

Atypical Hemolytic Uremic Syndrome: Atypical Course and Atypical Mutations Combination

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Case Report

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Abstract

Atypical hemolytic uremic syndrome (aHUS) is a rare thrombotic microangiopathy, associated with dysregulation of the alternative pathway of the complement system, characterized by hemolytic anemia, thrombocytopenia, and acute renal failure. In this report, we discuss the case of a woman with aHUS that carries heterozygous C3, complement factor H and membrane cofactor protein mutations, with an uncommon presentation, clinical course and outcome.

Keywords: Atypical hemolytic uremic syndrome; Complement C3 mutation; Complement factor H; Eculizumab; Membrane cofactor protein

Introduction

Atypical hemolytic uremic syndrome (aHUS) pathogenesis is strongly associated with complement dysregulation [1,2]. HUS is a thrombotic microangiopathy (TMA) clinically defined by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. Mutations and polymorphisms in genes encoding regulatory proteins such as complement factor H (CFH), complement factor I (CFI) and membrane cofactor protein (MCP/CD46), and the complement activating components C3 and factor B (CFB) have been identified. Atypical hemolytic uremic syndrome associated mutations in regulators are loss-of-function, while mutations in activators are gain-of-function [2,3]. Penetrance of the disease is 50%, which explain that the age at onset and

severity of the disease may vary among family members [2]. It is still unclear why aHUS has incomplete penetrance among the mutation carriers.

Eculizumab is a recombinant, humanized, monoclonal immunoglobulin G antibody that targets C5 and has reduced the magnitude of the thrombotic microangiopathy, restored kidney function and improved quality of life but, however, is expensive [2,4,5].

In this report, we discuss the case of a patient with aHUS that carries a pathogenic C3 mutation combined with a CD46 variant and the risk haplotypes MCPggaac and CFH-H3, with an atypical presentation, clinical course and outcome.

Case Report

A 20-year-old female presented in our hospital with abdominal pain with no previous history of diarrhea or fever. She had one uneventful pregnancy at the age nineteen and no other relevant past medical history. She had no family history of kidney disease or aHUS.

On examination she had a blood pressure of 118/56 mmHg, pulse of 60, respiratory rate of 18, oliguric and temperature of 36.5°C. On auscultation of lungs and heart was normal. There was no jugular venous distention. There was any skin rash or purpura. In the abdominal examination there was no distension, rigidity or rebound tenderness, but with generalized pain with deep palpation. Neurological exam result was normal.

The initial investigation revealed: Hemoglobin 9.5 g/L normocytic normochromic, reticulocytosis of 1.40%, platelet count $79 \times 10^6/\mu\text{L}$ and serum creatinine 3.1 mg/dL accompanied by hematuria (>25 per high-power field) and proteinuria (400 mg/dL). A peripheral blood smear revealed schistocytes, lactate dehydrogenase (LDH) level was elevated to 1229U/L and haptoglobin was <0.243 g/L. Direct Coombs test was negative. Renal Ultrasound showed normal renal size, normal

parenchyma-sinus differentiation and excluded obstruction. Abdominal ultrasound documented cholecystitis, which was assumed to be ischemic as a consequence of TMA.

Her complement levels (reference ranges) were as follows: Low C3, 43.9mg/dL (81–167mg/dL); normal C4, 30.2mg/dL (11–42mg/dL); CFB 44.8 mg/dL (19.1 - 38.2) and CHF 73.7 mg/dl (34.5 – 59.0). She had a negative ANA and anti-DNA assays. Stool culture results were negative for E. coli O157. ADAMTS13 activity levels, prior to PE initiation, were 101% (40-130%); CH50, 92.1% (69-129%) and AH50, 65.8% (30-113%).

Due to the clinical presentation with microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury with oliguria supported by hemodialysis, suspicion was high for atypical hemolytic-uremic syndrome and plasma exchange (PE) was started.

Following ten sessions of PE over a period of 11 days, her parameters improved (haemoglobin 10.5 g/dL, platelet count $148 \times 10^6/\mu\text{L}$, LDH 229U/L) and she recovered renal function after 9 days of haemodialysis (**Table 1 and Figure 1**).

	Hospital Admission Hospital Discharge					Follow Up			
	D1	D4	D11	D18	D26	2 months	3 months	6 months	10 months
Hemoglobin (g/ dL)	9,7	6,6	10,5	10,4	12,6	12,5	10,4	11,9	12,4
Platelet count (cells $\times 10^6/\mu\text{L}$)	79	63	148	209	250	238	188	207	221
LDH (U/L)	1229	525	297	366	339	199	367	169	171
Serum creatinine (mg/dL)	3,1	2,18	1,87	1,44	1,16	0,74	0,84	0,75	1,72
Complement C3 (mg/dL)	43,9		51,9	87,4			51,4		
Complement C4 (mg/dL)	30,2		25,9	48,8			35,9		

Table 1: Biochemical parameters variations.

C3 normal range: 81–167mg/dL; C4 normal range: 11–42mg/dL.

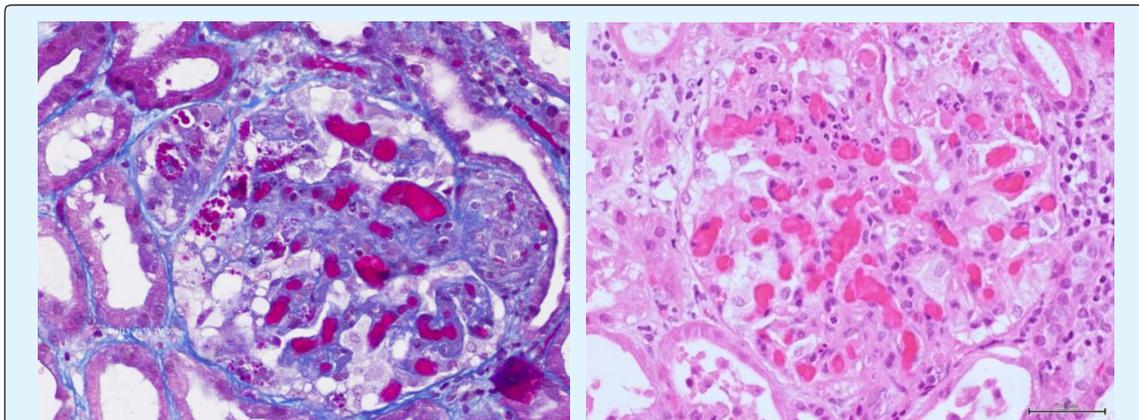
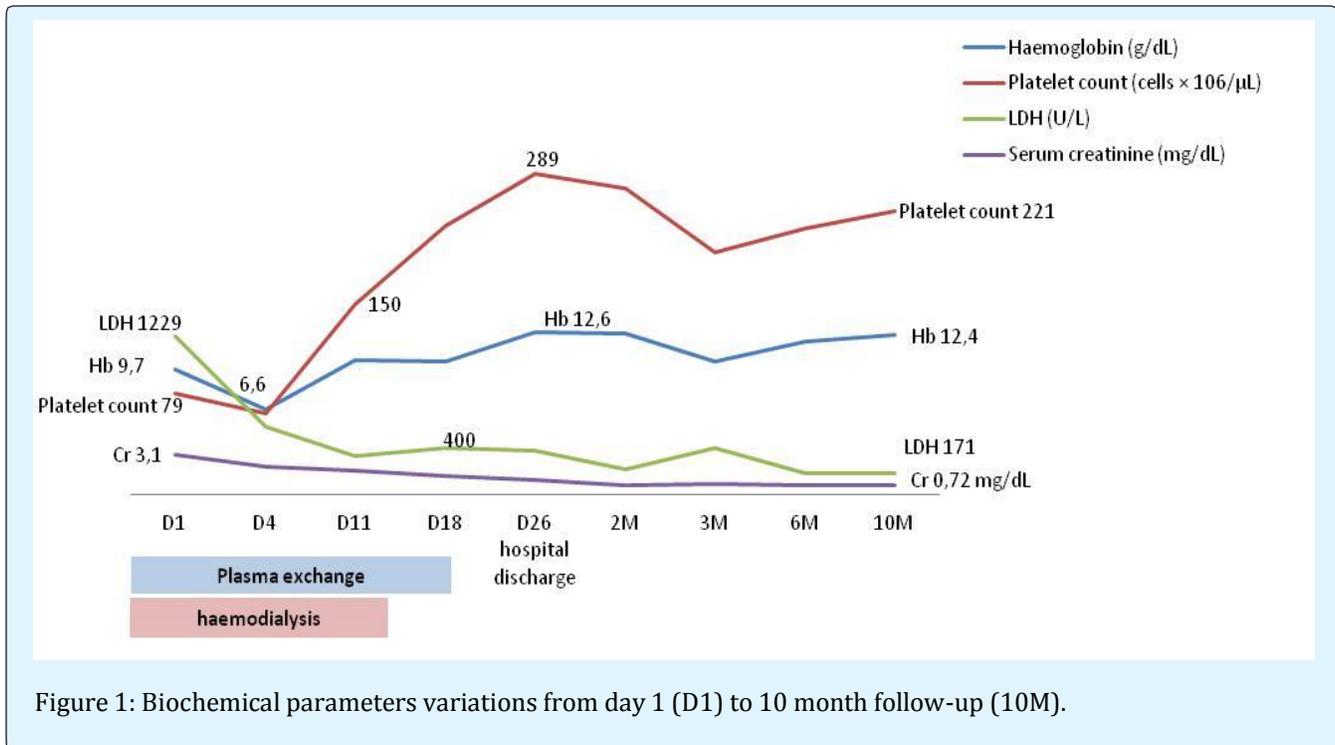


Figure 2: Renal biopsy.

Masson's trichrome stain (200x) showing *afferent arterioles* with fibrinoid necrosis and fragmented erythrocytes (on the left) and hematoxylin and eosin stain with *congestion* filling up *capillary loops* and *mesangiolysis* (on the right).

After 10 months of follow up she is clinically stable and without disease relapses. The molecular studies of complement genes were performed by a next generation sequencing (NGS) - based gene panel (*ADAMTS13*, *CFH*, *CFHR1*, *CFHR3*, *CFHR4*, *CFHR5*, *CFI*, *CFB*, *C3*, *THBD* and *DGKE*) using Ion Hi-Q™ Sequencing 200 Kit chemistry on an Ion Torrent™ PGM sequencing system (Thermo Fisher Scientific).

As recommended by the practice guidelines for the evaluation of pathogenicity recently published by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [6].

- i) We checked the frequency of variants found in the Exome Aggregation Consortium (ExAC), Exome Variant Server (EVS), Human Gene Mutation Database and FH aHUS Mutation Database

- ii) Variants were classified as pathogenic, likely pathogenic, uncertain significance, likely benign or benign based on the available evidence [6].

This molecular study approach found in this patient a C3-combined variant: A pathogenic missense mutation in *C3*, c.1775G>A, p.Arg592Gln; a benign missense variant of *CD46*, c.686G>A, p.Arg229Gln; and also had the risk haplotypes *MCPggaac* and *CFH-H3*. All variants were in the heterozygous state.

The benign variant *CD46* p.Arg229G in was only found in a population database (MAF: ExAC-EA, 0.0063) and therefore has an uncertain significance.

After 10 months follow up she is clinically stable and without disease relapses. Despite having infectious complications (tonsillitis with high fever) there was no recurrence of HUS. C3 levels remained persistently low. Given her clinical stability after a good response to plasmapheresis and without disease relapse, the patient hasn't started eculizumab.

Discussion

Mutations in *C3* gene were first associated with aHUS in 2008 by Frémeaux-Bacchi and colleagues, including the complement *C3*p.Arg592Gln mutation [7]. Since then, other mutations are being found [8]. *C3* mutations cause a defect of the ability of *C3* to bind to regulatory protein MCP and are an indirect gain of function mutation leading to an increased capacity for CFB to bind to *C3b* and an increased formation of the *C3* convertase. Converge at the cleavage of the terminal complement protein *C5* leading to endothelial injury, and finally thrombotic microangiopathy. Plasma *C3* levels are low in about 70% of patients that correlated with disease severity [8].

Lhotta, et al. suggests that, apart from aHUS, p.Arg592Gln mutation can be related with hypertension, haematuria and chronic kidney [9]. This pathogenic *C3* mutation was located near the *C3*/*CFH* binding sites, *CFH*-*SCR3* and *CFH*-*SCR4*, affecting interactions with *CFH* [8]. In addition, this study has shown that p.Arg592Gln as the variants p.Arg161Trp and p.Arg592Trp were associated with decreased MCP cofactor activity [8]. The worst prognosis is associated with *CFH* or *C3* mutations, with mortality rates or progression to chronic kidney disease during the first year following the initial event of approximately 50-70% (the risk associated with *CD46* mutation is 0-6%)[2,4]. Plasma treatment could remove mutant *C3* and provide regulatory plasma proteins to act against complement activation induced by mutant *C3* [10].

Richards et al in 2003 were the first to report mutations of *CD46* [11]. The loss of function mutation of *CD46* leads to a low *C3b*-binding and cofactor activity.

The haplotypes *MCPggaac* and *CFH-H3* are the most relevant polymorphisms associated with aHUS [4]. Clinical evolution varies widely depending on the patient's mutation; the one related to the best prognosis is the *CD46* mutation [2].

C3 levels in *CD46*-mutated patients are most often normal. If *C3* levels are low, it is likely that another mutation responsible for the activation of complement in the fluid phase is present. As MCP is not a circulating protein, a beneficial effect of plasmatherapy is unlikely to be expected in these patients. Of note, at least ninety per cent of patients undergo remission from acute episodes, whether or not they receive plasmatherapy [2,12].

CFH mutations were the first identified, more than 200 different mutations are described. Most mutations are heterozygous, but homozygous mutations can be observed and are typically associated with very low *C3* and *CFH* plasma concentrations [2].

Patients with mutation in *CD46* and *CFI* frequently had a second mutation in other complement genes. Patients with *CFH*, *C3* or *CFB* mutations are less frequently associated with other mutations. This observed fact can suggest that these mutations alone may be sufficient to cause aHUS[13]. The concurrence of *C3* mutations with risk polymorphisms in the *CFH* and *CD46* genes modulates penetrance and clinical severity of disease in aHUS [3,13].

Our patient clinical course was clearly distinct from patients with other *C3*p.Arg592Gln or *CFH-H3* mutations and was similar to patients with *CD46* mutations described previously. We can induce that even when an unfavorable group of risk factors co-segregates, the disease may not manifest in childhood. A trigger is necessary to initiate the disease, in 50-90% aHUS is preceded by infection and the presence of protective or risk polymorphisms will influence the progression of the disease.

To the best of our knowledge, only two cases with *C3* mutations have been reported, with favorable long term outcome [3,14]. Eculizumab is a monoclonal antibody that inhibits the activation of *C5* and the formation of the cell membrane attack complex, which is responsible for the damage produced to native cell structures in aHUS. In prospective studies, eculizumab effectively interrupted the process of TMA, associated with long-term favorable

outcomes, being know accept as a first line of treatment in aHUS [2,4,5].

Our patient was and still is a challenging case, because it was not clear what the triggering event was, she did not manifest the disease when she was exposed to possible triggers (pregnancy, infection), and presented extra-renal involvement (ischemic acute cholecystitis). Our patient illustrates the complexity of aHUS, which depends on multiple predisposing factors: gene mutations, polymorphisms and environmental precipitating factors. In the future, better understanding of the pathophysiology will have implications in the diagnosis, treatment and prognosis of these patients.

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