CASE IN POINT

Anti-Tumor Necrosis Factor Treatment for Glomerulopathy: Case Report and Review of Literature

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Background: Glomerulopathy involves damage to the glomerular filtration barrier for several reasons, resulting in idiopathic nephrotic syndrome (NS). Treatment options are limited and often include steroids with varying levels of response.
Case Presentation: A 7-year-old male with a history of NS at age 2 years that developed following a respiratory tract infection was found to have a heterozygous variant of uncertain significance in *COL4A4* and *TRPC6* genes. Biopsy findings included podocytopathy and changes in the basement membrane. Upon initial response to steroids, the patient was treated with a brief course of anakinra followed by adalimumab for > 2 years as steroid-sparing biological response modifiers. After a gradual taper, the patient remains in remission and has not received treatment in the last 12 months.
Conclusions: This case shows the complex nature of biologically predetermined cascading events in the emergence of glomerular disease with environmental triggers and genetic factors. Downregulation of somatic tissue-driven proinflammatory milieu originating from the constituents of the glomerular microenvironment can help in recovery from emerging podocytopathy. Blocking tumor necrosis factor-α early in the disease course, even temporarily, may allow time for the de novo regenerative process to prevail. Additional research is warranted to test this hypothesis and minimize steroid use.

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Podocytes are terminally differentiated, highly specialized cells located in juxtaposition to the basement membrane over the abluminal surfaces of endothelial cells within the glomerular tuft. This triad structure is the site of the filtration barrier, which forms highly delicate and tightly regulated architecture to carry out the ultrafiltration function of the kidney.[1] The filtration barrier is characterized by foot processes that are connected by specialized junctions called slit diaphragms.

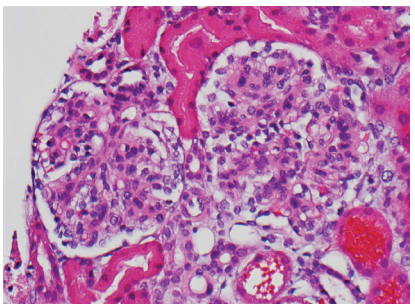
Insults to components of the filtration barrier can initiate cascading events and perpetuate structural alterations that may eventually result in sclerotic changes.[2] Common causes among children include minimal change disease (MCD) with the collapse of foot processes resulting in proteinuria, Alport syndrome due to mutation of collagen fibers within the basement membrane leading to hematuria and proteinuria, immune complex mediated nephropathy following common infections or autoimmune diseases, and focal segmental glomerulosclerosis (FSGS) that can show variable histopathology toward eventual glomerular scarring.[3,4] These children often clinically have minimal, if any, signs of systemic inflammation.[3-5] This has been a limiting factor for the commitment to immunomodulatory treatment, except for steroids for the treatment of MCD.[6] Although prolonged steroid treatment may be efficacious, adverse effects are significant in a growing child. Alternative treatments, such as tacrolimus and rituximab have been suggested as second-line steroid-sparing agents.[7,8] Not uncommonly, however, these cases are managed by supportive measures only during the progression of the natural course of the disease, which may eventually lead to renal failure, requiring transplant for survival.[8,9]

This case report highlights a child with a variant of uncertain significance (VUS) in genes involved in Alport syndrome and FSGS who developed an abrupt onset of proteinuria and hematuria after a respiratory illness. To our knowledge, he represents the youngest case demonstrating the benefit of targeted treatment against tumor necrosis factor-α (TNF-α) for glomerulopathy using biologic response modifiers.

CASE DESCRIPTION

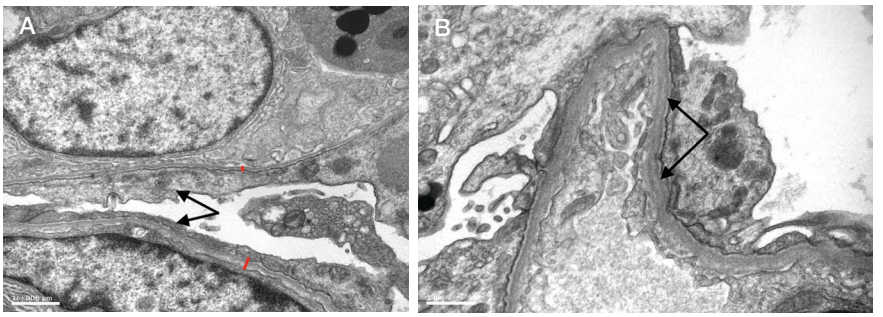
This is currently a 7-year-old male patient who was born at 39 weeks gestation to gravida 3 para 3 following induced labor due to elevated maternal blood pressure. During the first 2 years of life, his growth and development were normal and his immunizations were up to date. The patient's medical history included upper respiratory

FIGURE 1 Hematoxylin and Eosin Stain 40x



Glomeruli with mild mesangial matrix expansion and hypercellularity; no endocapillary hypercellularity or crescent formation can be seen.

FIGURE 2 Electron Microsscopy



A, Superior red bar indicates extreme thinning of the peripheral capillary basement membranes compared with the relatively normal thickness as seen with the inferior red bar), black arrows indicate effacement of foot processes in these areas; B, Black arrows indicate splitting of the lamina densa into multiple, discrete, electrondense layers within the glomerular capillary basement membrane.

tract infections (URIs), respiratory syncytial virus, as well as 3 bouts of pneumonia and multiple otitis media that resulted in 18 rounds of antibiotics. The child was also allergic to nuts and milk protein. The patient's parents are of Northern European and Native American descent. There is no known family history of eye, ear, or kidney diseases.

Renal concerns were first noted at the age of 2 years and 6 months when he presented to an emergency department in Fall 2019 (week 0) for several weeks of intermittent dark-colored urine. His mother reported that the discoloration recently progressed in intensity to cola-colored, along with the onset of persistent vomiting without any fever or diarrhea. On physical examination, the patient had normal vitals: weight 14.8 kg (68th percentile), height 91 cm (24th percentile), and body surface area 0.6 m^2. There was no edema, rash, or lymphadenopathy, but he appeared pale.

The patient's initial laboratory results included: complete blood count with white blood cells (WBC) 10 x 10^3/L (reference range, 4.5-13.5 x 10^3/L); differential lymphocytes 69%; neutrophils 21%; hemoglobin 10 g/dL (reference range, 12-16 g/dL); hematocrit, 30%; (reference range, 37%-45%); platelets 437 10^3/L (reference range, 150-450 x 10^3/L); serum creatinine 0.46 mg/dL (reference range, 0.5-0.9 mg/dL); and albumin 3.1 g/dL (reference range, 3.5-5.2 g/dL). Serum electrolyte levels and liver enzymes were normal. A urine analysis revealed 3+ protein and 3+ blood with dysmorphic red blood cells (RBC) and RBC casts without WBC. The patient's spot urine protein-to-creatinine ratio was 4.3 and his renal ultrasound was normal. The patient was referred to Nephrology.

During the next 2 weeks, his protein-to-creatinine ratio progressed to 5.9 and serum albumin fell to 2.7 g/dL. His urine remained red colored, and a microscopic examination with RBC > 500 and WBC up to 10 on a high powered field. His workup was negative for antinuclear antibodies, antineutrophil cytoplasmic antibody, antistreptolysin-O (ASO) and anti-DNase B. Serum C3 was low at 81 mg/dL (reference range, 90-180 mg/dL), C4 was 13.3 mg/dL (reference range, 10-40 mg/dL), and immunoglobulin G was low at 452 mg/dL (reference range 719-1475 mg/dL). A baseline audiology test revealed normal hearing.

Percutaneous renal biopsy yielded about 12 glomeruli, all exhibiting mild mesangial matrix expansion and hypercellularity (Figure 1). One glomerulus had prominent parietal epithelial cells without endocapillary hypercellularity or crescent formation. There was no interstitial fibrosis or tubular atrophy. Immunofluorescence studies showed no evidence of immune complex deposition with negative staining for immunoglobulin heavy and light chains, C3 and C1q. Staining for α 2 and α 5 units of collagen was normal. Electron microscopy showed patchy areas of severe basement membrane thinning with frequent foci of mild to moderate lamina densa splitting and associated visceral epithelial cell foot process effacement (Figure 2).

These were reported as concerning findings for possible Alport syndrome by 3 independent pathology teams. The genetic testing was submitted at a commercial

laboratory to screen 17 mutations, including *COL4A3*, *COL4A4*, and *COL4A5*. Results showed the presence of a heterozygous VUS in the *COL4A4* gene (c.1055C > T; p.Pro352Leu; dbSNP ID: rs371717486; PolyPhen-2: Probably Damaging; SIFT: Deleterious) as well as the presence of a heterozygous VUS in *TRPC6* gene (c2463A>T; p.Lys821Asn; dbSNP ID: rs199948731; PolyPhen-2: Benign; SIFT: Tolerated). Further genetic investigation by whole exome sequencing on approximately 20,000 genes through MNG Laboratories showed a new heterozygous VUS in the *OSGEP* gene [c.328T>C; p.Cys110Arg]. Additional studies ruled out mitochondrial disease, CoQ10 deficiency, and metabolic disorders upon normal findings for mitochondrial DNA, urine amino acids, plasma acylcarnitine profile, orotic acid, ammonia, and homocysteine levels.

Figure 3 summarizes the patient's treatment response during 170 weeks of follow-up (Fall 2019 to Summer 2023). The patient was started on enalapril 0.6 mg/kg daily at week 3, which continued throughout treatment. Following a rheumatology consult at week 30, the patient was started on prednisolone 3 mg/mL to assess the role of inflammation through the treatment response. An initial dose of 2 mg/kg daily (9 mL) for 1 month was followed by every other day treatment that was tapered off by week 48. To control mild but noticeably increasing proteinuria in the interim, subcutaneous anakinra 50 mg (3 mg/kg daily) was added as a steroid-sparing agent at week 39 and increased to 100 mg daily by week 41. His urine protein to creatinine ratio decreased from 1.720 to 0.575, and serum albumin normalized by week 53. At that time, due to the patient's up-trending proteinuria after a URI, as well as concerns for injection site skin reaction and quality of life on daily subcutaneous treatment, anakinra was substituted with subcutaneous adalimumab 20 mg every 2 weeks.

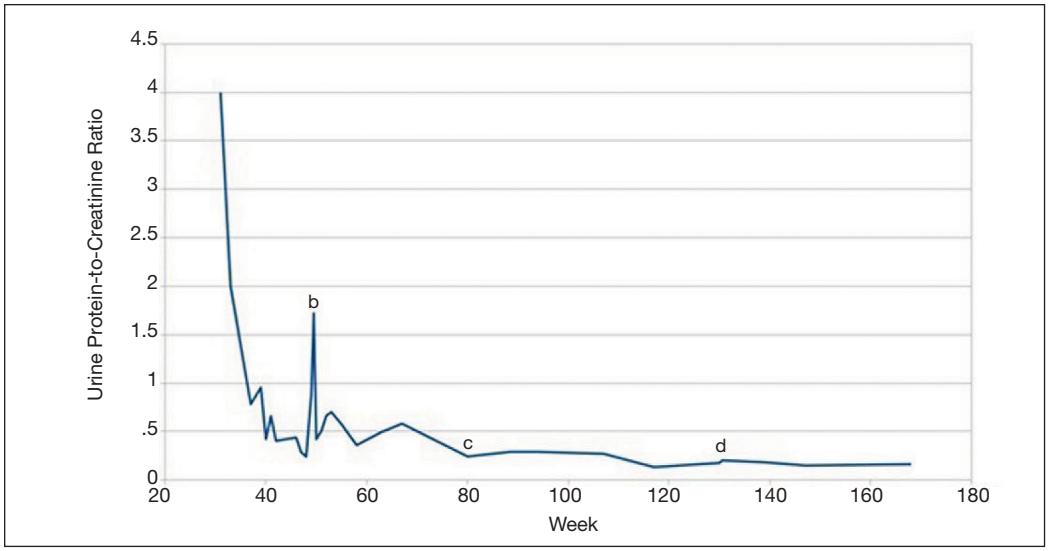
By week 80, the patient's urine protein to creatinine ratio normalized (< 0.2). This was followed by normalized urine microalbumin to creatinine ratio, and by week 130 his microscopic hematuria resolved. While on adalimumab, he remained well and was able to mount an immune response to viral infections uneventfully, including COVID-19. He tolerated a gradual wean of adalimumab to every 3 weeks by week 139 and discontinuation at week 151. At week 204, the patient has normal renal function and urine findings; his growth parameters are at 20.3 percentile for weight and 15.3 percentile for height.

DISCUSSION

This case describes a child with rapidly progressive proteinuria and hematuria following a URI who was found to have VUS mutations in 3 different genes associated with chronic kidney disease. Serology tests on the patient were negative for streptococcal antibodies and antinuclear antibodies, ruling out poststreptococcal glomerulonephritis, or systemic lupus erythematosus. His renal biopsy findings were concerning for altered podocytes, mesangial cells, and basement membrane without inflammatory infiltrate, immune complex, complements, immunoglobulin A, or vasculopathy. His blood inflammatory markers, erythrocyte sedimentation rate, C-reactive protein, and ferritin were normal when his care team initiated daily steroids.

Overall, the patient's clinical presentation and histopathology findings were suggestive of Alport syndrome or thin basement membrane nephropathy with a high potential to progress into FSGS.[10-12] Alport syndrome affects 1 in 5000 to 10,000 children annually due to S-linked inheritance of *COL4A5*, or autosomal recessive inheritance of *COL4A3* or *COL4A4* genes. It presents with hematuria and hearing loss.[10] Our patient had a single copy *COL4A4* gene mutation that was classified as VUS. He also had 2 additional VUS affecting the *TRPC6* and *OSGEP* genes. *TRPC6* gene mutation can be associated with FSGS through autosomal dominant inheritance. Both *COL4A4* and *TRPC6* gene mutations were paternally inherited. Although the patient's father not having renal disease argues against the clinical significance of these findings, there is literature on the potential role of heterozygous *COL4A4* variant mimicking thin basement membrane nephropathy that can lead to renal impairment

FIGURE 3 Urine Spot Protein-to-Creatinine Ratio[a]



[a]Patient treatments included enalapril from week 3; steroids from week 31 (ratio 4.0) to week 48; anakinra from week 39 (protein-to-creatinine ratio, 0.95) to week 53 (protein-to-creatinine ratio, 0.7); adalimumab week 53 to week 151.
[b]A viral infection at week 50 led to transient increase in proteinuria.
[c]Protein-to-creatinine ratio normalized and remained < 0.3 since week 80 without flares despite respiratory infections.
[d]Microscopic hematuria resolved at week 130.

upon copresence of superimposed conditions.[13] The patient's rapidly progressing hematuria and changes in the basement membrane were worrisome for emerging FSGS. Furthermore, VUS of *TRPC6* has been reported in late onset autosomal dominant FSGS and can be associated with early onset steroid-resistant nephrotic syndrome (NS) in children.[14] This concern was voiced by 3 nephrology consultants during the initial evaluation, leading to the consensus that steroid treatment for podocytopathy would not alter the patient's long-term outcomes (ie, progression to FSGS).

Immunomodulation

Our rationale for immunomodulatory treatment was based on the abrupt onset of renal concerns following a URI, suggesting the importance of an inflammatory trigger causing altered homeostasis in a genetically susceptible host. Preclinical models show that microbial products such as lipopolysaccharides can lead to podocytopathy by several mechanisms through activation of toll-like receptor signaling. It can directly cause apoptosis by downregulation of the intracellular Akt survival pathway.[15] Lipopolysaccharide can also activate the NF-αB pathway and upregulate the production of interleukin-1 (IL-1) and TNF-α in mesangial cells.[16,17]

Both cytokines can promote mesangial cell proliferation.[18] Through autocrine and paracrine mechanisms, proinflammatory cytokines can further perpetuate somatic tissue changes and contribute to the development of podocytopathy. For instance, TNF-α can promote podocyte injury and proteinuria by downregulation of the slit diaphragm protein expression (ie, nephrin, ezrin, or podocin), and disruption of podocyte cytoskeleton.[19,20] TNF-α promotes the influx and activation of macrophages and inflammatory cells. It is actively involved in chronic alterations within the glomeruli by the upregulation of matrix metalloproteases by integrins, as well as activation of myofibroblast progenitors and extracellular matrix deposition in crosstalk with transforming growth factor and other key mediators.[17,21,22]

For the patient described in this case report, initial improvement on steroids encouraged the pursuit of additional treatment to downregulate inflammatory pathways within the glomerular milieu. However, within the COVID-19 environment, escalating the patient's treatment

using traditional immunomodulators (ie, calcineurin inhibitors or mycophenolate mofetil) was not favored due to the risk of infection. Initially, anakinra, a recombinant IL-1 receptor antagonist, was preferred as a steroid-sparing agent for its short life and safety profile during the pandemic. At first, the patient responded well to anakinra and was allowed a steroid wean when the dose was titrated up to 6 mg/kg daily. However, anakinra did not prevent the escalation of proteinuria following a URI. After the treatment was changed to adalimumab, a fully humanized monoclonal antibody to TNF-α, the patient continued to improve and reach full remission despite experiencing a cold and the flu in the following months.

Literature Review

There is a paucity of literature on applications of biological response modifiers for idiopathic NS and FSGS.[23,24] Angeletti and colleagues reported that 3 patients with severe long-standing FSGS benefited from anakinra 4 mg/kg daily to reduce proteinuria and improve kidney function. All the patients had positive C3 staining in renal biopsy and treatment response, which supported the role of C3a in inducing podocyte injury through upregulated expression of IL-1 and IL-1R.[23] Trachtman and colleagues reported on the phase II FONT trial that included 14 of 21 patients aged < 18 years with advanced FSGS who were treated with adalimumab 24 mg/m^2, or ≤ 40 mg every other week.[24] Although, during a 6-month period, none of the 7 patients met the endpoint of reduced proteinuria by ≥ 50%, and the authors suggested that careful patient selection may improve the treatment response in future trials.[24]

A recent study involving transcriptomics on renal tissue samples combined with available pathology (fibrosis), urinary markers, and clinical characteristics on 285 patients with MCD or FSGS from 3 different continents identified 3 distinct clusters. Patients with evidence of activated kidney TNF pathway (n = 72, aged > 18 years) were found to have poor clinical outcomes.[25] The study identified 2 urine markers associated with the TNF pathway (ie, tissue inhibitor of metalloproteinases-1 and monocyte chemoattractant protein-1), which aligns with the preclinical findings previously mentioned.[25]

CONCLUSIONS

The patient's condition in this case illustrates the complex nature of biologically predetermined cascading events in the emergence of glomerular disease upon environmental triggers under the influence of genetic factors. Observations on this child's treatment response suggest that downregulation of somatic tissue-driven proinflammatory milieu originating from the constituents of glomerular microenvironment can help in recovery from emerging podocytopathy. The prolonged time span and stepwise resolution of proteinuria, followed by microalbuminuria (data not shown), and finally microscopic hematuria, supports the delicate balance and presence of reciprocal feedback loops between the podocytes and mesangial cells. Within this framework, blocking TNF-α, even temporarily, may allow time for the de novo regenerative process to prevail.

Chronic kidney disease affects 7.7% of veterans annually, illustrating the need for new therapeutics.[26] Based on our experience and literature review, upregulation of TNF-α is a root cause of glomerulopathy; further studies are warranted to evaluate the efficacy of anti-TNF biologic response modifiers for the treatment of these patients. Long-term postmarketing safety profile and steroid-sparing properties of adalimumab should allow inclusion of pediatric cases in future trials. Results may also contribute to identifying new predictive biomarkers related to the basement membrane when combined with precision nephrology to further advance patient selection and targeted treatment.[25,27]

Acknowledgments

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Ethics and consent

This case report is compliant with the rules and regulations of the Health Insurance Portability and Accountability Act. The content of this report was reviewed and approved by the Walter Reed National Military Medical Center's Public Affairs Office and approved by its institutional review board (ED)-2020-0493). Verbal and written consent was provided by the parent of this child described in this case report.

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