Mini review on the laboratory diagnosis of amyloidosis: An overview of methods, applications, and trends in analytical approaches

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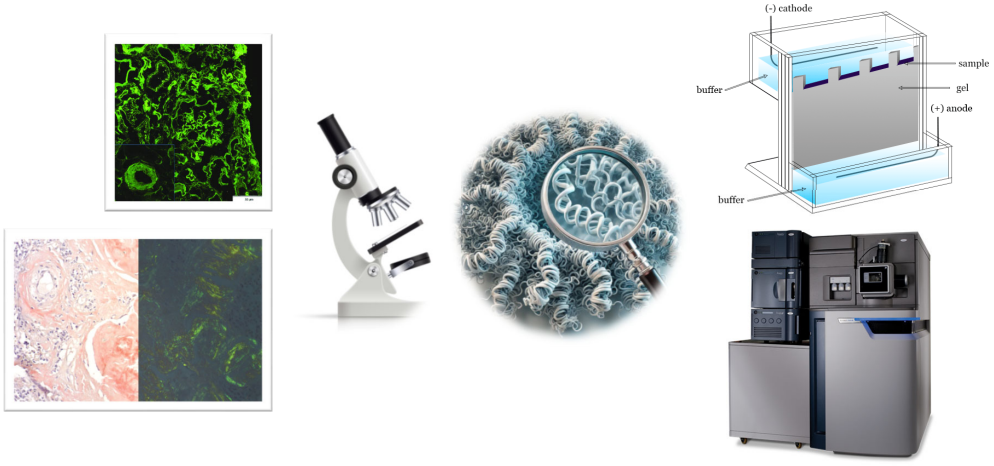
Amyloidosis represents a clinically and molecularly heterogeneous group of serious, potentially life-threatening disorders characterized by the extracellular deposition of insoluble amyloid fibrils derived from misfolded precursor proteins. These deposits disrupt tissue architecture and function, often affecting vital organs such as the heart and kidneys. Accurate diagnosis and subtyping of amyloidosis are essential for effective clinical management and personalized therapeutic interventions. This review provides an integrated overview of modern approaches to the laboratory diagnosis of amyloidosis, divided into three main areas: (1) histological and immunological methods (including Congo red staining, immunohistochemistry, immunofluorescence, and immunoelectron microscopy) for the initial detection and characterization of amyloid deposition, (2) electrophoretic techniques (capillary electrophoresis, isoelectric focusing, immunofixation electrophoresis) used primarily for the analysis of amyloid-associated proteins in serum and urine, and (3) mass spectrometry-based proteomic analyses that have significantly improved subtype specificity and clinical decision-making. By emphasizing the complementary roles of these techniques, the review aims to support timely, accurate, and subtype-specific diagnosis, ultimately improving clinical outcomes and treatment strategies for patients affected by amyloidosis.

Mini Review On The Laboratory Diagnosis Of Amyloidosis



Structured integration of histological, electrophoretic, and proteomic tools enhances diagnostic precision, enables accurate subtyping, and supports timely, personalized treatment.

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INTRODUCTION

Amyloidosis is an umbrella term for a range of systemic and organ-specific diseases characterised by the accumulation of insoluble amyloid fibrils formed from misfolded precursor proteins in extracellular spaces[1-3]. Although historically considered rare, amyloidosis is now being increasingly recognized due to advances in diagnostic techniques such as mass spectrometry (MS) and refined imaging modalities[4]. Light chain amyloidosis (AL) is the most common systemic form of the disease in Western countries. A study published in 2022 estimated the crude incidence rate of AL amyloidosis to be 10.44 cases per million person-years in various countries, including those in Europe and North America[5]. This clonal plasma cell disorder is characterized by the deposition of misfolded light chain proteins in various organs, leading to significant clinical manifestations, particularly in the heart and kidneys[6].

The systemic nature and complexity of amyloidosis necessitate a multidisciplinary approach to both diagnosis and management. Effective care therefore requires close collaboration among hematologists, cardiologists, nephrologists, pathologists, and other specialists, ensuring comprehensive evaluation and treatment that address the diverse organ-specific effects of the disease and improve patient outcomes[7,8].

To date, more than 40 amyloidogenic proteins have been identified in humans, with approximately 30 recognized as clinically relevant[9,10]. Among these, immunoglobulin light chains, transthyretin (ATTR), serum amyloid A (SAA), and β2-microglobulin are of particular importance. AL amyloidosis, most commonly associated with plasma cell dyscrasias, often presents with nephrotic syndrome, cardiomyopathy, or peripheral neuropathy. ATTR amyloidosis includes both hereditary (mutant ATTR) and wild-type (senile systemic) forms, the latter typically manifesting as a restrictive cardiomyopathy in older adults. AA amyloidosis, linked to chronic inflammation, frequently affects the kidneys and liver, while β2-microglobulin amyloidosis is associated with long-term dialysis. These diverse clinical presentations underscore the importance of accurate amyloid typing for appropriate management[10,11].

Amyloidosis classifications – primary (AL), secondary (AA), hereditary, wild-type ATTR, dialysis-related, and localized – provide a framework for understanding disease pathophysiology and guiding therapeutic decisions. Historically, diagnosis has relied heavily on histopathologic evidence, with Congo red staining revealing the characteristic apple-green birefringence under polarized light[12]. Immunohistochemistry (IHC) and serum protein electrophoresis have also played key roles in the identification and characterization of amyloid precursor proteins. However, these methods may lack the necessary sensitivity and specificity, particularly for complex or less common amyloid subtypes. Emerging evidence highlights the limitations of traditional diagnostics and the need for more accurate and efficient methods of amyloid typing[13].

Diagnostic strategies have evolved significantly in recent years. Electrophoretic techniques, while long-standing tools for protein characterization, often fall short in the detection and typing of amyloidogenic proteins in complex biological matrices[14]. In contrast, mass spectrometry has changed the diagnostic landscape by providing unprecedented sensitivity, specificity, and the ability to identify multiple amyloidogenic proteins from minimal or degraded samples[12,15]. This shift from traditional diagnostic tools to mass spectrometry-based proteomic approaches promises to improve diagnostic accuracy, reduce delays, and enable more personalized therapeutic strategies.

Modern approaches to the laboratory diagnosis of amyloidosis include a number of complementary techniques, each of which contributes to a more accurate and clinically relevant assessment of the disease. These methods can be broadly categorized into three main areas. First, pathologic diagnosis typically begins with classical approaches such as histochemical staining (e.g., Congo red), immunohistochemistry, and related immunologic methods including immunofluorescence (IF) and immunoelectron microscopy (IEM), which are essential for the detection and characterization of amyloid fibrils and their precursor proteins in tissues. Second, electrophoretic techniques - such as capillary electrophoresis (CE), isoelectric focusing (IEF), and immunofixation electrophoresis (IFE) – play a critical role in the identification of amyloid-associated proteins in body fluids such as serum and urine. Third, mass spectrometry has become an indispensable tool for in-depth molecular profiling of amyloid deposits, allowing definitive determination of their protein composition. Together, these diagnostic strategies provide a robust framework that supports accurate typing and timely diagnosis, thereby informing prognosis and guiding therapeutic decisions in patients with amyloidosis.

OVERVIEW OF HISTOLOGICAL AND IMMUNOLOGICAL METHODS FOR AMYLOID DETECTION AND TYPING

The pathologic diagnosis of amyloidosis has evolved significantly over the past several decades, driven by conceptual insights and technological innovation. Historically, the identification and classification of amyloid deposition relied primarily on histologic techniques, with Congo red staining serving as the cornerstone. The authors have also addressed this topic in several reviews[16]. This century-old gold standard allowed pathologists to identify the characteristic apple-green birefringence of amyloid under polarized light, providing a diagnostic baseline applicable to a wide range of tissues and clinical contexts. Over time, advances in immunological and molecular methods have greatly improved diagnostic accuracy. Techniques such

as immunohistochemistry and immunofluorescence, using antibodies directed against specific amyloidogenic proteins, provided a link from morphologic detection to biochemical classification. These methods significantly improved both sensitivity and specificity, allowing more nuanced categorization of amyloid subtypes and aiding in prognostic evaluation and therapeutic planning. For these reasons in particular, these techniques are used, for example, in article[17] where authors describe the use of immunohistochemistry in the typing of amyloid protein in a patient sample. The increasing recognition of numerous amyloidogenic proteins involved in human disease has further underscored the importance of accurate subtype classification.

In recent years, immunoelectron microscopy has emerged as a powerful tool that combines ultrastructural visualization with targeted immunolabeling to improve diagnostic accuracy. Together, these different methodologies – including Congo red staining, IHC, IF, and IEM – form a robust diagnostic framework. Each technique builds on the foundation laid by its predecessors, addressing specific challenges in amyloid detection and enabling comprehensive assessment of amyloid fibrils in both clinical and research settings. This chapter reviews these diagnostic modalities, including histological techniques, Congo red staining, IHC, IF, and IEM, and highlights their contributions to the evolving field of amyloid pathology.

Key studies that employed histological, immunohistochemical, immunofluorescence, and immunoelectron microscopy methods for amyloid detection and typing are summarized in Table 1.

Congo red staining

Congo red staining remains a cornerstone in the histopathologic diagnosis of amyloidosis and is widely considered the gold standard for the detection of amyloid deposition. The dye binds selectively to amyloid fibrils and produces a characteristic apple-green birefringence under polarized light, a diagnostic hallmark of amyloid structures[18,19]. This method is routinely applied to a variety of tissue types, including kidney, liver, heart, abdominal fat pad, and rectal biopsies, which may contain various amyloidogenic proteins such as immunoglobulin light chains, serum amyloid A, transthyretin, and others[18,19].

The sensitivity of Congo red staining is tissue dependent, with higher detection rates reported in organs with greater amyloid burden, such as the kidney or heart, where sensitivities range from 87% to 98% (ref.[19,20]). In contrast, tissues such as the abdominal fat pad have lower sensitivities (typically 55–85%), although specificity remains consistently high[19,21].

Despite its diagnostic value, Congo red staining does not allow differentiation of amyloid subtypes. Therefore, further immunohistochemical or proteomic analyses are required for definitive typing. Importantly, in cases where amyloidosis is clinically suspected but Congo red staining is negative, immunohistochemistry should be used to confirm or exclude the diagnosis[20,21]. While Congo red staining continues to serve as a reliable initial diagnostic tool, its limitations underscore the need for complementary techniques to ensure accurate subtype identification and guide appropriate therapeutic strategies.

Immunohistochemistry

Immunohistochemistry is a key technique for amyloid typing, especially in formalin-fixed paraffin-embedded samples (FFPE). Using antibodies against specific proteins, IHC can identify common amyloid types such as AL, AA, and ATTR (ref.[22,23]). Its utility extends to various tissues-renal, hepatic, cardiac, and gastrointestinal-with performance influenced by amyloid density and distribution[23,24]. Sensitivity and specificity are generally high, with specificity often exceeding 90% (ref.[24,25]), although false negatives can occur due to masked epitopes or fragmented deposits[22,24]. While common forms are readily detected, rarer subtypes (e.g., fibrinogen (AFib), apolipoprotein A1 (AApoA1)) may be more difficult to identify due to limited antibody availability[22,25]. Additional challenges include the need for antigen retrieval, potential cross-reactivity, and problems due to poor sample quality[23,25]. In complex cases, complementary methods such as mass spectrometry help to confirm results. Ultimately, combining IHC with advanced analytical techniques improves diagnostic accuracy and broadens the scope of amyloid typing[26,27].

Immunofluorescence

Immunofluorescence uses fluorescently labeled antibodies to detect and subtype amyloidogenic proteins – including AL, AA, and ATTR – in frozen tissue sections. While historically focused on renal pathology, IF is increasingly being applied to cardiac and other organ biopsies[28,29]. Under optimal conditions, IF offers superior sensitivity and specificity compared to immunohistochemistry, particularly for AL amyloidosis, with sensitivities up to 85% reported in renal specimens[29,30]. However, IF requires well-preserved frozen tissue, which is not always available, and can be affected by non-specific binding or background fluorescence[26,29]. These factors, together with the limited availability of specific antibodies for rare amyloid subtypes, highlight the limitations of the method. Consequently, IF is best used as part of an integrated diagnostic strategy, complementing techniques such as mass spectrometry and paraffin-based immunofluorescence to improve diagnostic accuracy and broaden its applicability[28,30,31].

Immunoelectron microscopy

Immunoelectron microscopy is a high-resolution diagnostic tool that combines the ultrastructural visualization capabilities of electron microscopy (EM) with the specificity of immunolabeling, making it essential for amyloid detection and typing in difficult cases[17,32]. Using gold-labeled antibodies, IEM directly visualizes 8–10 nm non-branching amyloid fibrils and precisely localizes proteins such as kappa and lambda light chains, serum amyloid A and transthyretin[25,26]. This technique offers greater sensitivity than light microscopy and reduces false positives compared to immunohistochemistry, especially in tissues with minimal deposits or inconclusive results from con-

Table 1. Overview of histological and immunological methods for amyloid detection and typing.

Method	Analytes	Type of biological matrices	Type of analysis	Additional information	Ref.
Congo red staining	Amyloid (non-typed, Congo red positive in kidney only)	Bone marrow, fat pad, renal tissue, blood vessels (radial artery, brachial vein)	Qualitative; Congo red staining (polarized light), immunofluorescence, confirmed by LC-MS/MS	Not quantified; amyloid detected in renal biopsy only; other tissues Congo red negative; MS confirmed kappa light chain in bone marrow	19 Bowen et al. (2012)
Congo red staining	Amyloid (non-typed; Congo red positive with and without TRFM)	FFPE tissue biopsies: kidney, bone marrow, fat pad, heart, colon, maxillary sinus	Qualitative; Congo red staining under light microscopy and Texas Red–filtered fluorescence microscopy (TRFM)	Not quantified; TRFM improved amyloid detection specificity from 21% to 83%; sensitive to scant congophilia	20 Shehabeldin et al. (2022)
Congo red staining	Amyloid (non-typed; Congo red positive with and without fluorescence microscopy)	FFPE tissue: heart, liver, kidney, small intestine, fat pad, thyroid, lymph node, urinary bladder, vocal cord, lung, cornea, colon, pancreas, tongue	Qualitative; Congo red staining under LM and polarized light, enhanced by fluorescence microscopy with Texas red filter	Not quantified; FM improved detection in 22/48 cases (esp. those with scant amyloid); diagnostic confirmation in cases missed by LM	21 (Clement CG, Thruong LD, 2014)
Immunohistochemistry	ALλ, ALκ, ATTR, AA, ALys, Aβ2M	FFPE and AFA-fixed tissues; multiple organs (kidney, salivary glands, fat, etc.)	Qualitative; diagnostic interpretation based on pattern and intensity	Not reported; intensity-based visual assessment	22 Barreca et al. (2021)
Immunohistochemistry	AL (κ, λ), ATTR, AA, AFib, AApoAI, ALys	FFPE tissues from various biopsies; used tissue microarrays for controls	Qualitative (pattern recognition and exclusion approach)	Not reported; diagnosis based on uniform reactivity	23 Schönland et al. (2012)
Immunohistochemistry	ALλ, ALκ, ATTR, AA, Aβ2M, AFib, AApoAI, ApoAII, ALys, AGel, ACys	FFPE tissues including kidney, liver, fat; validated prototype series	Qualitative and semi-quantitative (scoring 0–3)	Not reported; interpretation via consistent immunoreactivity	24 Linke RP (2012)
Immunohistochemistry	AL amyloid, ATTR, AA (contextual IHC use)	GI biopsies (stomach, small and large intestine)	Qualitative (based on histology and IHC marker presence)	Not reported; presence/absence-based qualitative diagnosis	25 Pezhouh et al. (2024)
Immunofluorescence	Primary: Kappa, Lambda (light chains); IgG, IgA, IgM (heavy chains); Serum amyloid A (SAA) Additional: Transthyretin (TTR); Apolipoprotein A-I (ApoA-I); Fibrinogen; Beta-2 microglobulin (β2M)	Frozen sections of cardiac and renal biopsy specimens	Qualitative; direct immunofluorescence for typing amyloid deposits (AL, AH, AA)	Not specified; interpretation based on fluorescence distribution and intensity	28 Collins et al. (2009)

Table 1. *(Continued)*

Method	Analytes	Type of biological matrices	Type of analysis	Additional information	Ref.
Immuno-fluorescence	Kappa, Lambda (light chains); Serum amyloid A (SAA); Transthyretin (TTR)	FFPE and frozen tissue biopsies: salivary glands, digestive tract, skin, fat pad, bladder, lung, lymph nodes, bone marrow	Qualitative and semi-quantitative; paraffin and frozen tissue immunofluorescence with enzymatic pretreatment; intensity graded 0–3+	Not numerically quantified; detection based on fluorescent signal intensity; Pk-based IF reached 90% sensitivity versus MS	29 Gibier et al. (2021)
Immuno-fluorescence	Kappa, Lambda (light chains); IgG, IgA, IgM, IgD, IgE (heavy chains); C3, C4, Properdin; Alpha-2 macroglobulin; Fibrinogen; Albumin; Transferrin	Fresh frozen renal tissue (needle biopsies and necropsy specimens)	Qualitative; direct immunofluorescence on frozen renal biopsies using FITC-conjugated monospecific antisera; evaluated by fluorescence intensity and pattern	Not quantified; interpreted via presence/absence and fluorescence pattern in mesangial regions	30 Jerath et al. (1980)
Immuno-fluorescence	Kappa, Lambda (light chains); IgG, IgA (heavy chains)	Fresh frozen renal biopsy specimens (native kidney)	Qualitative and semi-quantitative; retrospective evaluation of IF staining intensity on frozen renal biopsies using a 0–3+ scale; applied for distinguishing immunoglobulin-derived amyloidosis	Not quantified; positivity defined as ≥2+ for one light chain and ≤ trace for the other	31 Gonzalez Suarez et al. (2019)
Immunoelectron Microscopy	Kappa, Lambda (light chains); Serum amyloid A (SAA); Transthyretin (TTR); Apolipoprotein A-I (ApoA-I); Beta-2 microglobulin; Fibrinogen; Lysozyme	FFPE or fresh tissue biopsies: abdominal fat, salivary glands, skin, heart, lung, kidney, other involved organs	Qualitative; postembedding immunogold labeling for amyloid typing using electron microscopy	Not quantified; detectable in focal deposits; sensitivity >99% when amyloid fibrils are present	32 Verga et al. (2012)
Immunoelectron Microscopy	Kappa, Lambda (light chains); Transthyretin (TTR); Serum amyloid A (SAA)	FFPE tissue biopsies: heart, kidney, lung, GI mucosa, skin, bone marrow	Qualitative; postembedding immunogold electron microscopy using antibodies specific to amyloid fibril proteins	Not quantified; classification based on ultrastructural immunogold labeling of amyloid fibrils; IEM sensitivity reported as 91.6%	33 Abildgaard et al. (2020)
Immunoelectron Microscopy	Kappa, Lambda (light chains); Serum amyloid A (SAA); Transthyretin (TTR); Apolipoprotein A-I (ApoA-I); Fibrinogen; Lysozyme	FFPE or fresh tissue biopsies: abdominal fat aspirates; kidney, liver, salivary gland, endomyocardial tissue	Qualitative; postembedding immunogold electron microscopy using amyloid-specific antibodies	Not quantified; classification based on fibril labeling; IEM identified amyloid type in >99% of confirmed cases; sensitivity ~76.1%, specificity 100%	34 Fernández de Larrea et al. (2015)

AA, Amyloid A; AApoAI, Amyloid apolipoprotein A-I; Aβ2M, Amyloid beta-2 microglobulin; ACys, Amyloid cystatin C; AFib, Amyloid fibrinogen Aα-chain; AGel, Amyloid gelsolin; AL, Amyloid light-chain; ALκ, Amyloid light-chain kappa; ALλ, Amyloid light-chain lambda; ALys, Amyloid lysozyme; ApoAII, Apolipoprotein A-II; AFA, Alcohol, formalin, acetic acid; ATTR, Amyloid transthyretin; CR, Congo Red; FFPE, Formalin-fixed paraffin-embedded; IEM, Immunoelectron microscopy; IF, Immunofluorescence; IHC, Immunohistochemistry; LCM-MS/MS, Laser capture microdissection followed by tandem mass spektrometry; TRFM, Texas Red–filtered fluorescence microscopy.

ventional methods[33,34]. IEM is applicable to a variety of tissue types, including kidney, heart, liver, and abdominal fat aspirates, with fresh or optimally preserved specimens yielding the best results. Although paraffin-embedded specimens can be used, their sensitivity is reduced compared to fresh or frozen tissue[32,34]. The ability of the method to resolve ultrastructural details and provide molecular specificity makes it the gold standard for diagnosing atypical or mixed amyloid presentations. However, IEM requires specialized equipment and expertise, and is both labor intensive and time consuming. Preparation, labeling, and imaging often take several days, limiting its routine use to advanced diagnostic centers[33,34]. Despite these challenges, IEM remains a critical tool for resolving uncertain diagnoses and identifying minimal amyloid deposition. When integrated with complementary techniques such as mass spectrometry, IEM improves diagnostic accuracy and informs targeted treatment strategies[32,34]. Key studies that employed histological, immunohistochemical, immunofluorescence, and immunoelectron microscopy methods for amyloid detection and typing are summarized in Table 1.

Taken together, histological and immunological techniques provide a complementary, stepwise framework for amyloid detection and typing. Congo red staining remains the essential initial screening tool across a wide range of tissues, whereas IHC and IF refine the diagnosis by linking morphological deposits to specific precursor proteins, thereby guiding prognosis and therapy. IEM offers the highest diagnostic resolution in challenging or atypical cases by combining ultrastructural confirmation with molecular specificity and is particularly valuable in specialised centres for resolving equivocal findings. Clinicians should understand the strengths and limitations of each method to ensure appropriate tissue selection, interpretation of negative or borderline results, and timely referral for advanced testing. For laboratory professionals, integrating these techniques into standardised diagnostic algorithms helps ensure accurate and reproducible amyloid typing that directly informs patient management.

OVERVIEW OF ELECTROPHORETIC METHODS FOR THE CHARACTERIZATION OF AMYLOID

Electromigration methods are essential tools in the modern clinical laboratory for accurate and rapid protein characterization. Techniques such as capillary electrophoresis, capillary isoelectric focusing (cIEF), and immunofixation electrophoresis are critical for diagnosing and subtyping amyloidosis, monitoring disease progression, and guiding therapy. These methods separate proteins based on size, charge, and conformation using advanced detection systems such as UV, fluorescence, or mass spectrometry for high sensitivity and specificity[35-37]. In amyloidosis, these techniques effectively detect key pathological proteins such as serum amyloid A in AA amyloidosis or monoclonal light chains in AL amyloidosis. The combination of electromigration methods with histopathological approaches, such as Congo red staining, improves diagnostic accuracy by allowing precise identification and localization of amyloid deposits[36,37]. Electromigration methods, despite challenges such as sensitivity to low-abundance proteins and technical complexity, continue to improve with advancements in automation and instrumentation. These techniques have increasingly supplemented or even replaced traditional histological methods for diagnosing and subtyping amyloid proteins, offering greater precision and efficiency. Developmentally, they precede the use of liquid chromatography coupled with mass spectrometry (LC-MS) but remain vital for their ability to provide rapid and detailed analysis. An overview of key electrophoretic and isoelectric focusing methods applied for the detection and typing of amyloid and monoclonal proteins is provided in Table 2.

Capillary electrophoresis

Matrices used for amyloid protein determination by CE include serum, urine, and tissue extracts, each of which presents unique challenges in sample preparation and analysis. Tissue analysis often requires complex pretreatment steps, such as digestion or buffer exchange, to deal with heterogeneity and high background protein concentrations[38-41].

CE has been shown to be effective in identifying a variety of amyloidogenic proteins, such as immunoglobulin light chains (κ and λ), transthyretin, serum amyloid A, and amyloid beta (Aβ). In urine, for example, CE is often used to detect free light chains in AL amyloidosis, while in tissue samples it has been used to study amyloid beta peptides in Alzheimer's disease and amyloid fibrils in kidney biopsies[40-42]. Tissue matrices often require advanced methods, such as CE coupled with mass spectrometry (CE-MS) or laser-induced fluorescence (CE-LIF), to achieve high-resolution separation, which is critical for distinguishing small differences in the isoelectric points of amyloid precursor proteins[38,42].

Most applications focus on analyzing the intact molecular weight of proteins, as seen in serum and urine studies of monoclonal light chains. However, for highly complex proteins such as Aβ, digestion into smaller peptide fragments is necessary for high-resolution characterization[43].

The efficiency of CE is enhanced by its fast analysis time, with runs often completed within an hour, and minimal sample preparation requirements. For example, the Sebia Capillarys 2 system is particularly effective for quantifying monoclonal proteins in AL amyloidosis[40]. Despite these advantages, challenges remain, such as matrix interference from complex biological samples and the detection of low-abundance proteins. Advanced pretreatment techniques, such as sample concentration or buffer exchange, are often used to mitigate these problems. In addition, while instrument costs and the need for trained personnel can limit accessibility, recent innovations such as automated CE systems have increased its usability in routine clinical diagnostics[39,41,42].

CE has been used effectively in a number of studies. For example, Alper et al.[38] demonstrated the utility of CE in the study of Aβ 1-40 degradation using synthetic

Table 2. Overview of electrophoretic methods for the characterization of amyloid.

Method	Analytes	Type of biological matrices	Type of analysis	Additional information	Ref.
Capillary Electrophoresis	Amyloid beta (Aβ) 1–40	In vitro buffered reaction mixture containing Aβ 1–40 and insulin-degrading enzyme (IDE)	Quantitative; capillary electrophoresis used to evaluate kinetics of Aβ 1–40 degradation by IDE	Not reported	38 Alper BJ, Schmidt WK (2013)
Capillary Electrophoresis	IgG-kappa, IgG-lambda; Bence Jones proteins (kappa, lambda)	Serum and urine	Qualitative and semi-quantitative; capillary electrophoresis with immunosubtraction (CE/IS) for detection and immunotyping of monoclonal proteins	Not reported	39 Miyazaki K, Suzuki K (2016)
Capillary Electrophoresis	Free light chains (kappa or lambda)	Urine	Quantitative; capillary electrophoresis (UPCE) with immunotyping	Not reported	40 Russo et al. (2017)
Capillary Electrophoresis	IgA-lambda; IgG-kappa	Serum	Qualitative and semi-quantitative; capillary electrophoresis with immunotyping (immunosubtraction)	Not reported	41 Verma et al. (2021)
Capillary Electrophoresis	Amyloid beta (Aβ 1–40, Aβ 1–42); monomers, oligomers, fibrils	In vitro (Tris buffer with synthetic Aβ peptides)	Qualitative and semi-quantitative; capillary electrophoresis with laser-induced fluorescence detection (CE–LIF)	Not reported	42 Picou et al. (2012)
Isoelectric Focusing	Transthyretin (TTR) – wild-type and variants (Leu12Val, Thr40Asn, Phe64Val)	Serum	Qualitative and semi-quantitative; isoelectric focusing (IEF) under partially denaturing conditions in urea-PAGE	Not reported; variant bands detectable at ~0.05 g/L TTR concentration	44 Hinderhofer et al. (2019)
Isoelectric Focusing	Transthyretin (TTR); variants: Val30Met, Val122Ile, Thr60Ala, Leu58His, and others	Serum	Qualitative; native PAGE followed by IEF to visually detect presence of variant TTR isoforms based on band patterns	Not quantified	45 Connors et al. (1998)
Isoelectric Focusing	Serum amyloid P component (SAP); disialo, monosialo, asialo isoforms	Serum and plasma	Qualitative and semi-quantitative; capillary isoelectric focusing (cIEF) with MALDI-MS for SAP microheterogeneity profiling	Not quantified; detection shown at ~100 ng/µL SAP in 5 µL injection	46 Weiss et al. (2011)
Isoelectric Focusing	Free light chains (FLC): kappa and lambda	Serum and urine	Qualitative and semi-quantitative; isoelectric focusing (IEF) on agarose and polyacrylamide gels followed by affinity immunoblotting (AIB)	Not quantified; detection limit ~0.05–0.1 mg/L for FLC kappa and ~0.1–0.2 mg/L for FLC lambda	48 Zeman et al. (2024)

Table 2. *(Continued)*

Method	Analytes	Type of biological matrices	Type of analysis	Additional information	Ref.
Isoelectric Focusing	Transthyretin (TTR) variants: T60I and V122I	Human serum and adipose tissue	Qualitative and semi-quantitative; isoelectric focusing (IEF), immunohistochemistry, LC-MS/MS, biophysical analysis (Circular Dichroism (CD), fluorescence, Thioflavin T binding)	Not quantified in absolute units; semi-quantitative ratio	49 Prokaeva et al. (2023)
Isoelectric Focusing	Amyloid beta peptides (Aβ 1–38, Aβ 1–40, Aβ 1–42)	Human cerebrospinal fluid (CSF) and plasma	Qualitative and semi-quantitative; microfluidic isoelectric focusing (IEF) followed by micropillar MALDI-TOF-MS	Not directly quantified; method enabled detection of Aβ peptides at pg/mL levels after IP and ~10^5-fold enrichment	50 Mikkonen et al. (2016)
Immunofixation Electrophoresis	Monoclonal immunoglobulin light chains (κ and λ); serum free light chains (FLC)	Human serum	Qualitative and semi-quantitative; immunofixation electrophoresis (IFE) using Epalyzer2 and HYDRASYS systems; serum FLC assay	Not quantified in absolute units; semi-quantitative with detection thresholds (≥25–50 mg/dL)	51 Kawano et al. (2024)
Immunofixation Electrophoresis	Monoclonal immunoglobulin free light chains (κ and λ)	Human serum and urine	Qualitative; immunofixation electrophoresis (IFE) using Titan Gel system	Not quantified in absolute units	52 Zhou et al. (2013)
Immunofixation Electrophoresis	Monoclonal immunoglobulins and free light chains (κ and λ)	Human serum and urine	Qualitative and semi-quantitative; SPEP, IFE (serum and 24h urine), serum free light chain (FLC) assay	Not quantified in absolute units; FLC assessed semi-quantitatively	53 Rubinstein & Stockerl-Goldstein (2021)

Aβ, Amyloid beta; AIB, Affinity Immunoblotting; CD, Circular Dichroism; CE, Capillary Electrophoresis; CE–LIF, Capillary Electrophoresis with Laser-Induced Fluorescence Detection; CE/IS, Capillary Electrophoresis with Immunosubtraction; cIEF, Capillary Isoelectric Focusing; CSF, Cerebrospinal Fluid; FLC, Free Light Chains; IDE, Insulin-degrading enzyme; IEF, Isoelectric Focusing; IFE, Immunofixation Electrophoresis; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IP, Immunoprecipitation; LC-MS/MS, Liquid Chromatography–Tandem Mass Spectrometry; MALDI-MS, Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry; MALDI-TOF-MS, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry; PAGE, Polyacrylamide Gel Electrophoresis; SAP, Serum Amyloid P Component; SPEP, Serum Protein Electrophoresis; ThT, Thioflavin T; TTR, Transthyretin; UPCE, Urinary Protein Capillary Electrophoresis; urea-PAGE, Urea Polyacrylamide Gel Electrophoresis.

peptide solutions. Their analysis focused on the intact protein, providing insight into aggregation dynamics[3]. Miyazaki and Suzuki[39] applied CE with immunosubtraction to serum samples to identify and quantify monoclonal proteins in AL amyloidosis, focusing on the detection of intact proteins. Russo et al.[40] used CE to quantify κ and λ free light chains in urine, highlighting its efficiency in clinical workflows. Picou et al.[42] used CE-LIF to analyze Aβ aggregates, using digestion to separate smaller peptide fragments and characterize dynamic oligomers. Finally, Verma et al.[41] used CE in kidney biopsies to detect amyloid fibrils, focusing on identification without digestion.

Isoelectric focusing

Isoelectric focusing is a highly sensitive analytical technique that is widely used to separate and identify amyloidogenic proteins. The method is based on the separation of proteins by their isoelectric points (pI), making it a valuable tool in amyloidosis research and diagnostics. IEF has been particularly effective in identifying amyloid precursor proteins, detecting sequence variants, and distinguishing between wild-type and mutant forms of key proteins such as transthyretin and serum amyloid P (ref.[44-46]).

IEF has been applied to various biological matrices, including serum, plasma, cerebrospinal fluid (CSF), and tissue samples[47]. For serum and plasma, IEF has demonstrated exceptional sensitivity in the detection of monoclonal free light chains (FLCs) associated with AL amyloidosis. Zeman et al. (2024) showed that IEF combined with affinity immunoblotting (AIB) could detect FLCs in 24% of samples that were negative by conventional electrophoresis and immunofixation, highlighting its superior sensitivity[48]. Similarly, Connors et al.[45] used IEF to analyze serum samples from 110 patients with amyloidosis and identified amyloidogenic TTR variants in 74 cases with 96% sensitivity and 100% specificity[49].

In tissue samples, IEF has been used to detect amyloid deposition in rectal biopsies and to characterize the composition of amyloid fibrils. Prokaeva et al.[49] demonstrated the ability of the technique to identify compound TTR mutations, such as T60I and V122I, furthering the understanding of hereditary ATTR amyloidosis. For CSF, Mikkonen et al.[50] developed a microfluidic IEF method to analyze amyloid beta peptides, including isoforms Aβ1-40 and Aβ1-42, which are important biomarkers for Alzheimer's disease. This innovative approach significantly improved the sensitivity of subsequent MALDI-MS analysis.

IEF is primarily used to identify amyloidogenic proteins by resolving them into distinct pI bands. It has proven particularly effective in detecting TTR sequence variants such as Thr40Asn and Phe64Val, which have destabilizing effects associated with amyloidogenicit[44,49]. Coupling IEF with advanced detection systems, such as Affinity Immunoblotting (AIB) or mass spectrometry, allows the quantification of proteins. For example, Zeman et al.[48] reported detection limits of 1–2 ng per lane for monoclonal FLCs, highlighting the sensitivity of the method for clinical applications.

While IEF typically analyzes intact proteins, some applications require enzymatic digestion to examine smaller peptide fragments. For example, Mikkonen et al.[50] used digestion to resolve Aβ peptide aggregates in CSF, facilitating the study of aggregation dynamics and post-translational modifications. In contrast, Weiss et al.[46] used capillary IEF to analyze the microheterogeneity of SAP in intact form, revealing its isoform consistency across populations.

The applications of IEF continue to expand, demonstrating its versatility in amyloidosis research. Connors et al.[45] highlighted its use in the identification of TTR variants such as Val122I and Thr60Ala, which are critical for the diagnosis of hereditary ATTR amyloidosis[49]. Prokaeva et al. (2023) further emphasized the role of IEF in characterizing the additive destabilizing effects of compound TTR mutations, providing new insights into amyloidogenesis[49]. Meanwhile, Mikkonen et al.[50] applied microfluidic IEF to detect Aβ peptides in CSF and plasma, achieving a 10-fold improvement in detection sensitivity compared to conventional methods.

In summary, IEF has become a cornerstone technique for analysing amyloidogenic proteins, providing high-resolution, sensitive identification and quantification of key biomarkers. Integrating it with advanced technologies such as AIB and MALDI-MS has broadened its applications, establishing it as an indispensable tool in amyloidosis diagnostics and research.

Immunofixation electrophoresis

Immunofixation electrophoresis is a cornerstone technique in the detection and analysis of amyloidogenic proteins, particularly the monoclonal immunoglobulin light chains associated with AL amyloidosis. By combining electrophoresis with specific immunological detection, IFE offers high specificity and sensitivity, making it an essential tool in clinical laboratories for the diagnosis and monitoring of amyloidosis[48,51-53].

IFE has been widely applied to various biological matrices, including serum, urine and tissue extracts[54]. Serum and urine are the primary samples for the detection of monoclonal FLCs, including kappa (κ) and lambda (λ) light chains. For example, Zeman et al.[48] demonstrated that IFE, combined with isoelectric focusing and affinity immunoblotting (AIB), detected monoclonal FLCs in 24% of serum samples that were negative by conventional electrophoresis and immunofixation techniques. In addition, tissue extracts, particularly from kidney and heart biopsies, have been analyzed by IFE to confirm the presence of amyloid deposition and to provide molecular insights into amyloid fibril composition[53,55].

Although IFE is primarily a qualitative method used to identify monoclonal proteins, it also has a semi-quantitative nature that allows for the assessment of protein intensity based on band visibility. Zhou et al.[52] highlighted the utility of IFE in identifying monoclonal FLCs with a sensitivity of 81.3% and specificity of 98.6% in patients with AL amyloidosis and nephrotic-range proteinuria. Kawano et al.[51] further demonstrated that automated IFE systems, such as the HYDRASYS 2, improve sensitivity

and reproducibility compared to traditional electrophoresis systems, particularly for the detection of monoclonal light chains in patients with low serum FLC concentrations.

Despite its high specificity and sensitivity, IFE faces certain challenges. One of the main issues is the detection of low concentration monoclonal proteins, especially in complex matrices such as serum, which often requires sample concentration for effective analysis. Zeman et al.[48] highlighted the importance of advanced sample preparation to overcome this limitation[48]. In addition, high background protein levels in serum and urine can obscure monoclonal protein bands, complicating interpretation and requiring careful optimization of protocols[52,53]. Variations between IFE platforms also affect detection results. For example, Kawano et al.[51] reported significant variability in detection rates between automated and traditional systems, highlighting the need for standardization across platforms[48,55].

IFE is a labor-intensive technique, typically requiring 3–6 hours per run, depending on sample complexity and workflow. Automated platforms such as the HYDRASYS 2 and the Blue Horizon system have significantly improved reproducibility and reduced operator dependency, streamlining the process and increasing throughput. Sample requirements for IFE are minimal, with as little as 5–10 μL of serum or urine required for analysis. These advances make IFE a robust and efficient tool for routine diagnostic workflows[48,51,52].

The clinical applications of IFE in amyloidosis diagnosis are extensive. It is the gold standard for the detection of κ and λ FLCs in serum and urine, which are critical for the diagnosis of AL amyloidosis. Rubinstein et al.[53] emphasized its importance in identifying monoclonal light chains associated with cardiac amyloidosis, highlighting its diagnostic value in systemic amyloidosis[55]. In addition, IFE complements histopathological methods in tissue analysis by characterizing amyloid fibril composition, which helps to subtype amyloidosis and guide treatment strategies[48,55].

Thus, immunofixation electrophoresis remains a key tool for the detection and identification of amyloidogenic proteins. Its high specificity and sensitivity make it indispensable in amyloidosis research and diagnosis. However, challenges such as matrix interference and system variability require the use of complementary techniques and advanced instrumentation to optimize its clinical utility.

Overall, electrophoretic methods constitute a complementary panel for the detection and characterisation of amyloidogenic proteins. Capillary electrophoresis provides rapid, high-throughput analysis of serum, urine, and tissue extracts, enabling efficient screening for monoclonal immunoglobulins and other amyloid precursors. Isoelectric focusing offers superior analytical sensitivity and resolution for free light chains, transthyretin variants, and Aβ isoforms, making it particularly valuable in diagnostically challenging cases. Immunofixation electrophoresis remains the reference method for confirming and subtyping monoclonal immunoglobulins and is central to the diagnosis and monitoring of AL amyloidosis. For clinicians, an understanding of the specific strengths and limitations of each technique is crucial for appropriate test selection and interpretation of borderline or discordant results, whereas for laboratory professionals, integrating these methods into standardised, automated workflows helps ensure robust, clinically meaningful data that can be effectively combined with histopathology and mass spektrometry – based approaches.

MASS SPECTROMETRY IN AMYLOIDOSIS DIAGNOSTICS

Mass spectrometry has become a transformative technology in the diagnostic evaluation of amyloidosis, offering unparalleled analytical capabilities for the identification, characterization, and quantification of amyloidogenic proteins. The rise of proteomics, driven in large part by advances in MS instrumentation and bioinformatics, has redefined the standards of clinical diagnostics in this field. Traditional techniques such as Congo red staining, immunohistochemistry, capillary electrophoresis, or immunofixation, although historically valuable, are often limited by low specificity, poor resolution of protein variants, and susceptibility to interference[27,56]. An outline of key mass spectrometry applications in the detection and classification of amyloid proteins is presented in Table 3.

One of the major shortcomings of conventional methods is their inability to accurately subtype amyloid deposition. For example, IHC has been shown to correctly classify amyloid types in only 76% of cases, leading to potential delays or errors in treatment decisions[56]. In contrast, MS-based proteomics can identify specific protein sequences and post-translational modifications (PTMs) responsible for amyloid formation, achieving subtype specificity greater than 99% (ref.[57]). This capability is critical for clinical decision making, as treatment options vary significantly depending on whether the patient has AL, ATTR, AA, or another less common form of amyloidosis. The strength of MS lies in its versatility and depth. It can process a variety of biological matrices, including serum, plasma, cerebrospinal fluid, and formalin-fixed paraffin-embedded tissue. In FFPE samples, which are routinely archived in pathology departments, laser microdissection (LMD) is used to isolate amyloid deposits, followed by bottom-up proteomic analysis using tryptic digestion and tandem mass spectrometry (LC-MS/MS). This workflow, which has been applied in numerous studies, consistently provides high sensitivity and specificity[58-60]. The bottom-up approach is particularly suitable for proteomic analysis of complex samples, allowing the identification of a wide range of peptides with high reproducibility. For example, Brambilla et al.[61] used multidimensional protein identification technology (MudPIT) to analyze adipose tissue aspirates and successfully subtyped systemic amyloidosis, even identifying associated chaperone proteins such as clusterin and apolipoprotein.

Dasari et al.[57] demonstrated the diagnostic power of MS in a large-scale study analyzing 16,175 clinical samples over a decade. This effort revealed that 58.99% of cases

Table 3. Mass spectrometry in amyloidosis diagnostics.

Method	Analytes	Type of biological matrices	Type of analysis	Additional information	Ref.
Mass spectrometry	AL (κ, λ), ATTR, AA, AApoAI, Aβ2M, AFib, AGel, ALECT2, ALys, AH, AApoAIV, AANF	FFPE tissue (multiple organ types)	Qualitative; LMD + tandem MS (LDMS); compared with IHC	Not quantified in absolute units; based on peptide identification scores	56 Gilbertson et al. (2015)
Mass spectrometry	Amyloid signature proteins (ASP), amyloid fibril proteins	FFPE biopsy tissue	Qualitative; LDMS, compared with IHC and CR histology	Not quantified in absolute units; based on ASP presence	27 Rezk et al. (2019)
Mass spectrometry	21 amyloid types including AL (κ, λ), ATTR (wild-type and variant), ALECT2, AA, AH, AIns, AFib, AApoAI, AApoAIV, ALys, Aβ2M, ASem1, AGel, APro, AEnf, AIAPP, AApoCII, AANF, etc.	FFPE tissue, fat aspirates	Qualitative; LMD + LC-MS/MS; shotgun proteomics	Not quantified in absolute units; based on peptide identification	57 Dasari et al. (2020)
Mass spectrometry	Amyloidogenic proteins (IGK, IGL, TTR, SAA, SAP)	FFPE biopsy tissue	Qualitative; LMD-MS	Not quantified in absolute units; based on MS spectra	59 Vrana et al. (2009)
Mass spectrometry	>1,700 CSF proteins incl. OMD, CD44, PRL, CTSS, HLA-class II	CSF (cerebrospinal fluid)	Quantitative; DIA-LC-MS/MS with machine learning	Not quantified in absolute units; relative quantification over wide dynamic range	60 Karayel et al. (2022)
Mass spectrometry	AL (κ, λ), ATTR, AA amyloid	Subcutaneous adipose tissue (fat aspirate)	Qualitative and semi-quantitative; MudPIT MS with biomarker algorithm	Not quantified in absolute units; semi-quantitative via spectral counts	61 Brambilla et al. (2012)
Mass spectrometry	Aβ1–38, Aβ1–40, Aβ1–42	CSF (cerebrospinal fluid)	Quantitative; UHPLC–MS/MS with micro-elution SPE and isotope-labeled standards	0.1–20 ng/mL; patient CSF: Aβ1–38 ≈ 1.15 ng/mL, Aβ1–40 ≈ 3.7 ng/mL, Aβ1–42 ≈ 0.33 ng/mL	63 Lin et al. (2017)
Mass spectrometry	Amyloid fibril proteins: AL (κ, λ); SAP, ApoE, ApoA-IV	FFPE tissue (heart, liver, kidney, tongue, intestine)	Qualitative and semi-quantitative; LMD + LC-MS/MS; semi-quantitation via spectral counting	Not quantified in absolute units; based on spectral abundance	67 Holub et al. (2019)

AANF, Atrial Natriuretic Factor; AA, Amyloid A; AApoAI, Amyloid Apolipoprotein A-I; AApoAIV, Amyloid Apolipoprotein A-IV; AApoCII, Amyloid Apolipoprotein C-II; Aβ1–38, Amyloid Beta 1–38; Aβ1–40, Amyloid Beta 1–40; Aβ1–42, Amyloid Beta 1–42; Aβ2M, Amyloid Beta-2 Microglobulin; AEnf, Amyloid Enfurvitide; AFib, Amyloid Fibrinogen Aα-chain; AGel, Amyloid Gelsolin; AH, Amyloid Heavy Chain; AIns, Amyloid Insulin; AIAPP, Amyloid Islet Amyloid Polypeptide; AL, Amyloid Light Chain; ALκ, Amyloid Light Chain Kappa; ALλ, Amyloid Light Chain Lambda; ALECT2, Amyloid Leukocyte Chemotactic Factor 2; ApoA-IV, Apolipoprotein A-IV; ApoE, Apolipoprotein E; ASP, Amyloid Signature Proteins; CD44, Cluster of Differentiation 44; CR, Congo Red; CSF, Cerebrospinal Fluid; CTSS, Cathepsin S; DIA-LC-MS/MS, Data-Independent Acquisition Liquid Chromatography–Tandem Mass Spectrometry; HLA-class II, Human Leukocyte Antigen Class II; IGK, Immunoglobulin Kappa Light Chain; IGL, Immunoglobulin Lambda Light Chain; LC-MS/MS, Liquid Chromatography–Tandem Mass Spectrometry; LDMS, Laser Dissection Mass Spectrometry; LMD, Laser Microdissection; MudPIT MS, Multidimensional Protein Identification Technology Mass Spectrometry; OMD, Osteomodulin; PRL, Prolactin; SAA, Serum Amyloid A; SAP, Serum Amyloid P Component; SPE, Solid-Phase Extraction; TTR, Transthyretin; UHPLC–MS/MS, Ultra-High-Performance Liquid Chromatography–Tandem Mass Spectrometry.

were AL-type amyloidosis and 28.44% were ATTR-type, supporting the notion that these two forms comprise nearly 90% of all clinical cases. MS identified not only common types but also rare amyloid variants, making it invaluable for centers managing hereditary amyloidosis or ambiguous clinical cases.

In clinical laboratories, MS is also used to analyze biofluids such as serum and cerebrospinal fluid[62]. For example, quantification of amyloid beta peptides (Aβ1-38, Aβ1-40, Aβ1-42) in CSF by nano-LC-MS/MS using isotope-labeled internal standards has been shown to be effective in the diagnosis of Alzheimer's disease and monitoring of disease progression[63]. This high-resolution approach allows detection at the picogram level and facilitates differentiation between disease states based on specific peptide isoform concentrations. In cardiac amyloidosis, quantification of transthyretin from serum or tissue by LC-MS/MS can correlate with disease severity and guide therapeutic intervention[61,64].

One of the unique advantages of MS is its ability to reveal structural information about proteins through advanced dissociation and ion separation methods. Techniques such as electron capture dissociation (ECD) and electron transfer dissociation (ETD) preserve PTMs during fragmentation, making them essential for characterizing glycosylation, phosphorylation, and other relevant modifications[65,66]. In addition, ion mobility spectrometry (IMS) enhances separation by exploiting the shape and size of ions, aiding in the resolution of isobaric or conformationally distinct species. Spectral libraries and isotopic resolution further increase confidence in peptide identification and provide unparalleled depth of molecular insight.

Proteomics workflows in amyloidosis diagnostics typically follow one of two strategies: bottom-up or top-down analysis. The former is widely used due to its robustness and scalability, especially in routine diagnostic settings using FFPE or adipose tissue. The latter, although less common, has the advantage of preserving intact protein structures and analyzing full-length proteoforms a useful feature for detecting PTMs or sequence variants without prior digestion[63].

Sample preparation protocols are critical for accurate MS-based proteomics. For FFPE tissue, most protocols include deparaffinization followed by heat-induced antigen retrieval and trypsin digestion. While these steps can introduce variability, standardization efforts have shown that consistent use of buffers such as Tris/EDTA with zwittergent and controlled temperature conditions (e.g., 98°C for 90 min) significantly improves peptide yield and quality[59,67]. In adipose tissue, additional steps such as acetone defatting or buffer maceration are required to minimize lipid interference[61,64]. Brambilla et al.[61] optimized these protocols to obtain high quality peptide extracts suitable for high throughput MS analysis using MudPIT.

In addition to protein identification, quantification is an integral part of MS-based workflows. The use of labeled standards, such as 15N-labeled peptides, allows absolute quantification of key biomarkers. For example, isotope dilution MS has been used to quantify Aβ peptides in cerebrospinal fluid with high precision, a critical function in the diagnosis and staging of neurodegenerative diseases[63]. Similarly, serum amyloid A and light chains κ and λ can be quantified to monitor systemic inflammation or to track clonal burden in AL amyloidosis.

The growing clinical relevance of mass spectrometry is further highlighted by its adoption in major academic and reference laboratories. Automation and workflow integration have reduced hands-on time and improved reproducibility. Platforms such as Q-Exactive and Orbitrap offer high-resolution data acquisition, while tools such as data-independent acquisition (DIA) and machine learning-based spectral matching enable comprehensive proteome coverage. These innovations allow thousands of proteins to be monitored simultaneously across patient cohorts, opening up new opportunities for biomarker discovery and disease stratification[66,68].

Still, MS faces challenges. The complexity of plasma and serum matrices, which contain proteins that vary in concentration by 12 orders of magnitude, requires careful sample preparation to avoid signal suppression. In amyloidosis, therapeutic monoclonal antibodies can interfere with protein measurements, leading to false positives when using immunoassays, a problem that MS can circumvent[27,64]. However, cost, the need for trained personnel, and infrastructure requirements still limit broader adoption. In addition, interpretation of complex MS datasets requires advanced bioinformatics tools and access to comprehensive protein databases such as UniProt[69].

Mass spectrometry-based proteomics has become a central component of contemporary amyloidosis diagnostics. For clinicians, its ability to provide detailed structural, functional and quantitative insights into amyloidogenic proteins enables them to distinguish between AL, ATTR and AA amyloidosis with greater confidence, and to stratify risk more precisely and make genuinely personalised therapeutic decisions. For laboratory professionals, implementing standardised LMD–LC–MS/MS workflows, rigorously controlling sample preparation and using validated quantitative assays is essential to translate these capabilities into robust, reproducible results that can be integrated with histopathology and electrophoretic studies. Despite the remaining hurdles, the trajectory of MS-based proteomics in clinical diagnostics is clearly upward. MS has established itself as not only a diagnostic powerhouse, but also a key enabler of personalised medicine. Its integration into routine workflows is expected to increase as protocols become more standardised, instrumentation becomes more accessible, and awareness among clinicians continues to grow.

CONCLUSION

Because amyloidosis presents in both systemic and organ-specific forms with substantial clinical risk, optimal evaluation hinges on a structured, multidisciplinary diagnostic approach. This review systematically summarizes key diagnostic methods in three complementary areas: classical histological and immunological techniques, ad-

vanced electrophoretic approaches, and state-of-the-art mass spectrometry-based proteomics. While traditional techniques remain indispensable for the initial detection of amyloid, the clinical implementation of mass spectrometry (MS) has significantly improved diagnostic precision and amyloid subtype specificity, thereby directly influencing therapeutic choices and patient management[12,26,57,70].

Looking ahead, the integration of MS into standardized diagnostic algorithms across clinical laboratories is essential to ensure analytical consistency, sensitivity, and reproducibility. MS-based proteomic typing has become the reference method for amyloid subtyping, allowing reliable identification of all amyloid types in a single assay and enabling correlation with organ tropism and genetic findings[12,57].

In this context, AI-assisted proteomic interpretation is beginning to augment MS workflows by improving peptide identification and quantification. Deep-learning models (e.g., Prosit and DeepMass) predict fragmentation spectra and retention times. Meanwhile, machine learning (ML) re-scoring stabilizes DIA performance and reproducibility[71,72]. Plasma proteomics has been applied to the early detection and staging of Alzheimer's, Parkinson's, and oncologic diseases[73-75], and AI-assisted native/top-down MS coupled with structure prediction resolves disease-relevant proteoform conformations[76]. At present, however, AI-driven interpretation is not used for clinical amyloid typing; its anticipated contribution is to automate subtype recognition and support standardized, high-throughput diagnostic pathways once validation and harmonization are achieved – thereby complementing the integration of MS into routine algorithms outlined above[71-76].

Despite these advances, important challenges remain in standardization and harmonization of pre-analytical, analytical, and computational processes. Variability in sample preparation, data acquisition, and bioinformatic pipelines currently limits inter-laboratory comparability and large-scale clinical implementation [12,57,70]. Consensus guidelines, shared spectral libraries, and transparent data-processing frameworks will be essential to achieve full clinical integration.

Search strategy and selection criteria

We searched PubMed, Web of Science, and Scopus for English-language articles on amyloidosis diagnostics (histology/IHC/IF/IEM, electrophoretic methods, and MS-based proteomics, including AI-assisted interpretation and standardization) published 1980–2025; searches were updated October 23, 2025. Keywords combined terms such as "amyloidosis diagnosis", "amyloid typing", "Congo red", "immunohistochemistry", "immunofluorescence", "capillary electrophoresis", "isoelectric focusing", "immunofixation", "mass spectrometry", "proteomics", "AI/machine learning", and "diagnostic algorithm". We included peer-reviewed original studies, clinical series, and reviews reporting methodological or clinical performance; we excluded non-peer-reviewed supplements, editorials without primary data, and conference abstracts without full text. Reference lists of key papers were hand-searched; preprints were cited only when methodologically pertinent and clearly identified as preprints.

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