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ANTI-FACTOR H ANTIBODY-ASSOCIATED ATYPICAL HEMOLYTIC UREMIC SYNDROME: A CASE REPORT

Nora Abazi-Emini[1], Emilija Sahpazova[1], Jovana Putnik[2], Velibor Tasic[1]

[1] University Clinic for Children's Disease, Department of Nephrology, Medical Faculty, Skopje, N Macedonia

[2] Institute for Mother and Child Health Care of Serbia "Dr Vukan Čupić", Department of Nephrology, University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Corresponding author: Nora Abazi-Emini, MD, Department of Nephrology, University Clinic for Children's Disease, Mother Teresa 17, 1000 Skopje, Republic of North Macedonia

ABSTRACT

Introduction: Atypical hemolytic uremic syndrome (aHUS) is a rare form of thrombotic microangiopathy, caused by dysregulation of the complement alternative pathway. Deletion of the complement factor H–related genes, CFHR1 and CFHR3, together with the presence of CFH autoantibodies are reported in aHUS patients, representing 10% of cases of patients with aHUS.

Case presentation: We report here on a case of 4-year-old girl with anti-CFH antibody-associated aHUS. The measurement of complement factors and anti-factor H antibodies, was the main guideline for making an accurate diagnosis and providing the appropriate therapy, with the patient responding positively to plasma exchanges (PEs) and cyclophosphamide pulses. We then, one year after disease onset, continued with glucocorticoids and mycophenolate mofetil (MMF), as maintenance therapy. There were no complications during the therapy other than neutropenia. Now, one year after the cessation of the immune suppression therapy, she is in remission with normal kidney function, no signs of hemolysis, normal C3 levels, and normal range proteinuria. The anti-factor H autoantibody titer decreased but still remained positive, the factor H antigen values remained low all throughout. Close follow-up is applied with frequent urine testing and complete blood count with an intention for early detection of relapse of the disease.

Conclusion: The purpose of this case report is to emphasize the value of complement factor measurements and also to separate anti-CFH antibody-associated aHUS as an entity, because immunosuppressive therapy provides an excellent response..

Keywords: atypical hemolytic uremic syndrome, complement factor H autoantibodies, plasma exchanges, cyclophosphamide, mycophenolate mofetil

INTRODUCTION

Hemolytic uremic syndrome (HUS) is a severe condition with a characteristic triad of microangiopathic hemolytic anemia (Hemoglobin <100 g/l), thrombocytopenia (platelet count < 150 000/mm3), and kidney failure (serum creatinine > normal value for age). The typical form of HUS or post-diarrheal (D+) HUS is most common, resulting after a Shiga toxin-producing Escherichia coli (STEC) infection, mostly Escherichia coli 0157:H7. Atypical HUS (aHUS) is a consequence

of a disorder of the alternative complement pathway regulation. Typical HUS is manifested in infants and young children and generally has a good prognosis, whereas atypical HUS, which can manifest at any age, even in a newborn, and has a recurrent course with poor renal outcome. 5% to 10% of HUS in children are atypical HUS. Even in atypical HUS, the triggering factor is a preceding infection in up to 80% of pediatric cases [1]. Among the reported cases, 50% of aHUS patients had genetic mutations. These mutations included: loss of function mutations of complement regulators - FH, FHRs, MCP/CD46, FI, and gain of function mutations of complement activators – FB and C3. Also, forms of thrombomodulin mutations are confirmed. Non-complement-related genetic mutations, like DGKE mutations, are reported in few cases [2]. Deletion of the complement factor H–related genes CFHR1 and CFHR3, together with the presence of CFH autoantibodies, are reported in aHUS patients, representing 10% of the cases with aHUS. These patients show complete absence or reduction of CFHR1 and CFHR3 in plasma, but the pathogenesis of how this absence leads to the production of CFH autoantibodies is unknown. Factor H autoantibodies bind to the C terminal of factor H and impair its inhibiting activity to C3b. Not all patients with CFHR gene deletions develop antibodies, significant autoantibody response to CFH, however, can develop in the presence of normal CFHR1 [3,4,5].

We report here on a case of anti-CFH antibody-associated aHUS, with a positive response to plasma exchanges (PEs) and cyclophosphamide pulses, continued with glucocorticoids and mycophenolate mofetil (MMF) as maintenance therapy one year after disease onset. Now, one year after therapy cessation, our patient is still in remission.

CASE PRESENTATION

OA previously healthy 4-year-old girl was referred to our hospital. She was vaccinated regularly according to her vaccination calendar. The family did not have a history of kidney or inherited diseases. Three days before admission, she had pallor, icterus and diffuse ecchymoses. She had no preceding diarrhea or infection. Upon admission she was conscious, afebrile, with stable vital signs, BW 20 kg, BH 108 cm, skin and mucosa were pale and icteric with diffuse ecchymosis, normal arterial pressure (85/50 mmHg), normal neurological status, no organomegaly, or other organ involvement.

The initial laboratory finding revealed severe anemia (Hgb 46 g/L, RBC 1.67 x106/µl, Hct 12.7%) and severe thrombocytopenia (14x103/µl). A peripheral smear showed the presence of schistocytes. From the serum, chemistry electrolytes were within the normal range, urea 26.6 mmol/l, creatinine 120 µmol/l, uric acid 440 µmol/l, serum iron 40 µmol/l, ferritin 1040 µg/l, total bilirubin 63 µmol/l, unconjugated bilirubin 48 µmol/l, AST 130 U/L, ALT 25 U/L, LDH >1995 U/l, CRP 10mg/L. The complement C3 level was mildly decreased at 0.77 g/l, her C4 level was normal 0.26 g/l. Stool culture was negative for Shiga toxin and entero-hemorrhagic Escherichia coli 0157:H7. Upon bone marrow aspiration, no signs of malignancy were noted. The urine finding revealed macroscopic hematuria, proteinuria (nephrotic range 5 gr/diuresis) with dysmorphic erythrocytes and cylinders in microscopic evaluation of the sediment. An ultrasound showed that the kidneys were hyperechogenic. All these findings suggested a diagnosis of hemolytic uremic syndrome, and because of absent data for diarrhea and negative stool tests for Shiga toxin, the suspicion for atypical HUS was great.

Further diagnostic evaluation involved complement factors measurement, antibodies and genetic testing. They were performed in the Research Laboratory at Semmelweis University, Budapest Hungary. (Table 1)

Complement analysis confirmed deficient haptoglobin levels, thus supporting intravascular hemolysis. The factor I antigen, Factor B antigen, and classical pathway activity were within a reference range indicating no complement consumption. The terminal pathway activation marker sC5b9 was elevated, indicating in vivo complement activation. Anti-FH autoantibody clear was positive, with decreased FH antigen levels. These results confirmed the diagnosis of autoimmune atypical HUS.

Genetic analysis

The patient was found to be homozygous for a common deletion of CFHR1 and CFHR3 genes that are associated with the anti-factor H autoantibody-positive form of the hemolytic uremic syndrome. To reveal deletions or duplications in

Table 1. *Complement factors and antibody workup*

ADAMTS13 metalloprotease activity	**108 % (**reference range 67-150 %)
Total complement activity, classical pathway (hemolytic test):	**80 CH50/ml** (ref range 48-103 CH50/ml)
Total complement activity, alternative pathway (WIELISA-Alt):	**110 %** (reference range 70-125%)
Complement C3:	**1,11 g/L (**reference range 0,9-1,8 g/L)
Complement C4:	**0,18 g/L** (reference range: 0,15-0,55 g/L)
Factor H antigen:	**118 mg/L** (reference range 250-880 mg/L)
Complement factor I antigen:	**133** % (reference range 70-130%)
Complement factor B antigen:	**104** % (reference range 70-130%)
Anti- factor H IgG autoantibody:	**984 AU/mL (ref <110)**
C1q antigen	**111 mg/L** (ref: 60-180)
Anti-C1q IgG autoantibody	**3** U/mL (ref <52)
sC5b-9 (terminal complement complex)	**826 ng/mL** (ref 110-252 ng/mL)
Haptoglobin	**0,03** g/L (ref: 0,3-2,0 g/L)

CFH, CFHR-1, -2, -3 and -5 genes, a multiplex ligation-dependent probe amplification (MLPA) was performed. No additional risk variations were identified in the genes of CFH, CFI, and MCP (CD46). The DGKE gene appeared also without mutations or rare variations. This was determined via direct DNA sequencing of polymerase chain reaction (PCR) and products amplified from total genomic DNA.

Treatment

During the first three weeks of hospitalization, blood and fresh frozen plasma transfusions were administered. The patient had stable vital parameters, normal diuresis but with persistent thrombocytopenia, hematuria, high degradation products, and proteinuria of nephrotic range. When the diagnosis of autoimmune atypical HUS was confirmed, plasma exchanges were started, performed every day, for 5 days, using a double lumen femoral dialysis catheter. Substitution was done with 5% human albumin and fresh frozen plasma, and the volume of substitution was 1.5 times the plasma volume. After plasma exchanges, treatment was continued with intravenous cyclophosphamide pulses 200 mg (0.5 g /1.73 m2, BSA 0.77 m2) every month, 5 pulses in total, together with oral prednisone 40 mg/day (2 mg/kg/day) which was gradually reduced to 5mg/48h.

Soon after PEs and the first intravenous cyclophosphamide pulse, the patient displayed significant improvement with hematological remission and normal kidney function with mild proteinuria. After finishing cyclophosphamide pulses, immune suppression therapy was continued with mycophenolate mofetil (MMF) at 500 mg b.i.d. and corticosteroids at 5mg/48h for 6 months. During therapy she manifested neutropenia and the dose of MMF was reduced by 50% (250 mg b.i.d.). She had no other complications. The laboratory findings were stable at all time.

Now, one year after the cessation of the immune suppression therapy, she is in remission with normal kidney function, no signs of hemolysis, normal C3 levels, and normal range proteinuria. The anti-factor H autoantibody was decreased but still positive, and the values of factor H antigen remained low all throughout. (Table 2)

Table 2. *Laboratory parameters during the course of the disease*

	Onset of the disease	**PEs Cyclophosphamide Prednisone**	**M M F Prednisone**	**No immune suppression**
Period from disease onset	0-1 month	1-6 months	6-12 months	12-24 months
Biological parameters				
Hemoglobin (g/l) NR (110-150)	46	120	121	124
Haptoglobin (g/l) NR (0.3-2.0)	0.03	0.02	-	0.03
Platelets (x10^3/µl) NR (150-400)	14	239	354	293
LDH (U/l) NR (0-500)	>1995	263	234	225
Creatinine (µmol/l) NR (20-70)	120	41	43	42
eGFR (ml/min/1.73m^2) NR (90-150)	32	97	94	95
Complement				
C3 (g/l) NR (0.8-1.9)	0.77	1.15	1.02	0.84
CFH antibody (AU/ml) NR <100	984	431	328	287
Factor H antigen (mg/l) NR (250-880)	118	129	-	114
Urine				
Proteinuria NR (0-150 mg)	5 g/diuresis	250 mg/diuresis	30 mg/diuresis	60 mg/diuresis
Hematuria	macroscopic	Blood +	No	No

DISCUSSION

Atypical hemolytic uremic syndrome is a rare form of thrombotic microangiopathy, caused by dysregulation of the complement alternative pathway (OMIM #235400). Complement alternative pathway dysregulation is, in most cases, genetic but it can also be acquired secondary to Igg antibodies. Dragon-Durey et al. [6] from a cohort of 48 patients with aHUS, reported the presence of anti-CFH autoantibodies in three children, confirming for the first time that aHUS can be an autoimmune disease leading to acquired FH deficiency. This form of aHUS is present in 6-10% of all cases with aHUS. In another study, clinical features of patients with anti-CFH autoantibodies at disease onset are very similar with D+ HUS, with abdominal pain and vomiting (84%) and diarrhea (53%). Extrarenal complications are frequent. The evolution of the

disease is characterized by frequent relapses, but of the three patients who were treated by plasma exchanges, together with immunosuppressive treatment, not one relapsed, and all fully recovered [7]. Józsi et al. [3] identified a correlation between the presence of anti-CFH autoantibodies and the absence or reduction of plasma CFHR1/CFHR3. Of 148 patients with aHUS, 22 had complete absence or reduction of plasma CFHR1/CFHR3 and 16 of those patients were positive for anti-CFH antibodies (72%). Similar results were reported by Moore et al. [8].

For our patient, suspicion for atypical HUS was high due to the absence of diarrhea, stool tests with negative results for Shiga toxin, and the age of the patient. Immediately after admission, she was treated with plasma and blood transfusions, but without significant improvement. Complement work revealed deficient haptoglobin levels, suggesting intravascular hemolysis. ADAMTS13 activity was in the normal range, thus thrombotic thrombocytopenic purpura and congenital form Upshaw Shulman syndrome were excluded. Despite the level of C3, in further evaluations, it was within the normal range, yet that did not exclude the diagnosis of atypical HUS. According to the literature, C3 levels are low in just 30 - 40% of cases of aHUS. The terminal pathway activation marker sC5b9 was elevated, indicating in vivo complement activation. Anti-FH autoantibody was a clear positive, with decreased FH antigen level. Further treatment was redirected to plasma exchanges, concomitant with immunosuppressive therapy (corticosteroids and cyclophosphamide) with rapid clinical improvement and normalization of laboratory parameters. Several reports in recent years have recorded successful treatment, including plasma exchange and immunosuppressive agents (e.g., glucocorticoids, cyclophosphamide, mycophenolate mofetil, and rituximab) [6, 9-11].

Although the initial level of anti CFH antibodies was not too high (984 AU/ml), the clinical course was severe, so we wanted to emphasize that an antibody titer does not always indicate the severity of the disease.

Genetic tests confirmed homozygous common deletion of CFHR1 and CFHR3 genes. Thus, the exact term for this disease is DEAP – HUS (deficiency of CFHR plasma proteins and factor H autoantibody positive hemolytic uremic syndrome) [12].

The importance of early diagnosis with complete complement work is crucial for initiating an appropriate treatment. In developing countries, like ours, diagnostic and therapeutic limitations are present. The complement analysis was done in Budapest, Hungary, which lead us directly to the targeted treatment. Limited access to Eculizumab (monoclonal antibody to C5) prevents us from the implementation of international guidelines for atypical HUS. In this case, Eculizumab was not indicated because of successful treatment with PEs and immunosuppressive agents [13,14].

During the follow-up, after the cessation of immunosuppressive treatment, the titer of anti CFH antibodies was still above the limit, but our patient was stable without signs of hemolysis, with normal kidney function and normal range proteinuria. In some of the recent publications, the duration of immunosuppressive treatment after initial stabilization was shorter than in our case [9]. The international consensus approach to the management of atypical hemolytic uremic syndrome in children recommends treatment withdrawal after 1 year in patients who are in remission, with normal C3 level and anti-CFH antibody titer < 1000 AU/ml. All these criteria were fulfilled in our case [14].

According to Hofer et al. [15], patients who had no relapse within the first 6 months were relapse-free until the end of the observation period of 5 years. In our case, the immunosuppressive therapy was continued for one year, so the relapses were prevented. Now, however, one year on without any treatment, she is still relapse-free. Close follow-up is applied with frequent urine testing and complete blood count with an intention for early detection of relapse of the disease.

CONCLUSION

The purpose of this case report is to emphasize the value of complement factors measurement. It is important to separate anti-CFH antibody-associated aHUS as an entity, because immunosuppressive therapy provides an excellent response. It should also be emphasized how important it is for all countries to have access to Eculizumab as a new agent, a mainstream therapy for aHUS.

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Резиме

АНТИФАКТОР Х АНТИТЕЛА АСОЦИРАН АТИПИЧЕН ХЕМОЛИТИЧЕН УРЕМИЧЕН СИНДРОМ: ПРЕЗЕНТАЦИЈА НА СЛУЧАЈ

Нора Абази-Емини[1], Емилија Шахпазова[1], Јована Путник[2], Велибор Тасиќ[1]

[1] Универзитетска Клиника за детски болести, Оддел за нефрологија, Медицински факултет, Скопје, РС Македонија

[2] Институт за здраствена заштита на мајка и дете на Србија „Д-р Вукан Чупиќ“, Оддел за нефрологија, Медицински факултет, Белград, Р Србија

Вовед: Атипичниот хемолитичен уремичен синдром (аХУС) е ретка форма на тромботична микроангиопатија, која е предизвикана од дисрегулација на алтернативниот пат на комплементот. Делецијата на комплемент фактор Х поврзани гени CFHR1 и CFHR3, заедно со присуството на антитела кон комплемент фактор Х, се опишани кај пациенти со аХУС и претставуваат 10 % од случачите на атипичен хемолитичен уремичен синдром.

Презентација на случај: Прикажуваме случај на четиригодишно девојче со аХУС асоциран со антитела кон комплемент фактор Х. Мерењето на факторите на комплементот и антителата кон факторот Х беше главен водич за да се постави точна дијагноза и да се даде соодветна терапија, со добар одговор кон плазмафереза и циклофосфамидни удари, потоа продолжено со одржувачка имуносупресивна терапија со кортикостероиди и микофенолат мофетил во текот на една година по почетокот на болеста. За време на терапијата не се појавија несакани ефекти, освен неутропенија. Сега, една година по прекинот на имуносупресивната терапија, девојчето е во клиничка ремисија со нормална бубрежна функција, без знаци за хемолиза, нормално ниво на Ц3 и нормални вредности на протеинурија. Титарот на антитела кон факторот Х се намали, но сè уште е позитивен, вредностите на фактор Х антигенот останаа ниски цело време. Детето се следи со чести контроли на крвната слика и на уринатa, со намера рано да се открие релапсот на болеста.

Заклучок: Со овој презентиран случај сакаме да ја потенцираме важноста на мерењето на факторите на комплементот и, исто така, да го одвоиме антикомплемент фактор Х антитела асоциран аХУС како посебен ентитет, бидејќи имуносупресивната терапија дава одличен резултат.

Клучни зборови: атипичен хемолитичен уремичен синдром, антитела кон комплемент фактор Х, плазмафереза, циклофосфамид, микофенолат мофетил