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

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Abstract

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 end stage renal disease. We describe 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 (p.V50M) transthyretin (TTR) variant, who presented with nephrotic syndrome 7 months after commencement of inotersen. Renal histology demonstrated focal segmental glomerulosclerosis 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 III NEURO-TTR clinical trial, 3% of patients in the treatment arm developed a crescentic glomerulonephritis. All affected patients carried the (p.V50M) TTR variant which is known to be associated with renal amyloid deposition. This case adds to spectrum of renal disease associated with inotersen and indicates that discontinuation of the drug alone may result in resolution of renal complications without additional immunosuppression. Monitoring of renal function is essential in patients with ATTRv receiving inotersen, particularly if there is evidence of existing renal amyloid.

Key words

Amyloidosis, transthyretin, glomerulonephritis, inotersen, antisense oligonucleotide inhibitor

Introduction

Hereditary transthyretin amyloidosis (ATTRv) is a rare form of systemic amyloidosis caused by more than 130 pathogenic transthyretin (*TTR*) mutations which result in production of abnormal 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, especially in patients with the (p.V50M) variant (c.148G>A) who may range from having no nephropathy to proteinuria and/or end stage renal disease.[1-4] Advances in RNA targeted therapies have revolutionised the treatment of ATTRv although the impact on renal 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 III 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 the first time, the development of nephrotic syndrome associated with focal segmental glomerulosclerosis on renal histology 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 two week history of shortness of breath, facial swelling and leg oedema. Eight months previously she had been diagnosed with ATTRv

amyloidosis with dominant neuropathic involvement after presenting with a two 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 carried the known pathogenic (p.V50M) TTR variant. Radiolabelled ^{123}I-SAP scintigraphy at the time of diagnosis of amyloidosis revealed amyloid in the kidneys; however, there was no evidence of cardiac amyloidosis on ^{99m}Technetium labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) scintigraphy or echocardiography. Biochemical investigations at the same time were as follows; serum creatinine 53umol/L, estimated glomerular filtration rate >90mls/min, urinary protein creatinine ratio 73mg/mmol and albumin creatinine ratio 44mg/mmol. There was no prior history of renal disease in the patient or any of her affected relatives. Medications included amitriptyline 10mg once daily for neuropathic pain, inotersen 284mg weekly by subcutaneous injection commenced 7 months previously, and vitamin A supplementation.

On examination the blood pressure was 118/78mmHg, heart rate 90 beats per minute, oxygen saturation 95%, respiratory rate 16 breaths per minute and temperature 37.1 degrees centigrade. Full systems examination highlighted periorbital and peripheral oedema to the mid shins.

Urinary dipstick demonstrated protein 3+, blood 3+, leucocytes 2+, nitrites negative and a pH of 5.0; there were no cellular casts or dysmorphic red cells on urinary sediment analysis. Further investigations are summarized in Table 1 supporting a diagnosis of nephrotic syndrome. Native renal biopsy was performed and demonstrated 14 glomeruli; one glomerulus was globally sclerosed; three demonstrated segmental sclerosis with collapse of the glomerular tuft and capsular adhesion, while the remaining glomeruli demonstrated variable changes with mesangial

expansion (Figure 1A, 1B, 1C). Podoctyes 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 oedema was present. Immunofluorescence showed focal staining for immunoglobulin G and complement factor 3 in areas of amyloid deposition, and non-specific 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, 1E, 1F). Laser microdissection of the amyloid and tandem mass spectrometry confirmed it to be of ATTR type. Electron microscopy demonstrated thin glomerular basement membranes (177-245nm); it did not capture a segmental lesion or amyloid fibrils, and there was no podocyte foot process effacement.

Supportive treatment with 80mg 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 40g/L and urinary protein creatinine ratio fell to 88mg/mmol. Furosemide was discontinued. A further month later, urinary protein creatinine ratio was 35mg/mmol. Following a break from therapy, during which there was gradual progression of neuropathy, patisiran was commenced.

Discussion

To our knowledge, this is the first case report of focal segmental glomerulosclerosis 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 phase III clinical trial of inotersen, three patients (3%) in the treatment arm developed glomerulonephritis

thought to be due to the study drug. All three patients carried the (p.V50M) TTR variant which is known to be associated with renal amyloidosis.[6] There is a spectrum of nephropathy in patients with (p.V50M)-associated ATTRv amyloidosis;[1] one Portuguese study suggesting presence of renal amyloidosis in one third of affected individuals, with 10% progressing to end stage renal disease. Smaller Swedish studies have identified nephropathy in up to 50%.[1,4] At least fourteen 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 renal 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 the renal decline. This case indicates that agents such as patisiran, or the recently approved vutrisiran, which are not associated with nephrotoxicity, may be preferred in patients with renal ATTRv amyloidosis. A second generation ASO, eplontersen is currently undergoing a phase III clinical trial for ATTRv amyloidosis with promising efficacy and safety profiles reported on interim analysis. It is notable that renal dysfunction is common in patients wild-type ATTR amyloidosis, although this appears to be due to Type V cardiorenal syndrome rather than renal amyloidosis and is typically non-proteinuric.

The patient presented here had minimal proteinuria prior to commencing inotersen but did have evidence of renal ATTR amyloid deposits on SAP scintigraphy. Subsequent renal histology 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 our patient's presentation. The three reported

glomerulonephritides reported in the NEURO-TTR trial were all associated with glomerular crescents on histology. Each patient received over three months of inotersen treatment prior to presentation. One patient had renal recovery with glucocorticoids, cyclophosphamide and inotersen discontinuation, one did not receive immunosuppressive therapy and commenced permanent haemodialysis, 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 (p.V50M) TTR variant which is known to be associated with renal amyloid deposition, as opposed to most of the other ~130 pathogenic TTR variants which do not cause renal amyloid, raises the possibility of a contribution to the renal pathology from pre-existing amyloid deposits. In addition, our case and two of the cases reported in NEURO-TTR 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 anti-drug antibodies. It is noteworthy that inotersen clearance occurs renally and preclinical models of ASOs have shown tubular injury to be associated with anti-drug antibodies. Thrombocytopenia, often associated with antiplatelet antibodies, is also associated with inotersen treatment and together with the clinical response to glucocorticoids in the two trial patients who developed glomerulonephritis, further supports an immune contribution to renal injury.

Antisense oligonucleotide therapies are approved for many other indications including cytomegalovirus retinitis, Duchenne muscular dystrophy and familial hypercholesterolaemia. Clinical trials of newer agents are ongoing for indications as broad as type 2 diabetes mellitus and Alzheimer's disease, as well as renal diseases such as Alport's syndrome, autosomal dominant polycystic kidney disease and delayed graft function.[9] Thus far, glomerulonephritis has

not been associated with other approved ASO therapies; although is reported in animal models.[10] Low level proteinuria and kidney dysfunction have been demonstrated in pre-clinical and clinical trials although rarely result in cessation of therapy, and vary between agents.[11-15]

Treatment of ATTRv amyloidosis has been revolutionised in recent years by development of TTR stabilisers such as tafamidis, RNA targeted therapies such as patisran 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 focal segmental glomerulosclerosis complicating inotersen therapy for ATTRv amyloidosis which may have been contributed to by pre-existing renal ATTR amyloid deposits and recovered fully after withdrawal of inotersen. Risk of inotersen-induced nephrotoxicity should be borne in mind when considering TTR-lowering therapy for ATTR amyloidosis.

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Figure Legend

Figure 1. Renal histology under 400x magnification: A) Silver-methanamine stain positive segmental sclerosis; B) Haematoxylin and 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 polarised 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.

Table 1: Further investigations.

Investigation	**Results**		
	Amyloid diagnosis	**Nephrotic presentation**	**Follow up**
Haemoglobin (g/dL)	132	122	119
White cell count (x10^9/L)	10.4	5.3	7.4
Eosinophils (x10^9/L)	0.08	0.07	0.03
Platelets (x10^9/L)	300	237	329
Sodium (mmol/L)	142	138	139
Potassium (mmol/L)	3.6	4.1	4.3
Creatinine (micromol/L)	53	101	77
eGFR (ml/min/1.73^3)	>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) (umol/L)	8	5	5
Adjusted calcium (mmol/L)		2.33	
Immunoglobulins (g/L) IgA IgG IgM	 1.5 7.6 3.1	 1.38 5.29 3.98	 1.7 8.9 4.1

Serum electrophoresis and immunofixation		Not detected	
Hepatitis C RNA, hepatitis B core-antibody and surface antigen, human immunodeficiency virus antibodies		All negative	
ANA, ANCA, anti-GBM antibodies, dsDNA and rheumatoid factor		All negative	
Complement factor C3 (g/L)		0.85	
Complement factor C4 (g/L)		0.20	
Urine PCR (mg/mmol)	73	217	88
Ultrasound kidneys		10.9cm and 10.6 cm unobstructed kidneys with normal corticomedullary differentiation	

Results are reported from the time of amyloidosis diagnosis, nephrotic syndrome presentation and a follow up evaluation occurring two months after inotersen cessation. eGFR: estimated glomerular filtration rate as calculated by the modification of diet in renal disease formula; RNA: ribonucleic acid; ANA: antinuclear antibodies; ANCA: anti cytoplasmic neutrophil antibodies; GGB: glomerular basement membrane; dsDNA: double stranded deoxyribonucleic acid; PCR: protein creatinine ratio.