KIDNEY WEEK 2023

November 1-5 | Philadelphia, PA

Renal Biopsy

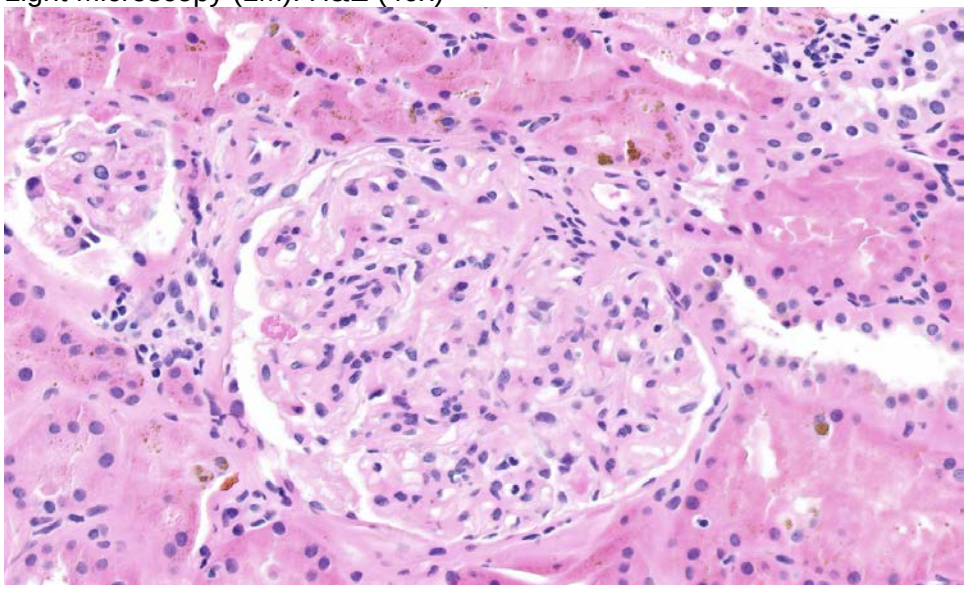
Clinical Correlations

Case 1 from Anatoly Urisman, MD, PhD, University of California San Francisco

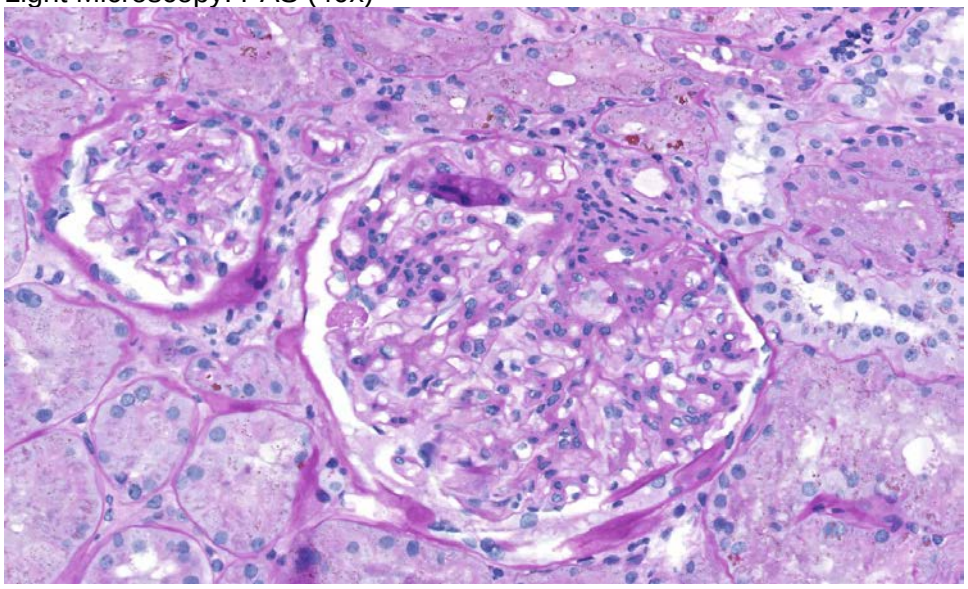
A 30-year-old woman with history of subnephrotic proteinuria was diagnosed with SLE as a teen when she developed Reynaud's and serology showed +ANA, +dsDNA, +SAA, +SSB, +APLA IgM, and low complements. She was treated with hydroxychloroquine and prednisone with reasonable control. At age 24, she developed life-threatening MSSA bacteremia and endocarditis, which required AVR and MVR (both mechanical valves) and anticoagulation with warfarin. More recently, she was switched to methotrexate and belimumab for arthralgias and rashes, initially with prednisone, which was then tapered. She also has a history of prior UTIs and non-obstructing nephrolithiasis. She continues to have arthralgias, and recent lab work shows microscopic hematuria and subnephrotic proteinuria. A renal biopsy is performed.

Labs: sCr 0.74, Hbg 7.7-8.8, INR 2.2. UA: +RBC (>50/hpf), moderate heme, UPCR 0.5 g/g. Positive dsDNA, normal C3, mildly decreased C4.

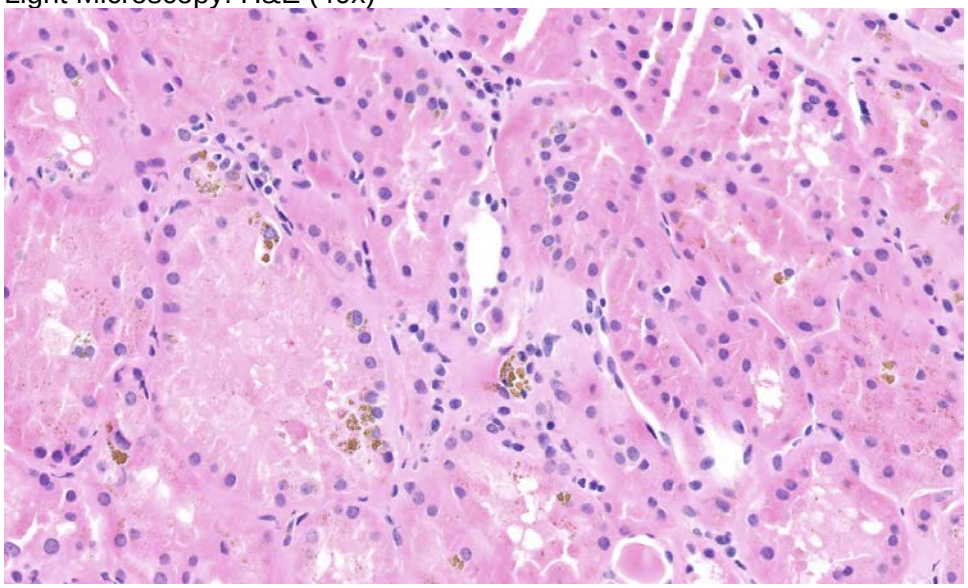
Light Microscopy (LM): H&E (40x)



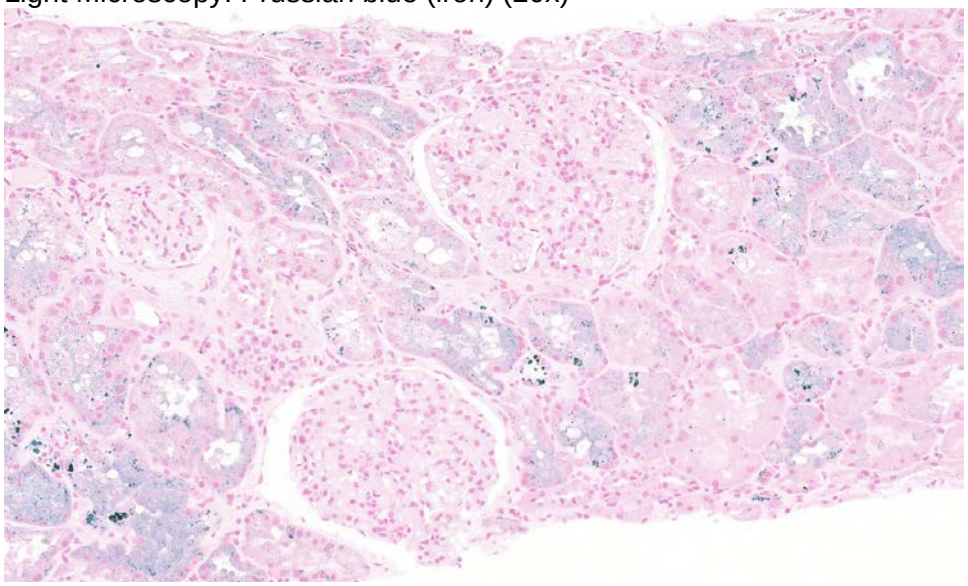
Light Microscopy: PAS (40x)



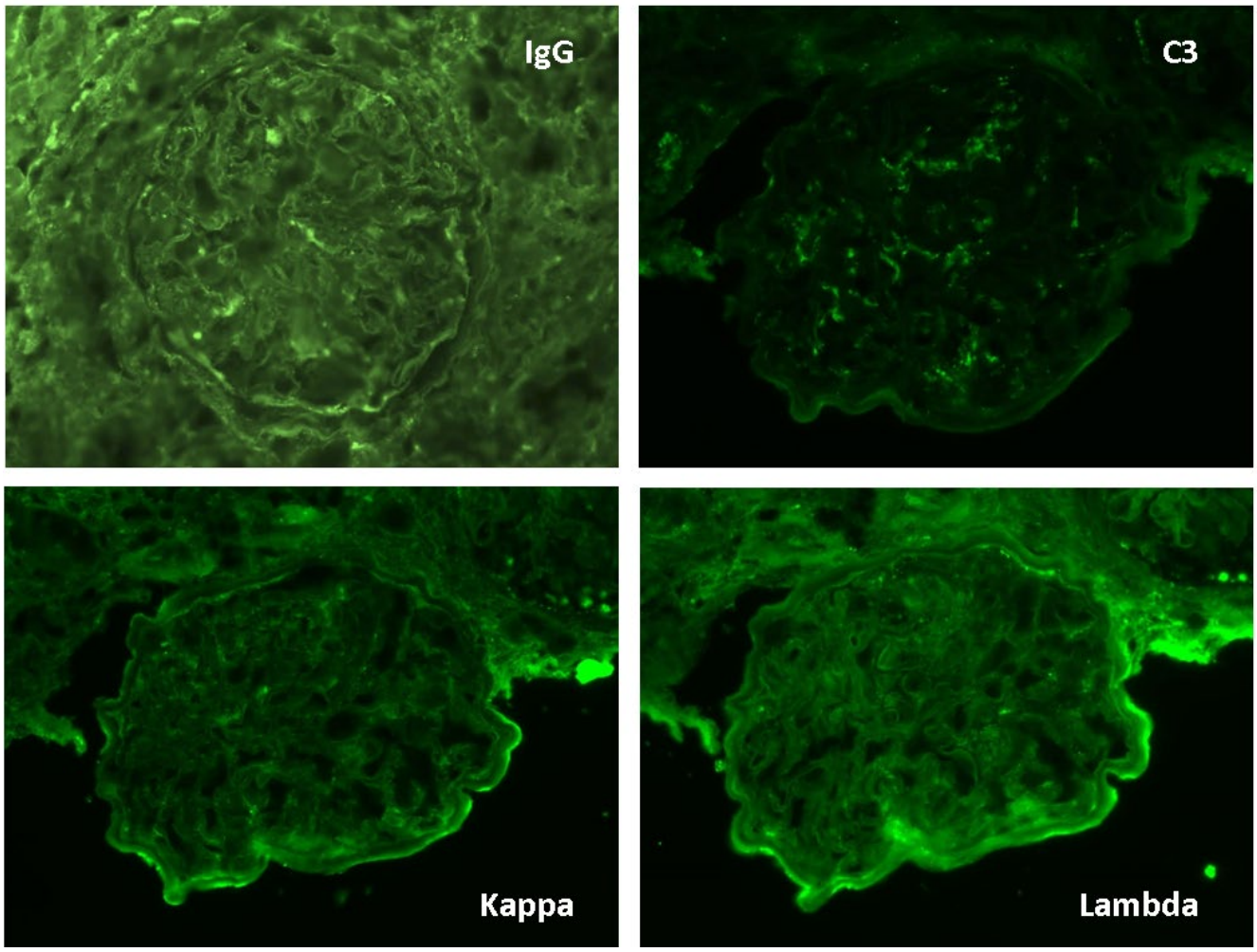
Light Microscopy: H&E (40x)



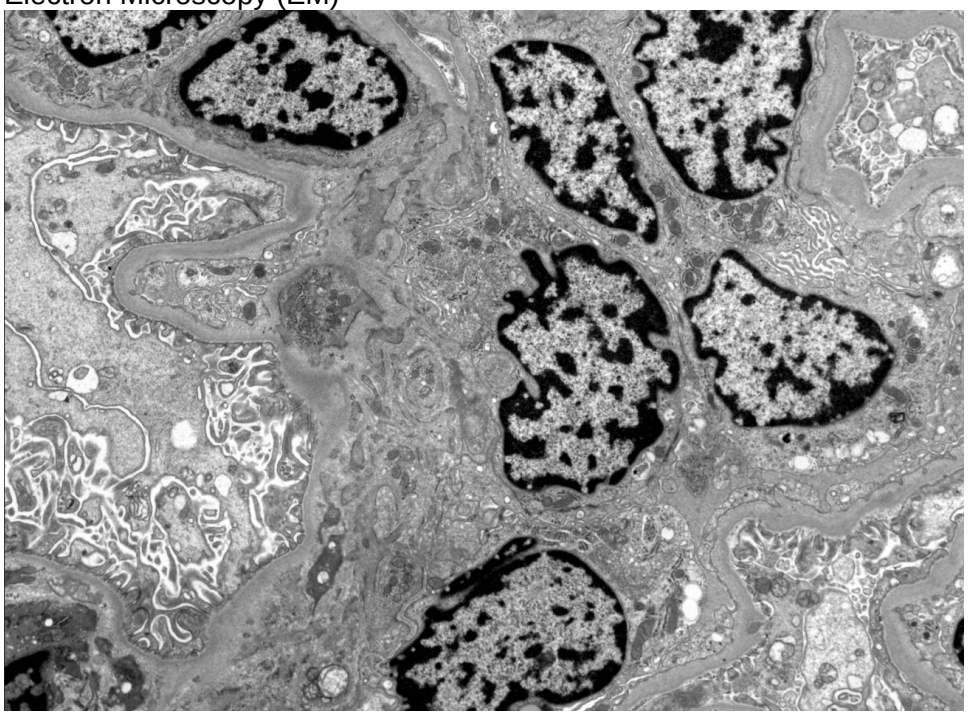
Light Microscopy: Prussian blue (iron) (20x)



Immunofluorescence Microscopy (IF)



Electron Microscopy (EM)



Pathologic Findings

Light Microscopy (LM): The specimen consists of 2 cores of renal cortical tissue with up to 14 glomeruli per section, none of which are globally sclerotic. The glomeruli show mild mesangial widening with mesangial hypercellularity. The capillary lumina are patent and free of significant endocapillary hypercellularity. Rare segments show segmental mesangial sclerosis, with partial capillary loop sclerosis and possible glomerular basement membrane double contours. No wire loop deposits, crescents, or necrotizing lesions are identified. The tubulointerstitium shows occasional foci of tubular atrophy and interstitial fibrosis, involving approximately 15% of the sample, with associated mild chronic interstitial inflammation. Some proximal tubules appear hyperplastic and contain increased protein reabsorption droplets. Scattered foci of hemosiderin deposition within the tubular epithelial cytoplasm are noted (confirmed by Perls' iron stain). A partially sampled interlobular caliber artery shows mild intimal fibroplasia. At least one arteriole demonstrates segmental hyaline sclerosis.

Immunofluorescence Microscopy (IF): Frozen sections contain renal cortical tissue with 8 glomeruli, 2 of which are globally sclerotic. The stain results are as follows:

IgG: 2+ diffuse punctate staining in glomeruli, tubules, and interstitium
IgM: +/- mesangial (punctate)
IgA: Negative
C3: 2+ mesangial (punctate)
C1q: Negative
Fibrinogen: Negative
Albumin: Negative
Kappa: 1+ diffuse punctate staining in glomeruli, tubules, and interstitium
Lambda: 1+ diffuse punctate staining in glomeruli, tubules, and interstitium

Electron Microscopy (EM): Toluidine blue-stained semi-thin sections contain renal cortical tissue with 1 glomerulus present; 46 electron microphotographs were taken. The images show mostly patent glomerular capillary lumina. Some capillary loops show endothelial cell swelling, but no significant endocapillary hypercellularity is identified. Mild mesangial matrix increase is noted in some segments, and rare capillary loops appear partially sclerotic. The glomerular capillary basement membranes are within normal thickness. Rare mesangial and subepithelial small electron-dense foci consistent with immune complex deposits are noted, but no larger or widespread deposits are seen. The foot processes of the visceral epithelial cells show focal effacement. No definite tubuloreticular inclusions are identified. The tubules and the interstitium are largely unremarkable.

Diagnosis and Interpretation

1. Mesangial lupus nephritis, (ISN/RPS Class II).
2. Tubular epithelial hemosiderin deposits (renal hemosiderosis).
3. Mild interstitial fibrosis and tubular atrophy (15%).
4. Mild arteriosclerosis and hyaline arteriolosclerosis.

Clinical Follow-Up

No changes were made to the SLE therapy, and the patient continued on MTX + HCQ regimen. The presence of hemosiderosis suggested chronic hemolysis with the following differential diagnosis:

- Autoimmune hemolytic anemia
- Prosthetic heart valves
- Warfarin ("anticoagulant-related nephropathy")
- Other forms of hemolysis (e.g., genetic)

A retrospective review of the chart demonstrated that hemolytic anemia was recognized soon after AVR/MVR surgery and initiation of anticoagulation, and hematology workup concluded that the most

likely cause was prosthetic valves. No changes were made to the patient's anti-coagulation regimen, and she continued with regular INR checks.

Questions for Case 1

1. What is the main pattern of glomerular injury seen by light microscopy?

A. Endocapillary proliferative
B. Mesangiopathic
C. Membranoproliferative
D. Crescentic

The glomeruli show segmental mesangial hypercellularity. There is no significant endocapillary hypercellularity (A) or duplication of the glomerular basement membranes (C). No crescents are present (D).

2. What is the brown pigment accumulating in the tubular epithelial cells?

A. Monoclonal light chains
B. Immune complexes
C. Hemosiderin
D. Lipofuscin

Hemosiderin has granular/globular golden-brown appearance and is positive for iron on Prussian blue (Perls') stain. The IF stains do not reveal significant staining in the tubular epithelial cells corresponding to the pigment (A, B). Lipofuscin (a.k.a. lipochrome) has a finely granular appearance, lighter gray-yellow color, and is negative for iron (D).

3. What is the most likely significance of the tubular pigment observed in the biopsy?

A. It is the result of chronic hematuria due to lupus nephritis.
B. It is the result of chronic hemolysis.
C. The patient has a family history of anemia.
D. Proteinuria is not related to the pigment.

Prominent hemosiderin accumulation (renal hemosiderosis), as in this case, suggests chronic hemolysis. Hematuria from lupus nephritis (or another glomerulonephritis) is typically associated with blood and RBC casts in tubular lumens, with or without associated tubular epithelial injury; significant hemosiderin accumulation is generally not observed (A). While genetic hemolytic conditions (e.g., sickle cell disease or another RBC disorder) should be considered, it is less likely in this patient with a history of prosthetic valves and warfarin therapy (C). While proteinuria due to lupus nephritis cannot be ruled out, even in mesangial (Class II) lupus nephritis, an association between chronic hemolysis and subnephrotic proteinuria is well supported in the literature.

Discussion

Renal hemosiderosis is a histologic pattern characterized by accumulation of hemosiderin in renal tubules, in peritubular interstitium, and sometimes glomeruli[1,2]. Hemosiderin is an endogenous (produced by the body) pigment that has characteristic golden-brown granular to globular appearance in histologic sections. It is composed predominantly of ferric iron (Fe^{3+}) bound to proteins like ferritin[3]. The presence of hemosiderin in the tissue sections can be confirmed with a Prussian blue (Perls') stain, which highlights iron deposits (blue color) in the pigment[4].

Renal hemosiderosis is associated with variable degrees of acute tubular epithelial injury, interstitial inflammation, which long-term may lead to significant tubulointerstitial scarring. This pattern of renal injury is most often encountered in conditions marked by intravascular hemolysis and hemoglobinuria. The differential diagnosis includes the following[5]:

- Autoimmune hemolytic anemia
- Drug or toxin-induced hemolysis
- Prosthetic cardiac valve (and other vascular devices)
- Genetic RBC and hemoglobin disorders (e.g., sickle cell disease, G6PD, PNH, etc.)
- Infection (e.g., malaria, parvovirus B19, etc.)
- Conditions with thrombotic microangiopathy (TTP/HUS, aHUS, other)
- Transfusion reactions

Numerous cases of renal hemosiderosis secondary to hemolysis in patients with prosthetic cardiac valves have been reported, some of which present with AKI, and others show more indolent loss of kidney function[6,7]. While mechanical destruction of RBCs is thought to be the main mechanism, other risk factors contribute to the development of hemolysis[8,9].

Of note, most patients who develop prosthetic valve-associated hemolysis also receive anticoagulation therapy. While warfarin has not been definitively established as a cause of hemolytic anemia, renal injury in patients on warfarin has been recognized for more than a decade[10] and more recently in patients on newer generation anticoagulants[11]. The biopsies of patients with “anticoagulant-related nephropathy” (ARN) show some histologic overlap with renal hemosiderosis, including tubular hemosiderin deposits, but tend to have more prominent acute tubular epithelial cell injury, accumulation of dysmorphic RBCs, and tubular RBC casts[10]. Thus, hematuria in the setting of over-coagulation has been proposed as the underlying mechanism in ARN. A possible role of intravascular hemolysis in ARN remains to be investigated.

References

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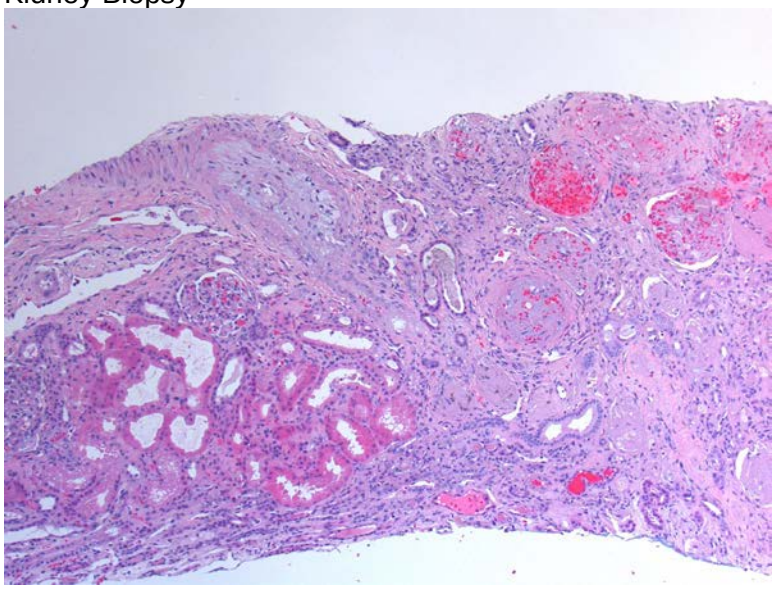
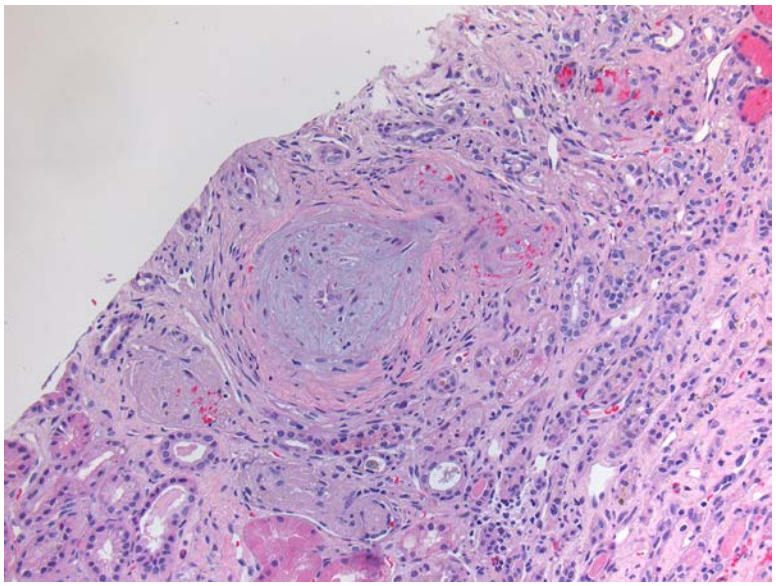
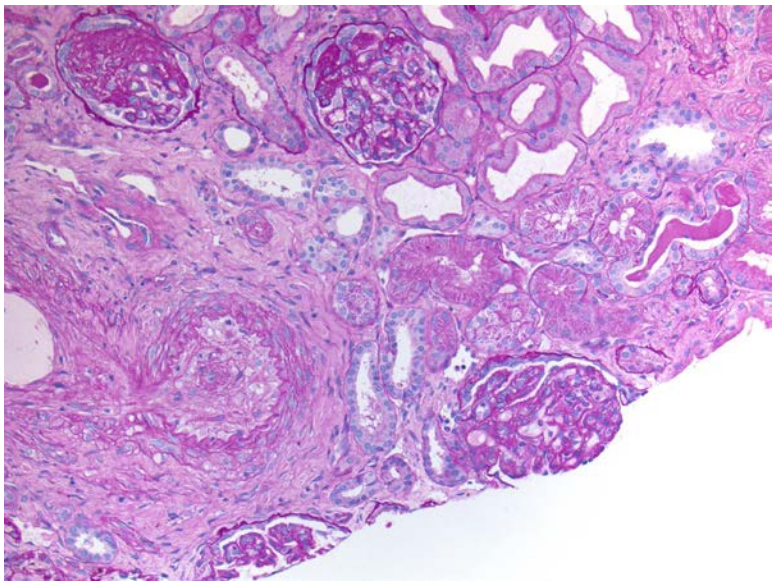
Case 2 from Matthew Palmer, MD, PhD, University of Pennsylvania

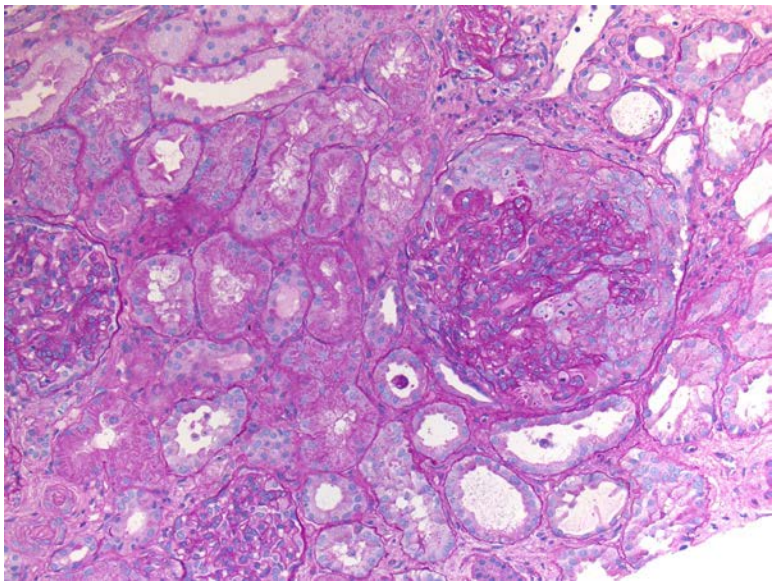
A previously healthy 5-year-old child developed cyclic fever and flu-like symptoms while visiting West Africa. He was treated for presumed malaria. He was admitted one week later with renal failure and altered mental status, including seizures and vision loss. His blood pressure was elevated, systolic to 170s. He was hospitalized in Ivory Coast for one month. He returned to the United States and presented with persistent CNS symptoms and renal insufficiency with Cr 2.75. Labs showed WBC slightly elevated at 13.9, normal hemoglobin and platelets, and UPCR 12, ANA and ANCA negative, C3/C4 normal. MR of the brain showed scattered microhemorrhages, small cortical infarcts, and small areas of parenchymal hemorrhage.

Kidney Biopsy









Pathologic Findings

Approximately 20% of the sampled cortex demonstrated coagulative necrosis consistent with infarction. Vessels at the interface with viable cortex showed marked intimal expansion with mucoid edematous change, red cell fragments within the wall, luminal narrowing, and a rare thrombus. Glomeruli showed variable ischemic retraction, segmental double contours, focal segmental sclerosis, and one with marked extracapillary epithelial hyperplasia consistent with florid collapsing lesion. Acute tubular injury was present in the viable cortex. The interstitium showed mild fibrosis. Immunofluorescence was negative for all reactants. Electron microscopy was non-contributory due to the necrotic nature of the sample.

Kidney biopsy diagnosis:

- Thrombotic microangiopathy with focal cortical necrosis
- Focal segmental glomerulosclerosis with collapsing features
- Mild interstitial fibrosis

Clinical Follow-Up

This previously healthy 5-year-old child hospitalized in Ivory Coast for management of presumed malaria initially presented with fever, neurologic compromise, and renal failure requiring hemodialysis. Brain imaging was consistent with scattered infarction and hemorrhage, and kidney biopsy demonstrated thrombotic microangiopathy (TMA) with cortical infarct and collapsing-type FSGS. Although malaria testing was negative, the test was performed after treatment. The leading differential diagnosis is severe malarial infection with residual neurologic and renal sequelae.

Questions for Case 2

1. Pathophysiologic mechanisms implicated in AKI from malaria disease include:

A. RBC aggregation leading to microvascular obstruction
B. Altered RBC surface antigen expression activates monocytes, which lead to helper T cell proliferation and acute interstitial nephritis
C. Hemolysis and cast nephropathy
D. All of the above

There are multiple possible mechanisms by which malaria can cause AKI. Aggregation of parasitized red cells due to altered expression of cell surface receptors can cause vessel obstruction and activation of monocytes, endothelium, and T helper cells. This inflammatory response can cause AIN. Parasitized red cells undergo lysis with resulting metabolic injury from hemoglobinuria or cast nephropathy.

2. Pathologic lesions described in the kidneys in patients with malaria include all except:

A. Thrombotic microangiopathy
B. Acute interstitial nephritis
C. Acute tubular injury
D. Urothelial carcinoma
E. Immune complex glomerulonephritis

Kidney pathology in malarial disease varies along with the variety of pathophysiologic mechanisms. Described lesions include TMA, AIN, ATI, and immune complex glomerulonephritis. Malignancy such as urothelial carcinoma has not been associated with malarial infection; however, schistosomiasis of the bladder has been.

3. Which has been linked with collapsing glomerulopathy?

A. Viral infection
B. Drugs, including bisphosphonates, heroin, and anabolic steroids
C. Lupus
D. Ischemia in the setting of TMA
E. All of the above

Collapsing glomerulopathy is a severe form of podocytopathy associated with several etiologies, including viral infection, lupus, drug toxicity, and TMA/ischemia. Linking some of these etiologies appears to be conditions with increased interferon and presence of high-risk APOL1 alleles.

Discussion

Malaria is a disease caused by parasitic infection of red blood cells by one of six species of *Plasmodium* protozoa. These parasites have coevolved with humans for millions of years and in response to selection pressures have developed a complex life cycle and gene expression program. The corresponding evolutionary consequences in humans are interesting conditions like sickle cell disease, hemoglobinopathies, and enzyme deficiency. The majority of individual infections are of little clinical impact. More significant disease occurs due to varied interaction between parasite and host response; therefore, clinical manifestations are variable[1].

One form of malarial disease relates to the hemolysis associated with release of parasites from ruptured red cells. Another pathologic pathway can unfold as infection of red cells by *Plasmodium falciparum*, which results in altered expression of adhesion molecules on the red cell surface, conferring a “sticky” phenotype. Infected cells adhere to one another to form rosettes and clumps, as well as to platelets and endothelial cells. Interaction with endothelial cells can lead to endothelial activation and expression of leukocyte adhesion molecules, endothelin, and inflammatory cytokines[1]. The combination of blood cell aggregation and endothelial activation/inflammation can cause thrombotic microangiopathy[2], vascular compromise, and ischemia. Organs commonly affected include the liver, brain, and kidneys.

AKI is present in approximately 40% of severe *P. falciparum* infections[3]. The spectrum of injury includes tubular injury (related to hemolysis or ischemia), interstitial nephritis, cortical necrosis, and thrombotic microangiopathy[4]. Although *P. vivax* infections have generally been considered to be clinically mild, several series of patients with renal TMA linked to *P. vivax* have been reported[5]. The pathology in these reports demonstrates marked vascular wall edema and luminal occlusion similar to the lesion classically associated with accelerated hypertension. Immune complex glomerulonephritis has also been described[6], most commonly in patients infected by *P. malariae*. The chronic infection typical of *P. malariae* and associated Th2 immune response with complement activation are thought to precipitate immune complex deposition in the kidney. A Th1 response is hypothesized to potentially cause interstitial nephritis.

Nephrotic syndrome is only rarely described in patients with malaria. One possible route could be a minimal change-type podocytopathy, which has been rarely reported[7]. The present case illustrates another possible mechanism involving collapsing-type focal segmental glomerulosclerosis as a consequence of TMA and bears similarity to another report[2]. TMA is one of several causes of collapsing glomerulopathy, likely a function of ischemia from compromised circulation[8]. It is also plausible that an immune response with elevated interferon, a condition associated with collapsing glomerulopathy, may contribute to this phenotype[9]. Finally, the collapsing phenotype is made more likely by the presence of APOL1 high risk alleles, which are more prevalent in areas with high malarial disease burden.

References

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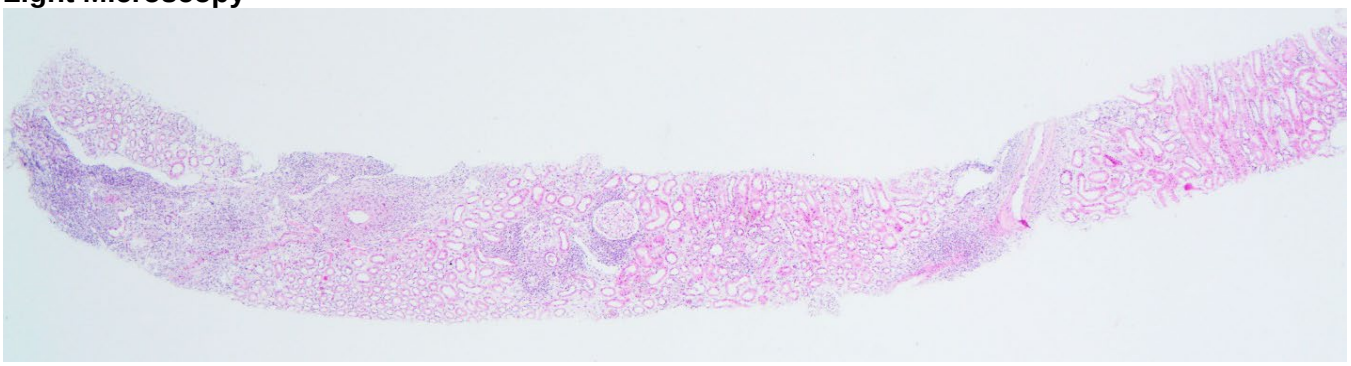
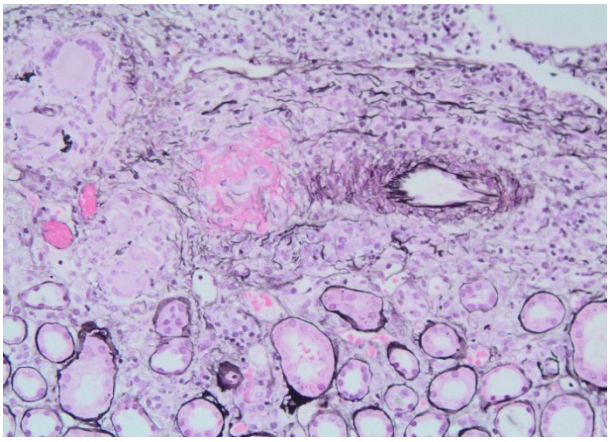
Case 3 from Laura Biederman, MD, Nationwide Children's Hospital/Ohio State Wexner Medical Center

A 46-year-old woman with a medical history of malignant melanoma with metastatic disease to the lungs, hypothyroidism, and major depression disorder was admitted to the hospital with symptoms of weakness, headache, nausea, and vomiting. She is nivolumab and ipilimumab for her melanoma. She reports ongoing nausea since her last treatment with immunotherapy 2 weeks ago. She has no prior history of kidney disease. She denies diarrhea but has had a recurrent headache for which she takes either take ibuprofen, naproxen, or acetaminophen for the pain. She states that she took 2 tablets of naproxen about 2 days ago. She has noted decreasing output over the past 2 days. She denies gross hematuria. In the ED, she was found to have an elevated serum creatinine up to 4.40 mg/dL (baseline 0.7 mg/dL). Her urinalysis showed greater than 500 mg/dL of protein, no blood, few WBCs in clumps, moderate transitional epithelial cells, and 2 granular casts. She also had abnormal liver function tests with an AST of 384 mg/dL, ALT of 154 mg/dL, and AP of 540 mg/dL. Her headaches and liver function tests were concerning for checkpoint inhibitor associated hepatotoxicity, and she was started on 80 mg of solumedrol and scheduled for a kidney biopsy for her ongoing renal dysfunction.

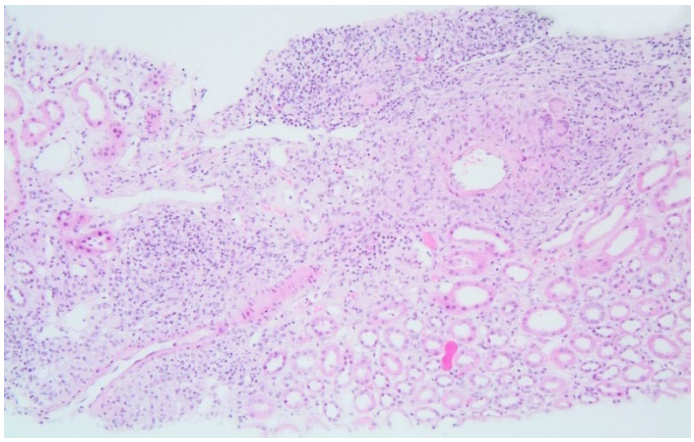
Light Microscopy



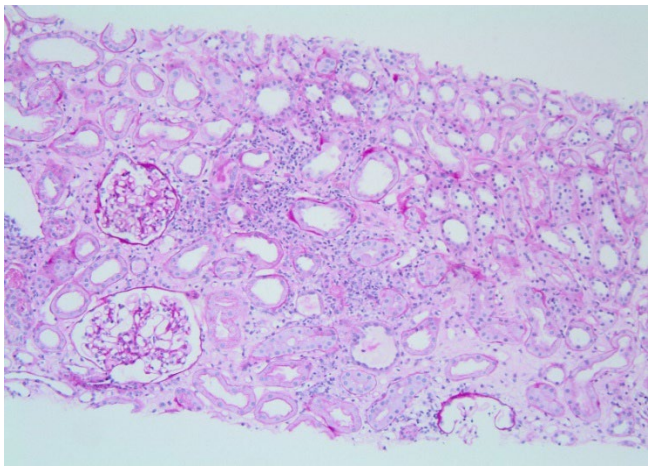
Low Power of Biopsy Core (H&E)



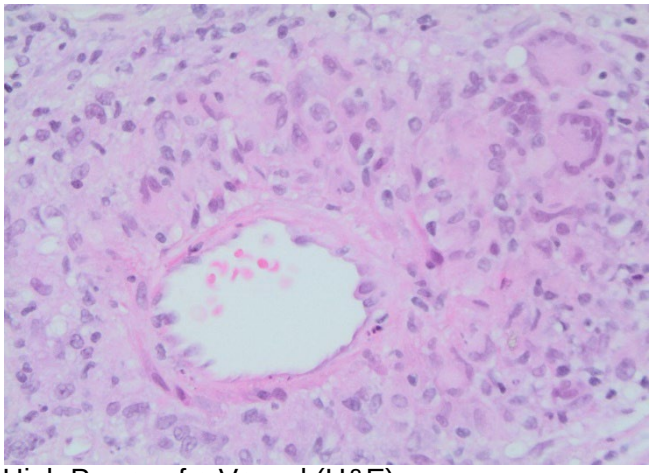
Vessel (Jones Silver Stain)



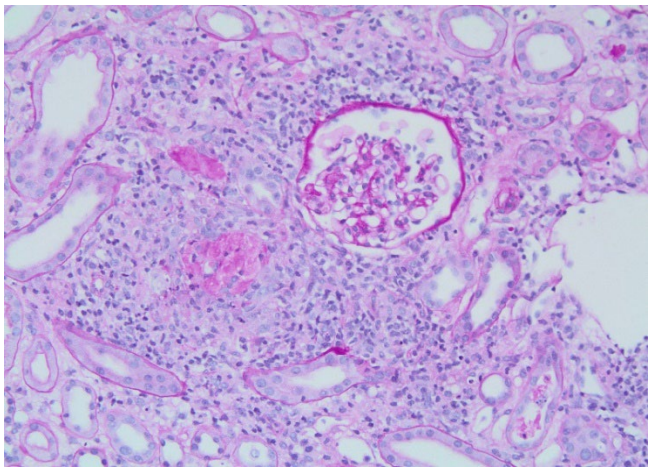
Vessel and Interstitium (H&E)

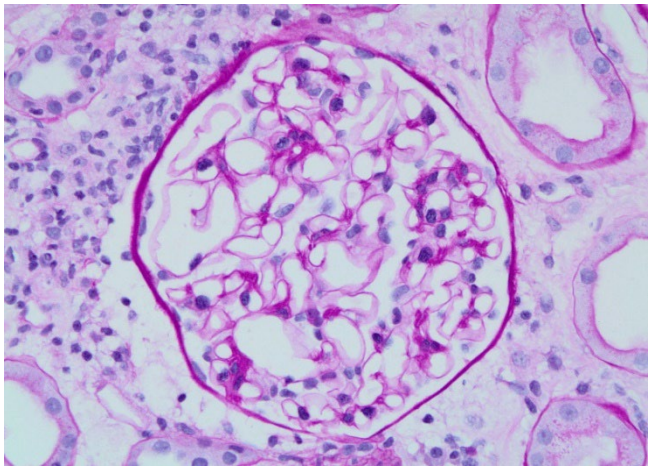


Interstitium (PAS Stain)

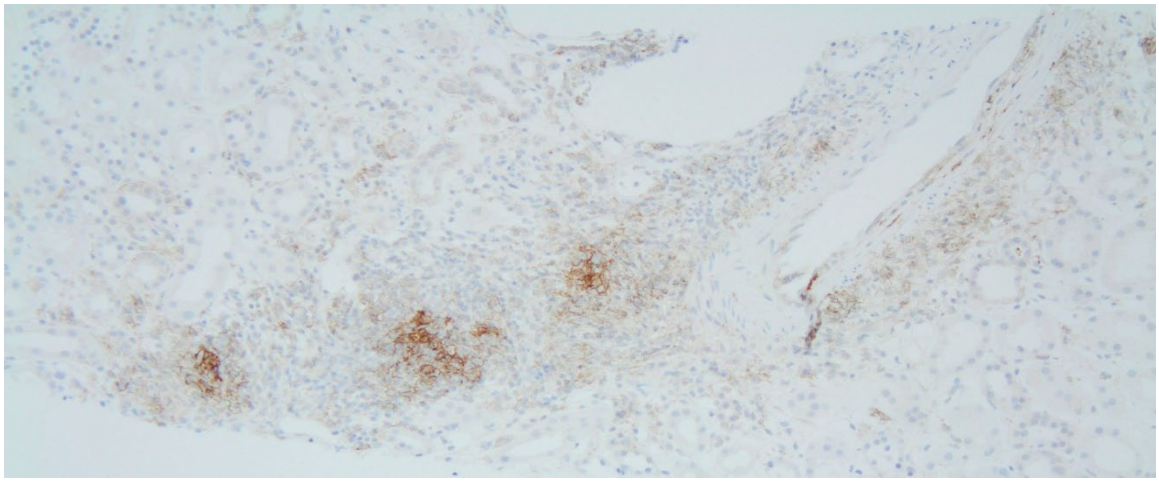


High Power of a Vessel (H&E)

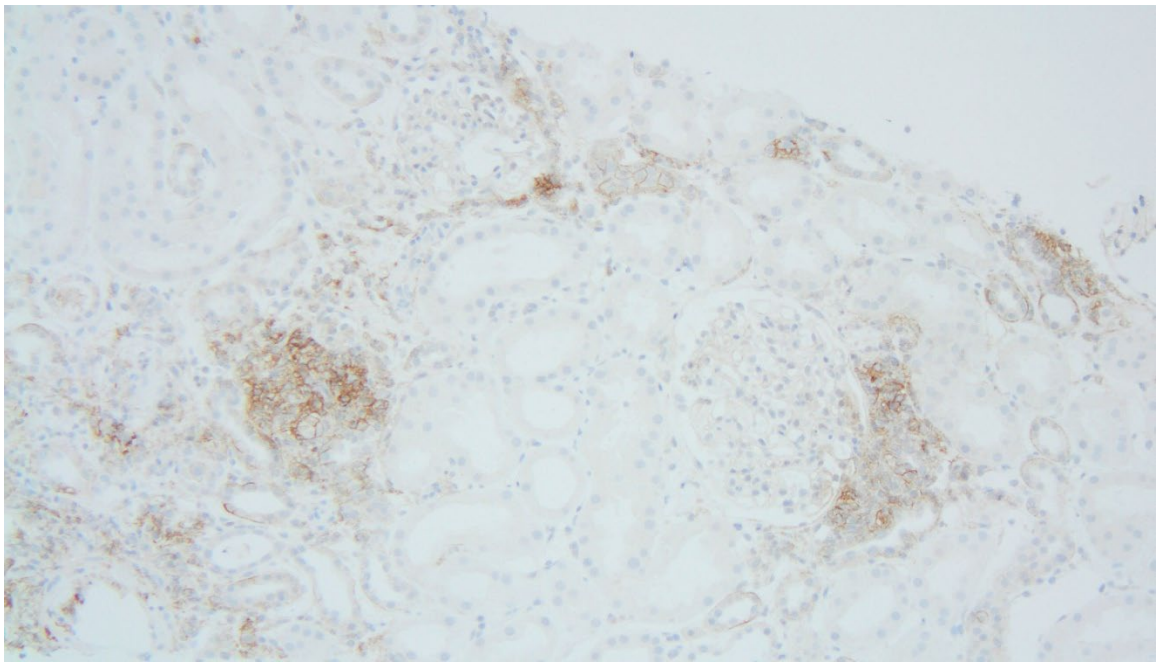




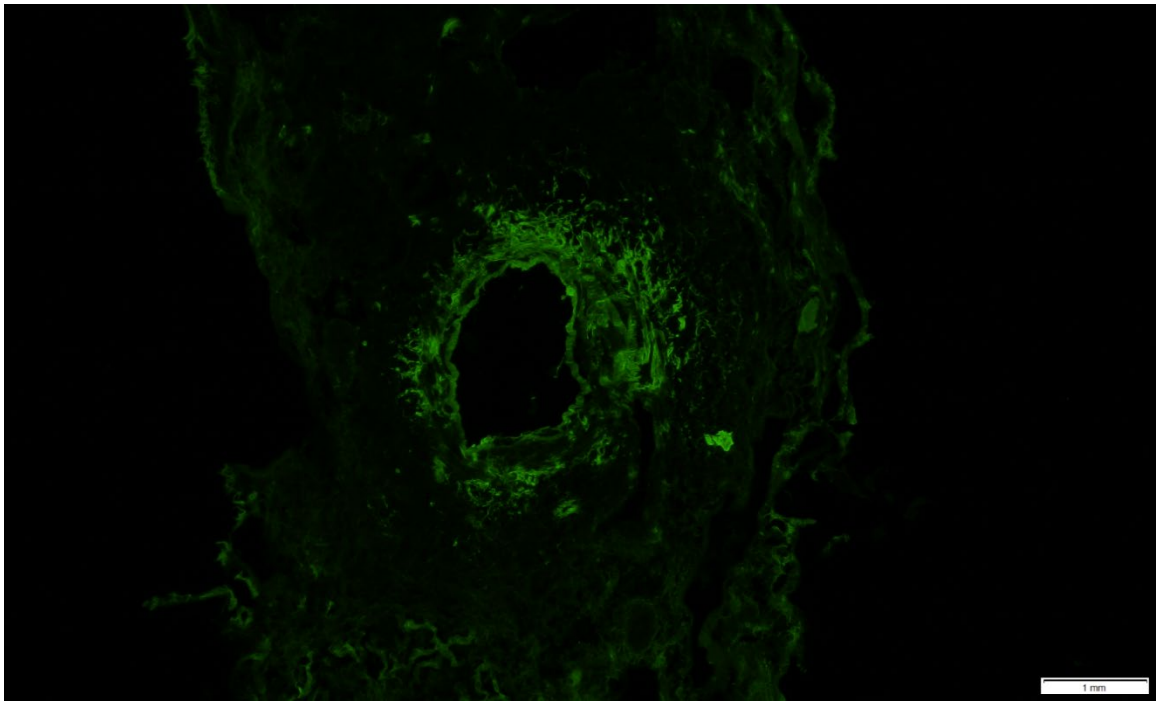
Glomeruli on PAS Stain



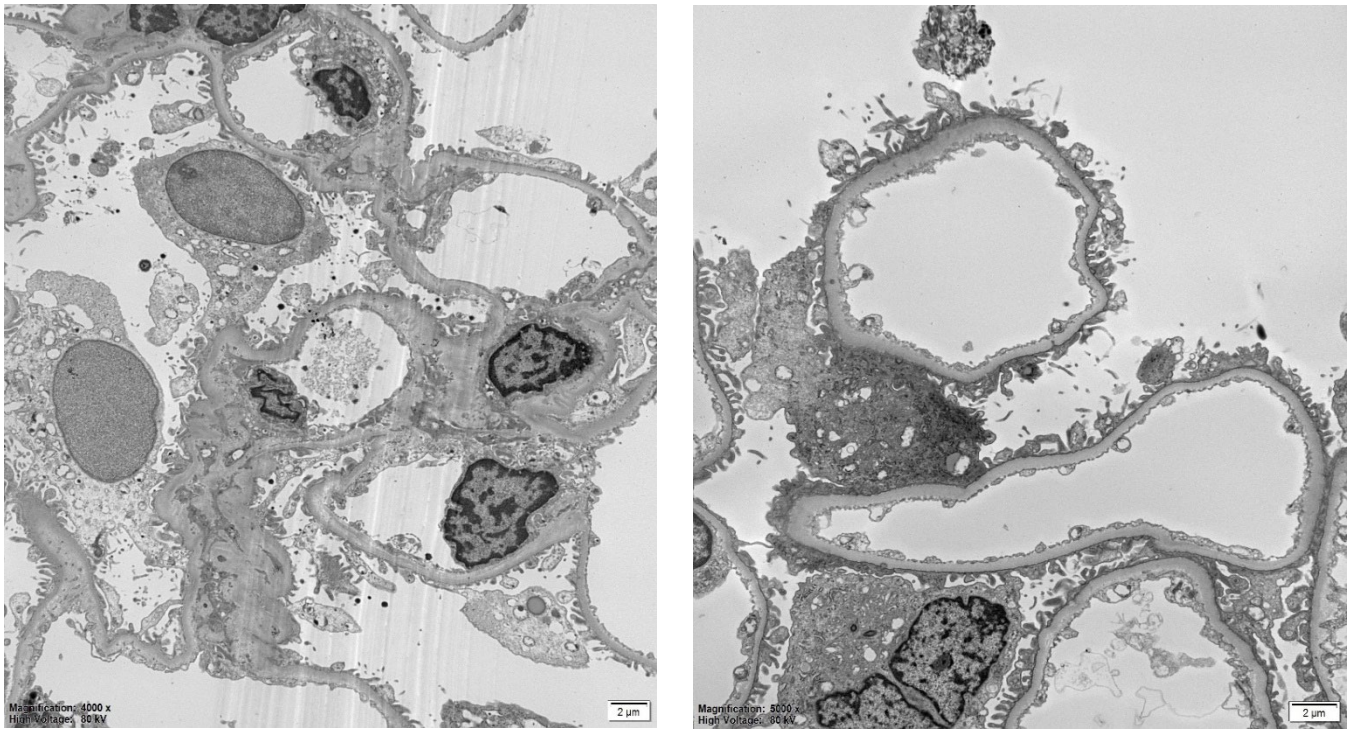
PDL-1 Immunohistochemical Stain



PDL-1 Immunohistochemical Stain



Fibrinogen staining in a vessel. All other fluorochromes showed no significant reactivity.



Representative pictures of ultrastructure of a glomerulus

Pathologic Findings

Light Microscopy: The kidney biopsy consisted of renal cortex and medulla with 30 glomeruli, 3 of which were globally sclerosed (10%). The glomeruli were normal size, cellularity, and architecture with open capillary loops and normal appearing mesangium. The tubulointerstitium showed patchy interstitial inflammation which on higher power consisted primarily mononuclear and granulomatous inflammation with occasional giant cells. The granulomatous inflammation was centered around larger arteries, and there was fibrinoid necrosis of an artery. Immunohistochemical staining for PDL-1 highlighted inflammatory cells in the areas of inflammation. There were also scattered tubular epithelial cells with membranous staining.

Immunofluorescence Microscopy: Standard immunofluorescence on frozen tissue (scale 0-3+) demonstrated no significant reactivity in the glomeruli but bright fibrin staining in a vessel, consistent with fibrinoid necrosis.

Electron Microscopy: Ultrastructural evaluation of the glomeruli revealed open capillary loops with no evidence of immune complex-type deposits. The mesangium was not expanded. Granulomatous inflammation and interstitial inflammation were not represented on the specimen submitted for electron microscopy.

Diagnosis

Granulomatous necrotizing vasculitis with associated interstitial nephritis related to immune checkpoint inhibitor therapy.

Clinical Follow-Up

She was continued on prednisone, and her creatinine trended down to 1.0 mg/dL (baseline 0.7 mg/dL). The steroids were tapered, and nivolumab and ipilimumab were stopped with plans to follow up with oncology for further treatment recommendations. Her other symptoms also improved.

Questions for Case 3

1. What is the most common renal side effect of immune checkpoint inhibitors (ICPIs)?

A. Interstitial nephritis
B. Vasculitis
C. Membranous nephropathy
D. Pyelonephritis

Although not the primary pattern seen in this biopsy, the most common renal side effect of ICPI therapy is interstitial nephritis. A PDL-1 immunohistochemical stain will show membranous reactivity on the tubular epithelial cells.

2. What is the best description of the vascular changes seen?

A. Thrombotic microangiopathy
B. Granulomatous vasculitis
C. Arteriosclerosis
D. Lupus vasculitis

This biopsy shows a granulomatous vasculitis pattern in the vessels. There is inflammation in and around the vessels, with giant cells as well as fibrinoid necrosis of the vessel walls.

3. Which is true about the PDL-1 immunohistochemical stain? (select all that apply)

A. It highlights PD-1, the receptor targeted by the ICPIs.
B. It is aberrantly expressed on some tumor cells.
C. The target it highlights functions as an immune inhibitor.
D. It is normally expressed on activated immune cells.

PDL-1 is an immunohistochemical stain that is specific to the target of ICPIs. PDL-1 is an immune checkpoint protein normally expressed on many white blood cells including effector T cells, B cells, and others. It is a co-inhibitory factor that binds to receptors of PD1 and B7-1 on activated T cells to downregulate the immune response. Some tumors aberrantly express it as an immune resistance mechanism. ICPIs down-regulate the expression of PDL-1 systemically as well as in the tumor. The resulting downregulation of an immune suppressing mechanism is likely what leads to many of the autoimmune side effects seen with these drugs. Notably, different immune checkpoint inhibitors have different receptor specificity and therefore different immunohistochemical stains.

Discussion

This case highlights an unusual adverse reaction of an immune checkpoint inhibitor (ICPI). ICPIs are a class of drugs that inhibit a ligand normally expressed on immune cells that functions to down-regulate the immune response. Some tumors aberrantly express these ligands as a method of immune evasion. Classically associated with interstitial nephritis in the kidneys, ICPIs have a broad range of toxicities over many different organ systems. There are two primary targets for checkpoint inhibition: programmed cell death receptor 1 (PDL-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). In this case, the patient was on both a PDL-1 (nivolumab) and a CTLA-4 inhibitor (ipilimumab). Many side effects, including autoimmune diseases, have been reported as a result of these medications. The most common renal side effect, interstitial nephritis and AKI, is considered a less common adverse event from ICPIs and is usually treated with glucocorticoids.

Vasculitis, including granulomatous vasculitis, is uncommonly reported with ICPIs. ICPI-related vasculitis is classically associated with large vessels and the central and peripheral nervous system[1]. However, it can involve any size vessel, and small vessel vasculitis—including vasculitis of the kidneys—has been described. ICPI-related vasculitis can have a classic appearance of pauci-immune,

ANCA-associated vasculitis, including positive ANCA testing[2–5]. However, some cases have a mixed appearance, including negative ANCA testing and vasculitis of larger sized vessels[6–10]. Interestingly, interstitial nephritis associated with ICPIs can also have granulomatous features[11,12]. Additionally, there are rare reports of ICPI-induced thrombotic microangiopathies (TMA), and some cases of vasculitis report “TMA-like features”[10,12].

The differential diagnosis would include other causes of vasculitis, granulomatous and otherwise, including giant cell arteritis, polyarteritis nodosa, sarcoidosis, and pauci-immune vasculitis. ANCA testing should be performed, as some cases of pauci-immune vasculitis can involve medium vessels, though the lack of any small vessel involvement in this case would be unusual. Additionally, as mentioned above, ICPIs can induce a positive ANCA and resulting pauci-immune necrotizing and crescentic glomerulonephritis.

Immunohistochemical staining for PDL-1 can be performed and is helpful in cases of ICPI-induced interstitial nephritis[13]. In this case, the presence of focal membranous staining in tubular epithelial cells suggests a component of ICPI interstitial nephritis is present in this patient as well. The strong PDL-1 staining in the areas of granulomatous inflammation is abnormal and is suggestive of a causative component of the ICPI.

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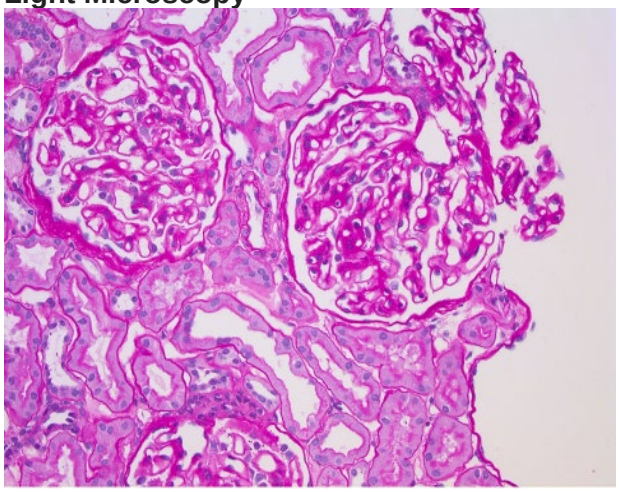
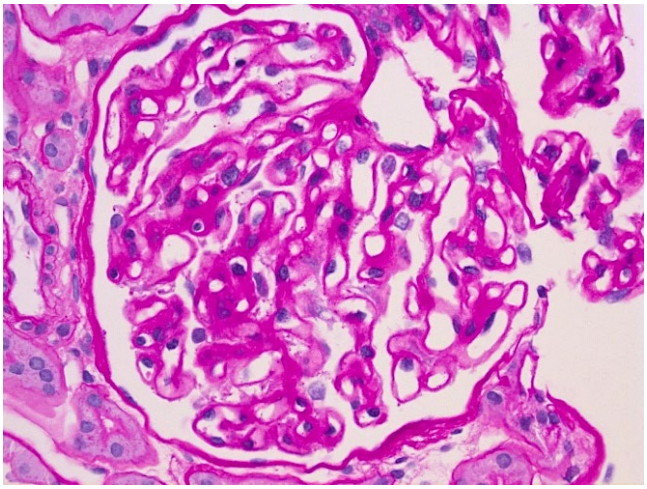
Case 4 from Carmen Avila-Casado, MD, PhD, University of Toronto

A 48-year-old woman referred with elevated ACR 56 found as part of initial assessment at new GI functional motility disorders. Past medical history: 11 miscarriages, antiphospholipid antibody positive (enrolled in a MSH study at the time), *H. pylori* eradication, Hepatitis C as a teenager, tubal ligation. She is taking a multivitamin and B complex. She works as high school education assistant. Prior smoking: quit 3 years before with 5-10 pack year history and 4-5 alcoholic beverages per week. Her family history includes: mother with Pendred syndrome and Parkinson disease. Past history of GI symptoms related to *H. pylori* and food allergies. No other relevant symptoms except generalized chronic mild arthralgias/body aches for many years with no recent change. No rashes or neurologic symptoms. No history of NSAIDs, other OTCs, or illicit drugs. At examination, Vitals - BP 90/60 (both arms), HR 60 and regular, no edema, good peripheral pulses. Chest - clear, Abdomen - normal. Laboratory investigations: SCr 50, ACR 40.8. Urinalysis: negative for blood, 0.3 g/L protein; urine sediment - bland. 24-hour urine collection - 0.34 g. Serology - ANA, dsDNA, RF, Hep B Ag, Hep B Ab, Hep C Ab, HIV - all negative; C3/C4 - normal. IgG - slightly low, IgA and IgM normal, no M protein, no free urinary monoclonal light chains.

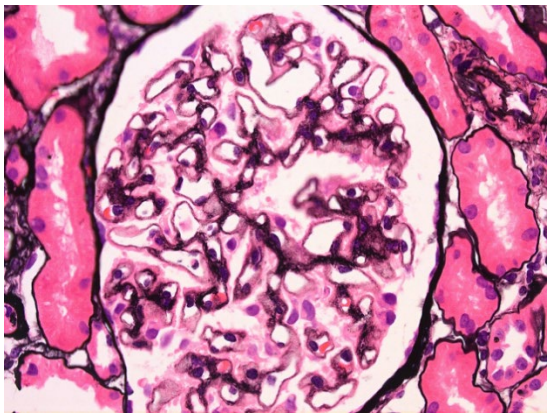
Management: Referred to rheumatology for assessment of arthralgias. In follow-up, ACR 67.8, patient declining conservative management, but there was a plan for repeat 24-hour urine collection and proceeding to renal biopsy. Repeat 24-hour urine - 0.84 g, ACR 337. Started on olmesartan medoxomil 20 mg daily and underwent renal biopsy.

Light Microscopy



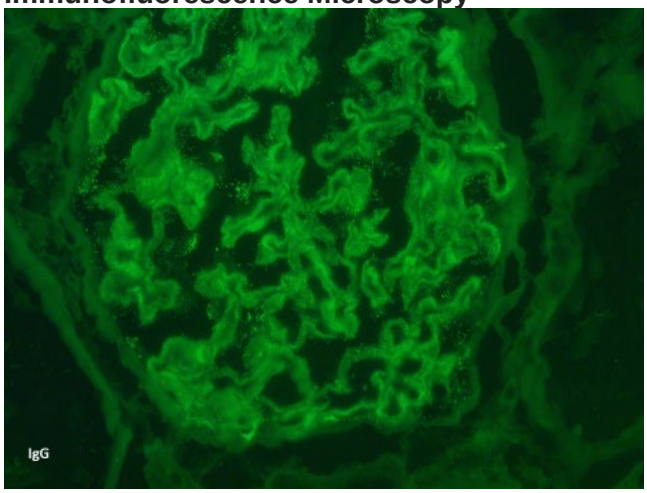


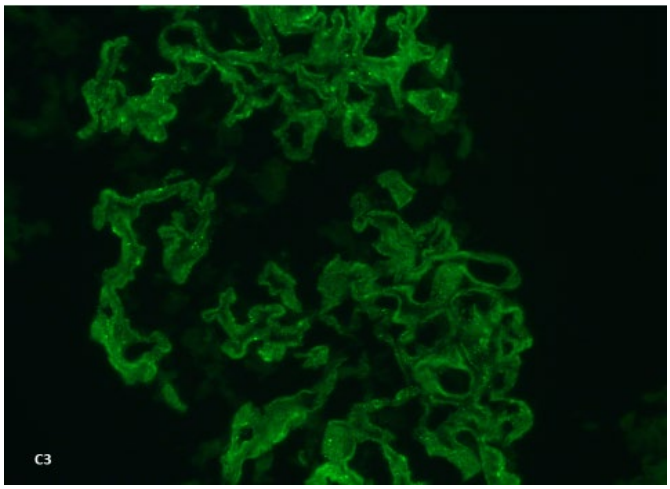
PAS Staining

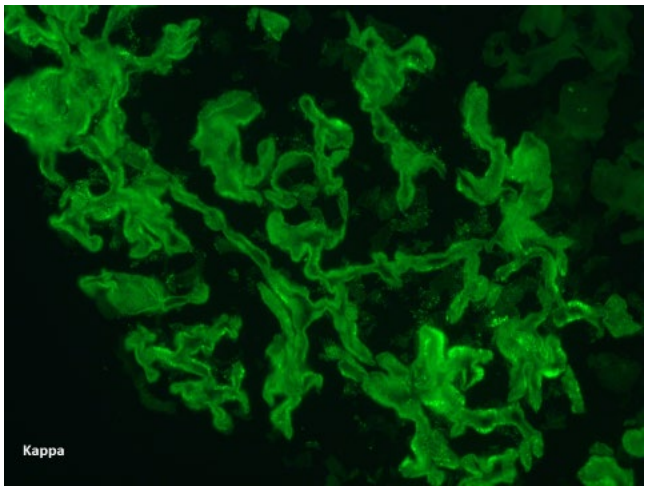


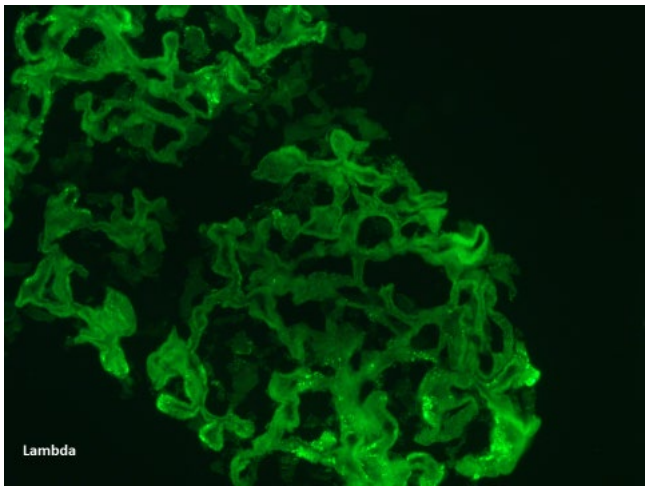
Silver Staining

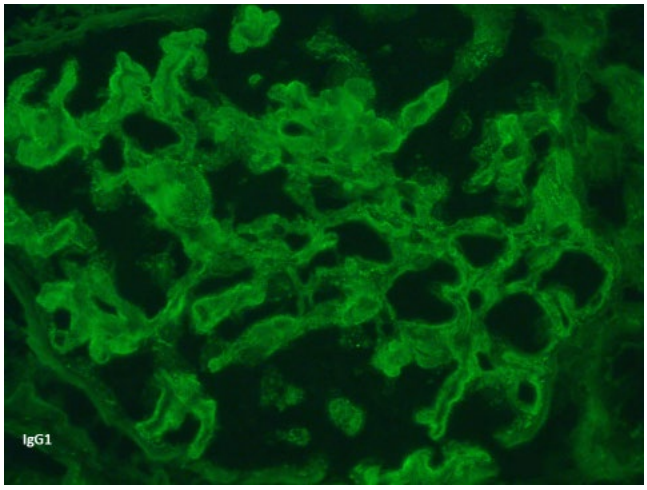
Immunofluorescence Microscopy

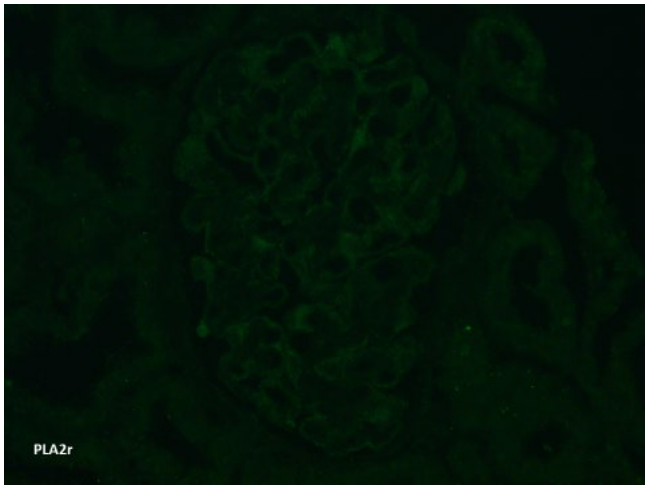




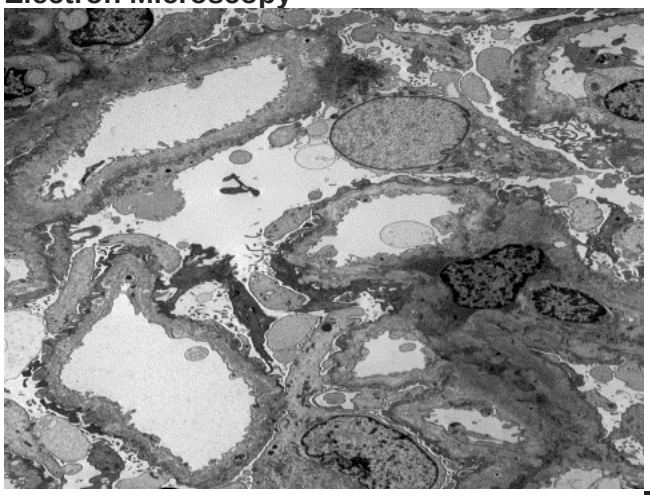


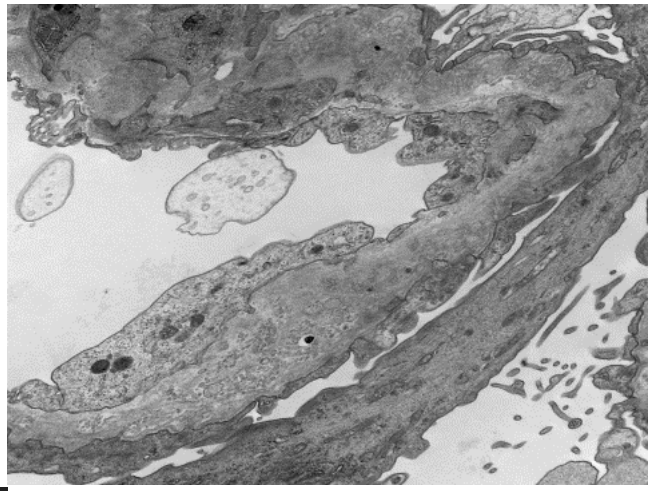


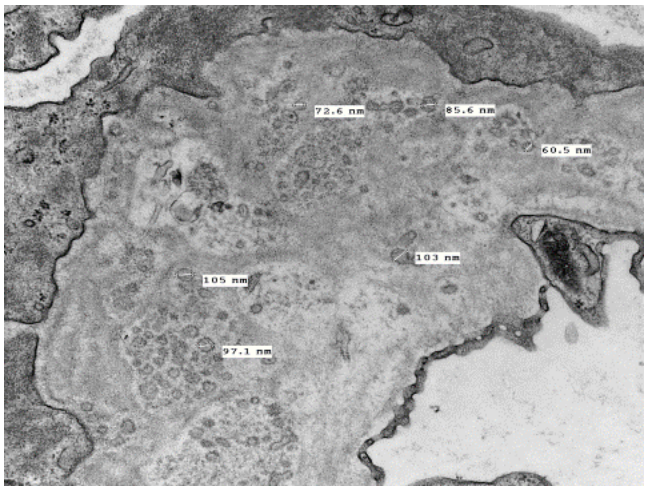




Electron Microscopy







Pathologic Findings

Light Microscopy (LM): The biopsy showed 41 glomeruli, 2 of them globally sclerosed. The remaining glomeruli display a membranous pattern of injury with diffuse thickening of the glomerular basement membrane (GBM) with mild mesangial expansion due to the segmental increase in the mesangial matrix without increase in cellularity. Silver staining does not show spikes, but the GBMs appear irregular, and some bubbling is identified. There is no interstitial fibrosis.

Immunofluorescence Microscopy (IM): The indirect IM shows mild trace linear staining for IgG, C3, kappa, and lambda along the GBMs with negative staining for IgM, IgA, and C1q. Staining for IgG subtypes showed mild linear trace staining along GBM for IgG1 and IgG4 with complete negative staining for IgG2 and IgG3. Staining for PLA2r was negative with a good positive control.

Electron Microscopy (EM): Ultrastructural examination of 3 glomeruli reveals irregular thickening and remodeling of the GBMs by the presence of clusters of microspherules ranging between 49 to 80 nm in diameter (not homogeneous in size). No evidence of immune type of electron dense deposits is seen. No tubuloreticular inclusions are identified. There is extensive foot-process effacement.

Questions for Case 4

1. How do you define the morphological pattern at light microscopy?

A. Mesangioproliferative
B. Membranous
C. Membranoproliferative
D. Diffuse proliferative
E. Early nodular pattern

This biopsy shows a membranous pattern with diffuse thickening of the GBM. There is no evidence of endocapillary or mesangial hypercellularity, ruling out mesangioproliferative, diffuse proliferative, and membranoproliferative pattern (no double contours). There is mild mesangial expansion due to the increase in mesangial matrix, but no nodular pattern is seen.

2. How do you interpret the immunofluorescence microscopy findings?

A. Mild trace linear positivity for IgG, IgG1, Kappa, Lambda, and C3
B. Finely granular staining positive for IgG, IgG1, Kappa, Lambda, and C3
C. Coarse granular staining positive for IgG, IgG1, Kappa, Lambda, and C3
D. Background staining for everything
E. Pseudolinear positivity for IgG, IgG1, Kappa, Lambda, and C3

There is mild trace linear staining for IgG, IgG1, Kappa, Lambda, and C3. Mild trace linear could be defined as not bright, in the scale of brightness 1+ out of 4+. No granularity is seen. This mild trace linear does not seem to be only background since it is completely negative for IgM, IgA, C1q, IgG2, IgG3, and PLA2r. It is not pseudolinear since there is no confluent granularity.

3. What are the ultrastructural findings in the GBM?

A. Immune complexes
B. Immunotactoids
C. Fibrils
D. Microspherules
E. Organized deposits

Ultrastructural examination reveals infiltration of the GBM by microspherules of different sizes—between 49 to 80 nm in diameter. Microspherules are not organized deposits; they are not immune complexes. Organized deposits are glomerular deposits organized as fibrils or microtubules. Immunotactoids are organized deposits forming microtubules ranging between 30-50 nm.

Diagnosis

Podocyte infolding glomerulopathy (PIG)

Discussion of Renal Pathology Findings

This biopsy shows a membranous pattern of injury with a lack of immune-complexes or organized deposits (no fibrils or microtubules). The features at IM examination are important to integrate the diagnosis. No granular staining supporting immune-complexes associated with membranous nephropathy is seen, ruling out the presence of immune-complexes. The presence of mild trace linear IgG1, IgG4, kappa, and lambda rules out any monoclonal gammopathy with a membranous pattern. The EM is the most important tool for the diagnosis, ruling out immune-type electron dense deposits and revealing massive infiltration by clusters of microspherules with extensive foot process effacement. Those findings are classic of PIG. Other differential diagnosis includes partially cleared deposits from previous membranous nephropathy. No evidence of immune complexes was found by IM or EM; to

support this fact, the patient never presented a nephrotic syndrome, and 24-hour proteinuria has always ranged between 0.3 and 0.8 g.

To integrate the diagnosis of PIG, we need to find:

1. LM: Thickening of the glomerular capillary walls due to the presence of non-argyrophilic intramembranous bubbles similar to those found in membranous glomerulopathy.

2. IM: The lack of immune-type electron-dense deposits of immune complexes by ultrastructural examination and by IM. IM usually shows a mild trace linear staining for IgG; however, no granular deposits are seen.

3. EM: The presence of clusters of translucent microspheres in different sizes (50-150 nm) generated by invagination of the podocyte cytoplasm into the basement membranes.

In its pure from, not associated with any other disease, light microscopy of PIG is characterized by thickening of the capillary walls due to the presence of non-argyrophilic intramembranous bubbles similar to those found in membranous glomerulopathy, but without electron-dense deposits of immune complexes by EM and IM. At ultrastructural examination, translucent microspheres generated by invagination of the podocyte cytoplasm into the basement membranes are observed. The infolding or protrusion of podocyte cytoplasm into the GBM results in intramembranous cytoplasmic microspherules and few microtubules resulting from the connection of the microspherule and the site of the infolding in the surface of the GBM. The pathogenesis of PIG remains poorly understood; therefore, its specific treatment and clinical course are unknown.

Proposed Mechanisms from Podocyte Infolding

The pathogenesis of PIG remains poorly understood; therefore, its specific treatment and clinical course are unknown.

PIG is a manifestation of a form of podocytopathy that leads to altered podocyte/basement membrane interactions with incorporation of podocyte cell membranes into the GBMs. The first published case of PIG data from 1985 is by Dale, *et al*, when they described what they thought were massive deposits of organelles, morphologically identical to nuclear pore complexes (NP), in the GBM of an individual with membranous nephropathy.

The presence of microspherules in other conditions, such as in association with membranous nephropathy, may suggest that some cases of podocyte infolding are driven or promoted by factors extrinsic to podocytes (such as immune complex deposition). However, those cases show the presence of immune complexes by IM and EM and should be considered as membranous nephropathy with focal podocyte infolding, a different entity from PIG alone, where no immune complexes are identified. Other proposed mechanism in patients with autoimmune diseases such as systemic lupus erythematosus (SLE) is the presence of antibodies against metalloproteinases of the GBM that may promote the invagination of the GBM.

Clinical Features in PIG

Up to now, 38 cases of PIG have been reported worldwide. It has been mainly described in Japanese literature but also described in white patients, usually presenting as subnephrotic range proteinuria (as in our patient). Nephrotic syndrome has been described to occur in approximately a third of patients. Podocyte infolding changes have also been described as being associated with autoimmune diseases such as SLE, Hashimoto thyroiditis, and Sjögren disease.

From the 38 cases with PIG described in the literature, proteinuria usually is in the subnephrotic range in the majority of cases, with 28.6% (10/35) that have proteinuria in the nephrotic range, and 27.7% (10/36) that are described as having nephrotic syndrome. Of the case reports that documented patient

outcomes, 45.2% (14/31) described complete remission of proteinuria, defined as a 24-hour urine protein of ≤0.3 g/day. Another 45.2% (14/31) of patients had partial improvement in proteinuria. Remission was usually in response to immunosuppressive therapy. However, there were 3 patients who had complete remission of PIG while not taking immunosuppressant medication.

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