Cardiac Amyloidosis

Diagnosis and Treatment

Michele Emdin
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Michele Emdin
Health Science Interdisciplinary Center
Scuola Superiore Sant'Anna
Fondazione Toscana Gabriele Monasterio
Pisa, Italy

Alberto Aimo
Health Science Interdisciplinary Center
Scuola Superiore Sant'Anna
Fondazione Toscana Gabriele Monasterio
Pisa, Italy

Giuseppe Vergaro
Health Science Interdisciplinary Center
Scuola Superiore Sant'Anna
Fondazione Toscana Gabriele Monasterio
Pisa, Italy

Marianna Fontana
National Amyloidosis Centre
University College London
London, UK

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Preface

As William Osler wrote in 1892 in his internal medicine book: "There are three phases to treatment: diagnosis, diagnosis, and diagnosis." We can then state: "There are three phases to diagnosis: knowledge, knowledge, and knowledge," and that this is particularly true for cardiac amyloidosis (CA), a once called rare disease, now increasingly recognized and treated thanks to the advances of knowledge most recently achieved by an impressive worldwide research effort.

CA is a disease caused by the accumulation of amyloid fibrils in the extracellular space of the myocardium, leading to profound changes in the heart's substrate, electrical properties, and function, which are the basis for the clinical syndrome, characterized by arrhythmias, conduction disturbances, and a progressive impairment of diastole and systole, progressively leading to overt heart failure. Until very recently, CA was almost always diagnosed very lately and after multiple hospitalizations in emergency medicine, cardiology, pulmonology, and internal medicine wards. Once the clinical suspicion was raised, the demonstration of tissue amyloid deposits was required for the final diagnosis, using highly invasive procedures such as endomyocardial biopsy. Furthermore, when the diagnosis was made, no effective therapeutic options could be offered to the patient. The perception of CA as a rare disease did not stimulate the scientific community and the pharmaceutical industry to support the research and development of new dedicated tools for either diagnosis or treatment.

Afterwards, the intuition and tireless activity of several masters, including Claudio Rapezzi in Bologna and Ferrara; Giampaolo Merlini in Pavia, Italy; Angela Dispenzieri and Martha Grogan at the Mayo Clinic School in Rochester; Mathew Maurer at the Columbia University, NYC, USA; Julian Gillmore and Philip Hawkins, joined by Marianna Fontana at the University College London, UK, and paralleled in Italy by the initiative of Gianfranco Sinagra in Trieste; and Michele Emdin and Giuseppe Vergaro in Pisa, prompted a true revolution in our current approach to CA.

The goals of a noninvasive diagnostic algorithm for transthyretin CA (ATTR-CA), and of the availability of safe, disease-modifying, and lifesaving therapies for light-chain and variant or wild-type transthyretin CA, were achieved. National and international scientific societies, such as the International Society of Amyloidosis, were born; recommendations on diagnosis and management were issued; as well as a specialty journal such as *Amyloid*, research consortia and forum, and patient support

groups were formed, worldwide. In turn, this interest in CA and the increased disease awareness in the medical community, favored by several educational initiatives, prompted the identification of an increasing number of cases diagnosed and treated.

Much work is still needed: the ability to recognize and treat patients with CA should become part of the core curriculum of cardiologists as well as internal medicine specialists, general practitioners, neurologists, nephrologists, and hematologists, and both individual and collaborative research should focus on the unsolved issues regarding CA pathophysiology, epidemiology, diagnostics, and therapeutics, as well as on novel strategies for promoting screening in high-risk populations and noninvasive, comprehensive diagnostic paths including novel answers at a local, regional, and national level through the creation of clinical and research networks supported by the scientific societies, as it is the case for Italy.

Our purpose is to give to all the clinicians and researchers interested in the field the integration of the state-of-the-art knowledge with the contribution of specialists from the leading centers in Europe and the United States.

The book is dedicated first to Claudio Rapezzi, to the man who paved the way for the current clinical and research approach to CA, as well as to the clinicians working all over the world to prolong survival and improve the quality of life of patients with CA, and, finally, to our patients, whose well-being is the final goal of all our efforts.

Pisa, Italy — Michele Emdin
Pisa, Italy — Giuseppe Vergaro
Pisa, Italy — Alberto Aimo
London, UK — Marianna Fontana

Contents

Tribute to Claudio Rapezzi

1

Gianfranco Sinagra and Aldostefano Porcari

There may be many ways to start a tribute to Claudio Rapezzi, no one is easy in the heart of those who have spent part of their life with him. He was a unique and extremely rare concentration of human and scientific qualities that cannot be expressed only with words. If we were to choose a single characteristic to describe Claudio, we would say that he was primarily a mentor with the unique ability to ignite the minds and the hearts of friends and colleagues with his scientific passion. Many have known him as a brilliant mind, many others as a trustful and distinguished partner in research with flashes of extraordinary intelligence, and many more as a close friend with extremely sharp irony and culture.

Although he was aware of his exceptional qualities, Claudio was extremely humble and had a natural disposition in human relationships, with his eyes wide open to the world of young physician and advancing medical knowledge. Claudio dedicated his life to the study of medicine and became a master in the art of observation. During a career spanning almost 50 years, he has deeply transformed the

G. Sinagra (✉)
Centre for Diagnosis and Treatment of Cardiomyopathies, Cardiovascular Department, Azienda Sanitaria Universitaria Giuliano-Isontina (ASUGI), University of Trieste, European Reference Network for Rare, Low Prevalence and Complex Diseases of the Heart-ERN GUARD-Heart, Trieste, Italy
e-mail: gianfranco.sinagra@asugi.sanita.fvg.it

A. Porcari
Centre for Diagnosis and Treatment of Cardiomyopathies, Cardiovascular Department, Azienda Sanitaria Universitaria Giuliano-Isontina (ASUGI), University of Trieste, European Reference Network for Rare, Low Prevalence and Complex Diseases of the Heart-ERN GUARD-Heart, Trieste, Italy

Division of Medicine, National Amyloidosis Centre, University College London, London, UK
e-mail: aldostefano.porcari@nhs.net

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cardiological and the amyloidosis community worldwide. Crossing the path of Claudio marked a fundamental moment in the career of many young researchers, sometimes in unexpected ways.

Claudio approached the many congresses on heart failure and cardiomyopathies as useful opportunities to connect with young physicians and discuss the grey areas encountered in clinical practice, the limitations of the official guidelines for the treatment of heart failure and the need to follow critical thinking when approaching uncommon clinical scenarios (Fig. 1.1). As a teacher, he embodied the qualities of curiosity, critical thinking, observation, passion and creativity. He inspired and feed deep passion for medical knowledge, analysis of details and inconsistencies in clinical profiles and presentation, and for deduction as preferred methodology to deal with the many problems faced in clinical practice of medicine. He had that exceptional quality of merging scientific knowledge with his passion for the philosophy of Kant, Popper, the nosological question raised by Umberto Eco, the renewed songs from Vasco Rossi and his love for Art. His friends and colleagues will never forget his ability to use iconic paintings such as Arcimboldo' self-portrait to explain

Fig. 1.1 Professor Claudio Rapezzi during a discussion at the "Advances in Heart Failure, Cardiomyopathies and Pericardial Diseases" held in Trieste (Italy)

the heterogeneous clinical phenotypes of patients presenting with systemic amyloidosis.

He was the exemplar of the physician and detective character in a contemporary version of Sherlock Holmes [1]. He shared with the cardiology community the "red flag approach" in cardiomyopathies and, particularly, in amyloidosis [2, 3]. He was among the first researchers to understand the key value of carpal tunnel syndrome as an early clinical marker of future development of cardiac amyloidosis [4, 5]. In the old days, through his expert interpretation of surface ECG, Claudio was able to characterise myocardial tissue composition and spot the presence of amyloid deposits. This is something that endomyocardial biopsy and cardiac magnetic resonance would have demonstrated many years later [6, 7]. He was very passionate on dissecting the heterogeneous clinical phenotype of ATTR amyloidosis [8, 9]. He has identified 3 main clinical phenotypes—cardiac, neurological and mixed—that have been implemented in clinical practice for diagnosing ATTR amyloidosis and for orienting therapeutic strategies worldwide. He was involved in the Transthyretin Amyloid Outcome Survey (THAOS), with the final aim of understanding and characterising the natural history of ATTR amyloidosis [10]. In seminal papers published in early 2000s, Claudio demonstrated the clinical applications of scintigraphy with bone tracers for the diagnosis of cardiac amyloidosis [11, 12]. Ten years later, that intuition paved the way for the development of a non-invasive algorithm for the diagnosis of transthyretin amyloid cardiomyopathy (ATTR-CM) in an international collaboration with the National Amyloidosis Centre (London, UK) which has deeply transformed the paradigm for diagnosing ATTR-CM [13–15].

Claudio coordinated the international phase 3 Safety and Efficacy of Tafamidis in Patients With Transthyretin Cardiomyopathy (ATTR-ACT) trial of tafamidis [16], which is the only drug ever tested in ATTR-CM with a proven impact on survival. In 2018, he presented the results of the ATTR-ACT at the European Society of Cardiology Congress held in Munich and ignited the audience with his passion and culture. Claudio considered tafamidis as the drug of the first four times:

- The first time that a drug is effective in ATTR-CM.
- The first example of precision medicine in the treatment of a cardiomyopathy.
- The first time a drug is effective in heart failure with preserved ejection fraction.
- The first time a drug without anti-neurohormonal activity is effective in heart failure.

The ATTR-ACT study has transformed the treatment of ATTR cardiomyopathy and represented a real revolution for patients and physicians worldwide (Fig. 1.2).

On top of his undisputed scientific expertise, Claudio was a great estimator of the writer Umberto Eco and the philosopher Karl Popper and was highly considered for his critical approach to the clinical methodology. A recent example is offered by the impossible interview between Sherlock Holmes and David Sackett about the fundamental question "how much can we trust the guidelines?" [17]. His pupils will never forget his positive approach to "error in medicine" as a source of thinking and a unique opportunity to overcome grey areas in medicine. In the field of classification

Fig. 1.2 Professor Rapezzi presenting the results of the ATTR-ACT study at the European Society of Cardiology congress held in Munich

and nosology, Claudio was part of an international group of researchers that defined the criteria for classification of cardiomyopathies in 2008 [18]. More recently, he identified the limitations of that classification among patients diagnosed and managed in the real world, especially in the setting of restrictive cardiomyopathy. Therefore, he has proposed a new definition of this specific form of heart disease [19].

In the latest years, Claudio moved the centre of his activity to Ferrara where he closely collaborated with Prof. Roberto Ferrari, estimated colleague and friend. At the Ferrara University, Claudio entered in a very fruitful phase of his career characterised by multicentre collaborations. With the national and international network that he built during 50 years of clinical and scientific activity, Claudio started a

number of research collaborations with friends and young physician with an interest in cardiac amyloidosis all around the world. In Ferrara, he conceived the design of the CAUSATIVE study, which is currently ongoing, with the aim of investigating the potential applications of computed tomography for the identification of cardiac amyloidosis among patients with severe aortic valve stenosis. With this project, he strenghtened the connections created with other Italian centres such as the cardiological amyloidosis community in Pisa under the leadership of Prof. Michele Emdin, close friend and partner in research, and his team of young physicians. The proposal for a re-definition of restrictive cardiomyopathy in the contemporary era was born from the intense cultural relationship between Claudio, Michele and the cardiological community in Trieste [19]. In Trieste, Claudio was used to attend the bi-annual congress "Incontri in Cardiologia" and give lectures on unmet needs in heart failure and cardiomyopathies. Young and adult cardiologists from Trieste keep a special memory of Claudio and, in particular, of the strong human and professional connection with him. With his unconventional spirit, he has inspired and encouraged, always with laughter, them and many young researchers to step into his path in the international amyloidosis community.

Claudio never forgot his old pupils from Bologna, grown in the research field of amyloidosis and disseminated in Italy and worldwide. His spirit and attitude were brighter when he met them during international congresses, he had that glimpse in his eyes when he had the opportunity of spending some time with them, a bond that time and life events could not weaken in any way.

Among the last quests of Claudio, there is definitively the foundation of the Italian Network for cardiac amyloidosis [20], that he has designed to promote collaboration among Italian centres for diagnosis and treatment of patients with suspected or confirmed cardiac amyloidosis. Sadly, Claudio has left us with many grey areas to untagle in the field of amyloidosis as well as with many ideas and research questions that awaits to be explored by his pupils disseminated worldwide. He was an extremely active part in many research projects, part of which are still ongoing such as the role of electrocardiography in the contemporary care of patients with cardiac amyloidosis [21], the cardiomyopathy-oriented interpretation of ECG findings [22, 23], the different behaviour of bone tracers validated for the diagnosis of ATTR-CM [24], the impact of tafamidis in ATTR-CM patients with NYHA class III, genotype-phenotype correlation in ATTR amyloidosis, gender differences in ATTR-CM [25–27], new treatment strategies and the possibility of combination therapy [28].

Professor Claudio Rapezzi was a giant of the amyloid field, an unattainable mentor and a unique friend.

We are close to his beloved Marinella, friends and colleagues in Ferrara, Bologna and all around the world.

He will be greatly missed and will continue being an inspiration for the next generations.

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Giampaolo Merlini and the Pavia School

2

Michele Emdin

The great advances in the diagnosis and treatment of cardiac amyloidosis (CA) are the result of a collective effort inspired by the pioneering work of a few masters. Among them Giampaolo Merlini (Fig. 2.1) is a giant, whose work and research contributed to advance modern clinical hematology and internal medicine. Giampaolo Merlini graduated in medicine and surgery at the University of Pavia as a student of the Ghislieri College and specialized in Hematology and Laboratory Medicine at the University of Pavia. Afterwards, he trained in clinical and laboratory investigations of monoclonal gammopathies at Malmö General Hospital, Lund University, Sweden under the supervision of Jan Waldenström. The teaching of Waldenström shaped his scientific interests, which have focused on the investigation of the molecular mechanisms of diseases, and namely on the biological activities of monoclonal proteins and related conditions. He further developed these research lines at the Institute of Cancer Research, College of Physicians & Surgeons, Columbia University, New York City, under the supervision of Elliott Osserman and together with chemist Elvin Kabat. Osserman introduced him to systemic amyloidoses and specifically to amyloidosis caused by misfolded monoclonal immunoglobulin light chains.

He was then the director of the center for the study and treatment of systemic amyloidosis and of the biotechnology research laboratories located in Pavia at the San Matteo Polyclinic Foundation, long recognized as a national referral center for the disease. This center, currently directed by Giovanni Palladini, a former student of Merlini, was founded in 1986 and employs the most advanced diagnostic tools and the most recent therapeutic resources, including experimental ones. This center

M. Emdin (✉)
Interdisciplinary Center for Health Sciences, Scuola Superiore Sant'Anna, Pisa, Italy

Cardio-thoracic Department, Fondazione Toscana Gabriele Monasterio, Pisa, Italy
e-mail: m.emdin@santannapisa.it

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Fig. 2.1 Giampaolo Merlini

is devoted to the care of patients with amyloidosis, has been instrumental in the introduction of new tools for diagnosis, risk prediction, and management, and has been one of the leading centers in the world in terms of scientific output [1]. He was full Professor (now *Emeritus*) of Clinical Biochemistry at the University of Pavia and President of the Postgraduate Course in Medical Biotechnology. Further, he directed the International Society of Amyloidosis and the Italian Society for Amyloidosis and was the Chairman of the Committee on Plasma Proteins of the International Federation of Clinical Chemistry and Laboratory Medicine.

Prof. Merlini's research interests included the pathogenesis, natural history, diagnosis, and treatment of monoclonal gammopathies, namely of immunoglobulin light chain amyloidosis. His research focused on the study of the molecular mechanisms of cardiac damage, the investigation of biomarkers for the assessment of prognosis and response to therapy, and on the development of new therapeutic agents and treatments. He was principal investigator of several research projects funded by the European Community and by national and international research agencies, and received several international awards: the Ham-Wasserman Lecture 2017 at the American Society of Hematology Congress, the Robert Kyle Award at the International Workshop on Waldenström's Macroglobulinemia in 2018, the Jan G. Waldenström Award of the International Myeloma Society in 2019, the Giampaolo Merlini Prize by the International Society of Amyloidosis in 2020, dedicated to his person, the "Standing on the Shoulders of the Giants" Award by the International Academy of Clinical Hematology in 2022.

His scientific output is extraordinary, with an H index of 102 (Scopus, June 2023) and over 650 publications; he is a highly cited researcher for the years 2021 and 2022. His most notable scientific achievements include a novel system to stage multiple myeloma [2]; the proposal of biphosphonates as effective drugs in multiple

myeloma [3]; the identification of 4′-iodo-4′-deoxy doxorubicin as a possible treatment for amyloidosis [4]; N-terminal pro-B-type natriuretic peptide as a possible biomarker for this condition [5]; the proposal of the melphalan-dexamethasone combination as an effective and safe treatment regimen for AL amyloidosis [6]; and the most recent evidence of the efficacy of birtamimab (a novel humanized monoclonal antibody designed to neutralize light chain aggregates and deplete organ-deposited amyloid via macrophage-induced phagocytosis) plus standard of care in Mayo stage IV light chain amyloidosis patients [7].

On a personal note, he was, and still is, always there, when I asked for an advice either on a difficult patient or on the research strategy and clinical governance: His words were always, and still are, enlightening. We share our faith in man and our commitment to the patient.

Curiositas felix, great culture and deep humanity, ability to organize and educate are some of the unique qualities of the man. Finally, Dante Alighieri's words "*Facesti come quei che va di notte, che porta il lume dietro e sé non giova, ma dopo sè fa le persone dotte" ("You acted like who goes at night carrying a light behind him, and does not help himself but makes people learned")* [8] best describe Giampaolo Merlini's attitude toward his disciples and his lesson to scholars, physicians, and researchers.

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A Brief History of Amyloidosis

3

Assuero Giorgetti, Angela Pucci, and Alberto Aimo

Abbreviations

AL	Amyloid light chain amyloidosis
ATTR	Amyloid transthyretin amyloidosis (v, variant; wt, wild-type)
ATTR-ACT	Tafamidis in transthyretin cardiomyopathy clinical trial
CA	Cardiac amyloidosis
PET	Positron emission tomography

Amyloidoses are considered rare diseases resulting from the extracellular deposition of amyloid, a fibrillar material derived from various precursor proteins that self-assemble with highly ordered abnormal cross β-sheet conformation. Deposition of amyloid can occur in the presence of an abnormal protein (e.g., variant transthyretin amyloidosis [ATTRv] and immunoglobulin light-chain [AL] amyloidosis), in association with prolonged and excessive secretion of a normal protein (e.g., reactive systemic amyloidosis and β2-microglobulin dialysis-related amyloidosis), or, in ageing process with unknown mechanisms (e.g., wild-type ATTR [ATTRwt] and atrial natriuretic peptide in isolated atrial amyloidosis) [1].

A. Giorgetti (✉)
Fondazione Toscana Gabriele Monasterio, Pisa, Italy
e-mail: asso@ftgm.it

A. Pucci
University Hospital of Pisa, Pisa, Italy
e-mail: a.pucci@ao-pisa.toscana.it

A. Aimo
Fondazione Toscana Gabriele Monasterio, Pisa, Italy

Scuola Superiore Sant'Anna, Pisa, Italy
e-mail: aimoalb@ftgm.it

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The term "amyloid" was introduced in the scientific literature by the German botanist Matthias Schleiden (1804–1881), who first applied the iodine-sulfuric acid test for starch in plants [2]. Schleiden demonstrated the presence of a starch-like substance, which he defined as "amyloid" in his book *Grundzige der wissenschaftlichen Botanik* ("Principles of Scientific Botany"), published in 1842–1843 [3]. The term derives from the Greek άμυλον and Latin "amylum", meaning "starch" [2].

Lesions attributable to amyloid deposits had already been described in the liver and spleen, already in 1639 [2]. The first use of the term "amyloid" in human disease is attributed to the physician and physiologist Rudolf Virchow (1821–1902) in his publication *Über eine in Gehirn und Rückenmark des Menschen aufgefundene Substanz mit der chemischen Reaction der Cellulose* ("About a substance found in the human brain and spinal cord with the chemical reaction for cellulose"), dating to 1854 [4]. In this text, Virchow described small roundish deposits in the gray matter of individuals with dementia, stating that those structures showed the same color reaction with iodine and sulfuric acid, i.e., a change from brown to blue, as starch. Virchow then proposed that these lesions had the same composition of starch, and defined them as "corpora amylacea." Over the following years, Virchow used the staining method with iodine and sulfuric acid on other amyloid-laden tissues [2].

In 1859, the German chemist August Kekulé (1829–1896) reported that organs infiltrated by amyloid had a high nitrogen content. Kekulé then proposed that the amyloid substance was composed mainly of protein, rather than carbohydrate, compounds [2]. Virchow did disagree with this conclusion, that he deemed wrong because whole tissue specimens were analyzed, rather than the lesions alone [5]. Virchow also did not agree with the use of methyl violet stain to detect amyloid, which was proposed independently by three scientists in 1875. Already in 1876, Soyka reported having found amyloid in the cardiac tissue with the use of this new method. Ackroyd and Ehrlich described methyl violet stain as "metachromatic" in 1878. Metachromatic stains challenged Virchow's iodine sulfuric acid test for decades, but were eventually replaced by Congo red [2, 5].

The Congo red dye was invented by the German chemist Paul Böttiger in 1884 as the first dye that did not require additional substances for fixation to the textile fibers [6]. In 1922, the German chemist Herman Bennhold discovered the Congo red ability to bind amyloid [7]. Reactivity with Congo red stain or "Congophilia with apple green birefringence" became the first diagnostic criterion for amyloid, introduced by the Belgian physician Paul Divry in 1927 [8]. The Puchtler's modification of Congo red staining, developed in 1962, is currently used to detect amyloid in histological specimens [9]. For histology, the samples are mostly formalin-fixed and paraffin-embedded, then 8–10 μm thick sections (such thickness increasing the staining sensibility) are stained with Congo red, and viewed in a light microscope under polarized light where amyloid is shown as green birefringent homogeneous material (red staining without light polarization is not specific of amyloid) [2]. Congo red is a symmetrical molecule with a hydrophobic center composed of two phenyl rings and two charged terminal naphthalene moieties; the terminal parts of Congo red contain sulphonic acid and amine groups. Although the interaction mechanisms between Congo red and amyloid fibrils have been intensively

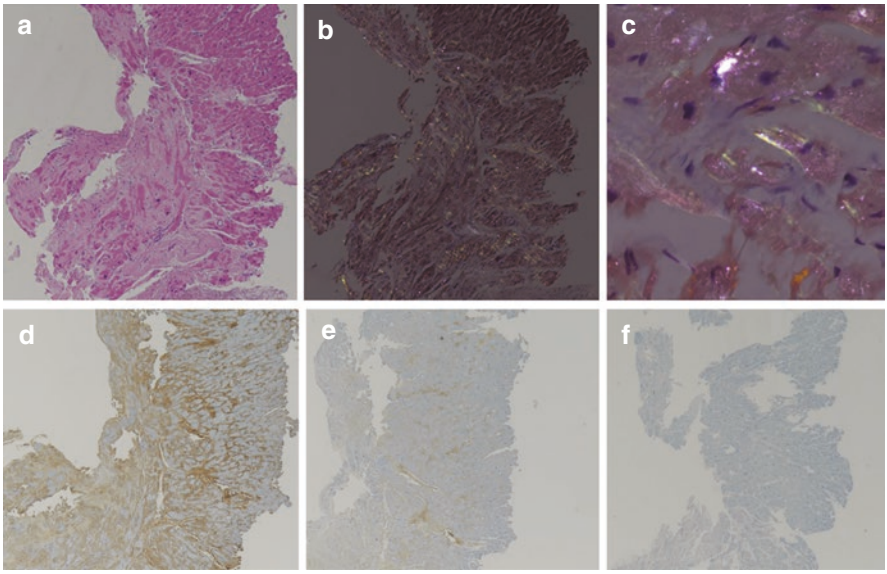

Fig. 3.1 Histology findings on an endomyocardial biopsy specimen. Routine histology showing enlarged interstitial spaces with amorphous and eosinophilic deposits (**a**). Amyloid deposits are demonstrated by green birefringence on the Congo red staining under polarized light (**b**), highlighting also very thin amyloid deposits surrounding single myocytes (**c**). Immunohistochemistry shows immunoglobulin light-chain Lambda immunoreactivity (**d**) with negative light-chain λ (**e**) and transthyretin (TTR) immunostaining (**f**). (**a**) Hematoxylin and eosin staining; (**b**, **c**): Congo red staining; (**d–f**): Immunoperoxidase staining and hematoxylin counterstaining. Original magnification: **a**, **b** and **d–f**: 4×; **c**: 40×

investigated, the process is not completely clarified. Congo red binding has been assumed to depend on the secondary, β-pleated configuration of the fibril, possibly mediated by hydrophobic interactions of the benzidine centers as well as the electrostatically charged terminal groups. The binding of Congo red to amyloid induces a characteristic shift in the maximal optical absorbance of the molecule from 490 nm to 540 nm, which causes the characteristic apple green birefringence under polarized light [2, 10] (Fig. 3.1).

3.1 Characterization of the Structure and Biochemical Composition of Amyloid Deposits

Besides the identification of amyloid in the brain of a patient with dementia by Alzheimer, in 1907 [11], no major breakthroughs in the research on amyloid came before the late 50 s. American researchers Alan S Cohen and Evan Calkins described the first extraction method that consisted of gentle physical separation and homogenization of the material in saline, followed by low-speed centrifugation [12]. Alternative methods involving the use of an alkaline solution of sodium glycinate

[13] and, most importantly, the "water extraction method" of Pras were proposed [14]. This last method has been widely used to extract almost all amyloid types except for Aβ and prion protein amyloid and enabled the identification of the β-pleated sheet configuration of amyloid proteins and the discovery of the biochemical structure of those proteins [2].

The secondary structure of amyloid consists of the polypeptide backbone, mostly in the β-pleated sheet conformation, oriented perpendicular to the fibril axis. This β-pleated sheet structure was revealed by X-ray diffraction analysis of isolated amyloid protein fibrils starting from 1968 [15, 16]. Glenner and coworkers also reported the relationship between "primary" amyloidosis and immunoglobulin light chains [17].

During the following years, many amyloid proteins were identified. Inflammation-associated amyloidosis, previously called the "secondary" and today AA amyloidosis, was shown to be caused by amyloid protein A, an acute phase protein in 1971 [18]. In 1978, prealbumin (now known as transthyretin, TTR) was found to be the protein constituent of amyloid deposits in familial amyloid polyneuropathy [19], the disorder described in 1951 by Corino Andrade in Portugal [20]. Similar disorders were found in the subsequent decades especially in Japan and Sweden. The Finnish type of familial amyloidosis, today known as AGel amyloidosis, was described in 1969 [21]. In 1980, TTR was characterized as the amyloid protein also in "senile cardiac amyloidosis" [22], later renamed as senile systemic amyloidosis, and now as ATTR. Over the following years, more than 30 circulating proteins were identified in tissue deposits in patients with amyloidotic disorders, including the Aβ peptide in Alzheimer disease and β2-microglobulin in dialysis-related arthropathy [2]. The development of molecular biology techniques allowed also to identify a growing number of mutations associated with hereditary forms and to establish some correlations between the genotype and clinical phenotype.

The identification of the amyloid precursors led to the development of the modern nomenclature of amyloidosis. The first official nomenclature committee was established in 1979 [2], and the latest nomenclature update was published in 2022 [23].

3.2 Novel Diagnostic Techniques

Thioflavin stain allows amyloid visualization using the fluorescence microscope. Thioflavin-T (Basic Yellow 1 or CI 49005) is a benzothiazole salt. When the dye binds to β sheets, it undergoes a 120 nm red shift of its excitation spectrum that may selectively be excited at 450 nm, resulting in a fluorescence signal at 482 nm. Thioflavin-S is a mixture of compounds resulting from the methylation of dehydrothiotoluidine with sulfonic acid. The fluorescence method is specific for amyloid similarly to Congo red and very sensitive [24]. Thioflavin stains are possible alternatives to Congo red staining. In both cases, the identification of the amyloid protein requires immunohistochemistry techniques, and then the use of antibodies targets the amyloid precursors. A definite diagnosis requires amyloid typing by

immunohistochemistry, immunoelectron microscopy, or mass spectrometry-based proteomic analysis, the latter one preferably after isolation of amyloid plaques by laser microdissection [25].

A major breakthrough in the field of diagnosis was the finding that technetium-based bone tracers bind ATTR deposits in the heart [26] (Fig. 3.2). In a multicenter center study on 1217 patients referred with suspected cardiac amyloidosis, an abnormal bone scintigraphy scan combined with a negative evaluation for a monoclonal gammopathy was shown to have a positive predictive value of 100% for ATTR cardiac amyloidosis (CA) [27]. An algorithm for the noninvasive diagnosis of ATTR-CA was then developed, in 2016 [27], and is now recommended by all the international documents to diagnose this condition [28].

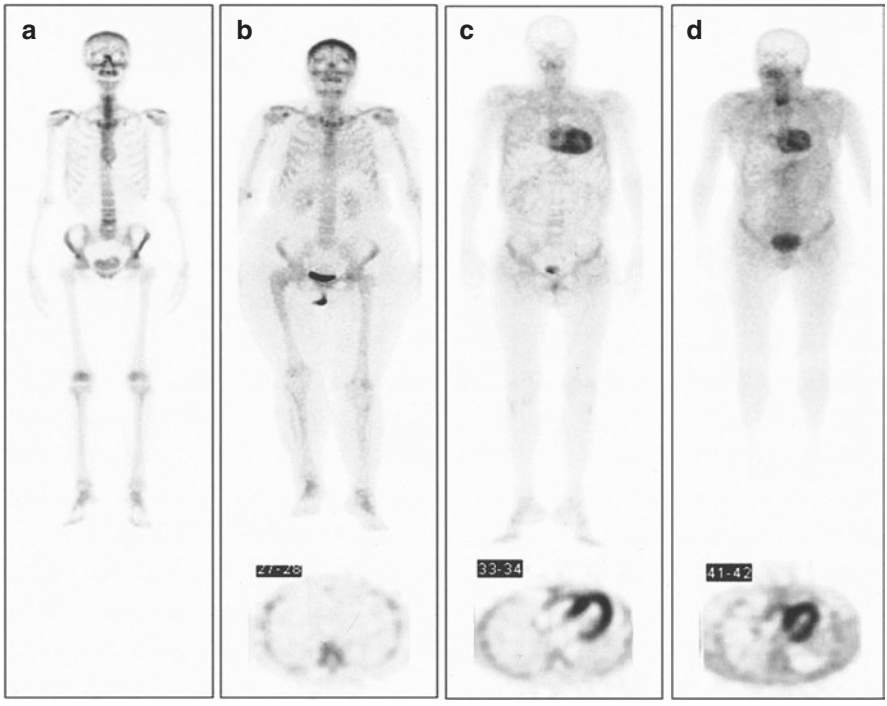

Fig. 3.2 Proposal of the Perugini scoring system. Representative examples illustrating the spectrum of ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) uptake among patients with transthyretin (TTR)-related or monoclonal immunoglobulin light-chain (AL) cardiac amyloidosis and unaffected controls (top row = whole-body scans, anterior view; bottom row = cross-sectional views of cardiac single-photon emission computed tomography in the same patients). A: Unaffected control subject without visually detectable uptake. B: Patient with AL amyloidosis and echocardiographic documentation of cardiac involvement without any visually detectable sign of myocardial ^{99m}Tc-DPD uptake; mild uptake of the tracer is visible only at the soft tissue level. C and D: Two patients with TTR-related amyloidosis and echocardiographic documentation of cardiac involvement, both showing strong myocardial ^{99m}Tc-DPD uptake (with absent bone uptake); in one of the patients (D), splanchnic uptake is also visible. Reprinted with permission from: Perugini et al. JACC [26]

In 1988, Hawkins described the use of antibodies targeting the amyloid serum P component (a protein found in all types of amyloid deposits) and labelled with ^{123}I, in a murine model of amyloidosis [29]; two years later, the same technique was employed successfully in human patients [30]. Another novel development was the discovery of Pittsburgh compound B, a positron emission tomography (PET) tracer labelled with ^{11}C and able to bind selectively the Aβ peptide. This tracer allowed to detect noninvasively the amyloid deposits in patients with suspected Alzheimer disease [31]. ^{18}F-labelled tracers, including ^{18}F-flutemetamol, ^{18}F-florbetapir, and ^{18}F-florbetaben are increasingly investigated in view of their longer half-time that avoids the need for on-site cyclotrons. Contrary to bone tracers, positron emission tomography amyloid-binding tracers demonstrate higher affinity for light-chain fibrils and may accurately distinguish patients with different types of CA. Specifically, patients with AL-CA display a persistent tracer uptake, while there is a rapid decrease in the signal in patients with ATTR-CA or those without CA [32]. Assessment of the diagnostic yield of PET imaging versus histological analysis is pending.

3.3 Therapeutic Approaches

AL and ATTR amyloidosis account for the vast majority of cases of CA. Their treatment will be analyzed in detail in dedicated chapters.

The therapy of AL amyloidosis has evolved in parallel with the therapy of multiple myeloma. The main stages of this process were the identification of melphalan as an effective therapy (in the 1970s and 1980s) [33], autologous stem cell transplantation (beginning of the 1990s) [34], the introduction of immunomodulatory agents and proteasome inhibitors (beginning of the 2000s) [35], and the recent identification of daratumumab as a possible first-line therapy [36].

As for ATTR amyloidosis, the attention was focused for a long time on hereditary forms, and particularly on patients with polyneuropathy. Liver transplantation was introduced at the beginning of the 1990s and was soon restricted to patients with early-onset, Val30Met-related disease (in whom cardiac involvement is much less common than polyneuropathy) [37]. The TTR tetramer stabilizer tafamidis was first characterized as an effective drug for patients with polyneuropathy [38]. In 2018, the phase 3 study tafamidis in Transthyretin Cardiomyopathy Clinical Trial (ATTR-ACT) demonstrated that tafamidis (a drug that stabilizes the TTR tetramer) improves the prognosis of patients with ATTR amyloidosis (either wild-type or variant) and cardiac involvement [39]. Following this study, tafamidis became the first approved drug for ATTR-related cardiomyopathy in May 2019. At present, tafamidis is the only therapeutic option for patients with ATTRwt-CA or ATTRv with isolated cardiac involvement, while those with cardiomyopathy and polyneuropathy may receive either tafamidis or patisiran (a blocker of TTR translation in the liver) [40]. Many therapies are under investigation, including gene editing [41] or the removal of tissue amyloid deposits through specific antibodies [42].

3.4 Conclusions

Our knowledge of amyloidosis has dramatically improved from the description by Virchow, but the term "amyloid" is still used to identify the abnormal substance whose tissue accumulation causes heterogeneous disease manifestations. Except for the noninvasive diagnosis of ATTR-CA by means of bone scintigraphy and the exclusion of a monoclonal protein, amyloidosis is always diagnosed by demonstrating tissue amyloid deposits and identifying the amyloidogenic protein. Major challenges are the need to perform often highly invasive biopsies (such as endomyocardial or renal biopsy), and the availability of immunohistochemistry, immunoelectron microscopy, or proteomics. An intriguing perspective is the introduction of an algorithm for the noninvasive diagnosis of AL-CA through PET tracers. While the mechanisms, whereby these tracers bind amyloid fibers, are still incompletely understood, the in-depth characterization of the amyloidogenic cascade has allowed the development of targeted therapeutic strategies such as TTR tetramer stabilizers, gene editing, or antibodies targeting tissue fibers.

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Pathophysiology, Classification, and Epidemiology of Amyloidosis

4

Alberto Giannoni, Chiara Arzilli, and Alberto Aimo

Abbreviations

AL	Amyloid light chain amyloidosis
ATTR	Amyloid transthyretin amyloidosis (vATTR, variant form; wtATTR, wild-type form)
ISA	International Society of Amyloidosis
SAA	Serum amyloid A protein
SAP	Serum amyloid P component
TTR	Transthyretin

4.1 Definition and Nomenclature

The definition of "amyloidosis" encompasses a group of disorders caused by tissue deposition, mainly extracellular, of misfolded proteins, which aggregate into insoluble fibrils that compose the amyloid substance [1]. Immunohistochemistry or proteomic analyses allow classifying the different types of amyloidosis based on the

A. Giannoni (✉) · A. Aimo
Interdisciplinary Center for Health Sciences, Scuola Superiore Sant'Anna, Pisa, Italy

Cardiology Division, Fondazione Toscana Gabriele Monasterio, Pisa, Italy
e-mail: alberto.giannoni@santannapisa.it; aimoalb@ftgm.it

C. Arzilli
Cardiology Division, Fondazione Toscana Gabriele Monasterio, Pisa, Italy
e-mail: carzilli@monasterio.it

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specific proteins that form the amyloid fibrils. The International Society of Amyloidosis (ISA) currently recognizes 42 different amyloidogenic proteins in humans. According to the nomenclature established by ISA, the amyloid protein is defined by the letter "A," followed by a suffix indicating the specific protein. This notation is also used to designate the different pathologies. The term amyloid refers to the specific protein involved, while amyloidosis refers to the disease caused by the amyloid protein. For example, when amyloid deposits are composed of immunoglobulin light chains, the amyloid protein is designated as AL and the disease is AL amyloidosis [1].

4.2 Amyloid Fibers

All forms of amyloidosis are characterized by tissue accumulation of insoluble fibrils composed of misfolded proteins. Amyloid fibrils have a 7–13 nm diameter and are composed by of 2–8 protofilaments, each having a 2–7 nm diameter, either intertwined or arranged side by side. Protofilaments are composed of β-sheet structures with hydrogen bonds between amino and carboxyl terminals of the amino acid chain [2]. The extremely regular structure of amyloid fibrils explains the characteristic apple green birefringence that can be seen on polarized light after Congo red staining [2].

The mechanisms underlying the formation of amyloid fibrils have not been completely characterized, but it is thought to depend on the intrinsic characteristics and local concentration of the amyloidogenic protein, its interaction with cell membranes and the extracellular matrix, and the insufficient removal of misfolded proteins by proteasomes (before protein release into the circulation) and macrophages [3–5]. The amyloidogenic potential of a protein is related to at least three factors, not mutually exclusive: (1) the intrinsic propensity of a protein to form amyloid deposits, (2) proteolytic changes, and (3) changes in the amino acid sequence. Some proteins are defined as "intrinsically misfolded" as they have at least a region without a fixed secondary or tertiary structure and can then change their conformation to better interact with their ligands [6]. Some examples are apolipoproteins AI, AII and the serum amyloid A protein (SAA) [7, 8]. The propensity to form amyloid deposits can be increased in some conditions, for example, when circulating concentration increases, as in the case of β2-microglobulin amyloidosis [9]. Alternatively, a normal protein can undergo proteolytic changes within the cell or in the extracellular spaces, and this process can increase its propensity to form amyloid deposits: this process occurs in many forms of amyloidosis [3] and has been well characterized in Alzheimer's disease [10]. Furthermore, a gene mutation can reduce protein stability, as in the familial forms of amyloidosis [11] or in mutations in the variable regions of immunoglobulin light chains [12–14] (Fig. 4.1).

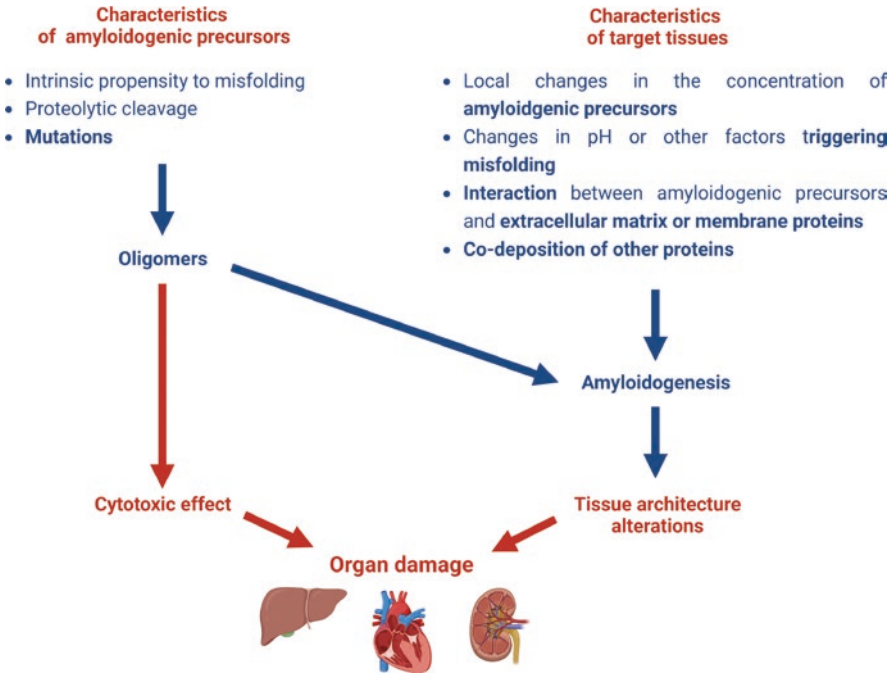

Fig. 4.1 Main characteristics of the amyloidogenic process and the resulting organ damage. The formation of amyloid deposits in tissues is attributed both to intrinsic characteristics of the amyloidogenic protein and to specific aspects of target tissues. Organ damage is caused by structural changes caused by amyloid deposition, and possibly also by a direct cytotoxic effect of protein oligomers

The process of amyloidogenesis is characterized by at least two phases. Indeed, all the interactions and conformational changes needed to form an initial amyloid deposit require a very long time to occur. After the so-called nucleation phase, the velocity of protein deposition increases markedly ("elongation phase" or "phase of sigmoidal growth"). During this phase, new nucleation processes can occur at the surface of fibrils or as a result of fibril fragmentation [3, 6].

In addition to amyloid fibrils, amyloid deposits include other protein components such as collagen [4], glycosaminoglycans, and proteoglycans (particularly dermatan sulfate and heparan sulfate), which form a scaffold promoting amyloid deposition [15] and may concur to cause organ tropism of amyloidogenic precursors [3]. Another protein very frequently encountered in deposits is serum amyloid P component (SAP), a pentraxin that has a high affinity for amyloid fibrils and protects them from proteolytic degradation and phagocytosis [16].