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Glucocorticoids Induce Partial Remission of Focal Segmental Glomerulosclerosis but Not Interstitial Nephritis in COVID-19 Acute Kidney Injury in an APOL1 Low-Risk Genotype White Patient

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Patient: Male, 34-year-old
Final Diagnosis: Focal segmental glomerulosclerosis
Symptoms: Acute kidney injury • nephrotic syndrome
Medication: —
Clinical Procedure: Kidney biopsy
Specialty: Nephrology

Objective: Rare coexistence of disease or pathology

Background: COVID-19 can be complicated by kidney disease, including focal segmental glomerulosclerosis (FSGS), interstitial nephritis, and acute kidney injury (AKI). Almost all known cases of COVID-19-associated glomerulonephritis have been in patients of African descent, with G1 or G2 apolipoprotein L1 (APOL1) risk alleles, and they presented collapsing type of FSGS.

Case Report: We report a case of biopsy-confirmed non-collapsing FSGS with secondary acute interstitial nephritis and AKI in a young White man with APOL1 low-risk genotype, who had COVID-19 pneumonia. His past history included arterial hypertension, anabolic steroids, and high-protein diet. He fully recovered from type 1 respiratory failure and AKI after transfusion of COVID-19 convalescent plasma and intravenous treatment with dexamethasone administered for 16 days in a dose reduced from 16 to 2 mg/day. Due to progressing severe nephrotic proteinuria (22.6 g/24 h), intravenous methylprednisolone was administered (1500 mg divided in 3 pulses over 3 days) immediately followed by oral prednisone (0.6 mg/kg body weight), with dose reduced 19 weeks later and switched to cyclosporine A (4 mg/kg body weight). Kidney re-biopsy, at that time, showed a decrease in proportion of glomeruli affected with podocytopathy, but progression of interstitial lesions. After 23 weeks of therapy, partial remission of FSGS was attained and proteinuria dropped to 3.6 g/24 h. After 43 weeks, proteinuria decreased to 0.4 g/24 h and the serum creatinine concentration remained steady.

Conclusions: High-dose glucocorticoid therapy was effective in the initial treatment of COVID-19-related non-collapsing FSGS, but had no effect on interstitial changes. Introduction of cyclosporine A to the therapy contributed to remission of disease.

Keywords: Acute Kidney Injury • COVID-19 • Cyclosporine • Glucocorticoids • Nephrotic Syndrome • Segmental Glomerulosclerosis

Full-text PDF: https://www.amjcaserep.com/abstract/index/idArt/933462

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Background

It is recognized that the SARS-CoV-2 virus can cause many adverse effects in organs other than the lungs, but it has been debatable whether these effects are directly mediated by presence of the virus or are secondary to immune system activation [1,2]. Acute kidney injury (AKI) can serve as an example of this. The details of the pathomechanism of its development in the course of COVID-19 have not been fully elucidated yet [2]. Data on its incidence vary considerably in different studies. One of the first observations was made in Wuhan, China and showed that AKI was present in 8.4% of infected patients and 3.6% of them required renal replacement therapy (RRT) [3]. Much higher numbers were presented in a retrospective study performed on a large cohort of 3993 patients with confirmed SARS-CoV-2 infection admitted to Mount Sinai Hospital; AKI was present in 46% of the patients and 19% required dialysis [4]. Regardless of its incidence, AKI greatly contributes to death in patients with COVID-19 [5,6]. Apart from a possible prerenal origin of AKI, more than half of patients with COVID-19-related AKI who required kidney biopsy were reported to have severe proteinuria, whereas hematuria was present in about 25% of cases [7]. These renal manifestations suggest the presence of glomerular injury in COVID-19-related AKI. The presence of glomerular disease was indeed confirmed in a series of patients, and most had podocytopathies. Focal segmental glomerulosclerosis (FSGS) with glomerular capillary collapse was the most common [7,8]. This disease was strongly associated with Black race and presence of APOL1 high-risk genotypes [7-9]. G1 and G2 high-risk alleles encoding the apolipoprotein L1 involved in innate immunity were found most frequently among patients of African descent [9,10]. West African ancestry determines increased probability of having the APOL-1 high-risk genotype [10]. Several reports indicated, however, that the occurrence of COVID-19 related glomerulopathy is not limited to people of Black race. One person of Hispanic ethnicity was also revealed to have a high-risk G2/G2 APOL1 genotype and collapsing FSGS [7]. The other patient whose APOL1 status was undetermined presented with FSGS without capillary collapse but required dialysis despite having the more favorable non-collapsing FSGS variant [7]. There are only a few reports of COVID-19-related FSGS in patients not of African descent [7,11]. Collapsing glomerulopathy is mostly associated with but not limited to the APOL1 high-risk genotype [7,8,10]. There are other genetic causes or epigenetic risk factors associated with collapsing FSGS, such as mutations in Scavenger Receptor Class B Member 2 (SCARB2) gene or Decaprenyl Pyrophosphate Synthetase Subunit 2 (PDSS2) gene, which is more frequent among White Americans than African Americans, or a decreased content of coenzyme Q10 in lymphoblastoid cell lines [12,13], but the majority of known COVID-19-related FSGS cases were in African Americans and had relatively short follow-up [7,8]. Information on COVID-19-associated glomerulopathies among non-Black people is scarce, which may reflect a lower incidence of this complication of SARS-CoV-2 infection in non-Blacks but does not exclude its existence. A case of an Asian Indian man with COVID-19 who had collapsing FSGS was reported, but variants of his APOL1 genes were not investigated and the patient was lost to follow-up [11]. Another Asian Indian man with undetermined APOL1 gene variants was initially diagnosed with minimal change disease, but as his renal function deteriorated and he became dialysis-dependent, despite prednisone therapy, kidney re-biopsy revealed collapsing FSGS [14]. A White man with COVID-19 had diffused crescentic Henoch-Schonlein purpura-associated nephritis, but information about his treatment and outcome is unknown [8]. A White female was diagnosed with minimal change disease, but because of low probability of having the APOL1 high-risk genotype, she was not tested for it [7], and she recovered after treatment with prednisone [7].

As presence or absence of SARS-CoV-2 RNA copies in the kidney parenchymal cells of 27 autopsied deceased patients with COVID-19 did not directly correspond with presence or absence of renal symptoms [1], one may conclude that a pathomechanism other than a direct viral activity is involved in kidney injury development. An intense immune response to SARS-CoV-2 infection with IFN-γ secretion, macrophage activation syndrome, and cytokine storm is one of the proposed mechanisms that may affect podocytes and trigger FSGS [2,15]. Therefore, immunosuppression with glucocorticoids aiming at reduction of circulating proinflammatory cytokine levels [16] may help in restoring proper podocyte function if an inflammation-related pathomechanism dominates.

In this report we present a case of a young White male who was diagnosed with AKI, FSGS, and interstitial nephritis in the course of COVID-19, manifesting with nephrotic syndrome, despite having the APOL1 low-risk genotype. He responded to glucocorticoid therapy with resolution of AKI and reduction of nephrotic proteinuria. A follow-up biopsy conducted after 5 months showed less intense glomerular involvement but progression of interstitial fibrosis and tubular atrophy.

Case Report

A 34-year-old White man presented to the emergency department reporting symptoms of mild diarrhea, headache, intermittent pain in the right hypochondriac and both lumbar regions, fever up to 39°C, non-productive cough, dyspnea on exertion, fatigue, anosmia, and ageusia. The symptoms gradually aggravated during the previous week. Three days prior to presentation, his initial real-time reverse transcription polymerase chain reaction (rRT-PCR) test for SARS-CoV-2 in a nasopharyngeal swab sample was negative. After online

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consultation with his primary care physician, he was treated with amoxicillin/clavulanate, with no improvement, and at the same time was screened for organ dysfunctions. A urine strip test 2 days before the current presentation showed 3+ protein and a blood test showed elevated serum creatinine (440 µmol/L). His baseline serum creatinine, measured 1 year earlier during a routine check-up, was 113 µmol/L. On presentation, serum creatinine further rose to 520 µmol/L, urinalysis showed protein concentration of 10.2 g/L with renal tubular epithelial cells and single hyaline-granular and granular casts in urinary sediment. Similar abnormalities were found in the next-morning spot urine, which showed his albumin/creatinine ratio (ACR) was 1982 mg/g. On presentation, the patient had serious hypoalbuminemia and mild hyponatremia. Serum potassium was in the reference range, and he was not anemic. Serum C-reactive protein (CRP) was highly elevated and aspartate transaminase (AST) was slightly increased. Detailed laboratory results are given in **Table 1** (blood tests) and **Table 2** (urine tests). Serologic tests for hepatitis B, hepatitis C, and HIV were negative. Serum complements testing results for C3 and C4 were normal. ANA and ANCA antibodies, monoclonal antibodies, κ and λ light chains were not detected.

A year ago, the patient was hospitalized due to an episode of acute kidney injury and acute heart failure accompanied by hypertension. These conditions had manifested with fluid retention, edema, proteinuria, rise of serum creatinine to 140 µmol/L, slight decrease of left ventricle ejection fraction to 50%, and its concentric hypertrophy. Proteinuria had not been quantified; therefore, it was impossible to assess the presence of potential baseline podocyte damage. The patient was a bodybuilder and was on a high-protein diet. He had been also taking anabolic steroids for several years, and at that time he had been on anastrozole, an estrogen synthase inhibitor. His previous health problems had been attributed to these habits. The next year he had been treated initially with loop diuretics, then indapamide, angiotensin-converting enzyme inhibitors (ramipril then perindopril), amlodipine, and nebivolol. He had not taken hormonally active medications since the previous hospitalization. The patient was a non-smoker and denied alcohol abuse.

On presentation, rRT-PCR testing for SARS-CoV-2 (DiaPlexQ, Novel Coronavirus (2019-nCoV) Detection Kit, SolGent, Daejon, Korea) in a nasopharyngeal swab sample was repeated, with positive result, and the patient was diagnosed with COVID-19. A physical examination showed a correct skin turgor and no edema, and he was euvolemic. Blood pressure was 130/80 mmHg, heart rate was 95 beats per minute, and body temperature was 38.5°C. His SpO_2 was 93% on room air, with a respiratory rate of 25 breaths per minute. He appeared ill but alert and cooperative. Lung auscultation revealed a bilaterally extended area of bronchial breathing sound with no crackles. Heart sounds were normal, and there was no murmur. The abdomen was soft on palpation, with active bowel sounds and tenderness in the right hypochondriac region. Costovertebral angles were painless. There were no pathological changes in the joints, and no skin rash was seen.

A chest CT scan without contrast enhancement revealed numerous areas of pulmonary consolidations and ground-glass opacities spreading bilaterally predominantly in bases of both lungs, and a thin layer of fluid in both intrapleural spaces with maximum thickness of 1 cm. A CT scan of the abdomen revealed both kidneys had decreased density and were enlarged to 14.5 cm length. There was no urinary retention or urolithiasis. His liver was enlarged to 22 cm in cranio-caudal dimension, and a small amount of fluid (a 6-mm layer) was present in the right flank of the peritoneal cavity.

For the following 2 days the patient was hospitalized in our nephrology department. He excreted 1000-1500 ml of urine per day, was able to drink water, and received intravenously 500 ml of multi-electrolyte fluid a day. Body temperature was controlled with metamizole and did not exceed 38°C. As peptic ulcer prophylaxis, 40 mg/day of pantoprazole was administered. Levofloxacin and ceftazidime were given against possible bacterial coinfection. Perindopril and indapamide were withheld. Blood pressure did not exceed 150/95 mmHg. The patient's serum creatinine concentration and CRP were increasing CRP (**Figure 1**). His general condition deteriorated; he became dyspneic at rest and required oxygen through a face mask in a gradually increasing flow up to 15 L/min, which maintained SpO_2 of 91%. Additionally, crackles became audible bilaterally over both lungs. Due to type I respiratory failure in the course of COVID-19 pneumonia, the patient received 2 units of COVID-19 convalescent plasma, and a treatment with intravenous dexamethasone in medium dose was introduced (**Figure 1**). No other antiviral therapy except for COVID-19 convalescent plasma was applied. The patient was transferred to the Intensive Care Unit (ICU), where ventilatory support with high-flow nasal cannula (HFNC, Airvo 2) was initiated. Besides ongoing pharmacotherapy, the patient received enoxaparin, fluconazole, cholecalciferol, morphine, torasemide, and intravenous fluids. Arterial blood gases were monitored 3 times daily, and the patient had no acidosis. Bicarbonate concentration and pCO_2 were within normal ranges, and he became gradually less hypoxemic for the following 4 days, with the minimum pO_2 of 57 mmHg and SpO_2 of 89% on the second day in the ICU. HFNC parameters were appropriately adjusted. The patient spent 7 days in the ICU, during which his general condition and pulmonary and kidney function improved, pain in the right hypochondriac region disappeared, and fever subsided. Liver parameters normalized. SpO_2 reached 97% but the patient still needed ventilatory support with HFNC, but less intense than initially. The patient was transferred back to the

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Table 1. Summary of blood laboratory evaluations and relevant trends during hospitalization.

Laboratory test	Reference	Hospital day							
		1	2	3	4	5	6	11	17
Creatinine	<105 µmol/L	520	530	570	540	450	320	126	112
Urea	2.8-7.2 mmol/L	18.0	20.3	22.5	23.5	25.0	24.1	12.2	9.7
Potassium	3.5-5.1 mmol/L	4.1	4.2	5.2	4.5	5.0	4.5	4.3	4.7
Sodium	136-146 mmol/L	134	135	138	137	138	138	139	140
Chloride	101-109 mmol/L	99	101	102	104	107	107	109	112
Bicarbonates	22-26 mmol/L	20.8	20.9	20.8	22.6	23.9	24.9		
Total calcium	2.2-2.65 mmol/L	1.75		1.8					
Corrected calcium*	2.2-2.65 mmol/L	2.2		2.3					
Phosphates	0.81-1.45 mmol/L		1.5			1.6		1.7	
pH	7.35-7.45	7.40	7.40	7.41	7.37	7.39	7.43		
Plasma lactate acid	0.5-2.2 mmol/L	0.8		0.6					
Albumin	35-55 g/L	22.5		19.3					18.3
Total protein	66-83 g/L		49.4	46.5					
Total cholesterol	3-5 mmol/L			4.2					6.1
Triglycerides	<1.7 mmol/L								2.2
Glucose	4.1-5.5 mmol/L		4.6						4.5
Total bilirubin	5-21 µmol/L	4.0	4.4						
ALP	30-12 U/L		35						
ALT	<50 U/L		41						
AST	<50 U/L	65	40						
GGT	<55 U/L	52	43						
CK	<171 U/L		130	107				33	
Myoglobin	28-72 ng/mL				92				
LDH	<248 U/L		510	560			415		
D-dimer	<0.5 mg/L	0.7							
Ferritin	30-400 ng/mL	3153							
Fibrinogen	150-450 mg/dL	699		518					
CRP	<5 mg/L	199	258	314	255	123	66	5	2
Procalcitonin	<0.5 µg/L	0.43	0.75	0.56				0.15	0.15
WBC	4-11×10³/µL	11.5	11.7	13.0	8.5	8.7	9.7	11.4	9.9
Platelet count	15-40×10³/µL	318	331	391	439	465	483	223	206
Hemoglobin	14-18 g/dL	15.3	13.9	14.2	13.7	13.2	13.4	12.5	11.1
Sars-CoV-2 rRT-PCR		Detected							Not detected
Anti-SARS-CoV-2 IgM									Detected
Anti-SARS-CoV-2 IgG									Detected

ALP – alkaline phosphatase; ALT – alanine transaminase; AST – aspartate transaminase; CK – creatine kinase; CRP – C-reactive protein; GGT – gamma-glutamyltransferase; IgM – immunoglobulin M; IgG – immunoglobulin G; LDH – lactate dehydrogenase; WBC – white blood cells; rRT-PCR – real-time reverse transcription polymerase chain reaction. * Calcium serum concentration corrected for hypoalbuminemia.

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Table 2. Summary of urine laboratory evaluations during hospitalization.

Laboratory test	Reference	Hospital day				
		1	2	12	17	18
24-hour proteinuria	<0.15 g/day			22.6		26.5
ACR	<10 mg/g		1982		4100	
Urinalysis						
Appearance	Clear	Hazy	Hazy		Clear	
Bilirubin	Negative	Negative	Negative		Negative	
Blood	Negative	+	Negative		+	
Color	Colorless, straw, yellow, pale yellow	Yellow	Yellow		Pale yellow	
Glucose	Negative	Negative	Negative		Negative	
Ketones	Negative	Negative	Negative		Negative	
Leukocyte esterase	Negative	Negative	Negative		Negative	
Nitrate	Negative	Negative	Negative		Negative	
pH	4.5-8.0	5.0	5.0		6.0	
Protein	Negative	10.2 g/L	9.5 g/L		4.9 g/L	
Specific gravity	1.005-1.030 g/mL	1.020	1.020		1.016	
Urobilinogen	<17 µmol/L	<17	< 17		<17	
Urine sediment						
Amorphous crystals	None seen, rare, occasional/HPF	None seen	Rare		None seen	
Bacteria	None seen, rare/HPF	Rare	Rare		Rare	
Casts	None seen/LPF	Rare: coarse granular, and hyline-granular; Occasional: fine granular	Occasional: fine granular and hyline-granular		Occasional, hyline casts	
Mucus	None seen/LPF	Rare	Rare		Rare	
RBC	0-2/HPF	Occasional	1-2		1-3	
Renal epithelial cells	None seen/HPF	Rare	Occasional		None seen	
Squamous epithelial cells	0-20/LPF	15	Occasional		Occasional	
WBC	0-5/HPF	10-15	1-3		1-2	
Yeast	None seen/HPF	None seen	None seen		None seen	

ACR – albumin/creatinine ratio; HPF – high-power field; LPF – low-power field; RBC – red blood cells; WBC – white blood cells.

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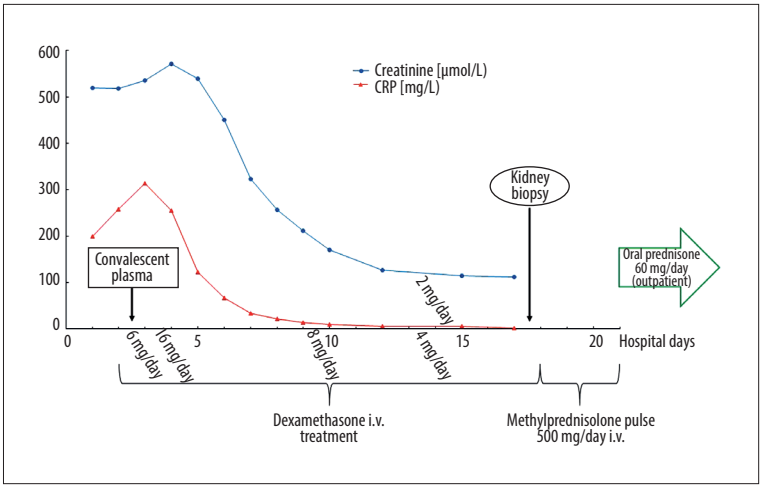

Figure 1. A decrease of serum creatinine and C-reactive protein during glucocorticoid treatment. Initial dexamethasone dose of 6 mg/day was followed by 16 mg/day for 6 days then it was gradually reduced to 8, 4 and 2 mg/day. 2 units of Covid-19 convalescent plasma was transfused on day 3. Methylprednisolone pulse was administered after obtaining first kidney biopsy results.

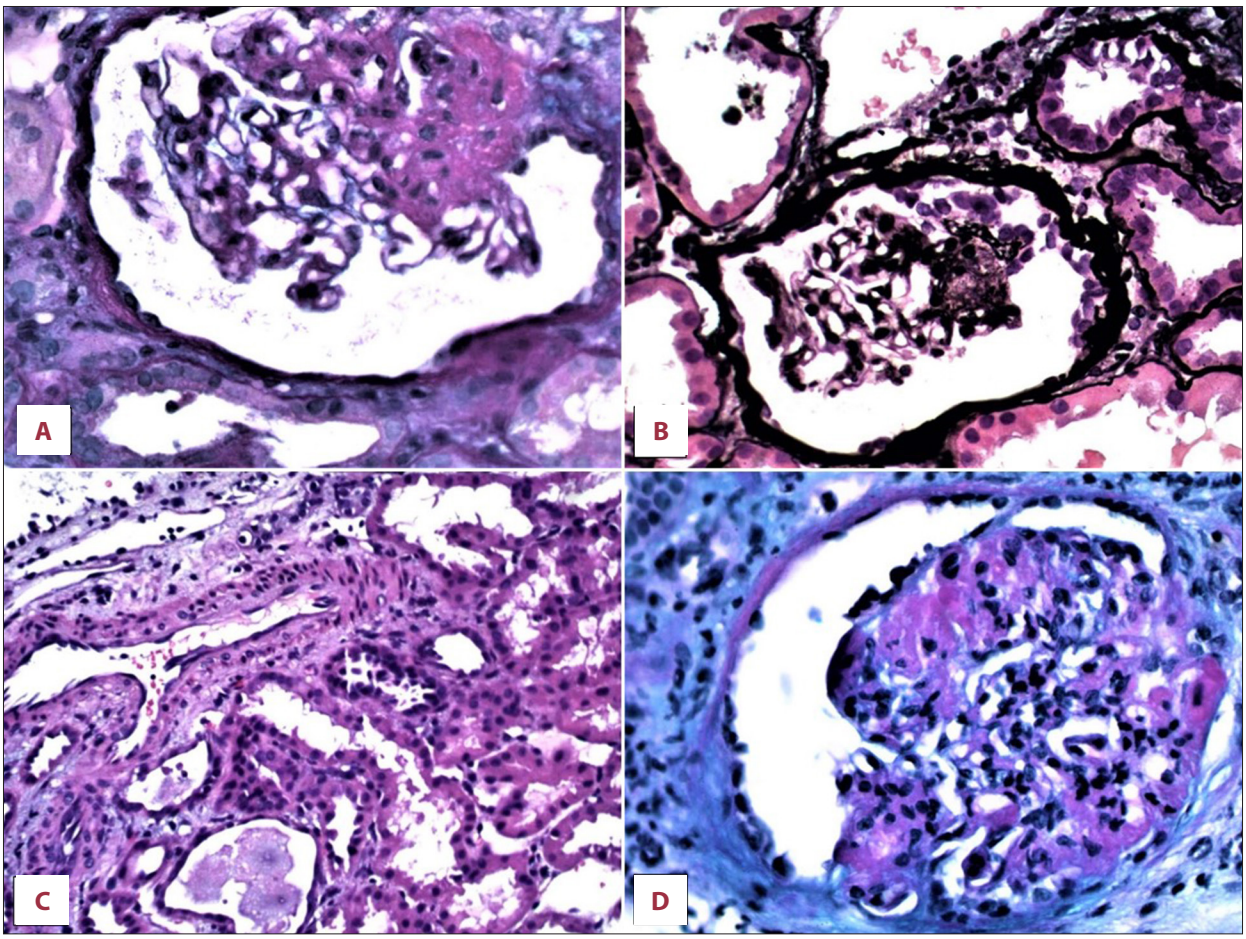

Figure 2. First kidney biopsy: (**A**) segmental sclerosis and hyalinosis of the glomerular capillary tufts (PAS staining, ×200); (**B**) a small focus of collapsed capillary loops and overlying slight epithelial hypertrophy and hyperplasia in the Bowman space of one glomerulus (Jones' methenamine-silver staining, ×200); (**C**) interstitial edema, severe degenerative changes of tubular epithelium and tubular microcysts, mild to moderate interstitial inflammation, composed of mononuclear leukocytes, some proximal tubular epithelial cells contained intracytoplasmic protein droplets positive for periodic-acid-Schiff (PAS staining, ×200). (**D**) The re-biopsy showed segmental sclerosis and hyalinosis of the glomerular capillary tuft, glomerular epithelial cells contained PAS – positive droplets (PAS staining, ×200).

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nephrology department. HFNC was discontinued after the following 2 days and for the next 4 days oxygen was supplied through a nasal canula until the patient refused to use it, having SpO_2 of 92% without oxygen supply. During dexamethasone treatment, the patient's serum CRP and creatinine concentration decreased by 30-fold and 5-fold, respectively (**Figure 1**). The patient gradually became polyuric, with diuresis reaching 5400 mL, allowing torasemide to be withdrawn. Despite gradual improvement in the patient's general condition, 24-hour urine collection showed proteinuria exceeding 22 g/day. Urine sediment was bland with single hyaline casts per low-power field (**Table 2**). ACR more than doubled since admission, while albumin concentration in serum further dropped (**Table 1**). Having considered the severity of nephrotic syndrome, a kidney biopsy was performed to investigate its cause. Meanwhile, a treatment with enalapril in gradually increasing dose was initiated, serum creatinine concentration remained stable, and blood pressure was good. The kidney biopsy (without electron microscope examination, due to technical problems) showed signs of both FSGS and interstitial nephritis. Therefore, dexamethasone was stopped and 1.5 g of methylprednisolone, divided in 3 intravenous pulses over 3 days, was administered, followed by oral prednisone in a dose of 60 mg/day (**Figure 1**). The patient's body weight was 88 kg and BMI was 25.4 kg/m^2. The patient was discharged home 21 days after initial presentation, for further follow-up in the outpatient clinic. He was prescribed several medications on discharge, including prednisone 60 mg/day, enalapril 20 mg/day, nebivolol 2.5 mg/day, and atorvastatin 10 mg/day. The summary of immunosuppressive therapy during hospitalization is as follows: 1) i.v. dexamethasone in modified doses from 3rd to 18th day since presentation, 2) i.v. methylprednisolone (500 mg/day) from 19th to 21st day (**Figure 1**).

The patient underwent the first kidney biopsy 14 days after initial presentation. A re-biopsy was conducted 20 weeks later during follow-up to reassess the extent of pathological changes and to perform ultrastructural examination.

In the first kidney biopsy, light microscopy showed 8 glomeruli. Two were completely sclerosed and 1 appeared normal. Other glomeruli displayed signs of segmental sclerosis and hyalinosis and focal segmental thickening of the capillary loops (**Figure 2A**). There was a small focus of collapsed capillary loops and overlying slight epithelial hypertrophy and hyperplasia in the Bowman space in 1 glomerulus (**Figure 2B**), but the extension of these glomerular changes was too small to unequivocally meet the criteria for collapsing variant of FSGS. No endocapillary hypercellularity or necrotizing lesions were seen in the glomeruli.

The interstitium showed edema, and inflammatory infiltrates consisted of lymphocytes. Simplification and degenerative lesions of tubular epithelium as well as scattered tubular microcysts were present (**Figure 2C**). The proximal tubules contained intracytoplasmic periodic-acid-Schiff (PAS)-positive protein resorption droplets. There were mild focal tubular atrophy and interstitial fibrosis involving approximately 5% of the cortical parenchyma. The interstitial changes were nonspecific for FSGS, but resorption droplets in the proximal tubule may have indicated glomerular proteinuria.

Immunofluorescence performed on 3 glomeruli showed no specific immune staining for IgG, IgA, IgM, C1q, C3, kappa, or lambda involving the glomeruli. The kidney tissue submitted for electron microscopy contained no glomeruli.

Based on light microscopy findings and immunofluorescence evaluation, focal segmental glomerulosclerosis and acute tubulointerstitial nephritis were diagnosed.

The second biopsy revealed glomerular changes similar to the ones found in the first biopsy. A total of 7 glomeruli were identified in the tissue submitted for evaluation. Light microscopy disclosed segmental sclerosis of the capillary tufts in 2 glomeruli. Glomerular epithelial cells contained PAS-positive droplets (**Figure 2D**).

Tubulointerstitial lesion in the re-biopsy kidney specimen showed more interstitial fibrosis and tubular atrophy compared to the first biopsy. Tubular atrophy and interstitial fibrosis accounted for about 15% of the cortical parenchyma. A direct immunofluorescence evaluation was negative for immune reactants in glomeruli, including IgG, IgA, IgM, C1q, C3, kappa, and lambda light chains. The kidney tissue submitted for the electron microscopy contained 4 glomeruli, including one that was globally sclerosed.

Ultrastructural examination showed focal thickening of the glomerular basement membrane and severe foot process effacement, involving more than 80% of the glomerular basement membrane surface area. No immune-type electron-dense deposits were identified (**Figure 3**). Based on light microscopy findings, immunofluorescence, and electron microscopy evaluation, the diagnosis of focal segmental glomerulosclerosis was confirmed with the progression of interstitial lesions.

Although both kidney biopsies met the criteria of representativeness, the low number of glomeruli could have contributed to the observed substantial difference in proportion of glomeruli affected by podocytopathy between both specimens, and this made it difficult to classify glomerular lesions that were on the border between collapsing and non-collapsing variant of FSGS.

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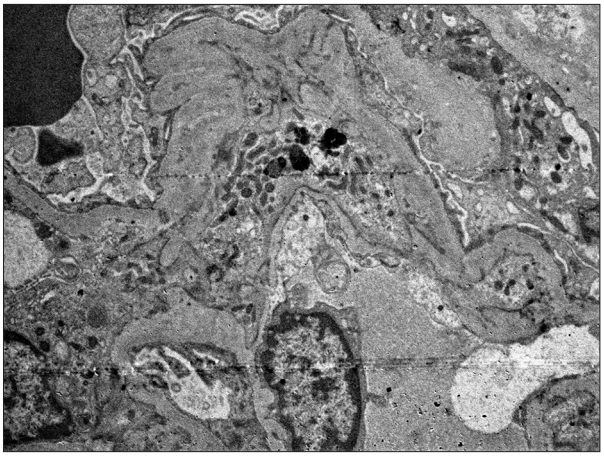

Figure 3. The re-biopsy, ultrastructural examination revealed focal thickening of the glomerular basement membrane, and foot process effacement. No immune-type electron-dense deposits were identified (original magnification ×4000).

Additional Studies

Two samples of urine and 2 fresh, mechanically homogenized specimens of kidney parenchyma taken during the first kidney biopsy (14 days after presentation) were examined with rRT-PCR (with the previously-mentioned detection kit) for the presence of SARS-CoV-2. All urine and tissue samples were negative for viral RNA.

After obtaining the patient's informed consent, genotyping for the apolipoprotein L1 (APOL1) G1 and G2 risk alleles was performed, as further described. Both risk alleles were absent and the gene sequence matched the hg19 reference sequence.

DNA was isolated from peripheral blood using a Maxwell® RSC instrument (Promega). Fragments of APOL1 gene (chr22: 36661747-36662448; 702bp) were amplified using forward GCCAGAGCCAATCTTCAGTC and reverse AATGTTTGCATTTGGGTCAA primer. PCR was performed in 25 µl with 1 µl of patient DNA, using standard reagents, including HotStarTaq DNA Polymerase (Qiagen), with a profile of 35 cycles and annealing at 59°C. Approximately 3 µl of the amplicons were visualized on 2.8% agarose gel. After enzymatic purification (EPPiC, A&A Biotechnology), PCR products were extended using the BigDye® 3.1 termination-ready reaction mix. Each sequencing reaction (20 µl) contained 4 µl of BigDye® mix, 30 ng of primer and 50 ng of the amplicon. The cycling conditions were as follow: initial denaturation at 95°C for 5 min was followed by 30 cycles at 95°C for 30 s, 58°C for 10 s, and 60°C for 4 min. Extension products were purified (BigDye XTerminator, Thermo Fisher Scientific) and analyzed using an ABI Prism 3130™ Genetic Analyzer. Sequences were edited and analyzed using BioEdit and MEGA 4: Molecular Evolutionary Genetics Analysis [17].

The patient was not tested for mutations in PDSS2 or SCARB2 genes since he had no neurological symptoms.

Follow-Up

The patient was followed up 6 times after discharge, at weeks 4, 12, 16, 19, 23, and 43. On the first visit to the clinic, he did not report any problems, and the physical examination was unremarkable. The lungs were clear to auscultation at every visit. His arterial blood pressure, given as a mean of home self-measurements from the week preceding the visit, was satisfactory (**Table 3**). His 24-h proteinuria was 50% lower 4 weeks after discharge but remained the same after another 8 weeks, when trace leg edema and a slight increase in blood pressure became notable (**Table 3**). Since then, the enalapril dose was increased to 30 mg/day. Serum creatinine concentration and eGFR were relatively stable at all visits (**Table 3**). Urine sediment was bland every time. On the third visit, trace leg edema was still present, although 24-h proteinuria decreased by 40% compared to the previous measurement (**Table 3**). The patient reported clearly visible purple abdominal striae and elevated fasting glucose in his self-monitoring with a glucose meter. Glucose concentration controlled in fasting venous plasma was 5.5 mmol/L. After 16 weeks of prednisone therapy, the patient was concerned by its adverse effects, while only 1 criterion of partial remission was achieved, which was proteinuria reduction of more than 50% compared to discharge point, whereas it was still in the nephrotic range (**Table 3**). Therefore, kidney re-biopsy was considered to be helpful in making further therapeutic decisions, as an electron microscope evaluation was missing in the initial kidney biopsy. The patient was readmitted to the hospital 19 weeks after initial discharge, and kidney re-biopsy was performed. His leg edema had disappeared, 24-h proteinuria decreased again by 40% to 41 mg/kg of body weight, mean ACR from 2 morning spot urine samples were 16% less than on initial presentation and 60% smaller than on initial discharge, and serum albumin concentration increased by 43% compared to discharge level, but the patient still had hypoalbuminemia (**Table 3**). Oral glucose tolerance testing confirmed an impaired fasting glucose (IFG), likely secondary to steroid therapy. Besides abdominal striae, there was a marked weight gain and adipose tissue redistribution. Therefore, after re-biopsy, which showed remarkable podocytopathy and progression of chronic interstitial lesions, the patient was switched to 400 mg of cyclosporine A (4 mg/kg/day) in 2 divided doses. The prednisone dose was decreased by 10 mg/day, and further reduction by 5 mg every 2 weeks was advised. Nebivolol was increased to 5 mg/day for better blood pressure control, and a treatment with 500 mg of metformin twice a day was initiated for IFG. Four weeks later, the patient was seen again. His cyclosporine trough level was 107 ng/ml, and 24-h proteinuria decreased further by 10%. The patient reported no problems, and a physical examination was unremarkable, kidney

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Table 3. Summary of relevant parameters and main treatment during follow-up.

Measure	Reference	Weeks after discharge					
		4	12	16	19	23	43
24-hour proteinuria	<0.15 g/day	11.7	11.4	6.8	4.0	3.6	0.4
ACR	<10 mg/g				1663		
Urine sediment	Bland	Bland	Bland	Bland, single hyaline casts	Bland	Bland, single hyaline casts	Bland
Serum creatinine	<105 µmol/L	101	108	99	119	115	119
CKD-EPI eGFR	>60 ml/min/1.73 m^2	83	77	85	68	71	68
Serum albumin	31-52 g/L				26.3		
Serum potassium	3.5-5.1 mmol/L	3.8	4.3	3.9	4.8	4.8	4.6
Serum sodium	136-146 mmol/L		139		141	142	
WBC in blood	4-11×10^3/µL	12.6	13.5	11.4	12.0	9.5	7.3
Platelet count	15-40×10^3/µL	196	264	235	210	218	234
Blood hemoglobin	14-18 g/dL	14.4	16.7	16.9	15.2	15.4	13.7
Arterial blood pressure*	<130/80 mmHg	135/75	139/81	136/82	138/85	130/79	128/76
Body weight [kg]		96	99	98	98	99	97
BMI**	18.5-25 kg/m^2	27.7	28.6	28.3	28.3	28.6	28
Prednisone dose [mg/day]		60	60	60	60→50	40	5
Cyclosporine dose [mg/day]		None	None	None	400***	400	400→300
Enalapril dose [mg/day]		20	20→30	30	30	30	30
Nebivolol dose [mg/day]		2.5	2.5	2.5	2.5→5	5	5
Kidney re-biopsy		–	–	–	Yes	–	–

ACR – albumin/creatinine ratio; CKD-EPI eGFR – chronic kidney disease epidemiology collaboration estimated glomerular filtration rate; WBC – white blood cells; BMI – body mass index. * Average home blood pressure from previous week; ** patient's muscle mass was above the average; *** therapy initiation; → dose change.

function was stable, and arterial blood pressure was well controlled (**Table 3**). The prednisone dose was further gradually reduced. The last visit took place 43 weeks after the initial hospital discharge; the 24-h proteinuria was close to the criterion of complete remission, while serum creatinine was stable (**Table 3**). The residual dose of 5 mg/day of prednisone was maintained, while cyclosporine A was reduced to 300 mg/day in 2 divided doses. The immunosuppressive therapy during the entire follow-up included: 1) prednisone administered enterally in a dose of 60 mg/day for 19 weeks followed by reduction to 50 mg/day and further reductions by 5 mg/day every 2 weeks until the dose of 5 mg/day was reached at 38 weeks of follow-up; and 2) cyclosporine A, 400 mg/day from the 19th week and 300 mg/day from the 43rd week (**Table 3**).

Discussion

The patient was diagnosed with COVID-19 pneumonia, FSGS, and interstitial nephritis. We suspected that glomerular disease might have led to secondary tubulointerstitial nephritis, which in turn resulted in loss of kidney function and acute kidney injury. This pathomechanism is suggested by the clinical course, laboratory results, and kidney biopsy findings. FSGS complicating COVID-19 is not an unexpected finding in a proteinuric patient with severe infection; however, it has been previously reported nearly exclusively in Black patients and was often associated with presence of risk alleles G1 or G2 of a gene encoding apolipoprotein L1 [8,9,18-24]. Those alleles in homozygous or G1/G2 combination are thought to modify innate

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immune response in a way that predisposes to podocytopathy and collapsing variant of FSGS and is frequently complicated with acute kidney injury [7,9,18-24]. The presence of these alleles may serve as the "first hit", while an intense immune response to SARS-CoV-2 infection can become the "second hit" triggering FSGS [8,21,22]. White patients, who very rarely have G1 and G2 alleles, and are not routinely screened for them, are unlikely to develop podocytopathy and FSGS in the course of COVID-19 and such cases have been very rare [7,11,14]. Our patient, who was genotyped and did not have the predisposing APOL1 alleles, might have other risk factors. Possibly, an episode of AKI and diagnosis of arterial hypertension 1 year earlier was a risk factor for COVID-19-related AKI, and until that time he had used anabolic steroids and was on a high-protein diet for body-building [25]. Anastrozole, which had been used by the patient, could have increased the risk for FSGS and arterial hypertension [26,27]. A variety of underlying diseases that predisposed to COVID-19-dependent kidney injury were also reported by other authors [7,14]. The final triggering role of SARS-CoV-2 infection is suggested by a rapid increase of serum creatinine shortly before and after the patient's admission to an emergency department, where a diagnosis of COVID-19 was made, and also by a marked decrease of serum albumin concentration and increase of urinary ACR and proteinuria during the 3-week hospitalization (**Tables 1, 2**). As the patient was not hypovolemic, prerenal AKI could be excluded. Instead, the intrarenal origin of the disease was indicated by active urine sediment on presentation, proteinuria, enlargement of both kidneys, and a decrease of kidney density in computed tomography. The patient was febrile on admission and had highly elevated acute phase markers such as serum CRP, ferritin, and fibrinogen concentration, without any marked increase of serum procalcitonin (**Table 1**), which is typical for virus-related cytokine storm. This could have an effect on podocytes, leading to their functional and ultrastructural changes, marked by the foot process effacement shown in electron microscopy. The good effects of immunosuppressive therapy support this theory. Other, earlier-reported cases of FSGS in COVID-19, although in APOL1 high-risk genotype patients, also suggested the immune reaction-related etiology of glomerulopathy. In most of these cases, SARS-CoV-2 was not found in renal parenchymal cells, which was verified by rRT-PCR, in situ hybridization for viral mRNA, or ultrastructural examination [7,8,18-24]. Based on these analyses, the probability of a direct viral infection is very low. In addition, in our case no viral material was found in homogenized samples of renal parenchyma and urine. In most FSGS cases in COVID-19 patients associated with the presence of G1 or G2 APOL1 risk alleles, biopsies showed glomerular capillary collapse and epithelial hypertrophy and hyperplasia in the Bowman space [18-23]. This subtype of FSGS is regarded as related to poor prognosis of renal survival. In our case, the patient was diagnosed with non-collapsing type of FSGS, but a single glomerulus in the first biopsy presented segmental capillary collapse and slight epithelial hypertrophy and hyperplasia in the Bowman space (**Figure 2B**). The intensity of lesions was low and not sufficient to diagnose a collapsing glomerulopathy. We may speculate that if glucocorticoid therapy had not been quickly introduced, this subtype of FSGS would have spread to most of the glomeruli and patient's renal function could have rapidly worsened. Although the initial dexamethasone therapy based on the findings of the RECOVERY trial was given for the treatment of severe pneumonia and type 1 respiratory failure [28], it allowed a restoration of kidney function. The patient also received 2 units of COVID-19 convalescent plasma, which might have also contributed to the good effect of the therapy [29]. Despite the acute course of disease on presentation, we should keep in mind the other possible situation, where a patient could have had asymptomatic FSGS before SARS-CoV-2 infection, which remained undiagnosed until its aggravation was triggered by severe COVID-19. The available data do not lead us to completely reject this theory. Nevertheless, a rapid deterioration of renal function together with the morphological changes of the kidneys and onset of nephrotic syndrome have a strong relation to COVID-19. Glucocorticoid therapy, however, most probably would exert its effects in both scenarios. We can also speculate that even if the patient had high-risk APOL1 genotype, the same treatment, as applied, would be able to mitigate the progression of FSGS.

After the results of the first kidney biopsy became available and respiratory symptoms of COVID-19 subsided, the patient re-tested negative for SARS-CoV-2. The virus was absent in nasopharyngeal swab, samples of urine, and kidney parenchyma; nevertheless, extensive nephrotic proteinuria remained remarkable and life-threatening. Therefore, we considered FSGS in this case to be a primary disease, which needs an immunosuppressive treatment. SARS-CoV-2 infection appeared only as a trigger for FSGS, and since the virus was eliminated from body but nephrotic syndrome persisted, glomerulopathy should not have been considered as secondary to viral infection. Therefore, we initiated therapy with intravenous methylprednisolone followed by oral prednisone. After 19 weeks of treatment, serum creatinine remained normal and a more than 50% reduction of proteinuria was achieved (**Table 3**). Moreover, adverse effects of glucocorticoid therapy became a concern, especially glucose intolerance. Because the ultrastructural examination of glomeruli was not performed, we decided to perform a re-biopsy to confirm the diagnosis and to look for some prognostic and therapeutic hints. Taking into account the adverse effects of prednisone, laboratory results, and the re-biopsy findings, we concluded that a better cost/benefit ratio for the patient would come from further treatment with cyclosporine than with prednisone. Four weeks from the initiation of cyclosporine, proteinuria further decreased and eGFR remained stable (**Table 3**). The patient's outcome was satisfactory, in contrast to many cases of COVID-19-associated FSGS, where patients

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had to initiate maintenance hemodialysis [7,8,14,18,21-23]. One study reported better prognosis related to less advanced interstitial fibrosis and tubular atrophy covering <30% of examined parenchyma [8]. The outcome in our patient, who had interstitial lesions covering 5% of parenchyma in the first biopsy and 15% in the re-biopsy, is in agreement with this observation. The progression of nonspecific tubulointerstitial changes after 19 weeks of glucocorticoid therapy may suggest reconsideration of the fact if this was only a case of tubulointerstitial nephritis secondary to FSGS or other concomitant tubulointerstitial disease. We cannot exclude the possibility that during the acute phase of COVID-19, cytokine storm, and hypoxia, before the first kidney biopsy was conducted, our patient had an acute tubular necrosis which led to the later aggravation of inflammation in the interstitium and enhanced fibrosis [30]. Autosomal dominant tubulointerstitial kidney disease (ADTKD) is the other possible condition which goes with interstitial fibrosis and tubular atrophy and, occasional, with microcystic dilatations. ADTKD, however, is an ultra-rare genetic disorder. It is confined to renal tubules and initially does not affect glomeruli. Glomerular secondary scarring is a relatively late symptom; therefore, proteinuria is minimal or mild, the opposite to the case of our patient. It tends to occur in families whereas our patient has a negative family history for kidney disease. On imaging studies, the kidneys are normal or small in size, while our patient had enlarged kidneys on presentation. In case of ADTKD, a slow progression of kidney disease is expected, whereas our patient presented a relatively steady serum creatinine concentration during follow-up visits [31]. Perhaps the third biopsy, if had been done after 43 weeks of follow-up (when the patient was close to complete remission), including 24 weeks of cyclosporine and slowly reduced glucocorticoid therapy, would have brought definitive information about disease activity and ultimate response to therapy, but it was not accepted by the patient.

Conclusions

The case presented shows that COVID-19 can evoke symptomatic non-collapsing subtype of FSGS in patients with risk factors for kidney disease other than the presence of G1 and G2 APOL1 alleles. It can manifest with nephrotic syndrome, interstitial nephritis, and AKI. Immunosuppressive treatment including glucocorticoids can induce partial remission of FSGS but not improve interstitial changes. The introduction of cyclosporine A to the therapy probably contributed to the remission of disease in this patient.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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