

KIDNEY BIOPSY TEACHING CASE

Subepithelial Humps and Microthrombi: Looking for a Mechanism

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INDEX WORDS: hemolytic uremic syndrome; acute post-streptococcal glomerulonephritis; factor V Leiden; nephritis-associated plasmin receptor.

HEMOLYTIC UREMIC syndrome (HUS) and acute poststreptococcal glomerulonephritis (APSGN) are 2 common causes of acquired renal disease in the pediatric population. Simultaneous development of these disorders clinically and/or histologically is rare, but has been reported in the literature.¹⁻⁵ The nature of the interaction between these 2 disease processes is unclear, as are potential predisposing factors. We present the case of a 5-year-old boy with the simultaneous occurrence of both disorders and suggest a possible pathogenic risk factor.

CASE REPORT

A 5-year-old boy with a history of mild reactive airway disease presented to a local emergency department with a 10-day history of an upper respiratory tract infection, anorexia, vomiting, decreased energy, and headache. There was no history of diarrhea. Oliguria and dark urine developed 3 days before admission. On initial evaluation, he was found to be hypertensive, anemic, and thrombocytopenic and was transferred to our institution for further evaluation and management. Vital signs showed a blood pressure of 160/120 mm Hg. Physical examination was remarkable for pallor, abdominal and lower-extremity petechiae, and mild facial edema. His father had died of a myocardial infarction at 46 years of age. There was no family history of renal disease or thrombosis.

Initial laboratory evaluation showed the following values: serum blood urea nitrogen, 166 mg/dL (59.3 mmol/L); creatinine, 4.1 mg/dL (362 μ mol/L); albumin, 2.1 g/dL (21 g/L); hemoglobin, 8.1 g/dL (81 g/L); platelets, 57,000/ μ L; lactate dehydrogenase, 5,322 IU/L; third component of complement (C3), 20 mg/dL (0.20 g/L); C4, 14 mg/dL (0.14 g/L); streptozyme, 1:400; and anti-streptolysin O antibody, 819 IU/L. Blood smear showed microcytic, polychromatophilic, and fragmented erythrocytes. Haptoglobin level was less than 11 mg/dL (0.11 g/L; normal, 74 to 332 mg/dL [0.74 to 3.32 g/L]). Antinuclear antibody, antineutrophil cytoplasmic antibody, and anti-glomerular basement antibodies were all negative. Coagulation studies showed normal prothrombin time, partial thromboplastin time, fibrinogen level, and thrombin time and an elevated d-dimer at 7.9 μ g/mL (normal, <0.5 μ g/mL). Urine showed 2⁺ blood, 3⁺ protein, and active sediment with erythrocyte, renal tubular, leukocyte, hyaline, and granular casts present. A renal ultrasound showed enlarged echogenic kidneys with a normal Doppler evaluation. He underwent hemodialysis on admission, received packed red blood cell transfusions, and, subsequently, a renal biopsy was performed.

Renal Biopsy Findings

The renal biopsy specimen contained 55 variably hypercellular glomeruli. The majority had global endocapillary proliferation with exudative infiltrates that obscured capillary lumens; cellular crescents occupied Bowman space in approximately 40% (Fig 1). Others had moderate mesangial hypercellularity associated with focal mesangiolysis, ectatic blood-filled capillary lumens, occasional fragmented erythrocytes, and few fibrin thrombi, either in peripheral capillaries or, uncommonly, at the hilum. Rare necrotic lobules were noted. Occasional glomeruli possessed features of both processes. There was mild edema without significant fibrosis and patchy lymphoplasmacytic infiltrates in the interstitium. Many tubules were filled with erythrocytes or protein casts and lined by mildly vacuolated and attenuated epithelium.

Immunofluorescence staining on frozen sections showed coarse granules along the peripheral capillary loop positive for immunoglobulin G (1⁺) and C3 (4⁺) that also strongly stained the mesangium. Granular immunoglobulin M (1⁺) staining of the mesangium was noted. Fibrinogen was present in Bowman space associated with crescents, but not readily detected in the glomerulus. There was no staining with antibodies directed toward immunoglobulin A, C1q, or C4.

Electron microscopic analysis showed occasional glomerular hump-like subepithelial electron-dense deposits plus scattered small subendothelial deposits (Fig 2). Capillary lumens were narrowed by subendothelial flocculent material and markedly swollen endothelial cells. Foot processes of visceral podocytes were diffusely effaced. The mesangium was hypercellular, and mesangial interposition was focal. Pathological findings were interpreted as postinfectious glomerulonephritis with thrombotic microangiopathy.

Follow-Up

The patient received pulse methylprednisolone therapy for 3 days, followed by a slow taper of oral prednisone dosage for approximately 8 weeks. He required intermittent hemodialysis therapy and antihypertensive medications for

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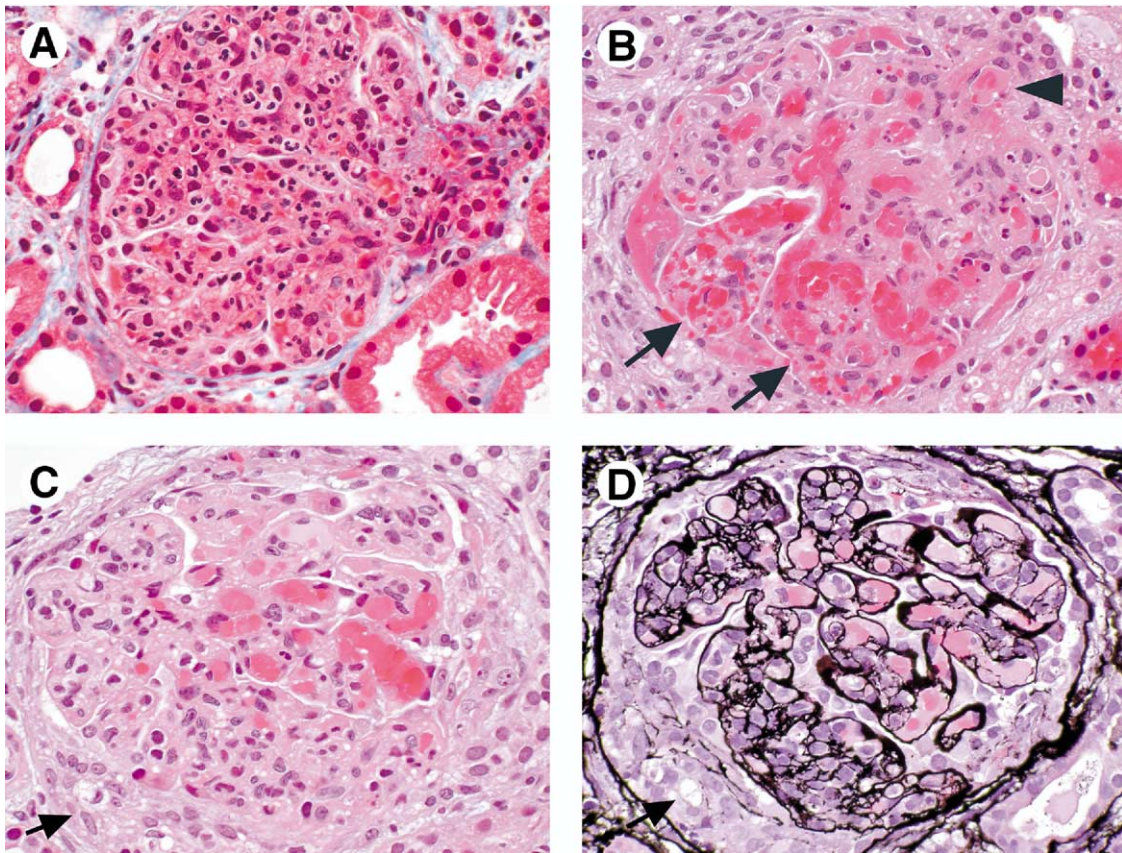


Fig 1. (A) A hypercellular glomerulus has endocapillary proliferation and an exudative infiltrate that imparts a lobular configuration and often obscures peripheral capillaries (trichrome; original magnification $\times 400$). (B) Capillaries in this moderately hypercellular glomerulus are distended with blood. Fibrin fills the lumen of a hilar arteriole (arrowhead). Fragmented erythrocytes are evident (arrows) (hematoxylin and eosin; original magnification $\times 400$). (C, D) Some glomeruli showed features of microangiopathy and APSGN. The markedly hypercellular lobules (left and lower) have neutrophilic infiltrates and an adjacent cellular crescent (arrows), whereas the opposite quadrants have congested capillaries and focal mesangiolysis (hematoxylin and eosin, Jones methamine silver; original magnification $\times 400$.)

3 weeks. Lactate dehydrogenase levels normalized within 3 months. C3 and C4 levels returned to normal 5 weeks after presentation, with levels of 133 and 25 mg/dL (1.33 and 0.25 g/L), respectively. Because of the known association between low serum C3 levels and factor H deficiency in a select subset of patients with atypical (diarrhea-negative) HUS,⁶ the patient's factor H level was measured and found to be normal. The patient's strong family history of early myocardial infarction combined with the unusual presence of microangiopathy in a biopsy specimen obtained from a patient with APSGN led to a suspicion that he may have an underlying hypercoagulable state. Evaluation of several coagulation proteins was performed. These studies showed a heterozygous factor V Leiden mutation and factor VIII activity level elevated at 158% (normal, 50% to 150%). He never had an episode of clinically overt thrombosis. Seventeen months after presentation, his creatinine level is 1 mg/dL (88 μ mol/L), and he still requires amlodipine, labetalol, and enalapril for blood pressure control.

DISCUSSION

This patient presented with several classic clinical features of APSGN, including an antecedent upper respiratory tract infection, gross hematuria, oliguria, and hypertension. He lacked bloody diarrhea associated with typical HUS, but had several laboratory findings indicative of thrombotic microangiopathy, including microangiopathic hemolytic anemia and thrombocytopenia. Although this is an unusual presentation, similar cases have been published previously (Table 1). A unifying pathogenetic mechanism is unknown, although 1 hypothetical paradigm is that thrombotic microangiopathy occurs as a secondary manifestation of endothelial injury

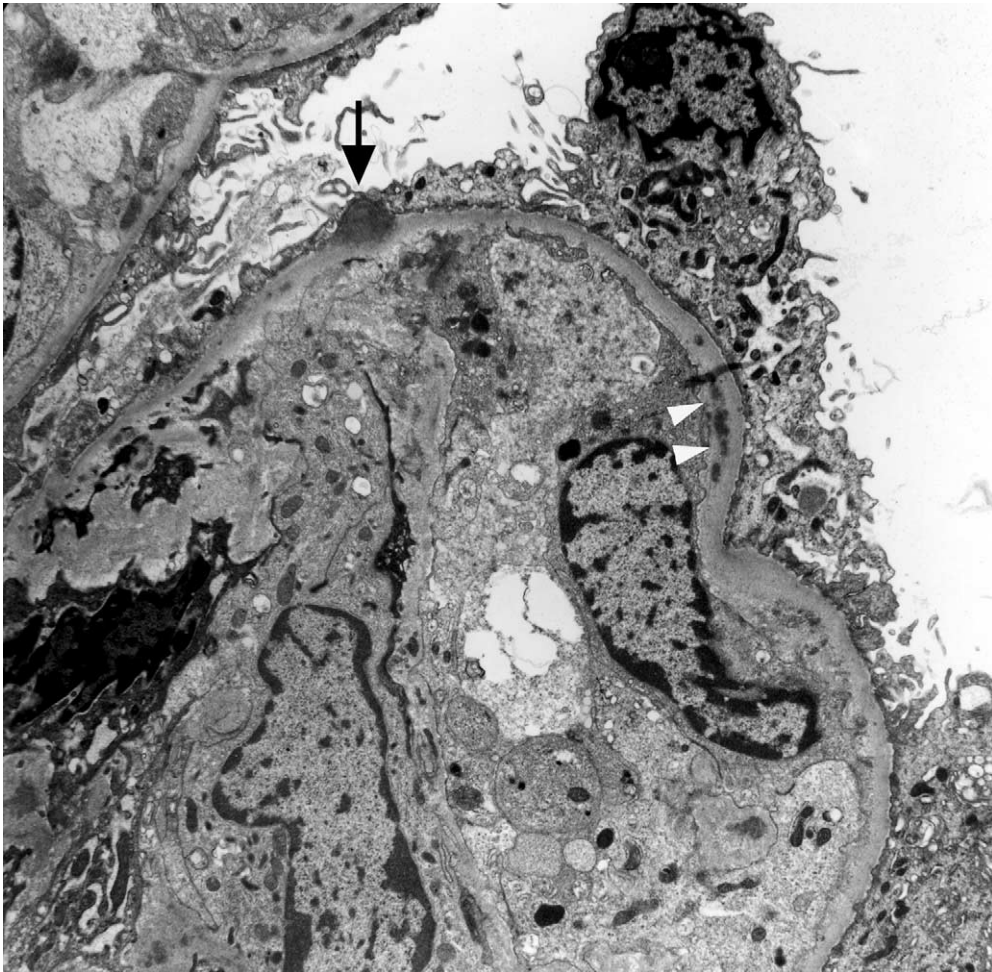


Fig 2. Electron microscopic findings. There is diffuse podocyte foot-process fusion and markedly swollen endothelial cells that narrow the lumen. Occasional small subendothelial electron-dense deposits are present (arrowheads). Large subepithelial humps are focally prominent (arrow).

induced by severe hypertension caused by APSGN.^{2,3,5}

The family history of early myocardial infarction in this case led to further investigations for genetic risk factors associated with hypercoagulability. The finding of a heterozygous factor V Leiden mutation is unique among reported patients with simultaneous APSGN and HUS, although these data were not reported for the other patients. Could this mutation be an unrecognized risk factor for the development of thrombotic microangiopathy in a patient with APSGN? We are aware of only 1 other report in the literature of a patient with atypical HUS and a factor V mutation. The described patient was a 29-year-

old woman administered oral contraceptives who developed atypical HUS.⁷

Recent work identified the nephritis-associated plasmin receptor (NAP1r) as a dominant nephritogenic antigen in patients with APSGN.⁸ This antigen, which is identical to glyceraldehyde-3-phosphate dehydrogenase of group A streptococcus, has been postulated to stabilize plasmin activity and trigger an enzymatic cascade involving the activation of metalloproteinases and subsequent damage to the glomerular basement membrane (GBM) and podocytes in patients with APSGN⁹ (Fig 3). Diagnostic renal biopsies usually are restricted to patients with atypical manifestations of APSGN because the clinical presen-

Table 1. Clinical Characteristics of Previous Case Reports

Features	Current Report	De Chadarevian et al ¹	Laube et al ²	Laube et al ²	Tan et al ³	Medani et al ⁴	Proesmans et al ⁵
Age (y)	5	5.5	12	6	10	13	14
Sex	M	M	M	F	F	M	M
Initial findings							
Creatinine (mg/dL)	4.1	2	14.6	2.2	15.8	7.2	1.1
Serum albumin (g/dL)	2.1	4.2	3.1	ND	2.6	2.9	ND
Hemoglobin (g/dL)	8.1	6.2	5	6.4	Hematocrit, 12%	11.2	11
Hemolysis?	Fragments	Fragments	Schistocytes	Schistocytes	ND	Fragments	ND
Platelets (1,000/ μ L)	57	132	44	60	148	97	99
C3 (mg/dL)	20	89	10	15	16	23.5	27
C4 (mg/dL)	14	16	10	24	18	35.3	18
ASOT	+	ND	+	+	+	+	ND
Proteinuria	3+	300 mg/dL	+	+	4+	4+	3+
Hematuria	+	15 RBC/HPF	+	+	3+	Gross	+
Casts	RBC, WBC	ND	Granular	+	+	Granular	ND
Blood pressure (mm Hg)	160/120	110/60	140/100	160/100	150/80	150/112	200/110

NOTE. To convert serum creatinine in mg/dL to μ mol/L, multiply by 88.4; albumin and hemoglobin in g/dL to g/L, multiply by 10; C3 and C4 in mg/dL to g/L, multiply by 0.01. Abbreviations: ND, not done; RBC, red blood cell; WBC, white blood cell; HPF, high-power field; ASOT, anti-streptolysin O titer.

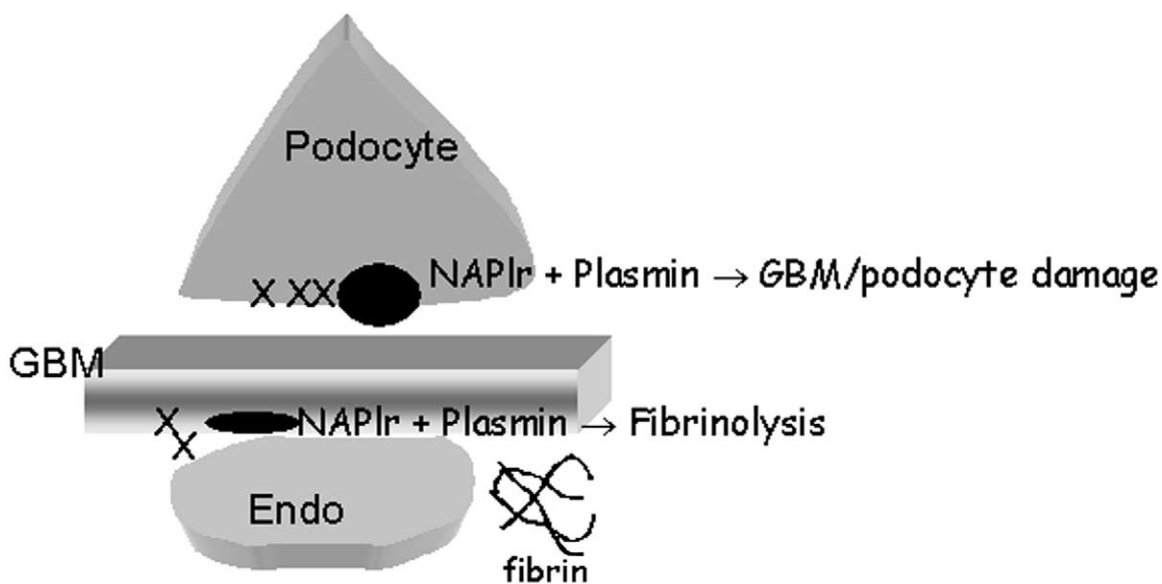


Fig 3. Proposed pathogenetic paradigm. The NAPlr antigen deposited in the subepithelial location may contribute to podocyte and GBM damage by concentrating plasmin and collagenase activity in the area. Conversely, when the NAPlr antigen deposits on the subendothelial aspect of the GBM, it may serve to enhance protease activity along the glomerular capillary wall and prevent the accumulation of fibrin strands caused by enhanced plasmin fibrinolytic activity. In the presence of a hypercoagulable state, local plasmin activity may be inadequate to prevent a microangiopathic process.

tation, positive streptococcal serological study results, and time-limited hypocomplementemia make it possible to presumptively diagnose APSGN in most patients. Fibrinogen deposition has been observed along the endothelial aspect of the GBM in 60% of renal biopsies performed during the acute phase of APSGN.⁸ Recent work by Oda et al⁹ identified intraglomerular plasmin activity in the kidneys of patients with APSGN, but not in normal control kidneys. Although this plasmin activity may promote protease-dependent damage to the glomerular capillary wall, both directly and indirectly through activation of latent metalloproteinases, plasmin activity also may enhance the recovery process through its fibrinolytic activity. Release of fibrin into Bowman space is thought to recruit macrophages and contribute to the formation of crescents, as observed in 40% of our patient's glomeruli. It is plausible that his factor V Leiden mutation led to more aggressive fibrin deposition within the glomerular capillaries in response to subendothelial immune complex deposition and/or hypertension-induced endothelial damage. Although large subepithelial immune deposit "humps" are considered a hallmark feature of APSGN, subendothelial

deposits often are detected when renal biopsies are performed during the early phase of the disease.

This patient had atypical HUS. Although several predisposing factors have been implicated in the pathogenesis of atypical HUS, most genetically determined factors involve abnormalities in the complement cascade, especially low levels of factor H. Although factor H level was normal in this patient, we cannot rule out the possibility of an anti-factor H antibody leading to depressed factor H activity, which has been detected in 3 patients with atypical HUS.¹⁰ Although this patient's glomerular C3 deposits were impressive, this is an expected finding in patients with uncomplicated APSGN. NAPlr itself is known to activate the alternative complement cascade *in vitro*.⁸

Because the risk for thrombosis is increased 5- to 10-fold in a patient with heterozygous factor V Leiden mutation,¹¹ it is possible that his naturally enhanced propensity for thrombosis could have triggered a unique interaction not usually seen in patients with APSGN who have a normal clotting cascade. It is tempting to speculate that in patients with typical APSGN, localized plasmin activity trapped within the subendothelial

space by the NAPlr antigen efficiently degrades small fibrin strands before microangiopathic injury can develop (Fig 1). When fibrin deposition is more aggressive, as in the case of factor V Leiden mutations, local plasmin activity may be overwhelmed and thrombotic microangiopathy ensues. Whether similar prothrombotic risk factors may have accounted for the simultaneous occurrence of APSGN and HUS in the other patients is unknown.

Factor VIII levels also were transiently elevated in this patient during his acute illness. Medani et al⁴ reported an elevated factor VIII level in their patient and postulated that this elevation could be a marker of severe endothelial injury that initiated platelet aggregation and erythrocyte fragmentation. Based on the current case, it seems reasonable to suggest that when a patient develops renal disease with features of both APSGN and HUS, a workup for evidence of an underlying hypercoagulable state should be considered, including evaluation for factor V Leiden mutation, prothrombin mutation, and antiphospholipid antibodies, as well as abnormal levels of protein C, protein S, and antithrombin III.

In summary, recent studies showed that APSGN is characterized by the deposition of NAPlr along the glomerular capillary wall and de novo generation of intraglomerular plasmin activity. Although such activity may have a role in the pathogenesis of glomerular injury in patients with APSGN, the associated features of extensive glomerular fibrin deposition and thrombotic microangiopathy in our patient in the face of factor V Leiden mutation suggests a potentially protective role for the NAPlr-plasmin interaction by preventing fibrin accumulation in the glomerular microcirculation and subsequent injury caused by thrombotic microangiopathy. This

pathogenetic paradigm remains hypothetical until further studies are performed in the unique setting of patients who develop HUS in association with APSGN.

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