

What is new in diagnosis of amyloidosis?

María A. Marco¹, Marcelina Carretero², Diego Pérez de Arenaza³, Eugenia Villanueva³, Erika B. Brulc⁴, Gisela Bendelman¹, Elsa M. Nucifora⁴, María S. Sáez⁵, Patricia B. Sorroche⁵, María A. Aguirre¹ and María L. Posadas Martínez¹

1. Servicio de Clínica Médica, Hospital Italiano de Buenos Aires. Buenos Aires, Argentina

2. Área de Investigación en Medicina Interna, Servicio de Clínica Médica, Hospital Italiano de Buenos Aires. Buenos Aires, Argentina

3. Servicio de Cardiología, Hospital Italiano de Buenos Aires. Buenos Aires, Argentina

4. Servicio de Hematología, Hospital Italiano de Buenos Aires, Buenos Aires. Buenos Aires, Argentina

5. Instituto de Medicina Traslacional e Ingeniería Biomédica (IMTIB), CONICET - Instituto Universitario del Hospital Italiano de Buenos Aires - Hospital Italiano de Buenos Aires. Buenos Aires, Argentina

ABSTRACT

Amyloidosis has always represented a diagnostic challenge. In 2020, the Amyloidosis Study Group (ASG) developed the "Clinical Practice Guideline for the Diagnosis of Amyloidosis". New lines of research have subsequently emerged. This narrative review aims to explore the state of the art in the diagnosis of amyloidosis diagnosis.

In patients with amyloidosis, protein typing by mass spectrometry is recommended, a technique hard to perform because it requires laser microdissection for sample preparation. Recent publications propose other methods to obtain the amyloid sample to be analyzed, making it possible to dispense with microdissection.

On the other hand, in patients with confirmed TTR amyloidosis (aTTR), the recommendation to sequence the amyloidogenic gene was intended for suspected cases of hereditary aTTR but has now been extended to all patients regardless of age.

Key words: amyloidosis, amyloidosis diagnosis, TTR amyloidosis, immunoglobulin light-chain amyloidosis, cardiac amyloidosis, amyloid neuropathies, amyloidogenesis.

¿Qué hay de nuevo en el diagnóstico de amiloidosis?

RESUMEN

La amiloidosis siempre ha representado un desafío diagnóstico. En el año 2020, el Grupo de Estudio de Amiloidosis (GEA), confeccionó la *Guía de Práctica Clínica para el Diagnóstico de Amiloidosis*¹⁻³. Nuevas líneas de investigación se han desarrollado posteriormente. Esta revisión narrativa tiene como intención explorar el estado del arte en el diagnóstico de la amiloidosis.

En pacientes con amiloidosis se recomienda la tipificación de la proteína mediante espectrometría de masa, técnica de difícil ejecución por requerir de microdisectores láser para la preparación de la muestra. Algunas publicaciones recientes proponen otros métodos para obtener la muestra de amiloide que se va a analizar, permitiendo prescindir de la microdissección.

Por otra parte, en pacientes con Amiloidosis ATTR confirmada, la recomendación de secuenciar el gen amiloidogénico se encontraba destinada a los casos sospechosos de ATTR hereditaria (ATTRv), pero actualmente esta se ha extendido a todos los pacientes sin importar la edad.

Author for correspondence: maria.marco@hospitalitaliano.org.ar, Marco MA.

Received: 12/22/22 Accepted: 11/13/23 Online: 12/29/2023

DOI: <http://doi.org/10.51987/revhospitalbares.v43i4.226>

How to cite: Marco MA, Carretero M, Pérez de Arenaza D, Villanueva E, Brulc EB, Bendelman G, Nucifora EM, Sáez MS, Sorroche PB, Aguirre MA, Posadas Martínez ML. What is new in diagnosis of amyloidosis? *Rev. Hosp. Ital. B.Aires.* 2023;43(4):209-213.

En lo que respecta a los estudios complementarios orientados al diagnóstico de compromiso cardíaco, se ha propuesto el uso de la inteligencia artificial para su interpretación, permitiendo la detección temprana de la enfermedad y el correcto diagnóstico diferencial.

Para el diagnóstico de neuropatía, las últimas publicaciones proponen el uso de la cadena ligera de neurofilamento sérica, que también podría resultar un indicador útil para seguimiento.

Finalmente, con referencia a la amiloidosis AL, la comunidad científica se encuentra interesada en definir qué características determinan el carácter amiloidogénico de las cadenas livianas. La N-glicosilación de dichas proteínas impresiona ser uno de los determinantes en cuestión.

Palabras clave: amiloidosis, diagnóstico de amiloidosis, amiloidosis TTR, amiloidosis AL, amiloidogénesis, amiloidosis cardíaca, neuropatía amiloide.

INTRODUCTION

Amyloidosis is considered a rare disease and has always posed a diagnostic and therapeutic challenge as such. However, significant progress has been made in recent decades regarding the diagnosis and treatment of various types of amyloidosis. In 2020, a multidisciplinary group from a private university hospital in Buenos Aires, the Amyloidosis Study Group (GEA), compiled the “Clinical Practice Guide for the Diagnosis of Amyloidosis”¹⁻³. The aim was to provide the medical community with fundamental guidelines based on the best available evidence and about the applicability of the recommendations..

From the year of its creation to the present date, new lines of research have gained strength or been initiated. This narrative review aims to explore the state of the art in topics related to amyloidosis diagnosis. To this end, we will expand on the available information, using the recommendations from the guidelines published by the Amyloidosis Study Group (GEA) as a starting point.

Recommendation 3

For patients with amyloidosis, we recommend protein typing by mass spectrometry.

Strong grade of recommendation, quality of evidence is high⁴.

UPDATES

Despite mass spectrometry becoming the preferred diagnostic method in reference centers such as the Mayo Clinic and the London Amyloidosis Centre in Argentina, the implementation of this technique is still not available as a standardized and named practice within the national healthcare system. The limitations to performing this technique are mainly two: a local shortage of mass spectrometers and laser microdissectors in the environment and the lack of expertise in the field to execute the technique correctly.

These limitations are not unique to our country, and several studies have been published in recent years proposing new ways to treat biological tissue samples to

avoid the need for laser microdissection in identifying amyloid.

Kamiie and colleagues proposed the treatment of various formalin-fixed, paraffin-embedded samples with organic solvents to selectively extract amyloid and differentiate it through electrophoresis, followed by mass spectrometry analysis on the obtained sample. Through different tests, they concluded that dimethyl sulfoxide could selectively extract amyloid from a conventionally treated pathological anatomy sample. The hypothesis supporting the ability of organic solvents to extract amyloid from a sample stems from their capacity to modify the secondary conformation of the amyloidogenic beta-folded protein to alpha⁴.

On the other hand, a team from the University of Washington, Seattle, Washington, proposed a manual, non-laser microdissection method that achieved 100% specificity in their results⁵.

Further research is needed to improve the accessibility of spectrometry by simplifying sample processing.

Recommendation 3

Confirmation of ATTRv amyloidosis by DNA sequencing of the amyloidogenic TTR gene of all four exons is suggested in patients with suspected ATTRv amyloidosis.

Weak grade of recommendation. Very low quality of evidence (consensus)¹.

UPDATES

In 2021, the Working Group on Myocardial and Pericardial Diseases of the European Society of Cardiology published its position on cardiac amyloidosis diagnosis and treatment. It suggests that genetic testing should be conducted once cardiac amyloidosis is confirmed in all patients, even in those of older age where ATTRv may not initially be suspected⁶. This suggestion is based on a Spanish retrospective descriptive study in which 116 patients with ATTR amyloidosis were included, 18 of whom had hereditary amyloidosis. The mean age of this group of patients was 69 ± 14.6 years. Of the 114 patients

diagnosed with ATTR over 60 years of age, a mutation was found in 14 of them (12%). These findings allowed the study of 57 first-degree relatives and the diagnosis in 20 of them of mutations in the TTR gene.

Recommendation 10

The performance of an electrocardiogram is recommended as the initial assessment for every patient with amyloidosis.

Strong recommendation, moderate quality of evidence².

Recommendation 11

Doppler echocardiography (conventional) is recommended as the initial imaging of choice for the diagnosis of cardiac amyloidosis in patients with suspected cardiac involvement due to amyloidosis.

Strong grade of recommendation, moderate quality of evidence².

Recommendation 13

Cardiac magnetic resonance imaging (MRI) with gadolinium is recommended for cardiac amyloidosis diagnosis in patients with previous studies suggestive or indeterminate of amyloidosis.

Strong grade of recommendation, moderate quality of evidence².

To date, the use of artificial intelligence and machine learning for the detection and diagnosis of amyloidosis is under development in multiple centers in many parts of the world. There are numerous publications on using these resources for diagnosing amyloidosis or organ involvement due to the disease, utilizing different diagnostic studies as analysis sources (laboratory measurements, imaging studies, 3-D motion studies, and information available in medical history). However, noteworthy is the use of artificial intelligence and machine learning for diagnosing cardiac amyloidosis, utilizing the electrocardiogram and cardiac imaging studies as sources of information.

All this builds on the already established advancements in the use of artificial intelligence in the diagnosis of cardiomyopathies, the growing diagnostic capability, the consequent increase in the prevalence of cardiac amyloidosis, and the prognostic impact that such involvement and its early diagnosis imply.

The application of machine learning and deep learning in the interpretation of electrocardiograms, echocardiograms, and cardiac MRIs stands out in the current literature on cardiac amyloidosis, reinforcing the predictor value that these studies possess. The development of these tools aims to achieve both early suspicion and detection through electrocardiographic and echocardiographic patterns and to contribute to the differential diagnosis of cardiomyopathies through cardiac MRI⁷.

Recommendation 21

Performing a skin biopsy with staining for fine fiber is strongly recommended for the early diagnosis of neuropathy in patients with a genetic diagnosis of TTR amyloidosis who present signs or symptoms suggestive of small fiber neuropathy.

Strong recommendation, very low-quality evidence³.

Recommendation 22

Performing a skin biopsy with staining for fine fiber is suggested for the early diagnosis of neuropathy in patients with suspected amyloidosis who present signs or symptoms suggestive of small fiber neuropathy.

Weak recommendation, very low-quality evidence³.

Recommendation 23

Conducting nerve conduction studies evaluating motor and sensory fibers is strongly recommended for coarse fiber neuropathy diagnosis in patients with a diagnosis or suspicion of amyloidosis.

Strong recommendation, very low-quality evidence³.

Recommendation 24

Performing the QST test is strongly recommended for the early diagnosis of neuropathy in patients with a genetic diagnosis of TTR amyloidosis who present signs or symptoms suggestive of small fiber neuropathy.

Strong recommendation, very low-quality evidence³.

Recommendation 25

Performing the QST test is suggested for the early diagnosis of neuropathy in patients with amyloidosis or suspected amyloidosis who present signs or symptoms suggestive of small fiber neuropathy.

Weak recommendation, very low-quality evidence³.

Recommendation 26

Based on the literature, Sudoscan is strongly recommended for the early diagnosis of peripheral autonomic neuropathy (even in asymptomatic patients) in those with suspected autonomic neuropathy due to amyloidosis.

Strong recommendation, very low-quality evidence³.

UPDATES

Peripheral neuropathy caused by ATTR or AL amyloidosis poses a diagnostic challenge. Early diagnosis may now involve changes in prognostic considerations, therapeutic decisions, or both. Currently, studies aimed at achieving early diagnosis are complex, and neuropathy is often confirmed when the patient presents frank symptoms.

Neurofilament light chain (NfL) is a specific protein of neuronal structure released into cerebrospinal fluid or blood in response to neuroaxonal damage. Specifically,

since the development of methods that enhance sensitivity in blood detection, this protein represents a potential biomarker for neuronal involvement in the diagnosis, monitoring, and prediction assessment of various neurodegenerative diseases, both in the central and peripheral nervous systems (multiple sclerosis, amyotrophic lateral sclerosis, Charcot-Marie-Tooth disease).

Between 2020 and 2021, three studies came out in support of the usefulness of measuring neurofilament light chain for diagnosing peripheral neuropathy due to amyloidosis.

Ticau et al. studied the behavior of neurofilaments in patients with ATTR amyloidosis. In an initial publication, they compared baseline levels of NfL in blood among patients with ATTRv cardiomyopathy participating in the ENDEAVOUR study (n=194), patients with ATTRv polyneuropathy participating in the APOLLO study (n=193), and healthy controls (n=53). Significantly elevated levels of neurofilament light chain were observed in patients diagnosed with amyloid cardiomyopathy compared to healthy controls (54.1 pg/mL vs. 16.3 pg/mL, $p < 0.001$). The difference remained significant when comparing controls with ATTR patients who did not present symptoms compatible with neuropathy. However, no differences emerged between those with a confirmed diagnosis of polyneuropathy and patients with compatible symptoms but without confirmation.

In a second study published in 2021, Ticau et al. assessed the evolution of NfL values in patients treated with patisiran vs. placebo in the phase 3 APOLLO study, revealing a frank decline in patients receiving patisiran and an increase in those who received placebo. This publication suggests that NfL could serve not only as an early biomarker for the presence of polyneuropathy but also as an indicator of its progression⁸.

On the other hand, a study conducted by the Amyloidosis Center of Expertise at the University of Groningen in the Netherlands compared the NfL values of patients with AL amyloidosis diagnosed with neuropathy and without neuropathy, patients with hereditary ATTR amyloidosis neuropathy, carriers of ATTRv mutations, and healthy controls. The serum NfL levels in patients with AL amyloidosis were significantly higher than in healthy controls, and those with AL amyloidosis and neuropathy had significantly higher values than those without neuropathy. Patients with ATTRv had higher levels ($p > 0.0001$) than gene carriers and healthy controls, with no significant differences between the latter two groups. Values were also compared in ATTRv patients with more advanced neuropathy against those with recent onset, noting that the former had higher values. This latest study not only aligns with the findings of the other two studies but also suggests that NfL may be useful for assessing peripheral nervous system involvement in AL amyloidosis⁹.

Further studies in this field are still required in order to define a standardized value, sensitivity and specificity for the test. As an aspect that facilitates the eventual

availability of this resource in health systems, NfL are being evaluated as biomarkers for other diseases of the central nervous system.

Other new developments with diagnostic implications

Much progress has been made on the road to the diagnosis of amyloidosis, including the availability of new diagnostic resources and the standardization and consensus of algorithms, as evidenced by the clinical practice guidelines published by the EAG. Currently, about AL amyloidosis, the scientific community is focused on understanding what gives a particular light chain its “amyloidogenic” character. Detecting the molecular determinants of amyloidogenesis would allow early detection of those cases of MGUS that are most likely to progress to AL amyloidosis.

N-glycosylation is one of these molecular determinants. Some years ago, glycosylation had already been postulated to contribute to amyloidogenesis and started from the MGUS stage. In 2019, Dispenzieri et al. from the Mayo Clinic and the University of Pavia demonstrated that kappa light chains from patients with amyloidosis had a 13-fold higher rate of glycosylation than patients without amyloidosis. In 2020, the Mayo Clinic published a retrospective cohort where they analyzed, through mass spectrometry, the light chains of multiple individuals with MGUS, showing that N-glycosylation constitutes an independent risk factor for progression to amyloidosis, with a hazard ratio of 10.1 (20-year risk of developing amyloidosis of 20% in those patients with glycosylated light chains vs. 3% in those with non-glycosylated chains). They also showed that N-glycosylation implied a higher incidence of other plasma cell dyscrasias, pointing out that in most patients who obtained alternative diagnoses to amyloidosis, the corresponding studies for diagnosing amyloidosis were not performed¹⁰.

The analysis of N-glycosylation as a predictor of progression to amyloidosis may become even more specific. In 2022, the University of Pavia published a paper analyzing the sequencing of 220 patients with amyloidosis and detecting particular patterns in the sequence and spatial pattern in which they arranged the N-glycosylation at issue.

At the last ISA (International Society for Amyloidosis) congress, this line of research was one of the protagonists of the sessions on basic research. Despite promising information, to date, no clear criteria or standardized methods have been defined for detecting amyloidogenic proteins or the diagnosis of AL amyloidosis in its early stages. Also, the proposal has limitations related to the lack of availability of spectrometers for studying light chains and methods for generalized sequencing of light chains.

By way of conclusion

In recent years, significant progress has been made in the amyloidosis diagnosis and treatment, particularly regarding protein typing, organ involvement assessment, and early diagnosis of ATTR amyloidosis. In this scenario,

new questions and needs related to diagnosis have emerged. With the availability of new and improved treatments, early and specific suspicion and diagnosis of amyloidosis, its subtypes, and organ involvement have gained greater importance.

However, its status as a “rare disease” associated with the difficulty in implementing and using new technologies poses a challenge globally and regionally that the scientific community must confront. Collaboration between research groups of different types, institutions, and regions is likely to be of great value in achieving further advancements.

Funding: Partial funding came from CELNOVA for the writing work; the GEA (Grupo de Estudios de Amiloidosis) receives funding through the institution for fellows, educational activities, and amyloidosis-related projects from CELNOVA, Pfizer, Janssen, and PTC BIOP.

Conflicts of interests: the authors declare no conflict of interests

REFERENCES

1. Posadas Martínez ML, Aguirre MA, Belziti C, et al. Guía de práctica clínica para el diagnóstico de la amiloidosis: Parte 1/3. Año 2020. Rev Fac Cien Med Univ Nac Córdoba. 2021;78(1):74-82. <https://doi.org/10.31053/1853.0605v78.n1.30824>.
2. Posadas Martínez ML, Nucifora E, Belziti C, et al. Guía de práctica clínica para el diagnóstico de compromiso orgánico de amiloidosis: Parte 2/3. Año 2020. Rev Fac Cien Med Univ Nac Córdoba. 2022;79(1):78-87. <https://doi.org/10.31053/1853.0605v79.n1.30897>.
3. Posadas Martínez ML, Aguirre MA, Greloni G, et al. Guía de práctica clínica para el diagnóstico de compromiso orgánico en amiloidosis: Parte 3/3 Año 2020. Rev Fac Cien Med Univ Nac Córdoba. 2022;79(4):391-399. <https://doi.org/10.31053/1853.0605v79.n4.30903>.
4. Kamiie J, Aihara N, Uchida Y, et al. Amyloid-specific extraction using organic solvents. MethodsX. 2020;7:100770. <https://doi.org/10.1016/j.mex.2019.100770>.
5. Phipps WS, Smith KD, Yang HY, et al. Tandem mass spectrometry-based amyloid typing using manual microdissection and open-source data processing. Am J Clin Pathol. 2022;157(5):748-757. <https://doi.org/10.1093/ajcp/aqab185>.
6. García-Pavia P, Rapezzi C, Adler Y, et al. Diagnosis and treatment of cardiac amyloidosis: a position statement of the ESC Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2021;42(16):1554-1568. <https://doi.org/10.1093/eurheartj/ehab072>.
7. Jiang S, Zhang L, Wang J, et al. Differentiating between cardiac amyloidosis and hypertrophic cardiomyopathy on non-contrast cine-magnetic resonance images using machine learning-based radiomics. Front Cardiovasc Med. 2022;9:1001269. <https://doi.org/10.3389/fcvm.2022.1001269>.
8. Ticau S, Sridharan GV, Tsour S, et al. Neurofilament light chain as a biomarker of hereditary transthyretin-mediated amyloidosis. Neurology. 2021;96(3):e412-e422. <https://doi.org/10.1212/WNL.00000000000011090>.
9. Louwsma J, Brunker AF, Bijzet J, et al. Neurofilament light chain, a biomarker for polyneuropathy in systemic amyloidosis. Amyloid. 2021;28(1):50-55. <https://doi.org/10.1080/13506129.2020.1815696>.
10. Dispenzieri A, Larson DR, Rajkumar SV, et al. N-glycosylation of monoclonal light chains on routine MASS-FIX testing is a risk factor for MGUS progression. Leukemia. 2020;34(10):2749-2753. <https://doi.org/10.1038/s41375-020-0940-8>.