Amyloid Typing by Liquid Chromatography-Tandem Mass Spectometry

Background Information

Systemic amyloidosis is a rare condition characterized by deposits of amorphous material that interfere with the normal structure and function of tissues. Most often amyloid deposits are identified in the heart, kidney and vascular walls, but virtually every organ system can be affected. All amyloid deposits are thought to be composed of proteins forming beta-pleated sheets, structures that render them insoluble and rigid.

Clinical Indications

Investigations leading to the diagnosis of systemic amyloidosis can be triggered by a variety of symptoms induced by heart or kidney failure, neuropathy or coagulation abnormalities. Abnormalities suggestive of cardiac amyloidosis have been described by ultrasonography. Amyloid deposits can be identified during workups for low-grade lymphomas, plasma cell myelomas, familial conditions or renal failure.

There are four major types of systemic amyloidosis: primary amyloidosis, familial amyloidosis, secondary amyloidosis and senile amyloidosis. The most common form of amyloidosis, primary amyloidosis, is the consequence of deposits of immunoglobulin light chains. Primary amyloidosis is usually diagnosed in patients with plasma cell neoplasms, from monoclonal gammopathy to plasma cell myeloma. Familial amyloidosis is a consequence of inherited mutations, most common in the transthyretin gene. Transthyretin, this time in its wild-type form, is the main component of amyloid in senile amyloidosis, a condition most often diagnosed in the heart. With the decrease in the incidence of chronic inflammatory diseases, mainly infectious, and with better management of autoimmune disorders, the incidence of secondary amyloidosis has markedly decreased and is currently a rare disease. The main amyloidogenic protein in secondary amyloidosis is Amyloid A. Overall, at least 28 proteins have been described to be responsible for the formation of amyloid deposits.

In addition to the proteins that are considered amyloidogenic, amyloid deposits in all types of systemic amyloidosis constantly include other proteins: Serum P component (SAP), Apolipo-protein E (ApoE), Apolipoprotein A-I (ApoA-I) and Apolipo-protein A-IV (ApoA-IV).

Methodology and Interpretation

In histologic sections stained with hematoxylin-eosin, amyloid deposits can be difficult to differentiate from serum or dense collagen. Several stains have been developed to assist in the diagnosis of amyloid, with the Congo Red stain currently considered the gold standard. The microfibrillar nature of the amyloid deposits can be confidently identified by electron microscopy, while the beta-pleated sheet structure of the amyloid can only be demonstrated with x-ray diffraction, a technique exclusively used in research.

While from a purely morphologic point of view there are no significant differences between different types of amyloid, the treatment of each type of amyloidosis is different. Immuno-histochemical stains show the amyloid deposits to be positive for SAP, but this does not always allow the differentiation of amyloid from serum. In many cases stains for Transthyretin, Amyloid A, kappa or lambda immunoglobulin light chains allow further characterization of the components of the amyloid deposits. However, in a significant number of cases these techniques fail to identify with confidence the amyloidogenic protein. Factors that prevent a confident diagnosis include abnormal protein folding and truncation, as well as the presence of many other endogenous proteins in the amyloid deposits. This results in a significant fraction of cases being inadequately typed and, as a consequence, treated.

Recent studies have shown liquid chromatography-tandem mass spectrometry (LC-MS/MS) to be a reliable method in the identification of amyloidogenic proteins and it is considered by some groups as the current gold standard. This technique was initially used on fresh or frozen tissue, but recently it has been shown that analysis of formalin- fixed, paraffin-embedded tissue (FFPET) can lead to similar results. The analysis usually begins with visual identification of amyloid deposits, followed by their dissection under the microscope (laser microdissection). The specimen is then digested with trypsin, resulting in the generation of peptide fragments. LC-MS/MS is then used to analyze these peptide fragments, resulting in an m/z spectrum. The specific m/z characteristics of the different peaks are compared to those in several databases, leading to the identification of the peptide fragments in the amyloid

digested by trypsin. These peptide fragments are quantified and the higher the number of peptide fragments originating from a particular amyloidogenic protein, the higher the degree of confidence that the particular protein is a component of the amyloid. Peptides from the structures of SAP, Transthyretin, ApoE, ApoAI and ApoA-IV are used as internal controls, indicating the adequate identification of amyloid. When more than one amyloidogenic protein is identified, comparison of the relative abundance of peptide fragments can indicate which protein is the most abundant in the sample.

This amyloid typing test allows typing of the amyloid deposits with a precision superior to the other available techniques. It incorporates multiple internal controls and allows amyloid typing to be performed in archived specimens. When necessary, in addition to the LC-MS/MS, alternative techniques such as immunohistochemical stains can be employed.

Limitations of the Assay

This test is not a substitute for a surgical pathology consult. The diagnosis of amyloidosis should not be made by mass spectrometry, as this method has not been developed as a substitute for a detailed morphologic analysis, Congo Red stain or immunohistochemistry. Amyloid analysis by tandem-mass spectometry requires that a sufficient amount of amyloid is microdissected. In a few cases the assay may fail due to the insufficient amyloid available. In some cases additional immunohistochemical stains are necessary in order to increase the confidence with which the diagnosis is rendered.

References

Sipe JD, Benson MD, Buxbaum JN, *et al*. Amyloid fibril protein nomenclature: 2012 recommendations from the Nomenclature Committee of the International Society of Amyloidosis. In *Amyloid*, 2012; 19(4):167-170.

Lavatelli F, Vrana JA. Proteomic typing of amyloid deposits in systemic amyloidoses. In *Amyloid*, 2011; 18(4):177-182.

Vrana JA, Gamez JD, Madden BJ, *et al*. Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. In *Blood*, 2009;114(24)4957-4959.

Test Overview

Test Name	Amyloid Typing by Tandem Mass Spectrometry
Ordering Mnemonic	*ATMS* R
Methodology	Affected areas are removed from paraffin-embedded tissues by laser microdissection. Protein digestion is performed, followed by liquid chromatography-tandem mass spectrometry (LC MS/MS).
Specimen Requirements	Formalin-fixed, paraffin-embedded tissue
Clinical Information	Profiling the presence or absence of amyloidogenic proteins in tissue section to aid in forming a pathologic diagnosis. Interpretation by pathologist.
Disclaimers or Notations	This test includes a surgical pathology consultation to confirm the presence of amyloid. The initial diagnosis of amyloidosis will be made by examination of routine stain, Congo Red stain, immunohistochemistry, etc. This test serves in the identification of amyloidogenic proteins only after the initial diagnosis has been reached.
Billing Code	89590
CPT Codes	83788; 88313; 88321; 88380; 88399(x10)

Technical Information Contact:
Linda McDonald
216.445.0394
mcdonal1@ccf.org

Scientific Information Contact:
Claudiu Cotta, MD
216.444.4588
cottac@ccf.org