

ALECT2 amyloidosis: a new type of systemic amyloid highly prevalent in the Hispanic population

Víctor H. Jiménez-Zepeda,* Nelson Leung**

* Tom Baker Cancer Center, Department of Medical Oncology and Hematology, Calgary, AB, CA.

** Mayo Clinic Rochester, Department of Medicine, Division of Nephrology and Hematology, Rochester, MN, USA.

ABSTRACT

Amyloidosis results from extracellular deposition of fibril-forming proteins and currently ~30 different proteins have been found to be amyloidogenic. Recently, a novel type of amyloidosis with a high incidence on Hispanic population has been described to be derived from leukocyte chemotactic factor 2 (ALECT2). The objective of the present article is to raise awareness on the presence of this entity for the medical community in México. ALECT2 is a clinical entity characterized by deposition of the LECT2 protein mainly on liver and kidney. Renal ALECT2 affects elderly Hispanics who present with chronic renal insufficiency and bland urine sediment, not always associated to proteinuria. No treatment guidelines are reported for this disease but support measures including organ transplantation when required are recommended. Further genetic and clinical characterization of this entity is needed to help understanding the mechanisms by which this protein becomes amyloidogenic and how to prevent organ damage related to its deposition.

Key words. LECT2. Amyloidosis. Mass spectrometry.

BACKGROUND

Amyloidosis is caused by extracellular deposition of proteins misfolded into an insoluble beta-pleated sheet physical format.¹ More than 25 different proteins are known to cause amyloidosis.² The disease is often devastating, and options for treatment are limited for certain types of amyloid.³ The clinical manifestations, prognosis, and therapy vary greatly depending on the specific type of amyloidosis.⁴

Amiloidosis asociado a LECT2, un nuevo tipo de amiloidosis sistémica con alta prevalencia en población hispana

RESUMEN

La amiloidosis es el resultado del depósito extracelular de proteínas fibrilares, con cerca de 30 diferentes tipos de proteína amiloidogénica identificados en la actualidad. Recientemente, un nuevo tipo de amiloidosis altamente prevalente en población hispana derivado de la proteína LECT2 (ALECT2) ha sido identificado. El objetivo de este artículo es informar sobre esta nueva variante a la comunidad médica de México. ALECT2 es una entidad clínica caracterizada por depósito de la proteína en el hígado y riñón primordialmente. ALECT2 afecta población hispana en edades tardías y se caracteriza por la presencia de insuficiencia renal y sedimento urinario blando, no siempre asociado a proteinuria. No existen guías de tratamiento para esta enfermedad y se sugiere implementar terapia de sostén de las funciones orgánicas afectadas. Estudios encaminados a caracterizar clínica y genéticamente esta enfermedad son esenciales para la detección temprana y la prevención de mayor depósito de la proteína en los diferentes órganos.

Palabras clave. LECT2. Amiloidosis. Espectrometría de masas.

Recently, a novel amyloid type leukocyte chemotactic factor 2 (LECT2) associated with systemic disease most commonly with kidney and liver deposition (ALECT2) has been described.^{5,6} LECT2 was originally isolated and characterized from culture media of PHA-activated human T-cell leukemia cells⁷ as part of studies to identify chemotactic factors important in the pathophysiology of neoplasia happened to be amyloidogenic. Although the original characterization of LECT2 proved to be of

bovine origin from the fetal calf serum used in media, these studies led to the cloning and structural characterization of the human analog.⁷ The protein was independently characterized from fetal bovine cartilage and named chondromodulin II.⁸ The human LECT2 gene is localized to chromosome 5 (5q31.1-q32) and consists of four exons and three introns.⁹

ALECT2 was first described in 2008 in a renal biopsy of an unidentifiable type of amyloid.⁵ Since then, the number of ALECT2 cases has increased substantially. In a study of 23,650 renal biopsies, ALECT2 was identified in 40 (~10%) of the 414 cases of amyloidosis.¹⁰ In this series, ALECT2 was the second most common type of amyloid behind only immunoglobulin light chain (AL) amyloidosis (83.3%) and ahead of serum amyloid A (AA) or secondary amyloidosis (5%). Another series based on 4,139 biopsies from all tissues placed ALECT2 third behind AL and AA amyloidosis.¹¹ In another study of 31 previously unclassified amyloid cases, 7 were later found to be ALECT2 making the most common type of previously unclassified amyloid.¹⁰

There is a high concentration of ALECT2 cases in the Southwestern region of United States (US). In the renal biopsy series, 54% of the amyloid diagnosed in the Southwestern US was ALECT2 vs. 5% for the rest of the country.¹⁰ No other type of amyloid showed this degree of difference. Most striking is that 88% of the patients were Hispanic. This was also reported in a larger series of 72 patients where 91.7% were Hispanic.¹² Majority of the remaining patients were Native American or of Middle Eastern descent.

Clinical characteristics

ALECT2 was initially reported as renal limited amyloidosis. Patients most commonly presented with varying degrees of impaired kidney function. Proteinuria is surprisingly low grade in comparison to other forms of amyloidosis.^{5,10} This may be explained by the location of the amyloid deposits within in the kidney. However, recent reports suggest that ALECT2 is a more systemic form of amyloidosis affecting liver, spleen, colon, and adrenal glands, among other organs.^{6,11,13} So far, no cases of cardiac involvement has been reported.

Kidney involvement

Said, *et al.*, recently reported the largest clinical characterization and outcomes of renal ALECT2.¹²

Seventy-two patients with renal ALECT2 were evaluated. As previously mentioned, 92% of patients were Hispanic and over half were elderly (≥ 65 years). Proteinuria was variable and was absent in a third, whereas nephrotic syndrome (~10%) and hematuria (16.1%) were rare. Hypertension was present in 68.1% and ~10% presented on dialysis. After a median follow-up of 26 months, about one-third of patients who were not on dialysis developed end-stage renal disease (ESRD). Median time to ESRD was 16 months. The median renal survival was 62 months and independent predictors of renal survival were: serum creatinine at diagnosis, with a value of 2 mg/dL as the cutoff to predict for ESRD, percentage of global glomerulosclerosis and presence of diabetes. Approximately 10% of patients tested for monoclonal protein had a monoclonal gammopathy. Four patients died during follow up and 4 more received kidney transplant as part of the treatment. The second largest study reported recently by Larsen, *et al.*, described a series of 414 cases of amyloidosis, from which 40 of them were ALECT2, representing the second most common type of renal amyloidosis.¹⁰ The majority of these cases presented with chronic renal disease and interestingly 40% of patients did not have proteinuria. Of the 21 patients with adequate follow-up, 2 presented with ESRD and 4 others progressed to ESRD over a mean of 120 months. The average deterioration in renal function in 19 patients with laboratory data showed a decline in estimate glomerular filtration rate (eGFR) of 0.5 mL/min/1.73 m² per month. This study interestingly, added on the possibility of ALECT2 being an inherited form of amyloid disease as manifested by the presence of 2 brothers suffering from the same condition.

In agreement with the above-mentioned reports, a case series presented by Murphy, *et al.*, notably confirmed the presence of renal ALECT2 in Mexican Americans (7/10) suggesting that this entity is highly frequent in this population.¹⁴

Liver involvement

We recently reported on the frequency of ALECT2 as a cause of hepatic amyloidosis in the United States.⁶ Patients with liver involvement had a median age at presentation of 60 years, which is slightly lower than what is seen in AL amyloidosis.¹⁵ Like renal ALECT2 amyloidosis, hepatic ALECT2 patients were usually of Hispanic ethnicity (n = 28). Most cases presented liver function test abnormalities attributed to liver involvement. Also, cases of

liver involvement were incidentally discovered during evaluations for conditions unrelated to the liver or was associated with other causes of liver disease such as chronic viral hepatitis, suggesting that ALECT2 liver involvement might not be as clinically evident as that seen in kidney ALECT2. All ALECT2-involved liver samples showed unusual globular amyloid deposits located in the periportal parenchyma or at the periphery of the portal triad, and sometimes surrounding the central venules. ALECT2 accounted for 25% of hepatic amyloidosis cases. These cases were misdiagnosed as AL or AA amyloidosis based on the clinical context.

Diagnosis

Like all amyloidosis, ALECT2 must be diagnosed by tissue biopsy. Amyloid deposits should develop the characteristic apple green birefringence when viewed by polarized light after staining with Congo red.¹⁶ On electron microscopy, randomly arranged fibrils with a diameter of 8-13 nm can be found. In the kidney, amyloid deposits are usually most commonly found in the glomerulus and less so in the vascular and interstitial space. In ALECT2 however, the interstitial deposits are one of its main features. In one study, deposits were found in 100% of the cortical interstitium whereas glomerular deposits were only found in 88% of cases.¹⁰ The lack of glomerular involvement has been associated with less proteinuria in other forms of amyloidosis.¹⁷

One of the most important components of the diagnosis in amyloidosis is the typing of the amyloid.^{15,18} In ALECT2, the protein was first identified by Direct Edman sequence analysis.⁵ Although antibodies to LECT2 is commercially available, ALECT2 is most commonly typed by laser microdissection combined with mass spectrometry (LMD/MS) based proteomics.¹⁹ Using this technique, amyloid deposits are precisely dissected and the protein profile can be compared with the protein profile of a normal glomerulus. There are many advantages of LMD/MS over conventional methods. The avoidance of the need for antibody is a major advantage. Availability of commercially available antibodies limits the type of amyloid that can be identified by immunohistochemistry.¹⁶ In addition, false positive and false negative are common with immunohistochemistry tests. The biggest advantage however is the ability to test for all known proteins simultaneously while immunohistochemistry can only test a single protein with each test.

Table 1. Renal amyloidosis based on mass spectrometry results.

Amyloid type	Percentage
AL kappa and lambda	42.51
LECT2	20.4
AA Amyloidosis	12.5
Heavy chain and heavy + light chain	10
Fibrinogen A-a amyloidosis	5.5

Adapted from Sethi, *et al.*¹⁹

The typing evaluation once amyloid should start with immunofluorescence study. This works best for identification of AL, and immunoglobulin heavy chain (AH). Immunohistochemistry tests are available for AA, fibrinogen α chain (Afib), transthyretin (ATTR), and beta-2-microglobulin (AB2M).²⁰ If these amyloid types are ruled out, ALECT2 should be strongly suspected. Immunohistochemistry with anti-LECT2 antibodies has been described in the original series of this disease and it has been proven to be useful.^{5,14} If possible though, tissue should be sent for LMD/MS for proteomic analysis to confirm the diagnosis particularly if the patient has a monoclonal gammopathy as up to 10% may.¹⁶ Table 1 summarizes the most common type of renal amyloid based on mass spectrometry results showing that ALECT2 is a highly prevalent type in this setting.

LECT2 gene analysis

The systemic amyloidoses are associated with mutated, as well as wild-type, amyloidogenic precursor molecules.²¹ Peripheral blood leukocytes were available from 4 out of 10 patients reported by Murphy, *et al.*, and analyses of their genomic DNA revealed no mutations in the LECT2 coding sequences. Interestingly, a G/A polymorphism involving nucleotide 172 in exon 3 that accounts for the presence of valine or isoleucine at position 40 in the mature protein was seen.¹⁴ This was confirmed in 100% of patients tested in the most recent series by Larsen, *et al.*¹⁰ Importantly, each of the 4 patients evaluated in this study¹⁴ (as well as the first case reported with ALECT2 and 11 other)¹³ was homozygous for the G allele. In principle, this replacement of the isoleucine (A allele) with valine could be the responsible for making the protein unstable and subsequently accounting for the amyloidogenic properties associated to LECT2.

Treatment options

Unfortunately, there is no consensus on the best way to treat ALECT2. Patients should receive support therapy based on the organ dysfunction seen and performance status. A recent study revealed that kidney involvement by ALECT2 is quite common but other vital organs such as heart has not been noted to be involved in majority of cases.¹² After a median follow-up of ~2 years, 30% of cases evolved into ESRD and some of them (n = 5) went on to receive kidney transplantation. Graft loss was not seen in any of these patients. However, recurrence of ALECT2 was registered in 1 case. With such as scarce information, it is hard to make clinical guidelines for treatment of this entity and rather patients should be assessed based on individual needs (i.e. kidney transplantation, dialysis, etc.). No targeted therapy able to remove this protein has been reported or suggested and additional information is needed to establish better treatment recommendations.

CONCLUSIONS

The recent use of novel techniques for the typing of amyloidoses has increased our ability to recognize new entities associated to amyloid deposition. ALECT2 amyloidosis has emerged as one of the most common types of systemic amyloidosis showing a high prevalence of renal and liver involvement.

LECT2-associated amyloidosis is a recently recognized and distinctive type of amyloid that requires further clinical and biological characterization. The remarkable finding of ALECT2 being associated to Mexican Americans raised concerns about the prevalence of this disease in México and even the possibility of hereditary nature. No cases have been reported to the best of my knowledge in México and presumably, many cases might be under-recognized due to either lack of awareness and/or complexity in making the adequate amyloid typing. Elderly patients presenting with chronic renal insufficiency and bland urine sediment, with or without proteinuria in México should be assessed under the possibility of ALECT2. The Mayo Clinic and our group are aiming to identify new cases of this entity and to perform deep sequencing analyses to better understand the biological aspects of this condition. I envision this as being a document to raise awareness on the presence of this entity and to guide the general approach for renal amyloid disorders in México.

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Reimpresos:

Víctor H. Jiménez-Zepeda, M.D.

Tom Baker Cancer Center
Department of Medical Oncology and Hematology,
Calgary, AB, Canada
Ph. (403) 521-3716
Correo electrónico:
victor.zepeda@albertahealthservices.ca

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