-modifying treatments were developed for ATTR CA, following three main mechanisms, as illustrated in Figure 5:

- The stabilization of TTR tetramers, in order to reduce the formation of amyloid fibrils (Tafamidis, Diflunisal, Acoramidis);
- The reduction of amyloid production through silencing the transthyretin gene (Patisiran, Vutrisiran, Inotersen, Eplotersen);
- The reabsorption and degradation of already deposited fibrils in tissues by using monoclonal antibodies.

**Tafamidis**

Tafamidis is a 5 benzoxazole derivative that binds to transthyretin with high affinity and selectivity, hindering tetramer dissociation and consequent amyloidogenesis. It was proven to reduce all-cause mortality, the number of heart failure admissions and the hospitalization days and improve the functional class in both cardiac and non-cardiac hereditary and wtTTR-CA with positive biopsy, mainly in NYHA class I and II groups at baseline (Maurer et al., 2018; McDonagh et al., 2021). Its greater limit is the cost-effectiveness.

**Acoramidis**

With the same premises, a safe and effective alternative is represented by the recently introduced Acoramidis (AG10), a strong and selective tetramer stabilizer of both mutated and wild type TTR. In the latest ATTRibute-CM trial, held on ATTR-CA patients with NYHA I-III heart failure, it was shown to improve cardiovascular-related hospitalizations, NT-proBNP levels and quality of life, thus representing a safe and effective alternative (Gillmore et al., 2024).

**Diflusinal**

Diflunisal also stabilizes the transthyretin tetramer, preventing its misfolding. It demonstrated slowing the progression of polyneuropathy in hATTR patients and improving laboratory and echocardiographic parameters in cardiac hATTR amyloidosis. However, being...
a nonsteroidal anti-inflammatory drug, it carries gastro side effects and nephrotoxic properties, limiting its chronic administration in the elderly population (Berk et al., 2013).

**Patisiran**

Patisiran is a small RNA-interfering molecule (siRNA), administered intravenously, that specifically inhibits hepatic synthesis of transthyretin, lowering its circulating levels. It was approved for the treatment of patients with FAP regardless of the presence of cardiac involvement, showing halting or reversion of disease progression and an improvement of neuropathy in patients with hereditary transthyretin amyloidosis, together with improvements in quality of life, walking, nutritional status, and activities of daily living (Adams et al., 2018).

**Vutrisiran**

A second siRNA, Vutrisiran, was recently approved, showing similar efficacy to Patisiran, good tolerability and better ease of use, as it can be administered subcutaneously (Adams et al., 2023).

**Inotersen**

Inotersen is an antisense oligonucleotide that cleaves the mRNA of TTR gene, leading to its degradation. It is approved to slow the progression of neurological amyloidosis. Its side effects are thrombocytopenia and glomerulonephritis (Dasgupta et al., 2020).

**NTLA-2001**

CRISPR-Cas9–based in vivo gene editing is a promising treatment option for amyloidosis. Preliminary findings demonstrated that administration of NTLA-2001 to patients with hATTR amyloidosis with polyneuropathy was associated with sustained reductions in the serum TTR protein concentration (Gillmore et al., 2021).

**Organ transplantation**

Liver and cardiac transplantation can be considered in the end-stage phase of familial aTTR cardiac amyloidosis.

**Tools for degradation of amyloid fibrils**

Tauroursodeoxycholic acid (TUDCA) is a biliary acid that reduces non-fibrillar TTR aggregates, needing further research (Cardoso et al., 2010; Cardoso and Saraiva, 2006; Teixeira and Saraiva, 2015). Anti-TTR monoclonal antibodies and anti-serum amyloid P component (SAP) antibodies (e.g., dezamizumab). They were studied with the aim of promoting the phagocytosis of TTR amyloid aggregates, but they have not yielded successful results so far (Galant et al., 2016; Higaki et al., 2016; Hosoi et al., 2016; Phay et al., 2014; Richards et al., 2022; A. Wechalekar et al., 2022).

In Figure 5 the present therapeutic strategies in amyloidosis are depicted.

### 7. ROS quenching and anti-inflammatory approaches

**Flavonoids**

Among the inhibitors of amyloid substance formation is the family of flavonoids, a subgroup of polyphenols, pleiotropic compounds also provided with strong antioxidant
properties. Its aromatic rings enable the prevention of amyloid fibrils aggregation by creating non-covalent bonds to the core structure of the amyloid proteins (Miyata et al., 2010). Generally, flavonoids have been found to stabilize the TTR tetramers, however, their effects vary among the family. The flavonoid epigallocatechin gallate (EGCG) is a natural compound found in green tea. It showed significant efficiency in the inhibition of TTR amyloid aggregation by binding the tetrameric non-misfolded transthyretin and stabilizing its structure while preserving its physiological function, including the T4-transportation. In addition, a potential role in the disaggregation of amyloid deposits and disruption of preformed fibrils was proposed, leading to a conversion into unstructured off-pathway oligomers. The large polymers theaflavins of tea catechins have shown significant inhibitory function as well (aus dem Siepen et al., 2015; Cappelli et al., 2018; Ferreira et al., 2009; Miyata et al., 2010).

Preliminary data from ATTR CA patients who were administered EGCG daily for one year showed significant reductions in interventricular septal thickness and left ventricular myocardial mass, probably due to a reduction of myocardial amyloid burden. Due to its different binding sites, a beneficial effect of the association between EGCG and tafamidis was suggested, with a potential synergistic effect on ATTR stabilization. However, its application may be restrained, as EGCG interacts with human serum albumin, affecting the bioavailability of tafamidis (aus dem Siepen et al., 2015; Cappelli et al., 2018; Ferreira et al., 2009; Miyata et al., 2010).

Catechin and epicatechin also seem to decrease oxidative stress on the endothelium by increasing nitric oxide release and bioavailability and causing vasodilation. This represents an important feature, as endothelial dysfunction is one of the mechanisms in heart failure associated with poor prognosis. Dark chocolate is also rich in flavonols and it was shown to decrease endothelial dysfunction and NT-proBNP levels when administered in chronic in patients with HF and was associated with a decrease in HF hospitalizations in small observational studies (Dural et al., 2022; Mostofsky et al., 2010).

Therefore, these substances represent a possible therapeutic strategy as they prevent the formation of amyloid fibrils and lead to non-toxic or less harmful products, with high tolerability and low toxicity, although survival improvements have not been demonstrated (aus dem Siepen et al., 2015; Cappelli et al., 2018; Ferreira et al., 2009; Miyata et al., 2010).

Although inflammation and oxidative damage were proven to play a pivotal role in the development and progression of HFpEF, particularly in cardiac amyloidosis, research on targeting suppression and modulation of immune responses in this specific setting was not yet conclusive.

Other antioxidants

N-acetylcysteine (NAC), a ROS blocker, has so far only been tested in patients with acute myocardial infarction, with consistently inconclusive and conflicting results (Pasupathy et al., 2017). A study of free radical scavenger treatment (vitamin C, vitamin E, and acetylcysteine) in patients with hereditary transthyretin amyloidosis with polyneuropathy showed no decrease in hydroxynonenal - a product of lipid peroxidation. However, an increased nutritional status was recorded for liver-transplanted patients, suggesting scavenger treatment may facilitate recovery after transplantation (Suhr et al., 2001).

Chelating agents
As previously mentioned, transition metal ions are involved in oxidative damage and light chain induced toxicity. The addition of metal chelator or metal-binding 8-hydroxyquinoline compounds (chelex, PBT2, and clioquinol) was proven to be effective in blocking the ROS production and preventing the cardiotoxic effects of amyloid light chains in simple multicellular models (Diomede et al., 2017), laying the foundation for total inhibition of the vicious cycle of redox damage in humans.

**Immunomodulatory and anti-inflammatory drugs**

Several immunosuppressive and immunomodulatory drugs have been repurposed for the treatment of cardiac amyloidosis. Broad immunosuppression with corticosteroids, methotrexate, cyclosporin A or intravenous immunoglobulins were hypothesized, mainly in the acute and ischemic setting, without significant results (Michels da Silva et al., 2019; Ton et al., 2014). Studies involving TNF-α were held on patients with HF. Small studies investigated etanercept and infliximab, two TNF-α receptor antagonists, registering positive effects such as improved LVEF, 6MWT, and NYHA class. However, recent trials (i.e. Recover, Renaissance, Attach trial) showed no clinical benefit in chronic HF with NYHA III and IV (Dural et al., 2022; Mostofsky et al., 2010; Pasupathy et al., 2017). Studies on inhibition of effector T and B lymphocytes are limited.

Soluble guanylyl cyclase (sGC), an enzyme activated by NO and involved in the cGMP-PKG signaling pathway, was recently shown to have beneficial effects on cardiac remodeling, thus becoming a target for therapy in HFrEF. Vericiguat, a sGC stimulator, was administered to patients with HFrEF, showing improvement in quality of life (Armstrong et al., 2020; McDonagh et al., 2021).

**Cardiac contraction modulation therapy (CCM)**

In this setting, cardiac contraction modulation therapy (CCM) may play a role. It is an experimental HF device therapy that delivers high amplitude non-excitatory biphasic electrical signals to the myocardium during its refractory period. Eligible patients are individuals with NYHA class II or III, LVEF < 50%, peak VO2 ≥ 10 mL/kg/min and without arrhythmias (< PVCs 10,000/day) (McDonagh et al., 2021). It appeared to have a modulating effect on gene expression thanks to DNA interactions via specific electromagnetic response elements (EMREs), with a positive effect on fibrogenesis and inflammatory stress (Butter et al., 2008). CCM reverses cardiac maladaptive gene modifications and regulates some important sarcoplasmic reticulum and stretch-response genes such as A- and B-type natriuretic peptides, α-MHC, SERCA-2a, phospholamban, and ryanodine receptors. Moreover, it inhibits the TGF-β1 pathway, with reduced collagen production and fibroblast differentiation, therefore attenuating interstitial fibrosis (Zhang et al., 2016). The normalization of diastolic Ca2+ levels might lead to reductions of ROS and activation of CaMKII (Tschöpe et al., 2019). The p38 mitogen activated protein kinase (p38MAPK) pathway is also down-regulated. Lastly, CCM seems to facilitate the production of chaperone proteins, promoting the balance of protein synthesis and degradation and protecting against cell death in response to amyloid fibrils (Marchese et al., 2023).

8. Conclusions

The role of oxidative stress in the development and perpetuation of heart failure in the cardiac amyloidosis has been extensively studied in the recent years and the consistent and sustained overproduction of ROS in such a diseased cardiac tissue, largely overwhelming...
the antioxidant defense system, has been outlined to be a primary pathogenic factor. In both light chain immunoglobulin and transthyretin amyloidosis, two major sources of ROS overproduction in the cardiomyocytes have been recognized to be the upregulation of the membrane-bound enzyme NADPH oxidase (NOX) and the altered membrane potential of mitochondria. The recognized pro-inflammatory effect of a condition of oxidative stress and damage is further enhancing the underlying inflammatory status, which in turn exacerbates the oxidative stress and this vicious cycle will result in the amplification of the overall cardiac tissue damage.

Therefore, an adequate therapeutic approach that would suitably consider such a vicious cycle implying a growing oxidative- and inflammatory-dependent heart damage in AL and ATTR amyloidosis looks really advisable. Although pre-clinical studies showed promising results by adopting various agents able to quench oxidative stress and damage, a significant impact on morbidity and mortality of AL and ATTR amyloidosis was not yet shown. Therefore, new clinical trials should evaluate therapeutic protocols that would more efficiently challenge the vicious cycle of oxidative damage and inflammation well evident in cardiac amyloidosis. One impelling reason for this is the fact that numerous patients with wtATTR amyloidosis, in particular, are elderly, an age group that shows a continuous percentage increase.
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Table 1. Main features of the light chain immunoglobulin and transthyretin amyloidosis.

Figure 1. Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS).
The colour spectrum of the arrow bar above the scheme varies with the reactivity of these species. The main classes of defence mechanisms against ROS and RNS are depicted briefly.
ET: electron transport; $\text{O}_2^{-}$: superoxide ion; $\text{H}_2\text{O}_2$: hydrogen peroxide; NO: nitric oxide; Cl-: chloride ion; ONOO$: peroxynitrite; HOCl: hypochlorous acid; OH$: hydroxyl radical; Fe$^{3+}$: ferric ion.

Figure 2. The molecular defensive system against ROS, in details.
$\text{O}_2^{-}$: superoxide ion; $\text{H}_2\text{O}_2$: hydrogen peroxide; NO: nitric oxide; ONOO$: peroxynitrite; HOCl: hypochlorous acid.

Figure 3. Main cellular and molecular sources of ROS and RNS in AL and ATTR amyloidosis.
gp91phox/p22phox/p40phox/p47phox/p67phox: subunits of NADPH oxidase (NOX); eNOS: endothelial nitric oxide synthase; ROS: reactive oxygen species; NO: nitric oxide.

Figure 4. Pathophysiological consequences of oxidative damage in heart failure with preserved ejection fraction.
HFpEF: Heart failure with preserved ejection fraction; ROS: reactive oxygen species; NO: nitric oxide; TGF-β: transforming growth factor β; Smad3: small mother against decapentaplegic 3; cGMP: cyclic guanosine monophosphate; PKG: cGMP-dependent protein kinase.

Figure 5. Therapeutic strategies in amyloidosis.
CRISPR: Clustered regularly interspaced palindromic repeats; Cas9: CRISPR-associated protein 9; NTLa-2001: an in vivo gene-editing therapeutic agent; TTR: transthyretin; ASCT: autologous stem cell transplant; BMDex: melphalan + dexamethasone + bortezomib; CyBorD: cyclophosphamide + bortezomib + dexamethasone; Dara-CyBorD: cyclophosphamide + bortezomib + dexamethasone + daracumab; TTR: transthyretin.
<table>
<thead>
<tr>
<th>AL amyloidosis</th>
<th>TTR amyloidosis</th>
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<td>Pathogenesis</td>
<td>Infiltration of a small plasma cell clone into bone marrows</td>
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<td>→ Production of amyloidogenic monoclonal immunoglobulin free light chains</td>
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<td>→ Ageing-associated failure of repair and proteostatic mechanism</td>
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<td><strong>Non-hereditary</strong></td>
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<td>Direct proteotoxicity of FLC</td>
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<td>Worse systolic dysfunction</td>
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FLC, free light chains; TTR, tranthyretin;
From Less Reactive to Highly Reactive Species

**ROS and RNS defense mechanisms**

- **Antioxidants and Scavenging Enzymes**
- **Free iron chelators**

**Pathways**

- **Nox/Duox**
- **Xanthine Oxidase (XO)**
- **Cyclooxygenase (COX)**
- **Mitochondrial ET Pathway**

**Reactive Species**

- **NO**
- **ONOO⁻**
- **HOCI**
- **HO•**

**Reactions**

- **Nitric Oxide Synthase (NOS)**
- **Super Oxide Dismutase (SOD)**
- **Myeloperoxidase (MPO)**
- **Fenton Reaction**
- **Haber–Weiss Reaction**

**From Less Reactive to Highly Reactive Species**

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Pathophysiological consequences of oxidative damage in HFpEF

Cardiomyocytes
ROS Production ↑

- ROS ↑
- TGF-β/Smad3 pathway Activation
- Promoting Myocardial Fibrosis ↑

ROS binding to NO
- NO availability ↓
- cGMP ↓
- PKG downregulation

Ca 2+ ion Regulation
- Suppression of Hypertrophic Changes
- Phosphorylation of
  - Troponin I
  - Titin
- Ventricular Diastolic Relaxation

Cardiomyocyte Contractility
**Transthyretin Amyloidosis (ATTR)**

- Liver Transplantation: (End Stage Phase)
- CRISPR-Cas9–based in vivo gene editing:
  - NTLA-2001
- TTR Gene Silencing:
  - Patisiran
  - Vutrisiran
  - Inotersen
  - Eplotersen
- TTR Tetramer Stabilization:
  - Tafamidis
  - Diflunisal
  - Acoramidis

**Light Chain Amyloidosis (AL)**

- Hematological Disease Treatment (Low-risk Patients):
  - High Dose Melphan + ASCT
- Hematological Disease Treatment (High-risk Patients):
  - BMDex
  - CyBorD
  - Dara-CyBorD

**Degradation of deposited amyloid fibrils:**
- Monoclonal Antibody

**Heart Transplantation:** (End Stage Phase)