Diagnosis, pathogenesis and outcome in Leucocyte chemotactic factor 2 (ALECT2) amyloidosis

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Leucocyte chemotactic factor 2 (ALECT2) amyloidosis

Leucocyte chemotactic factor 2 (ALECT2) amyloidosis is a newly identified form of renal amyloidosis which, according to US biopsy series, is the third most common form of renal amyloid. Here we detail for the first time the epidemiology and ethnic preponderance to this disease in a European country, which differs substantially from that in North America, and determine both renal and patient outcomes in a cohort followed prospectively for a prolonged period.

Abstract

Introduction: Renal biopsy series from North America suggest that leucocyte chemotactic factor 2 (ALECT2) amyloid is the third most common type of renal amyloid. We report the first case series from a European Centre of prevalence, clinical presentation, and diagnostic findings in ALECT2 amyloidosis and report long term patient and renal outcomes for the first time.

Methods: We studied the clinical features, diagnostic investigations and outcome of all patients with ALECT2 amyloidosis followed systematically at the UK National Amyloidosis Centre (NAC) between 1994 and 2015.

Results: Twenty-four patients, all non-Caucasian, were diagnosed with ALECT2 amyloidosis representing 1.3% of all patients referred to the NAC with biopsy proven renal amyloid. Diagnosis was made at median age 62 years, usually from renal histology; immunohistochemical staining was definitive for ALECT2 fibril type. Median eGFR at diagnosis was 33 ml/min and median proteinuria was 0.5 g/24 hr. Hepatic amyloid was evident on SAP scintigraphy in 11/24 cases but was not associated with significant derangement of liver function. No patient had evidence of cardiac amyloidosis or amyloid neuropathy. Median follow up was 4.8 (0.5-15.2) years during which 4 patients died and 4 progressed to ESRD. Mean rate of GFR loss was 4.2 (0.5-9.6) ml/min/year and median estimated renal survival from diagnosis was 8.2 years. Serial SAP scans revealed little or no change in total body amyloid burden.

Conclusions: ALECT2 amyloidosis is a relatively benign type of renal amyloid, associated with a slow GFR decline, which is reliably diagnosed on renal histology.

Neither the molecular basis nor the factors underlying the apparent restriction of ALECT2 amyloidosis to non-Caucasian populations have been determined.

Key Words: Amyloidosis, End Stage Renal Disease (ESRD), proteinuria, Chronic Kidney Disease (CKD), Leucocyte Chemotactic Factor 2 Amyloidosis (ALECT2)

Introduction

Renal amyloidosis results from the pathological deposition of amyloid protein fibrils in glomeruli and/or renal parenchyma. The diagnosis of amyloid is based on light microscopy demonstrating characteristic green birefringence in affected tissues that have been stained with Congo red and viewed under crossed polarized light. More than 30 different human proteins can form amyloid *in vivo,* which on electron microscopy appears as randomly orientated non-branching fibrils of 7-12 nm width. The commonest form of amyloid diagnosed in kidney biopsies is AL type, in which the fibrils are derived from monoclonal immunoglobulin light chain. Some 13 other proteins that can deposit as amyloid in the kidneys include serum amyloid A protein (SAA), apolipoproteins A-I, A-II and C-III, fibrinogen, lysozyme, gelsolin and transthyretin.[1]

Leucocyte chemotactic factor 2 (ALECT2) amyloidosis was first described in 2008 in a 61-year-old woman who had presented seven years earlier with nephrotic syndrome and glomerular amyloid deposits and later underwent nephrectomy for clear cell renal carcinoma.[2] Murphy *et al* subsequently described a series of ten adults with ALECT2 amyloid who presented with varying degrees of renal impairment, proteinuria and extensive interstitial and mesangial amyloid deposits. Interestingly, all affected individuals were homozygous for the G allele, a polymorphism which results in a substitution of isoleucine with valine at position 40 in the mature LECT2 protein.[3] Two

recent retrospective studies, one from the Mayo Clinic and the other from Nephropath which included 72 and 40 patients respectively have established the clinical and pathological characteristics and outcomes in renal ALECT2 amyloidosis in the U.S.[4-6] A retrospective U.S. renal biopsy series of 285 samples identified ALECT2 amyloid as the third most common type of renal amyloid at 2.5%.[7] In a retrospective study of 130 cases of hepatic amyloid, the Mayo Clinic investigators identified ALECT2 amyloid in 25% of cases making it the second commonest form of hepatic amyloid.[8] They identified unique pathological and clinical features, including the fact that the diagnosis of amyloid was often incidental, starkly contrasting with hepatic AL amyloid in which hepatomegaly and liver dysfunction are typical.

There have been few studies worldwide and none from Europe exploring long term follow up and outcome in ALECT2 amyloidosis. Here we report the prevalence, clinical presentation, diagnostic findings and long term outcome among all 24 patients who were diagnosed with ALECT2 amyloidosis at the UK National Amyloidosis Centre over a 21 year period.

Methods

Patients and Outcomes

We included in this study all patients with ALECT2 amyloidosis who attended the UK National Amyloidosis Centre over a period of greater than 20 years (1994 to 2015). Nine of 24 patients were identified to have ALECT2 amyloidosis retrospectively and the remainder were diagnosed with ALECT amyloidosis at the time of diagnosis of amyloid. Patients attended the NAC for their initial diagnostic evaluation and were followed up at regular (usually 12 monthly) intervals. Investigations undertaken at each visit to the centre included detailed blood and urine biochemistry, electrocardiography,

echocardiography and monitoring of visceral organ involvement as well as whole body amyloid load by ^{123}I-SAP scintigraphy. Additional investigations were undertaken when clinically indicated.

All patients were managed in accordance with the declaration of Helsinki and informed patient consent and institutional review board approval from the Royal Free Hospital Ethics committee were obtained for this study.

Histology and Immunohistochemistry

Renal (n=20), hepatic (n=4) and rectal (n=1) biopsies were processed, as previously described (one patient had both liver and renal biopsies).[9] Briefly, serial sections were cut from each formalin fixed paraffin embedded block (FFPE). Amyloid was diagnosed on Congo red staining by apple green birefringence when viewed under cross polarized light microscopy. Paraffin embedded sections of 2 µm thickness were used for IHC, and sections of 6 µm thickness were used for Congo red and IHC/Congo red overlay, as previously decribed.[9] Immunohistochemical staining of the amyloid deposits was performed using an extensive panel of monospecific antibodies reactive with serum amyloid A protein (SAA) (Euro Diagnostica AB), kappa and lambda immunoglobulin light chains (Dako), apolipoprotein A-I (Genzyme), fibrinogen Aα-chain (Calbiochem®), transthyretin (Dako), and LECT2 (R & D Systems). The goat anti-human LECT2 antibody (cat no AF722), was used at 1:600 dilution. Staining specificity was determined by prior absorption of the antiserum with purified human protein, in these cases with human LECT2, which resulted in complete abrogation of staining. IHC was performed on the Sequenza™ (Fisher Scientific, UK) platform using Impress™ (Vector laboratories UK) detection kit following the standard method, with the use of a metal enhanced DAB Substrate kit (Thermo Scientific, UK) for visualizing

the immuno compound. All slides were viewed on a DM4000 (Leica Microsystems, UK) and was interpreted independently by two experienced operators.

Proteomic analysis of amyloidotic tissue

Laser micro dissection (LMD) followed by liquid chromatography and tandem mass spectrometry (MS) (LC-MS/MS) was performed on Congo red-positive glomeruli or renal tubules as previously described by Dogan and colleagues.[10] For comparison, purified human LECT2 protein was trypsinized and analyzed by LC-MS/MS on a Velos orbitrap mass spectrometer. MS data files were analyzed using Mascot[11] to search the SwissProt database (SIB Bioinformatics Resource, Bethesda, MD). Searches were conducted based on trypsin as the digestion enzyme and oxidation of methionine set as a variable modification; mass tolerances were 10 ppm for precursor ions and 0.60 Da for fragment ions.

Genetic Analysis

Genomic DNA was extracted from whole blood treated with EDTA as previously described.[12] Coding regions of the *LECT2* gene (NCBI Ref NC_000005.10) were amplified by polymerase-chain-reaction assay (PCR) and analyzed by automated sequencing as previously described.[2] PCR products were purified with a QIAquick PCR purification kit (Qiagen) according to the manufacturer's protocol and sequenced with the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The electropherograms of the *LECT2* gene were analysed on the ABI 3130xl Genetic Analyser using Sequencing Analysis Software version 5.4.

^{123}I-Labelled SAP Scintigraphy

All patients underwent whole body anterior and posterior scintigraphic imaging 24 h after administration of ^{123}I-labelled serum amyloid P component (SAP) using a GE Infinia Hawkeye gamma camera (GE Healthcare, Iowa, MN), as previously described.[13] Labelled SAP studies were interpreted by a panel of physicians with experience of over 29 000 SAP scans.

Statistical Methods

Patient survival, time from diagnosis of amyloidosis to end-stage renal disease defined as commencement of renal replacement therapy (renal survival) and survival from commencement of renal replacement therapy were estimated by Kaplan-Meier analyses. Censor date was 1st December 2015. Rate of decline of renal function was analyzed in those patients who were not requiring renal replacement therapy at the time of diagnosis, and was expressed in ml/min/yr.

Results

Patients

Twenty-four patients were confirmed to have ALECT2 amyloidosis at the UK National Amyloidosis Centre (NAC), comprising 0.36% of 6739 patients with amyloidosis and 1.3% of 1853 patients with biopsy proven renal amyloid seen at the centre between 1994 and 2015. Of these 24 cases, 9 were diagnosed retrospectively after the discovery of ALECT2 amyloidosis in 2008 having previously been classified as amyloid with no immunospecific staining. Twenty-two patients had a renal presentation with proteinuria and/or impaired renal excretory function, including 20 who had been referred to the NAC on the basis of amyloid detected in a kidney biopsy. Four patients had amyloid detected on liver histology (including one with established amyloid

deposits in the kidneys), performed in 3 of the 4 cases to investigate chronic viral hepatitis infection and in the last case as work up for peritoneal pseudomyxoma. The diagnosis of ALECT2 amyloidosis was therefore established on renal histology in 20 cases, liver histology in 3 cases and on rectal histology in the remaining case (Table 1).

Median age at diagnosis was 62 years (range 37–75), and male to female ratio was roughly equal (14/10). Notably, 16 (67%) patients were from the Indian subcontinent (India and Pakistan), 7 (30%) patients were of Middle Eastern ancestry (Egyptian and Sudanese), and one was of Mexican origin. ALECT2 amyloidosis was not diagnosed in any British Caucasians. Patient demographics at diagnosis are shown in Table 1.

Two patients had established ESRD and were receiving renal replacement therapy in the form of hemodialysis at the time of diagnosis of ALECT2 amyloidosis. Neither had presented with nephrotic syndrome. Among the remaining 22 patients who remained dialysis independent at the time of diagnosis, median eGFR was 33 ml/min (range 15-110) and median proteinuria was 0.5 g/24 hr (range 0.1-2.9).

Liver function tests were entirely normal among 21 patients. There was mild elevation of transaminases (ALT 54 and 73 U/L) in 2 patients and mildly obstructive liver function in a single patient as evidenced by an alkaline phosphatase of 150 U/L. No patient had hyperbilirubinaemia or clinical evidence of jaundice.

No patient had clinical, echocardiographic or cardiac biomarker evidence of cardiac amyloidosis or clinical evidence of amyloid peripheral or autonomic neuropathy.

None of the 24 patients had evidence of a monoclonal gammopathy. Four patients had a concurrent diagnosis of type 2 diabetes mellitus.

Histology

Renal histology revealed amyloid throughout the kidneys, often with bright congophilia and 'sparkly glistening' apple green birefringence when viewed under crossed polarized light. Liver histology revealed amyloid deposits with a similar 'sparkly glistening' appearance under crossed polarized light.

Among 20 patients who underwent renal biopsies, the vast majority (85%) had amyloid in the renal cortical interstitium which was sometimes extensive and often associated with tubular atrophy. Amyloid deposits were identified in the tubular basement membranes in approximately one third of renal biopsies and in the vessel walls in two thirds (Table 2). Glomerular amyloid was present in more than 70% of renal biopsies, and was associated with mesangial matrix expansion which was usually diffuse and global (Figure 1A and 1B). Typically, a proportion of biopsied glomeruli were globally sclerosed. The burden of amyloid within each compartment of the kidney varied substantially between patients, and apart from the extensive interstitial amyloid observed in some patients, there was no 'characteristic' pattern of deposition or apparent relationship between ethnicity and localization of renal amyloid deposits.

Liver histology revealed amyloid deposits in vessel walls and globular deposits in the portal tracts (Figure 1D and 1E). Gastrointestinal histology showed amyloid in the submucosa. The amyloid stained specifically with anti-LECT2 antibody in every case (Figure 1C and 1F).

Proteomic Analyses

Proteomic analysis of the excised amyloid was performed in 14/24 samples. LECT2 was identified in 12/14 cases; there was insufficient material for valid interpretation of proteomic results in the two negative cases, each of which had <30 proteins identified

in the sample (both of whom were diagnosed on the basis of immunohistochemistry). A positive control sample of authentic LECT2 treated in the same way as the patient samples identified LECT2 with 95% protein coverage.

Genetic Analyses

All patients underwent genetic analysis and all except one affected individual were found to be homozygous for the G allele, a polymorphism which results in a substitution of isoleucine with valine at position 40 in the mature protein. The remaining affected individual was heterozygous for the G allele, which as far as the authors are aware, is the first report of ALECT2 amyloidosis in a patient who is heterozygous for this allele.

^{123}I-Labelled SAP Scintigraphy

SAP scintigraphy at diagnosis showed extra-renal amyloid deposits in most patients; 21/24 (88%) had splenic amyloid, 11/24 (46%) had hepatic amyloid and 9/24 (38%) had adrenal gland involvement by amyloid despite preserved adrenocortical function in every case. The adrenal glands were masked by heavy overlying liver and spleen amyloid deposits in 8 of the 15 remaining cases.

Serial SAP scintigraphy, with an interval between the diagnostic and latest scan of up to 10 years, revealed little change in total body amyloid burden. There was no evidence of amyloid regression from any organ in any patient but, interestingly and uncharacteristically for untreated systemic amyloidosis, nor was there evidence of accumulation of amyloid within the liver, kidneys or spleen over prolonged periods (Figure 2).

Renal and Patient Outcomes

Median follow up in the whole cohort was 4.8 years (range 0.5–15.2). During this time, 4 patients died. The cause of death was known in 3 cases who died from bronchopneumonia, cardiac arrest secondary to underlying ischaemic heart disease (IHD), and 'pump failure' secondary to aortic stenosis respectively. Median age at death was 74 years (range 63–77). Median estimated patient survival from diagnosis of ALECT2 amyloidosis was 15.1 years (Figure 3a).

Among 22 patients who were dialysis independent at diagnosis, mean rate of GFR loss was 4.2 ml/min/yr (range 0.5-9.6). Four patients progressed to ESRD during follow up. Median estimated time from diagnosis to ESRD by Kaplan Meier analysis among those 22 patients was 8.2 years (Figure 3b). Proteinuria remained sub-nephrotic and serum albumin remained within the normal range in all patients throughout follow up. Indeed there was no significant change in proteinuria over time among most patients (mean change <0.1 g/24hr/yr).

Discussion

LECT2 was reported to be a new amyloid fibril protein by Benson and colleagues in 2008 following discovery of unanticipated amyloid within a nephrectomy specimen from an individual with co-existing clear cell renal carcinoma.[2] LECT2 is a 16 kDa protein consisting of 151 amino acids, including an 18 amino acid signal peptide. The amyloidogenic fibril protein in ALECT2 amyloidosis is composed of the entire 133-residue peptide. Although there is no mutation in the *LECT2* gene associated with ALECT2 amyloidosis, nearly all affected individuals in our series, including two affected first degree relatives, were found to be homozygous for the G allele, a polymorphism which results in a substitution of isoleucine with valine at position 40 in the mature protein, consistent with previous reports.[2] The G/G genotype has an overall

frequency of 0.477 and in subjects of European descent it is found at a frequency range of 0.6-0.7,[2] although the frequency of this polymorphism in the studied population is unknown. Murphy *et al* described a clear ethnic distribution in their case series of ten patients with ALECT2 amyloidosis; 7 of the 10 patients were of Mexican American origin,[3] a finding corroborated by the Nephropath investigators who found that the majority of patients with ALECT2 amyloidosis were of Hispanic origin.[14] A recent biopsy series revealed ALECT2 amyloid to be the second commonest type of renal amyloid in Egypt, accounting for almost one third of cases.[15] Our cohort of 24 patients further supports an ethnic bias but includes individuals from the Indian Subcontinent and Egypt/Sudan as well as Hispanics, corroborating the recent findings of Larson et al.[14]

The aetio-pathogenetic mechanisms underlying ALECT2 amyloid formation require further evaluation. LECT2 is mostly produced by the liver and is known to be a chemotactic factor to neutrophils while it also stimulates the growth of chondrocytes and osteoblasts.[16] Lin Bin *et al* identified LECT2 as an acute phase reactant with a high level of induction upon infection,[17] and it appears to play a role in liver regeneration.[18] One might speculate therefore, that LECT2 is an acute phase reactant that is specifically triggered by a chronic hepatic infection or chronic hepatic inflammation and that the combination of the G/G genotype and excessive production of LECT2 by the liver predispose to development of amyloid. Other proposed mechanisms for ALECT2 amyloidosis included interference in the catabolic or transport pathways of LECT2 resulting in an increased local tissue concentration or alterations in a binding partner of the LECT2 protein in the serum resulting in increased free circulating LECT2 levels.[6] Due to the absence of a commercially available LECT2 assay, we were unable to

measure plasma LECT2 concentration among our patient cohort in order to further explore these possibilities.

The vast majority of patients diagnosed with ALECT2 amyloidosis had a renal presentation characterized by low level proteinuria and a reduced GFR. Given the frequent (>70%) glomerular involvement by amyloid in this cohort, the absence of heavy proteinuria is surprising, and contrasts other types of renal amyloid in which the glomeruli are involved which are typically characterized by the nephrotic syndrome. Around half of patients with ALECT2 amyloidosis had evidence of liver involvement by SAP scintigraphy but liver synthetic function was preserved in every case and liver function abnormalities were absent or mild, consistent with previous reports.[4] Three patients were diagnosed on liver histology, prompted in each case by another potential cause of liver dysfunction; presence of amyloid may have been incidental to the dysfunction since amyloid deposits were frequently present among those without derangement of liver function. No patient had cardiac amyloidosis at diagnosis and clinically significant pulmonary or neural amyloid was not observed.

Staining of formalin fixed paraffin embedded renal and liver sections with an anti-LECT2 antibody was unequivocally diagnostic in every case. There was no background or non-specific staining of the sections, and the amyloid deposits were avidly and specifically stained by the antibody in both tissues. The immunohistochemistry was corroborated by proteomic analysis of amyloid whenever this was undertaken on adequate samples. The authors would therefore recommend that tissue from all cases of suspected ALECT2 amyloidosis is stained immunohistochemically and/or analyzed by tandem mass spectrometry.

Despite absence of amyloid-specific therapy for ALECT2 amyloidosis, its natural history, described here in detail for the first time, is slow. Mean rate of GFR

loss was 4.2 mls/min/year contrasting the typical decline in other types of renal amyloidosis (Table 3), such as untreated renal AL amyloidosis in which median time from diagnosis to ESRD is only about 12 months;[19] only 6 patients in the whole cohort reached ESRD despite prolonged follow up. Serial SAP scans, performed up to a decade apart, revealed absence of amyloid accumulation and similarly, no patient developed cardiac or neuropathic amyloid during follow up. Consequently, median estimated patient survival from diagnosis was more than 15 years.

Given the discrepancy between the clinical and histological prevalence of ALECT2 amyloid and the often insidious nature of its clinical manifestations, [7] the true prevalence of this type of amyloid may be greater than is currently recognized. Since promising novel therapies to remove existing amyloid deposits are now under development,[20] the diagnosis of ALECT2 amyloidosis ought to be considered in any non-Caucasian patient with renal amyloid deposits who has low level proteinuria, even when a monoclonal protein is present.

Disclosures

None

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References

1. Sipe JD, Benson MD, Buxbaum JN, *et al.* Nomenclature 2014: Amyloid fibril proteins and clinical classification of the amyloidosis. *Amyloid* 2014; **21**: 221-224.

2. Benson MD, James S, Scott K, *et al.* Leukocyte chemotactic factor 2: A novel renal amyloid protein. *Kidney Int* 2008; **74**: 218-222.

3. Murphy CL, Wang S, Kestler D, *et al.* Leukocyte chemotactic factor 2 (LECT2)-associated renal amyloidosis: a case series. *Am J Kidney Dis* 2010; **56**: 1100-1107.

4. Said SM, Sethi S, Valeri AM, *et al.* Characterization and outcomes of renal leukocyte chemotactic factor 2-associated amyloidosis. *Kidney Int* 2014; **86**: 370-377.

5. Larsen CP, Kossmann RJ, Beggs ML, *et al.* Clinical, morphologic, and genetic features of renal leukocyte chemotactic factor 2 amyloidosis. *Kidney Int* 2014; **86**: 378-382.

6. Nasr SH, Dogan A, Larsen CP. Leukocyte Cell-Derived Chemotaxin 2-Associated Amyloidosis: A Recently Recognized Disease with Distinct Clinicopathologic Characteristics. *Clin J Am Soc Nephrol* 2015; **10**: 2084-2093.

7. Larsen CP, Walker PD, Weiss DT, *et al.* Prevalence and morphology of leukocyte chemotactic factor 2-associated amyloid in renal biopsies. *Kidney Int* 2010; **77**: 816-819.

8. Mereuta OM, Theis JD, Vrana JA, *et al.* Leukocyte cell-derived chemotaxin 2 (LECT2)-associated amyloidosis is a frequent cause of hepatic amyloidosis in the United States. *Blood* 2014; **123**: 1479-1482.

9. Tennent GA, Cafferty KD, Pepys MB, *et al.* Congo red overlay immunohistochemistry aids classification of amyloid deposits. In: Kyle RA, Gertz MA,

editors. Amyloid and Amyloidosis 1998. Pearl River, New York: Parthenon Publishing; 1999. p. 160-162.

10. Rodriguez FJ, Gamez JD, Vrana JA, *et al.* Immunoglobulin derived depositions in the nervous system: novel mass spectrometry application for protein characterization in formalin-fixed tissues. *Lab Invest* 2008; **88**: 1024-1037.

11. Perkins DN, Pappin DJ, Creasy DM, *et al.* Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* 1999; **20**: 3551-3567.

12. Talmud P, Tybjaerg-Hansen A, Bhatnagar D, *et al.* Rapid screening for specific mutations in patients with a clinical diagnosis of familial hypercholesterolaemia. *Atherosclerosis* 1991; **89**: 137-141.

13. Hawkins PN, Lavender JP, Pepys MB. Evaluation of systemic amyloidosis by scintigraphy with ^{123}I-labeled serum amyloid P component. *N Engl J Med* 1990; **323**: 508-513.

14. Larsen CP, Beggs ML, Wilson JD, *et al.* Prevalence and organ distribution of leukocyte chemotactic factor 2 amyloidosis (ALECT2) among decedents in New Mexico. *Amyloid* 2016; **23**: 119-123.

15. Larsen CP, Ismail W, Kurtin PJ, *et al.* Leukocyte chemotactic factor 2 amyloidosis (ALECT2) is a common form of renal amyloidosis among Egyptians. *Mod Pathol* 2016; **29**: 416-420.

16. Yamagoe S, Yamakawa Y, Matsuo Y, *et al.* Purification and primary amino acid sequence of a novel neutrophil chemotactic factor LECT2. *Immunol Lett* 1996; **52**: 9-13.

17. Lin B, Chen S, Cao Z, *et al.* Acute phase response in zebrafish upon Aeromonas salmonicida and Staphylococcus aureus infection: striking similarities and obvious differences with mammals. *Mol Immunol* 2007; **44**: 295-301.

18. Sato Y, Watanabe H, Kameyama H, *et al.* Changes in serum LECT 2 levels during the early period of liver regeneration after adult living related donor liver transplantation. *Transplant Proc* 2004; **36**: 2357-2358.

19. Gertz MA, Lacy MQ, Dispenzieri A. Immunoglobulin light chain amyloidosis and the kidney. *Kidney Int* 2002; **61**: 1-9.

20. Richards DB, Cookson LM, Berges AC, *et al.* Therapeutic Clearance of Amyloid by Antibodies to Serum Amyloid P Component. *N Engl J Med* 2015; **373**: 1106-1114.

21. Gillmore JD, Lachmann HJ, Rowczenio D, *et al.* Diagnosis, pathogenesis, treatment, and prognosis of hereditary fibrinogen A alpha-chain amyloidosis. *J Am Soc Nephrol* 2009; **20**: 444-451.

Table 1. Patient demographics

No	Age at diagnosis/ Sex	Ethnic Origin	Histology	Hepatitis B/C	Serum creatinine (µmol/L)	eGFR (ml/min)	Proteinuria (g/24hr)	SAP scintigraphy (Liver/Spleen/ Kidneys/Adrenals)
1	61 M	Egyptian	Renal and hepatic	C	201	30	0.17	L/S/K
2	62 M	Egyptian	Renal	-ve	245	24	0.10	L/S
3	58 F	Egyptian	Renal	-ve	116	44	0.47	L/S/K/A
4	62 M	Egyptian	Renal	-ve	219	27	0.48	K/A
5	57 M	Egyptian	Hepatic	-ve	94	76	1.11	L/S/K/A
6	66 M	Indian	Renal	-ve	379	15	0.90	L/S
7	37 M	Indian	Renal	-ve	74	110	0.10	Normal
8	74 M	Indian	Renal	-ve	181	33	0.10	S/K
9	63 M	Indian	Renal	-ve	156	40	2.52	S/K/A
10	62 M	Indian	Renal	-ve	239	25	2.98	S/K
11	68 F	Indian - Punjabi	Renal	-ve	186	24	2.97	S/K/A
12	75 F	Indian	Renal	-ve	183	25	0.10	L/S/K/A
13	69 M	Pakistani	Renal	-ve	178	34	0.10	L/S/K
14	66 M	Pakistani	Renal	-ve	424	HD	0.12	A
15	67 F	Pakistani	Renal	-ve	284	15	1.06	S
16	59 F	Pakistani	Renal	-ve	131	38	0.20	S/K/A
17	55 M	Pakistani	Renal	-ve	127	53	0.51	S/K
18	75 M	Pakistani - Kashmiri	Hepatic	B & C	114	56	0.10	L/S/K/A
19	70 M	Pakistani - Punjabi	Renal	-ve	213	20	0.65	S/K
20	71 F	Punjabi	Renal	B	143	32	1.74	S/K
21	53 M	Sudanese	Hepatic	C	80	93	0.10	S
22	54 F	Sudanese	Renal	-ve	562	HD	Anuric	L/S/K
23	56 F	Mexican	Rectal	C	57	100	0.10	L/S
24	73 F	Indian	Renal	-ve	206	21	0.18	L/S/K

Table 2. Renal histology in relation to clinical presentation

Patient Number	Interstitial Amyloid (+/-)	Glomerular Amyloid (+/-)	Tubular Amyloid (+/-)	Vascular Amyloid (+/-)	Diabetes (Y/N)	eGFR (ml/min)	24 urinary protein loss (g/24hr)	Hypertension (Y/N)
1	+	-	-	-	0	30	0.17	N
2	+	+	-	-	0	24	0.10	N
3	+	+	-	+	0	44	0.47	N
4	+	-	+	-	0	27	0.48	N
6	+	+	+	+	0	15	0.90	Y
7	+	-	-	+	0	110	0.10	N
8	+	+	-	+	0	33	0.10	N
9	-	+	+	+	0	40	2.52	Y
10	+	-	-	+	1	25	2.98	N
11	-	+	+	+	1	24	2.97	Y
12	-	-	-	+	0	25	0.10	Y
13	+	+	-	+	0	34	0.10	N
14	+	+	-	+	1	HD	0.12	N
15	+	+	-	+	0	15	1.06	Y
16	+	+	-	+	0	38	0.20	Y
17	+	+	-	-	0	53	0.51	N
19	+	+	-	-	0	20	0.65	Y
20	+	+	-	-	0	32	1.74	Y
22	+	+	-	+	0	HD	Anuric	Y
24	+	+	+	+	1	21	0.18	N

Table 3. Comparison of rate of GFR loss in ALECT2 amyloidosis and hereditary renal amyloidosis

Type of Amyloid	**Number**	**Mean eGFR loss per year ml/min/year**
Leucocyte chemotactic factor 2 amyloidosis (ALECT2)	22	4.2
Lysozyme (ALys)	7	4.7
Apolipoprotein A-1 (AApoAI)	24	6.2
Fibrinogen A α-chain (AFib)	23	11.5[21]

Figure Legends

Figure 1. Histology in ALECT2 amyloidosis: A) Congo red staining of a renal biopsy specimen showing amyloid in all renal compartments including the glomeruli, renal tubules, vessels and interstitium. B) Apple green birefringence when viewed under cross polarized light. C) Immunohistochemical staining with an antibody against LECT2 showing specific staining of the amyloid which is completely abrogated by prior absorption of the antibody with purified LECT2. D) Congo red staining of a liver biopsy specimen viewed under Brightfield light showing amyloid in vessels and portal tract. E) Characteristic immunofluorescence of amyloid after Congo red staining and F) Immunohistochemical staining with an antibody against LECT2 showing specific staining of the amyloid.

Figure 2. Serial SAP scintigraphy in ALECT2 amyloidosis. SAP anterior whole body SAP scans, taken 10 years apart, in a patient with ALECT2 amyloidosis showing absence of amyloid accumulation in the liver or spleen over this time period.

Figure 3. Outcome in ALECT2 amyloidosis. A) Patient survival in ALECT2 amyloidosis by Kaplan Meier analysis. Median estimated survival was 15.2 years. B) Renal survival, defined as time from diagnosis to renal replacement therapy, in ALECT2 amyloidosis in comparison to other untreated renal amyloidoses including AApoAI, ALys, and AFib amyloidosis.