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Case Report

Nephrotic-Range Proteinuria and Peripheral Edema in a Child: Not Only Idiopathic Nephrotic Syndrome

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Keywords

Atypical hemolytic uremic syndrome · Nephrotic-range proteinuria · Arterial hypertension · Hemolytic anemia · Thrombotic microangiopathy · Alternative complement pathway · Thrombomodulin gene mutation · Eculizumab

Abstract

Hemolytic uremic syndrome (HUS) is defined by the simultaneous occurrence of hemolytic anemia, thrombocytopenia, and acute kidney injury due to thrombotic microangiopathy (TMA) mainly occurring in renal and cerebral microvessels. Although the most common cause of HUS in children is Shiga toxin-producing *Escherichia coli*, atypical forms in which Shiga toxin is not the trigger may occur. Research over the last few years has shown that complement dysregulation secondary to mutations of genes coding for proteins involved in the regulation of the alternative pathway of complement account for most forms of atypical HUS (aHUS). Among these, thrombomodulin (THBD) gene mutations, representing 3–5% of all alternative pathway complement component abnormalities, correlate with early disease onset and rapid evolution to end-stage renal failure. aHUS onset is generally sudden, but

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occasionally the only manifestations of renal TMA are arterial hypertension, proteinuria, and a progressive increase in serum creatinine. Nephrotic syndrome at disease onset is exceptional. We describe the case of an adolescent female who presented with peripheral edema due to nephrotic-range proteinuria with bioptic evidence of TMA. Study of the alternative complement pathway showed a heterozygous missense THBD gene mutation (P501L variant) consistent with aHUS diagnosis. One year later she developed clinical signs of hemolytic anemia. Eculizumab, an anti-C5 monoclonal antibody, was started with rapid improvement. This case report highlights the phenotypic variability in aHUS due to THBD gene mutation. Early diagnosis by renal biopsy followed by genetic screening is required to optimize management in such a rare disease with a severe prognosis.

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Case Report

A girl aged 13 years and 9 months with no previous morbidity was referred to our department with a 2-week history of evident periorbital and pretibial edema, myalgia, and fatigue. Her blood pressure was 130/80 mm Hg, and urine dipstick testing showed proteinuria +++ and microhematuria ++. She had no recent history of infections and she was not taking any medication. She had a β-thalassemic trait. Her family history was unremarkable for renal diseases but positive for thrombosis. Laboratory investigations revealed mild renal failure with serum creatinine at 1.04 mg/dl (an estimated glomerular filtration rate of 85 ml/min/1.73 m^2 using Schwartz's formula), hemoglobin at 10.1 g/dl, and a normal platelet count. Serum electrolytes, albumin, and the lipid panel were within the normal range. Urine analysis showed proteinuria up to 2 g/24 h and presence of 107 erythrocytes/μl. Renal ultrasound was unremarkable. The complement system components C3 and C4, hemolysis indices, antinuclear and antineutrophil cytoplasmic antibodies, prothrombin time, and activated partial thromboplastin time were normal. Viral serologic markers for hepatitis B, hepatitis C, varicella, cytomegalovirus, Epstein-Barr virus, rubella, measles, and mumps excluded any infection. Thrombophilia screening tests showed mild hyperhomocysteinemia up to 15.5 μmol/l (normal value 4–13) and a methylenetetrahydrofolate reductase (MTHFR) gene homozygous mutation (C677T variant).

Renal biopsy demonstrated diffuse thickening and multilayering of glomerular capillaries with entrapment of red blood cell fragments. Hilar arterioles showed intimal fibromucoid hyperplasia; occasional thrombi occluded vascular lumina (fig. 1). On ultrastructural examination, glomerular capillaries and arterioles were thickened for subendothelial amorphous material deposition and endothelial cell hyperplasia (fig. 2). A routine immunofluorescence panel (IgA, IgG, IgM, C1q, C3, and fibrinogen) was substantially negative in glomeruli; C3 complement fraction stained arteriolar walls (fig. 3). These findings were consistent with thrombotic microangiopathy (TMA).

Normality of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) protease activity (55%; normal value 50–150) and the absence of antibodies against ADAMTS13 excluded thrombotic thrombocytopenic purpura. The C5b-9 membrane attack complex of complement had results in the normal range. Genetic analysis was undertaken to screen for atypical hemolytic uremic syndrome (aHUS) risk factors.

Fluid restriction, a low-sodium diet, and antihypertensive therapy with carvedilol and amlodipine were started with normalization of blood pressure. When the nephrosis had resolved, ramipril was introduced to control proteinuria. A thrombophilic status was treated by cobalamin and acetylsalicylic acid administration. Within 2 months, the proteinuria be-

came negative with persistence of only mild microhematuria. Serum creatinine also progressively returned to the normal range.

Screening for alternative complement factor 3 (C3), factor I (CFI), and factor B (CFB) and membrane cofactor protein (MCP) mutations, as well as for factor H (CFH) and CFI autoantibodies, resulted negative. Molecular analysis of the THBD gene, coding for thrombomodulin, showed a rare heterozygous missense mutation (P501L variant) inherited from the mother consistent with aHUS diagnosis. Furthermore, the patient was carrying 2 CFH gene heterozygous polymorphisms (2016G and 2808T variants) in short consensus region (SCR) 11 and SCR 16, respectively.

One year after disease onset, the patient presented severe microangiopathic hemolytic anemia [hemoglobin 7.2 g/dl, unconjugated bilirubinemia 1.62 mg/dl, lactate dehydrogenase (LDH) mildly increased to 500 U/l, haptoglobin <1 mg/dl, and reticulocytes 11%] with a normal platelet count (236,000/mm^3) and renal function. Circulating C3, C4, and C5b-9 levels were normal. Following vaccination against *Neisseria meningitidis*, eculizumab was introduced at 900 mg weekly for the first 4 doses, followed by 1,200 mg at week 5 and every 2 weeks thereafter (standard dosage). Rapid normalization of hemoglobin, haptoglobin, and LDH was observed. Currently, after more than 1 year of follow-up, the patient is doing well with no disease recurrence.

Discussion

aHUS is a genetic, chronic, and progressive inflammatory disease that represents 5–10% of pediatric cases of HUS and the majority of adult cases [1]. Onset during childhood appears slightly more frequent than during adulthood, and 70% of children have the first episode of the disease before the age of 2 years [2]. aHUS prognosis is generally poor, with a 10–15% mortality rate during the acute phase and up to 50% of cases progressing to end-stage renal disease (ESRD) [3].

The syndrome is caused by defects in complement system regulation which can be inherited, acquired, or both. These defects result in chronic, uncontrolled activation of the complement system, leading to platelet, leukocyte, and endothelial cell activation and systemic TMA [4]. The pathological lesions that define TMA include thickening of arterioles and capillary walls, prominent endothelial swelling and detachment, and subendothelial accumulation of proteins and cell debris. The ultimate outcome is fibrin- and platelet-rich thrombi obstructing vessel lumina with resultant tissue ischemia [5].

aHUS is, therefore, characterized by the triad of mechanical, nonimmune (negative Coombs test) hemolytic anemia (hemoglobin <10 g/dl), thrombocytopenia (platelet count <150,000/mm^3), and renal impairment. The presence of fragmented erythrocytes (schizocytes) and high LDH and undetectable haptoglobin levels confirms intravascular hemolysis [2].

Although the onset is generally sudden with pallor, fatigue, decreased urine output, and sometimes edema, the disease may occur progressively in approximately 20% of patients, often with fluctuating anemia and thrombocytopenia. Nonetheless, arterial hypertension, proteinuria, and a gradual increase in serum creatinine may represent the only manifestations of renal TMA [2, 6]. Extrarenal findings are also present in up to 20% of cases, and they include cardiac involvement (both acute and chronic in 3–10%) and neurologic abnormalities (in up to 16% of children) such as altered mental status, seizures, and focal neurologic deficits [7].

Extensive research has established an association between aHUS and dysregulation of the alternative complement pathway. Although 50% of patients have a heterozygous loss-of-function mutation in a regulatory gene such as CFH, CFI, or MCP, a gain-of-function mutation in an effector gene such as CFB or C3 and acquired CFH antibodies have been identified in patients with both familial and sporadic aHUS. More recently, mutations in the THBD gene have been associated with aHUS in about 5% of patients [8, 9].

Thrombomodulin is a ubiquitous transmembrane endothelial cell glycoprotein with anticoagulant, anti-inflammatory, and cytoprotective properties. In a study of 152 patients with aHUS, 7 patients had 6 different heterozygous mutations of the THBD gene. Analysis in vitro confirmed that wild-type thrombomodulin, in the presence of cofactors such as CFH or C4b-binding protein, negatively regulates complement by accelerating factor I-mediated inactivation of C3b. By binding to thrombin – thereby preventing it from activating C5 – and by promoting activation of the plasma procarboxypeptidase B, thrombomodulin also accelerates the inactivation of anaphylatoxins C3a and C5a, providing additional protection of the membrane surface. Cultured cells expressing thrombomodulin variants associated with aHUS show reduced capacity to inactivate C3b and to activate procarboxypeptidase B and are thus less protected from activated complement. Delvaeye et al. [8] described a male child with the same P501L THBD gene variant (patient S015 in their study) as found in our patient, who developed recurrent aHUS presenting at 6 months of life and resulting in ESRD. In vitro, this rare mutation moderately reduces thrombomodulin expression, inducing a predisposition to the endothelial injury and microvascular thrombosis typical of aHUS. Noris et al. [3] also showed that patients with CFH and THBD abnormalities have the earliest disease onset (0–1 years); however, in 12–50% of subjects, aHUS may occur after the age of 25 years. In our patient, a carrier of the THBD P501 variant, disease onset was around the age of 14 years with specific clinical manifestations. These data underline the phenotypic variability that can occur with the same THBD abnormality and suggest that a mutation of a single THBD allele is not sufficient to cause aHUS. In our patient, the coexistence of CFH polymorphic 2016G and 2808T variants – on SCR 11 and SCR 16, respectively – may have contributed to disease onset. The data show that common polymorphisms of the CFH gene are strongly associated with aHUS and may have a role in disease manifestations in subjects with or without CFH mutations [3, 10]. Therefore, additional genetic, epigenetic, or infectious factors (especially upper respiratory tract infection, fever, or diarrhea) are likely necessary to trigger an aHUS onset later in life [8, 11].

THBD P501L mutation has also been reported in patients with venous thrombosis [8]. Although the data show that the thermolabile variant (C677T) of MTHFR does not seem to be a significant risk factor for venous thromboembolic events [12], the coexistence in our patient of THBD abnormalities and moderate hyperhomocysteinemia may potentiate a systemic risk of thrombotic and ischemic events, worsening disease prognosis.

Recent data have demonstrated that patients with THBD mutations had a poor outcome, with evolution to ESRD in 46% of the patients after 1 year and 54% after 3 years of follow-up [3]. In theory, all patients with aHUS who have reached ESRD could be candidates for renal transplantation. The overall risk of post-renal transplantation aHUS relapse is up to 50%, while the risk of recurrence in patients with THBD mutation is not well documented [2, 7].

If we consider thrombomodulin as a transmembrane protein, we should not expect a disease recurrence after renal transplantation. Unexpectedly, Delvaeye et al. [8] reported that in 1 patient (S924), aHUS had developed for the first time in the allograft, and in a second patient (S015), the disease recurred 3 days after renal transplantation. This can be ex-

plained by the existence of a soluble thrombomodulin form generated by enzymatic cleavage of the intact protein. If mutated, this plasmatic form, which normally has vasculoprotective properties and anticomplement activity, may be inadequate in either amount or quality to provide sufficient protection or may actually contribute to the disease. Renal transplantation, in which wild-type thrombomodulin expression may also be downregulated, would not be expected to affect the composition of the soluble form. Thus, aHUS might develop or recur in an otherwise healthy allograft [3, 13].

Although plasma therapy has empirically been the treatment of choice for aHUS for several years, eculizumab now represents the gold standard of aHUS management also as rescue therapy or to prevent recurrence after renal transplantation. Eculizumab, a recombinant, monoclonal, humanized anti-C5 antibody, blocks C5 cleavage and the formation of the membrane attack complex C5b-9. This results in reduction of the terminal complement activation that occurs in patients with complement-mediated HUS, thereby reducing endothelial damage, thrombosis, and subsequent renal injury [6, 14].

Legendre et al. [4] recently reported on eculizumab's efficacy and safety in 2 prospective, phase II, multicenter trials carried out on patients ≥12 years of age with primary aHUS or recurrent aHUS after transplantation. In over 80% of these patients, eculizumab induced significant improvement in renal function and inhibition of complement-mediated TMA, resolving hemolysis, as has occurred in our patient. However, the long-term risks of therapy, such as meningococcal infection and the immune response to the drug, are still unknown. What is certain is that immunity against *Neisseria* meningitis depends on the lytic terminal complement complex C5b-9. Thus, to prevent fatal meningococcal infection, all children treated with eculizumab should be vaccinated or receive antibiotic prophylaxis [14].

Conclusion

In conclusion, we report a case of aHUS due to the THBD P501L variant that shows the important phenotypic variability that can occur with the same mutation and how, rarely, these patients can present with an extremely unspecific clinical manifestation. In our case, the bioptic evidence of TMA and the genetic analysis of alternative complement components were essential for the diagnosis of aHUS, as well as strict monitoring of the patient until microangiopathic anemia occurred. Once more eculizumab formed an effective, safe, and well-tolerated therapy, inducing rapid improvement of TMA and maintaining disease remission after more than 1 year of therapy.

Statement of Ethics

All the authors declare that the paper agrees with guidelines for human studies and animal welfare regulations. All the authors declare that the patient's parents gave their informed consent and that the study protocol was approved by the institute's committee on human research.

Disclosure Statement

All the authors have no sponsorship or funding arrangements related to their research. All the authors have no conflicts of interest to disclose.

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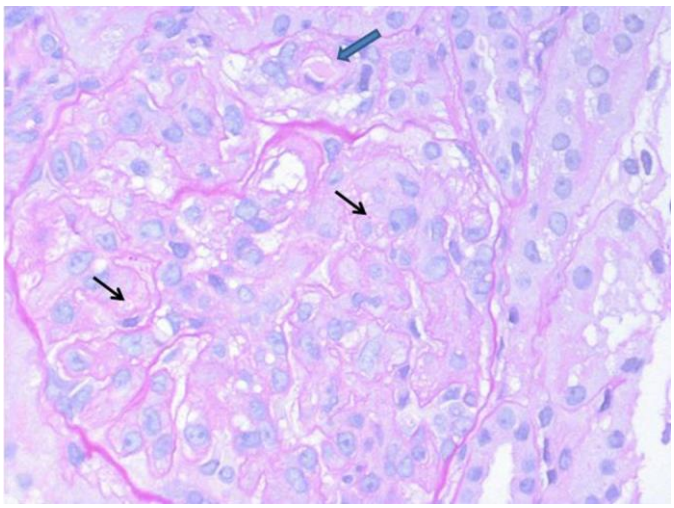

Fig. 1. Histology (F.D.-C.). Diffuse thickening and endothelial cell hyperplasia of capillaries with frequent luminal obstruction. Fragmented red blood cells were trapped in the capillaries' wall (black arrows). A small arteriolar thrombus is evident at the hilum (blue arrow). PAS. ×63.

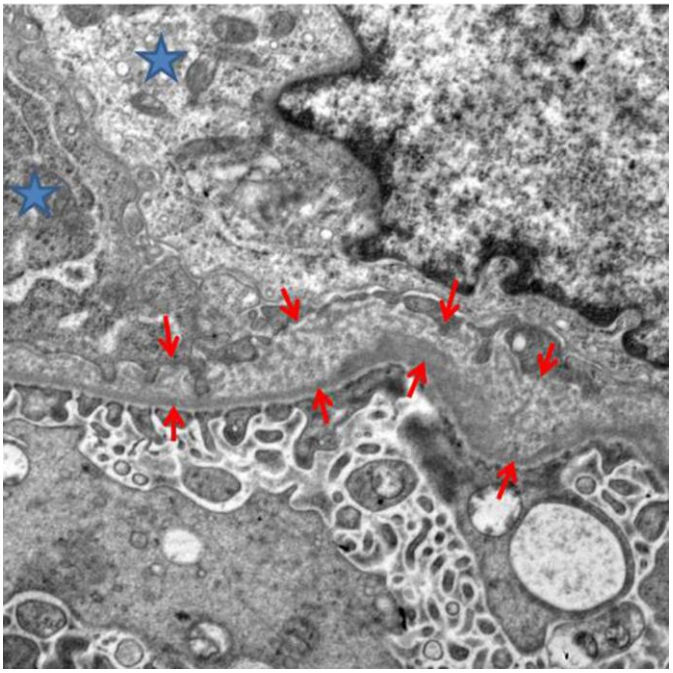

Fig. 2. Electron microscopy (F.D.-C.). Amorphous material deposition in the subendothelial space and multilayering of the glomerular basal membrane (red arrows). Cytoplasmic debris and deformed red blood cells occluding vessel lumina (blue stars).

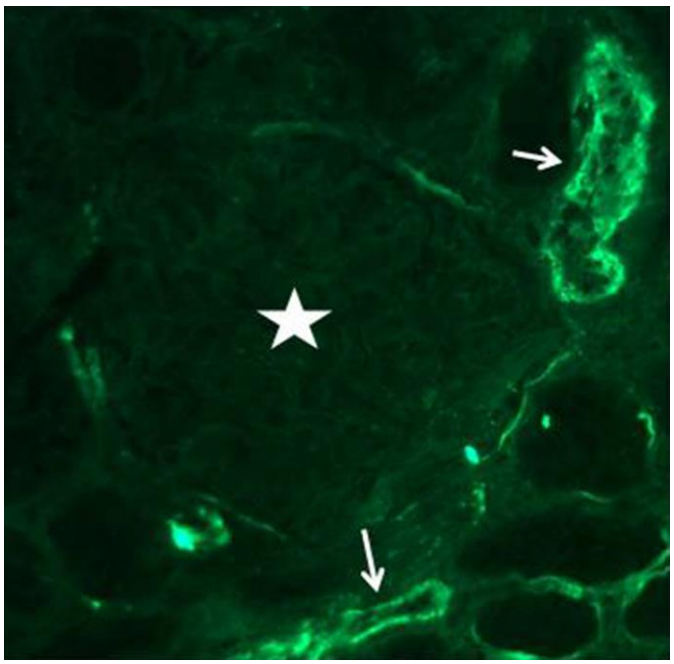

Fig. 3. C3 immunofluorescence (F.D.-C.). Glomerular substantial negativity (white star) and moderate staining of afferent and efferent arteriolar walls (white arrows).