

Focal Segmental Glomerulosclerosis Complicating Therapy With Inotersen, an Antisense Oligonucleotide Inhibitor: A Case Report

Steven Law, Julia Arnold, Muhammad U. Rauf, Lauren Heptinstall, Janet Gilbertson, Dorota Rowczenio, Jyoti Baharani, Gerald Langman, Marianna Fontana, and Julian D. Gillmore

Inotersen is an antisense oligonucleotide inhibitor licensed for the treatment of polyneuropathy complicating hereditary transthyretin amyloidosis (ATTRv). Nephrotoxicity has been reported with inotersen, including progression to kidney failure. We describe what is to our knowledge the first reported case of inotersen-associated nephrotic syndrome secondary to focal segmental glomerulosclerosis (FSGS) and review the literature concerning inotersen-induced nephrotoxicity. We report a woman in her early 30s with ATTRv associated with the V50M transthyretin (TTR) variant, who presented with nephrotic syndrome 7 months after commencement of inotersen. Renal histology demonstrated FSGS and scanty glomerular amyloid deposition. Discontinuation of inotersen alone resulted in complete clinical and biochemical resolution of nephrotic syndrome. Inotersen is associated with significant nephrotoxicity. In the phase 3 NEURO-TTR clinical trial, 3% of patients in the treatment arm developed a crescentic glomerulonephritis. All affected patients carried the V50M TTR variant, which is known to be associated with renal amyloid deposition. This case adds to the spectrum of kidney disease associated with inotersen and indicates that discontinuation of the drug alone may result in resolution of renal complications without additional immunosuppression. Monitoring of kidney function is essential in patients with ATTRv receiving inotersen, particularly if there is evidence of existing renal amyloid.

Complete author and article information provided before references.

Am J Kidney Dis. XX(XX):1-5. Published online month xx, xxxx.

doi: 10.1053/j.ajkd.2022.08.018

© 2022 The Authors.

Published by Elsevier Inc. on behalf of the National Kidney Foundation, Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Introduction

Hereditary transthyretin amyloidosis (ATTRv) is a rare form of systemic amyloidosis caused by more than 130 pathogenic variants in the transthyretin gene (TTR) that result in production of amyloidogenic TTR protein. Certain TTR variants are associated with distinct clinical phenotypes, although amyloid cardiomyopathy, peripheral neuropathy, and/or autonomic neuropathy predominate. Clinically significant renal amyloidosis in ATTRv is rare but can occur, and may range from having no nephropathy to proteinuria and/or kidney failure, especially in patients with the V50M variant of TTR (c.148G>A p.(V50M); that is, a guanine-to-adenine substitution at nucleotide 148 of the coding sequence, predicted to lead to a valine-to-methionine substitution at amino acid 50).¹⁻⁴

Advances in RNA-targeted therapies have revolutionized the treatment of ATTRv, although the impact on kidney outcomes is unknown.⁵⁻⁷ One such treatment is inotersen, an antisense oligonucleotide inhibitor (ASO), which reduces serum TTR concentration by approximately 80%, delays or halts neurologic progression,⁶ and may slow or halt progression of amyloid cardiomyopathy.⁷ In the phase 3 clinical trial of inotersen (NEURO-TTR), 3% of patients in the treatment arm developed crescentic glomerulonephritis compared to none in the placebo arm.⁶

We report, for what is to our knowledge the first time, the development of nephrotic syndrome associated with focal segmental glomerulosclerosis (FSGS) on kidney biopsy following commencement of inotersen. The patient's nephrotic syndrome resolved completely following discontinuation of inotersen without a need for immunosuppression.

Case Report

A woman in her early 30s presented with a 2-week history of shortness of breath, facial swelling, and leg edema. Eight months previously she had been diagnosed with ATTRv amyloidosis with dominant neuropathic involvement after presenting with a 2-year history of progressive peripheral and autonomic neuropathy. Two first-degree relatives had undergone liver transplantation during the pre-RNA therapy era for ATTRv amyloidosis; all affected family members had the V50M TTR variant, which is known to be pathogenic. Radiolabeled ¹²³I-serum amyloid P component (SAP) scintigraphy at the time of diagnosis of amyloidosis revealed amyloid in the kidneys; however, there was no evidence of cardiac amyloidosis on ^{99m}Tc-Technetium-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) scintigraphy or echocardiography. Biochemical investigations at the same time were as follows: serum creatinine, 53 μmol/L; estimated glomerular filtration rate, >90 mL/min/1.73 m²; urinary protein-creatinine ratio (UPCR), 73 mg/mmol; and urinary albumin-creatinine ratio, 44 mg/mmol. There was no history of kidney disease in the patient or any of her affected relatives. Medications included amitriptyline 10 mg once daily for neuropathic pain, inotersen 284 mg weekly by subcutaneous injection commenced 7 months previously, and vitamin A supplementation.

On examination the blood pressure was 118/78 mm Hg, heart rate 90 beats per minute, oxygen saturation 95%, respiratory rate 16 breaths per minute, and temperature 37.1 °C. Full systems examination highlighted periorbital and peripheral edema to the mid shins.

Table 1. Further Investigations

	Amyloid Diagnosis	Nephrotic Presentation	Follow-up ^a
Hemoglobin, g/dL	132	122	119
White blood cell count, 10 ⁹ /L	10.4	5.3	7.4
Eosinophils, ×10 ⁹ /L	0.08	0.07	0.03
Platelets, ×10 ⁹ /L	300	237	329
Sodium, mmol/L	142	138	139
Potassium, mmol/L	3.6	4.1	4.3
Creatinine, μmol/L	53	101	77
eGFR, mL/min/1.73 m ²	>90	55	79
Serum albumin, g/L	41	26	40
Alanine transaminase, IU/L	20	27	23
Alkaline phosphatase, IU/L	59	69	77
Bilirubin (total), μmol/L	8	5	5
Adjusted calcium, mmol/L		2.33	
Immunoglobulins			
IgA, g/L	1.5	1.38	1.7
IgG, g/L	7.6	5.29	8.9
IgM, g/L	3.1	3.98	4.1
Serum electrophoresis and immunofixation		Not detected	
Viral screening ^b		Negative	
Autoantibody screening ^c		Negative	
Complement factor C3, g/L		0.85	
Complement factor C4, g/L		0.20	
UPCR, mg/mmol	73	217	88
Kidney ultrasound		Unremarkable ^d	

Abbreviations: eGFR, estimated glomerular filtration rate (as calculated by the Modification of Diet in Renal Disease equation); UPCR, urinary protein-creatinine ratio.

^aEvaluation occurring 2 months after inotersen cessation.

^bFor hepatitis C virus RNA, hepatitis B virus core antibody and surface antigen, HIV antibodies.

^cAntinuclear antibodies, antineutrophil cytoplasmic antibodies, anti-glomerular basement membrane antibodies, anti-double-stranded DNA antibodies, and rheumatoid factor.

^dResults showed 10.9 cm and 10.6 cm unobstructed kidneys with normal corticomedullary differentiation.

Urinary dipstick demonstrated protein (3+), blood (3+), leucocytes (2+), pH of 5.0, and negative results for nitrites; there were no cellular casts or dysmorphic red cells on urinary sediment analysis. Further investigations are summarized in Table 1 and support a diagnosis of nephrotic syndrome. Native kidney biopsy was performed and demonstrated 14 glomeruli. One glomerulus was globally sclerosed; 3 demonstrated segmental sclerosis with collapse of the glomerular tuft and capsular adhesion, while the remaining glomeruli demonstrated variable changes with mesangial expansion (Fig 1A-C). Podocytes were prominent and a few were multinucleated. There was no significant interstitial fibrosis or tubular atrophy; a mild focal interstitial chronic inflammatory cell infiltrate with slight edema was present.

Immunofluorescence showed focal staining for immunoglobulin G and complement factor 3 in areas of amyloid deposition, and nonspecific low-level granular IgA and IgM staining. There was minimal mesangial and vascular amyloid by Congo red staining with immunospecific staining of the amyloid with anti-TTR antibody (Figure 1D-F). Laser microdissection of the amyloid and tandem mass spectrometry confirmed it to be of ATTR type. Electron microscopy demonstrated thin glomerular

basement membranes (177-245 nm); it did not capture a segmental lesion or amyloid fibrils, and there was no podocyte foot-process effacement.

Supportive treatment with 80 mg furosemide once daily was commenced and inotersen was immediately discontinued; no immunosuppression was given. Two months later, the patient's symptoms had resolved, serum albumin was 40 g/L, and UPCR fell to 88 mg/mmol. Furosemide was discontinued. A further month later, UPCR was 35 mg/mmol. Following a break from therapy, during which there was gradual progression of neuropathy, treatment was commenced with patisiran, a small interfering RNA directed against a target sequence in the 3' untranslated region of the TTR messenger RNA.

Discussion

To our knowledge, this is the first case report of FSGS complicating treatment of ATTRv amyloidosis with inotersen. Inotersen was licensed for the treatment of ATTRv with polyneuropathy in 2018.⁶ During the pivotal NEURO-TTR trial of inotersen, 3 patients (3%) in the treatment arm developed glomerulonephritis thought to be due to the study drug. All 3 patients carried the V50M TTR variant, which is known to be associated with renal

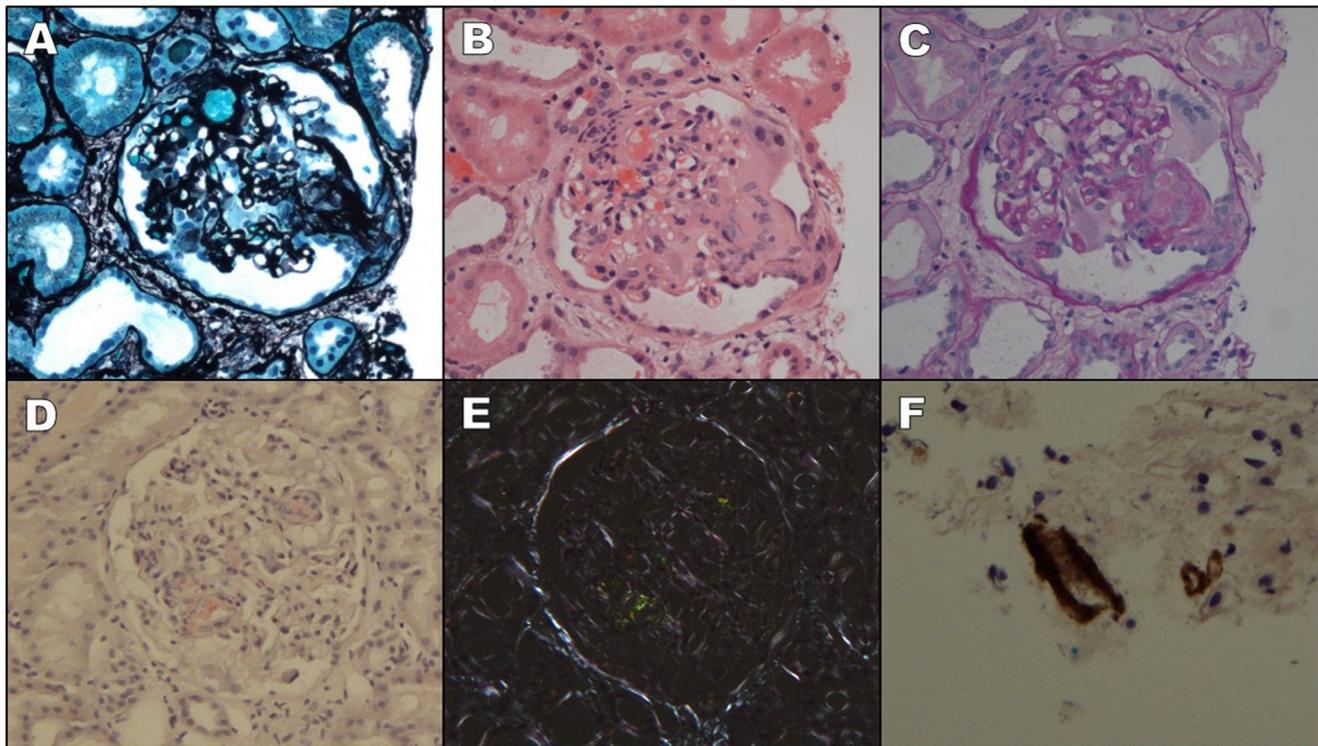


Figure 1. Kidney biopsy findings. (A) Silver methanamine stain–positive segmental sclerosis. (B) Hematoxylin-eosin stain confirming segmental sclerosis. (C) Periodic acid–Schiff stain–positive segmental sclerosis. (D) Congo red positivity in the mesangium. (E) Congo red staining viewed under cross-polarized light confirmed presence of minor glomerular amyloid deposition. (F) Positive immunohistochemical staining of amyloid with anti-TTR antibody confirming amyloid of ATTR type within a glomerular capillary loop. For all panels: original magnification, $\times 400$.

amyloidosis.⁶ There is a spectrum of nephropathy in patients with V50M-associated ATTRv amyloidosis¹; 1 Portuguese study suggested the presence of renal amyloidosis in one-third of affected individuals, with 10% progressing to kidney failure. Smaller Swedish studies have identified nephropathy in up to 50%.^{1,4} At least 14 other amyloidogenic TTR variants are associated with amyloid nephropathy, but these are extremely rare.¹ A study of 32 patients suggested a role for liver transplantation in halting the progression of amyloidotic kidney dysfunction in ATTRv amyloidosis,⁸ and it is conceivable that the novel TTR-lowering agents such as inotersen and patisiran might also prevent ongoing amyloid accumulation in the kidneys and prevent kidney dysfunction. This case indicates that agents such as patisiran or vutrisiran (another small interfering RNA–based drug that was recently approved), which are not associated with nephrotoxicity, may be preferred in patients with renal ATTRv amyloidosis. The second-generation ASO eplontersen is currently undergoing a phase 3 clinical trial for ATTRv amyloidosis and has promising efficacy and safety profiles reported on interim analysis. It is notable that kidney dysfunction is common in patients with wild-type ATTR as opposed to ATTRv amyloidosis (ie, not due to a TTR variant), although this appears to

be owing to type V cardiorenal syndrome rather than renal amyloidosis and is typically nonproteinuric.

The patient presented here had minimal proteinuria prior to commencing inotersen but did have evidence of renal ATTR amyloid deposits on SAP scintigraphy. Subsequent kidney biopsy identified only small amounts of mesangial amyloid deposition, and withdrawal of inotersen alone (without immunosuppressive therapy) led to resolution of nephrotic syndrome and a fall in proteinuria back to baseline levels within a few months, suggesting a causal relationship between inotersen and the patient's presentation. The 3 reported glomerulonephritides reported in the NEURO-TTR trial were all associated with glomerular crescents on histology. Each patient received more than 3 months of inotersen treatment before presentation. One patient had renal recovery with glucocorticoids, cyclophosphamide, and inotersen discontinuation; 1 did not receive immunosuppressive therapy and commenced maintenance hemodialysis; and the third had resolution of proteinuria with glucocorticoids alongside cessation of inotersen.⁶ The mechanism underlying the glomerulonephritis with inotersen remains unknown. The disproportionate number of renal adverse events in patients carrying the V50M TTR variant—which is known to be associated with renal amyloid deposition, as opposed to

most of the other ~130 pathogenic TTR variants that do not cause renal amyloid deposition—raises the possibility of a contribution to the renal pathology from pre-existing amyloid deposits. In addition, our case and 2 of the cases reported in the NEURO-TTR trial showed evidence of C3 and IgG deposition, raising the possibility of an immune-mediated process. The patient without IgG or C3 deposition had no pre-existing amyloid deposition but did have detectable antidrug antibodies. It is noteworthy that inotersen clearance occurs renally and preclinical models of ASOs have shown tubular injury to be associated with antidrug antibodies. Thrombocytopenia, often associated with antiplatelet antibodies, is also associated with inotersen treatment and, together with the clinical response to glucocorticoids in the 2 trial patients who developed glomerulonephritis, further supports an immune contribution to kidney injury.

ASO therapies are approved for many other indications, including cytomegalovirus retinitis, Duchenne muscular dystrophy, and familial hypercholesterolemia. Clinical trials of newer agents are ongoing for indications as broad as type 2 diabetes mellitus and Alzheimer disease, as well as kidney diseases such as Alport syndrome, autosomal dominant polycystic kidney disease, and delayed graft function.⁹ Thus far, glomerulonephritis has not been associated with other approved ASO therapies, although it is reported in animal models.¹⁰ Low-level proteinuria and kidney dysfunction have been demonstrated in preclinical and clinical trials but rarely result in cessation of therapy and vary between agents.¹¹⁻¹⁵

Treatment of ATTRv amyloidosis has been revolutionized in recent years by development of TTR stabilizers such as tafamidis, RNA-targeted therapies such as patisiran and inotersen (along with their second-generation counterparts vutrisiran and eplontersen, respectively), and novel in vivo gene editing therapeutics.¹⁶ Clinical trials of these agents in patients with ATTR amyloid cardiomyopathy are underway and, to our knowledge, there have been no reports of glomerulonephritis thus far. Increasing recognition of ATTR amyloid cardiomyopathy is likely to herald far more widespread use of these agents in the near future.¹⁷⁻²⁰

In summary, we report a case of FSGS complicating inotersen therapy for ATTRv amyloidosis that may have been contributed to by pre-existing renal ATTR amyloid deposits and that recovered fully after withdrawal of inotersen. Risk of inotersen-induced nephrotoxicity should be borne in mind when considering TTR-lowering therapy for ATTR amyloidosis.

Article Information

Authors' Full Names and Academic Degrees: Steven Law, MBBS, Julia Arnold, MBBS, Muhammad U. Rauf, MBBS, Lauren Heptinstall, FRCPath, Janet Gilbertson, MSc, Dorota Rowczenio, PhD, Jyoti Baharani, MB ChB, Gerald Langman, FRCPath, Marianna Fontana, PhD, and Julian D. Gillmore PhD.

Authors' Affiliations: National Amyloidosis Centre, Division of Medicine, University College London, United Kingdom (SL, MUR,

JG, DR, MF, JDG); Department of Renal Medicine, Heartlands Hospital, University Hospitals Birmingham Foundation Trust, Birmingham, United Kingdom (JA, JB); Department of Pathology, Royal Free Hospital, London, United Kingdom (LH); and Department of Cellular Pathology, Heartlands Hospital, University Hospitals Birmingham Foundation Trust, Birmingham, United Kingdom (GL).

Address for Correspondence: Steven Law, MBBS, Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, University College London, Rowland Hill Street, London, England NW3 2PF. Email: stevenlaw@nhs.net

Support: None.

Financial Disclosure: Dr Gillmore reports consultancy fees from Anlylam, Ionis, Eidos, and Intellia. The remaining authors declare that they have no relevant financial interests.

Patient Protections: The authors declare that they have obtained written consent from the patient reported in this article for publication of the information about her that appears within this Case Report.

Peer Review: Received April 1, 2022. Evaluated by 2 external peer reviewers, with direct editorial input from the Pathology Editor, an Associate Editor, and a Deputy Editor. Accepted in revised form August 7, 2022.

References

- Lobato L, Rocha A. Transthyretin amyloidosis and the kidney. *Clin J Am Soc Nephrol*. 2012;7(8):1337-1346. doi:10.2215/CJN.08720811
- Steen L, Wahlin A, Bjerle P, Holm S. Renal function in familial amyloidosis with polyneuropathy. *Acta Med Scand*. 1982;212(4):233-236. doi:10.1111/j.0954-6820.1982.tb03205.x
- Andersson R. Familial amyloidosis with polyneuropathy. A clinical study based on patients living in northern Sweden. *Acta Med Scand Suppl*. 1976;590:1-64.
- Lobato L. Portuguese-type amyloidosis (transthyretin amyloidosis, ATTR V30M). *J Nephrol*. 2003;16(3):438-442.
- Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med*. 2018;379(1):11-21. doi:10.1056/NEJMoa1716153
- Benson MD, Waddington-Cruz M, Berk JL, et al. Inotersen treatment for patients with hereditary transthyretin amyloidosis. *N Engl J Med*. 2018;379(1):22-31. doi:10.1056/NEJMoa1716793
- Dasgupta NR, Benson MD. Treatment of ATTR cardiomyopathy with a TTR specific antisense oligonucleotide, inotersen. *Amyloid*. 2019;26(sup1):20-21. doi:10.1080/13506129.2019.1582490
- Nowak G, Suhr OB, Wikström L, Wilczek H, Ericzon BG. The long-term impact of liver transplantation on kidney function in familial amyloidotic polyneuropathy patients. *Transpl Int*. 2005;18(1):111-115. doi:10.1111/j.1432-2277.2004.00015.x
- Roberts TC, Langer R, Wood MJA. Advances in oligonucleotide drug delivery. *Nat Rev Drug Discov*. 2020;19(10):673-694. doi:10.1038/s41573-020-0075-7
- Frazier KS, Obert LA. Drug-induced glomerulonephritis: the spectre of biotherapeutic and antisense oligonucleotide immune activation in the kidney. *Toxicol Pathol*. 2018;46(8):904-917. doi:10.1177/0192623318789399
- Janssen MJ, Nieskens TTG, Steevens TAM, et al. Therapy with 2'-O-Me phosphorothioate antisense oligonucleotides causes reversible proteinuria by inhibiting renal protein reabsorption. *Mol Ther Nucleic Acids*. 2019;18:298-307. doi:10.1016/j.omtn.2019.08.025

12. Van Poelgeest EP, Swart RM, Betjes MG, et al. Acute kidney injury during therapy with an antisense oligonucleotide directed against PCSK9. *Am J Kidney Dis*. 2013;62(4):796-800. doi:10.1053/j.ajkd.2013.02.359
13. Balwani M, Sardh E, Ventura P, et al. Phase 3 trial of RNAi therapeutic givosiran for acute intermittent porphyria. *N Engl J Med*. 2020;382(24):2289-2301. doi:10.1056/NEJMoa1913147
14. Mendell JR, Goemans N, Lowes LP, et al. Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. *Ann Neurol*. 2016;79(2):257-271. doi:10.1002/ana.24555
15. Goemans NM, Tulinius M, van den Akker JT, et al. Systemic administration of PRO051 in Duchenne's muscular dystrophy. *N Engl J Med*. 2011;364(16):1513-1522. doi:10.1056/NEJMoa1011367
16. Gillmore JD, Gane E, Taubel J, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. *N Engl J Med*. 2021;385(6):493-502. doi:10.1056/NEJMoa2107454
17. Ravichandran S, Lachmann HJ, Wechalekar AD. Epidemiologic and survival trends in amyloidosis, 1987–2019. *N Engl J Med*. 2020;382(16):1567-1568. doi:10.1056/NEJMc1917321
18. Gillmore JD, Maurer MS, Falk RH, et al. Nonbiopsy diagnosis of cardiac transthyretin amyloidosis. *Circulation*. 2016;133(24):2404-2412. doi:10.1161/CIRCULATIONAHA.116.021612
19. Rapezzi C, Guidalotti P, Salvi F, Riva L, Perugini E. Usefulness of 99mTc-DPD scintigraphy in cardiac amyloidosis. *J Am Coll Cardiol*. 2008;51(15):1509-1510; author reply 1510. doi:10.1016/j.jacc.2007.12.038
20. Maceira AM, Joshi J, Prasad SK, et al. Cardiovascular magnetic resonance in cardiac amyloidosis. *Circulation*. 2005;111(2):186-193. doi:10.1161/01.CIR.0000152819.97857.9D