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Original contribution

Leukocyte chemotactic factor 2 amyloidosis cannot be reliably diagnosed by immunohistochemical staining☆,☆☆

Paisit Paueksakon MD[a,*], Agnes B. Fogo MD[a], Sanjeev Sethi MD[b]

[a]*Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN 37232–2561, USA*
[b]*Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, USA*

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Summary We investigated the role of leukocyte chemotactic factor (LECT2) immunohistochemical staining in the diagnosis of type of renal amyloidosis. Fifty renal amyloidosis cases with available paraffin blocks in our 2002 to 2012 renal biopsy files were reviewed. Patients were designated as a defined amyloid, including amyloid light chain (AL) and amyloid-associated amyloid (AA), or a non-AL/non-AA amyloid group. LECT2-specific antibody immunohistochemistry was performed in all 50 cases. Laser microdissection and mass spectrometry (LMD/MS) were performed in 10 cases. Forty-five patients had amyloid classified as either AL (44) or AA (1), and 5 had undetermined amyloid. Three of the five non-AL/non-AA group patient biopsies showed positive LECT2 immunohistochemical staining, and of these, LECT2 was also identified by LMD/MS in 1 patient, fibrinogen-α was identified in 1 patient, and apolipoprotein IV was identified in 1 patient. Two of these non-AL/non-AA patients showed negative LECT2 staining, and LMD/MS showed apolipoprotein IV as a major protein component. Five of the 44 AL amyloid patients showed weakly positive LECT2 staining. However, LECT2 was not identified by LMD/MS in any of these 5 cases. The single patient with AA amyloid was negative for LECT2 by immunohistochemical staining. Among 5 non-AL and non-AA amyloidosis patients in our study, 1 had LECT2, 1 had fibrinogen-α, and 3 had apolipoprotein IV as a major protein component. The data from this study show that weak LECT2 staining should be regarded as indeterminate or a negative result and does not per se allow diagnosis of specific amyloid type. The diagnosis of LECT2 renal amyloidosis may require LMD/MS confirmation.

1. Introduction

Amyloidosis is a group of diseases characterized by excessive tissue deposits of proteins in an insoluble β-pleated sheet format. Amyloid deposits are identified based on their apple-green birefringence under a polarized light on Congo red staining and by the presence of randomly arranged, non-branching fibrils that measure 7 to 10 nm in diameter on electron microscopy [1]. The subtypes of amyloid are categorized by the chemical composition of the proteins. The most common form of renal amyloidosis is amyloid light chain (AL) or primary amyloidosis, in which fibrils are composed of monoclonal immunoglobulin light chains [2]. Reactive secondary amyloidosis is characterized by tissue deposition

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* Corresponding author. Department of Pathology, Microbiology and Immunology MCN C2318B Vanderbilt University Medical Center 1161 21st Avenue South Nashville, TN 37232–2561, USA.
E-mail address: paisit.paueksakon@vanderbilt.edu (P. Paueksakon).

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of serum amyloid A protein, which is derived from an acute-phase reactant protein synthesized by the liver. Reactive amyloidosis is seen in conditions associated with chronic immune activation including rheumatoid arthritis, ankylosing spondylitis, chronic draining infections (ie, osteomyelitis, chronic skin infections including decubitus ulcers, bronchiectasis), Crohn disease, tuberculosis, and familial Mediterranean fever [2]. Hereditary forms of amyloidosis comprise another group of amyloid that is now being diagnosed with more frequency and include amyloid derived from transthyretins, fibrinogen-α, lysozyme, gelsolin, and apolipoproteins [1,3].

Renal leukocyte chemotactic factor 2 (LECT2) amyloidosis has recently been described [4-6] and shows extensive congophilic deposits in the glomeruli, interstitium, and arteries. LECT2 is a chemotactic factor for neutrophils and has other physiologic roles, including cell growth promotion and repair after damage [5,7,8]. We performed LECT2 immunohistochemical staining on 50 cases of renal amyloidosis defined by Congo red positivity with available paraffin blocks in our laboratory over the past 11 years to assess the diagnostic utility of LECT2 staining in defining amyloid type.

2. Materials and methods

Fifty renal amyloidosis cases with available paraffin blocks in our 2002 to 2012 renal biopsy files at Vanderbilt University Medical Center were reviewed. Patients were designated as defined amyloid (AL and AA) or non-AL/non-AA (absence of monoclonal staining pattern for κ and λ by immunofluorescence study and negative amyloid-associated protein immunohistochemistry). To determine the nature of the non-AL/non-AA group and specificity of LECT2 protein staining in diagnosis of amyloidosis type, immunohistochemical study for LECT2-specific antibody was performed in all 50 cases of amyloidosis. Laser microdissection and mass spectrometry (LMD/MS) were performed at Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, in 5 AL patients and 5 non-AL/non-AA patients to determine and confirm subtype of amyloidosis.

To demonstrate specificity of LECT2 protein staining, LECT2 immunohistochemistry was also performed on 10 patients with arterionephrosclerosis and 5 patients with diabetic nephropathy with negative Congo red stains and no amyloid fibrils by electron microscopy.

2.1. Light microscopy

Briefly, renal biopsies were fixed in buffered formaldehyde, dehydrated in graded alcohols, and embedded in paraffin using standard techniques. Serial 2- to 3-μm-thick sections were cut and stained with hematoxylin and eosin, Jones methenamine silver, and periodic acid–Schiff reagent. In addition, a 7-μm-thick section was cut and stained with Congo red wherein the diagnosis of amyloid was confirmed by Congo red positivity with apple-green birefringence under polarizing microscopy. Known amyloid cases served as positive control.

2.2. Immunofluorescence and immunohistochemical studies

Samples were placed in Michel's media, washed in buffer, and frozen in a cryostat. Sections, cut at 2 μm, were rinsed in buffer and reacted with fluorescein-tagged polyclonal rabbit anti-human antibodies to IgG, IgA, IgM, C3, C1q, and κ and λ light chains (all from Dako, Carpenteria, CA) for 1 hour and rinsed, and a coverslip was applied using aqueous mounting media. For AA and LECT2 detection, 2-μm-thick paraffin-embedded sections were cut, deparaffinized, rehydrated, and blocked with normal horse serum for 5 minutes, followed by reaction for 1 hour with a 1:100 dilution of mouse anti-human amyloid A protein monoclonal antibody (mAb) (DakoCytomation, Glostrup, Denmark) or a 1:40 dilution of a goat anti-human LECT2 mAb (Vector Laboratories, Burlingame, CA). Immunoreactions were visualized using 3,3′-diaminobenzidine as the substrate (Dako, Carpenteria, CA). Liver tissue from a patient with cirrhosis was used as a positive control for LECT2 (Fig. 1). Negative controls were done omitting primary antibody.

2.3. Electron microscopy

Tissue was allocated to glutaraldehyde, dehydrated using graded alcohols, followed by embedding in Spurr embedding resin. Sections of 1 μm thickness were cut using an ultramicrotome and stained with toluidine blue. Glomeruli were selected, and sections of 70 to 100 nm were cut and examined in a Morgagni Philips FEI transmission electron microscope. Electron photomicrographs were routinely taken at 5600×, 7200×, and 56 000× magnifications, and amyloid fibril diameter was measured.

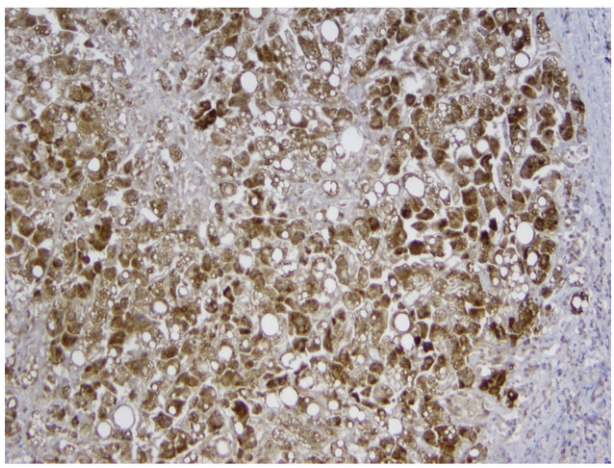

Fig. 1 Positive control for LECT2 using cirrhotic liver tissue (LECT2 immunostaining; original magnification ×200).