Liver manifestation of atypical hemolytic uremic syndrome associated with heterozygous polymorphism c-332T>C in the promoter of the complement factor Hgene – case report

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Background: Primary atypical hemolytic uremic syndrome (aHUS) is caused by complement dysregulation. Diagnosis is based on determining the presence of hemolytic anemia, thrombocytopenia, and the presence of schistocytes in the peripheral blood smear and damage to internal organs - most commonly the kidneys. Liver involvement as the main symptom of the disease is rare, making it difficult to recognize.

Case presentation: We present a case of a 65 year old woman whose clinical picture was dominated by weakness, vomiting, jaundice, disturbances of consciousness, as well as characteristics of liver and kidney damage. Laboratory tests showed typical features of hemolytic anemia Hb 9.3 g/dl, high LDH, low haptoglobin, thrombocytopenia 116x10^9/l, signs of renal damage: creatinine 3.6 mg/dl, and elevated bilirubin level 5.5 mg/dl. Manual differential blood smear stated 6-8% schistocytes. Establishing a diagnosis was possible after genetic testing, which showed only a heterozygous polymorphism in the promoter of the factor H gene. Eculizumab was not an option (lack of registration in Poland at the time), thus other methods of treatment were sought (infusions of plasma, plasmapheresis and corticosteroids).

To summarize atypical hemolytic uremic syndrome is not always acute. For carriers of the mutated gene, the image of the disease may be incomplete and proceed in a chronic way that delays diagnosis. Hepatic manifestation of aHUS is casuistic in medicine and probably results from damage to the vascular endothelial cells in small hepatic vessels.

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Key words:
- thrombotic microangiopathy
- atypical hemolytic-uremic syndrome
- complement factor H mutation

Manifestacja wątrobowa atypowego zespołu Hemolityczno-Mocznicowego związanego z heterozygotycznym polimorfizmem c-332T>C w promotorze czynnika dopełniacza genu H – opis przypadku.

Wstęp: Atypowy zespół hemolityczno-mocznicowy (aHUS) jest spowodowany rozregulowaniem układu dopełniacza. Postawienie diagnozy bazuje na ocenie obecności: niedokrwistości hemolitycznej, małopłytkowości oraz obecności schistocytów w rozmazie krwi obwodowej, a także uszkodzenia organów wewnętrznych - najczęściej nerek. Zajęcie wątroby będace głównym objawem jest rzadkie, co sprawia trudności diagnostyczne.

Opis przypadku: Przedstawiamy przypadek 65 letniej kobiety, w obrazie klinicznym dominowały: osłabienie, wymioty, żółtaczka, zaburzenia świadomości (będące również charakterystyczne dla niewydolności wątroby lub nerek). Badania laboratoryjne wykazały cechy anemii hemolitycznej - stężenie hemoglobiny 9,3 g/dl, podwyższone LDH, obniżone stężenie haptoglobiny; trombocytopenię 116x10^9/l; cechy niewydolności nerek: kreatynina 3,6 mg/dl; a także podwyższone stężenie bilirubiny 5,5 mg/dl. W rozmazie ręcznym krwi obwodowej stwierdzono 6-8% schistocytów. Postawienie diagnozy było możliwe po wykonaniu badań genetycznych, w których stwierdzono heterozygotyczny polimorfizm w promotorze czynnika genu H. Eculizumab, ze względu na brak wówczas rejestracji w Polsce, nie mógł być jedną z opcji terapeutycznych, dlatego poszukiwano innych metod (przetaczanie osocza, plazmaferezy oraz glikokortykosteroidy).

Podsumowując atypowy zespół hemolityczno-mocznicowy nie zawsze ma ostry przebieg. U nosicieli zmutowanego genu obraz choroby może być niekompletny i powodować postać przewlekłą, co opóźnia postawienie rozpoznania. Wątrobowa manifestacja jest kazuistyczna, prawdopodobnie jest to wynik uszkodzenia komórek endotelialnych małych naczyń wątroby.

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Słowa kluczowe:
- mikroangiopatia zakrzepowa
- atypowy zespół hemolityczno-mocznicowy
- mutacja czynnika dopełniacza H

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Background

Atypical hemolytic uremic syndrome (aHUS) is a rare disease associated with excessive complement activation [1-3]. Subsequent endothelial damage and disseminate coagulation lead to hemolytic anemia and thrombocytopenia. These symptoms lie at the center of thrombotic microangiopathies: aHUS, diarrhea-associated hemolytic uremic syndrome (STEC-HUS) and thrombotic thrombocytopenic purpura (TTP). Eculizumab, a monoclonal antibody against compliment component 5, is the new standard of care for aHUS. As eculizumab was not registered in Poland during patient's hospitalization, other methods of treatment were sought (plasma infusion, plasma exchange, glicocortycosteroids). Following case of aHUS is unique for the predominance of liver symptoms and the chronic clinical course.

Case presentation:

We present a case of a 65-year old woman, Caucasian race, body weight of 56 kg, who was admitted to Tertiary Nephrologic Center with postprandial vomiting, recurrent vertigo and general asthenia. Clinical examination revealed xanthochromia and liver enlargement (5 cm beneath the ribcage). Three years prior to first hospitalization a routine examination during a surgical provision of left arm fracture revealed a bilirubin level of 2 mg/dl. No chronic diseases in patient medical history. At the age of 31 patient gave birth to a healthy daughter, with no complications during pregnancy and childbirth. During present hospitalization laboratory tests showed: anemia (hemoglobin, Hb 9.3 g/l, hematocrit HCT 27%, red blood cell count RBC 2.84 $x10^{12}$/l), kidney and liver dysfunction (creatinine 3.6 mg/dl, urea 72 mg/dl, bilirubin 5.5 mg/dl, alanine transaminase ALT 20 U/l, aspartate transaminase AST 46 U/l), moderate thrombocytopenia (platelets PLT 116$x10^{9}$/l) and the following coagulation disorders: prolonged international normalized ratio (INR), an increased D-Dimer and a decreased antithrombin 3 value (ATIII). Abdominal ultrasonography revealed an oval focal lesion in the left liver lobe (decreased echogenicity, 14 mm in diameter) and numerous cysts in the right liver lobe. In CT scan, in both lobes of the liver visible several outbreaks of ~8 mm in diameter. Liver was slightly enlarged, with a reduced coefficient of extinction (approx. 45 J.H.) (Fig. 1). During the diagnostic process no biopsy or magnetic resonance imaging (MRI) was conducted due to thrombocytopenia and the presence of a metal plate in the left arm (Fig. 2). A whole body positron emission tomography (PET) showed no intensified glucose uptake. Tests for viral and autoimmune hepatitis and other autoimmune disorders were carried out (for systemic lupus erythematosus, dermatomyositis, antiphospholipid syndrome, systemic vasculitis): anti-double stranded DNA (anti-dsDNA) antibodies, lupus anticoagulant, antinuclear antibody (ANA), anti-liver-kidney microsome 1 (anti-LKM1), anti-neutrophil cytoplasmic antibody (ANCA), smooth muscle antibody (SMA). No antibodies were found.

Moreover, the patient suffered from recurrent episodes of impaired consciousness during hospitalization. A computer tomography of the head visualized a fresh cerebral ischemia in the right cerebral hemisphere (Fig. 3). Furthermore, despite blood transfusions anemia and thrombocytopenia intensified. The presence of schistocytes in the blood smear (6-8%), an increased level of lactate dehydrogenase (LDH) and reticulocyte count and an almost undetectable level of haptoglobin suggested a hemolytic microangiopathic anaemia. Considering the entire clinical picture, a diagnosis of atypical hemolytic uremic syndrome (aHUS) or thrombotic thrombocytopenic purpura TTP of unknown origin was considered. Blood serum was collected and showed a decreased complement component 3 (CC3) activity 80 mg/dl (90 - 180); and normal ADAMTS13 (disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity – 93% (N 40-130%). Shiga toxin was negative. Because of the deteriorating clinical condition, therapy was started. The infusions of 500 mg methylprednisolone for 3 consecutive days were followed by 40 mg of oral prednisone bringing a temporary improvement. Additionally, a total of 24 units of fresh frozen plasma (FFP) were infused. The clinical state improved, without renal replacement therapy a diuresis of over 2 liters per day was achieved with a slight deterioration of liver function.

Table I

Results of the genetic testing. NGS - next generation sequencing, MLPA - Multiplex Ligation-dependent Probe Amplification. No identified mutated gene was described in 25-30% cases [20].

Wyniki badań genetycznych; NGS, MLPA. Brak identyfikacji zmutowanego genu był opisywany w 25-30% przypadków [20].

Test	Method	Results	Comments
CFH gene screening	NGS	No mutations	Frequency:20-30%[20]
		Heterozygous polymorphism c.-332T>C	Polymorphism associated with aHUS[16]
MCP gene screening	NGS	No mutations	Frequency:10-15%[20]
C3 gene screening	NGS	No mutations	Frequency:5-10%[20]
CFI gene screening	NGS	No mutations	Frequency:4-10%[20]
CFB gene screening	NGS	No mutations	Frequency:1-2%[20]
		Heterozygous polymorphism p.G252S	
THBD gene screening	NGS	No mutations	Frequency:5%[20]
		Heterozygous polymorphism p.A473V	
ADAMTS13 gene screening	NGS	No mutations	
		Heterozygous polymorphism p.Q448E	
DGKE gene screening	NGS	No mutations	
CFH-CFHR1 Hybrid gene screening	MLPA	Negative	
Deletion of *CFHR1-CFHR3* genes	MLPA	Deletion in heterozygosity	Frequency:6%[18]

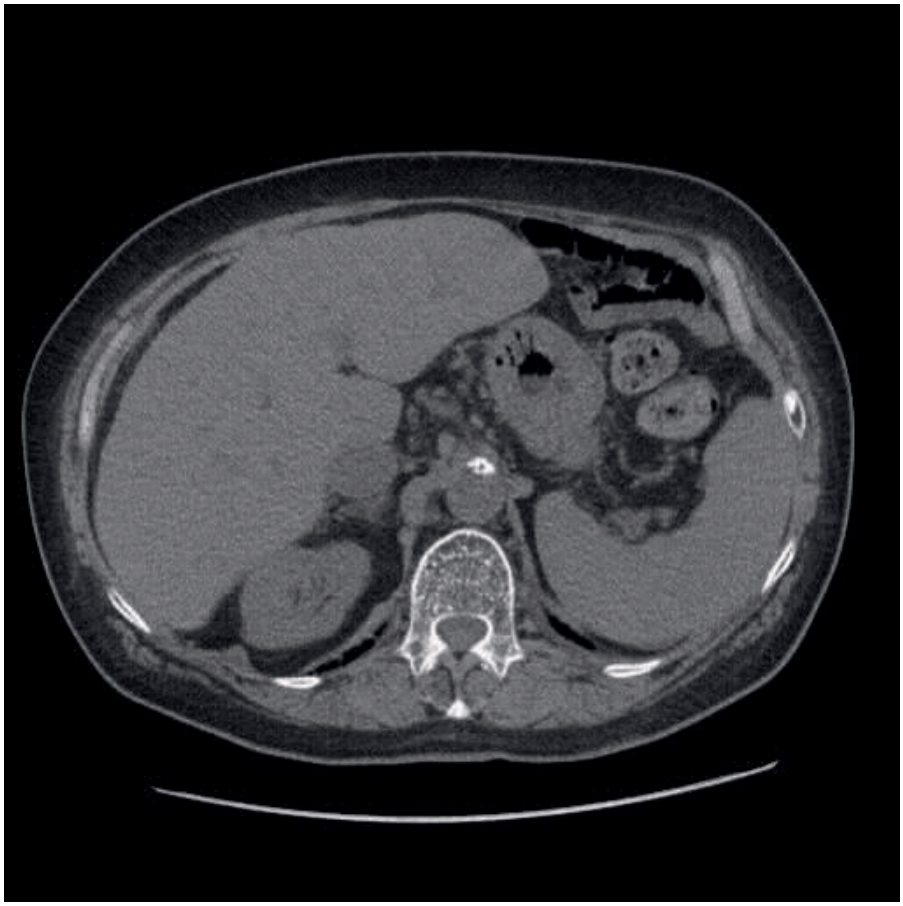

Figure 1
CT scan. In both lobes of the liver visible several outbreaks diameter of about 8 mm - cystis? other? In addition, in the segment IVB vaguely demarcated, low-hyper density change focal - 9 mm. Liver slightly enlarged.
Tomografia komputerowa. W obu płatach wątroby widocznych kilka ognisk średnicy około 8 mm - cysta? Ponadto w segmencie IVB niejasno rozgraniczona ogniskowa hypodensyjna zmiana - 9 mm. Wątroba lekko powiększona.

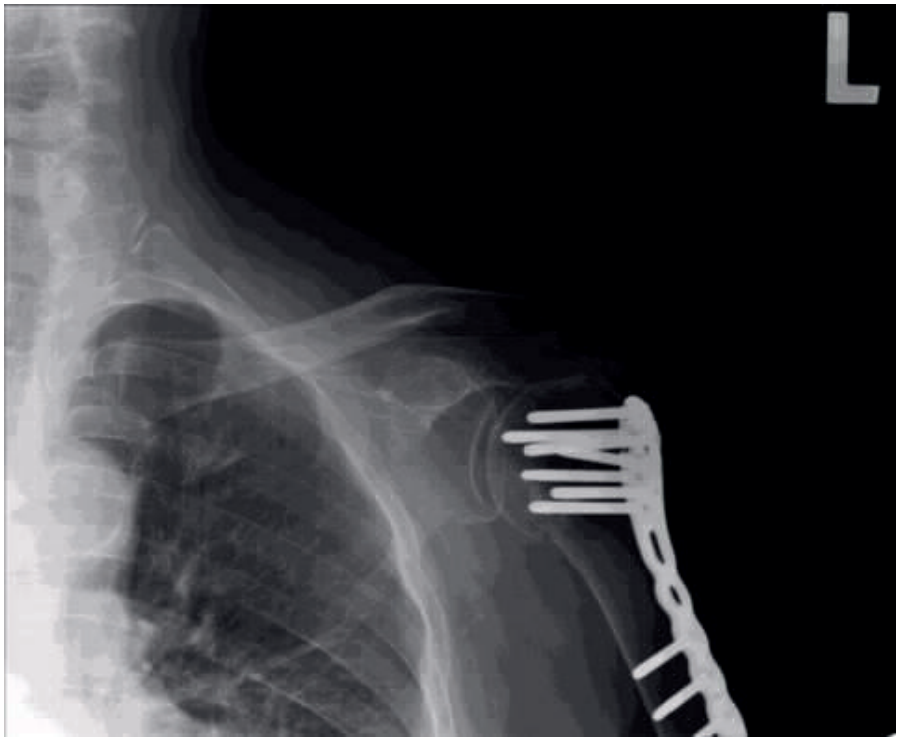

Figure 2
X-ray image. State after fracture of the humeral head left. Implanted titanium screws.
Zdjęcie rentgenowskie. Stan po złamaniu głowy lewej kości ramiennej. Wszczepione śruby tytanowe.

The patient was repeatedly hospitalized for impaired consciousness accompanied by anemia and a decline of kidney and liver function manifested by hepatic encephalopathy and dehydration. The patient received repeated therapeutic plasma exchanges. Three plasmaphereses were performed, each exchanging 4 litres of plasma with FFP bringing no improvement to the clinical state. Due to the ineffectiveness of treatment (plasmapheresis, steroids, FFP transfusion), recurrent relapses of aHUS and lack of targeted therapy with eculizumab, (not available in Poland at that time), the patient was offered a participation in a phase 2 clinical trial examining the safety of the monoclonal antibody anti-MASP 2 (OMS721-TMA-001, NCT02222545). Change in levels of creatinine, haemoglobin, bilirubin and platelets over time are shown on Figure 4.

Genetic testing was performed to assess the most common mutations associated with HUS/TTP development (Tab. I). Testing was performed in The Clinical Research Center for Rare Diseases “Aldo e Cele Daccò”, Mario Negri Institute for Pharmacological Research, in Italy. No mutations were found by Next Generation Sequencing (NGS) of complement factor H (CFH), membrane cofactor protein (MCP), complement component 3 (CC3), complement factor I (CFI), complement factor B (CFB), thrombomodulin (THBD), a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), diacylglycerol kinase ε (DGKE) genes. A heterozygous polymorphism c-332T>C in the promoter of complement factor H gene was found by multiplex ligation-dependent probe amplification (MLPA). The mutation is associated with aHUS development.

Discussion

Atypical hemolytic uremic syndrome is a rare disease with a prevalence of 2.1 cases per million [4-6]. Various complement abnormalities and excessive coagulation lead to consumptive thrombocytopenia. Hemolytic anemia is caused by damage to erythrocytes caused on their way through the narrowed vessels. The disease is associated with renal function impairment: 60% of children and 80% of adults eventually require renal replacement therapy [7]. Neurological (impaired consciousness, stroke, focal deficits and epileptic seizures) and cardiac involvement is present in 10-30% of cases [5,8]. The main symptom in our patient was liver malfunction, renal involvement. Hepatic manifestation of aHUS may have been a result of hemolysis in the endothelium of hepatic vessels and subsequent organ ischaemia. Only about half of the patients with typical presentation of aHUS have identifiable complement factor mutations. Our patient had no mutation of complement factor H but was heterozygous for the polymorphism c.-332C>T in the promoter of CFH gene. Such abnormality has already been identified in other patients with aHUS [9]. Patient was a carrier of the mutated gene, and this could be a reason

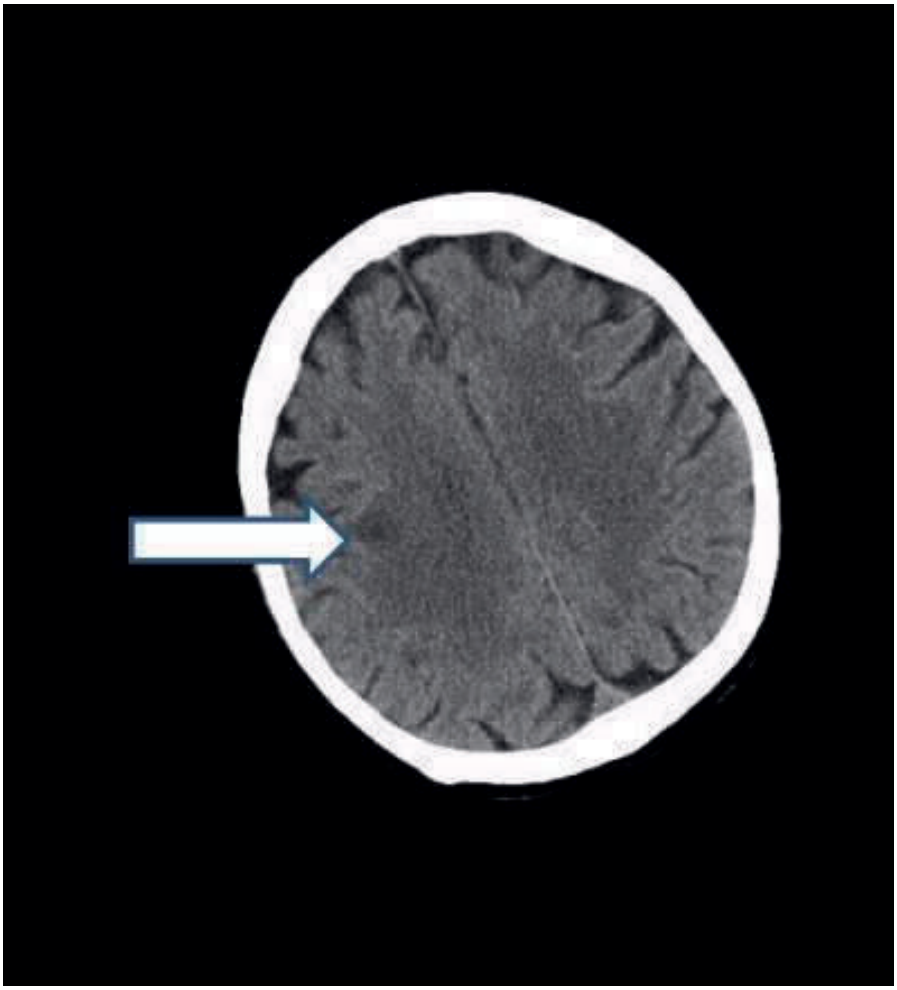

Figure 3
CT scan. A computer tomography of the head visualized a fresh cerebral ischemia in the right cerebral hemisphere.
Tomografia komputerowa. Tomografia komputerowa głowy uwidoczniła świeże zmiany niedokrwienne w prawej półkuli mózgowej.

for a milder and more chronic course of disease and subsequent difficulty in establishing diagnosis of aHUS. In order for the syndrome to develop a combination of factors must coincide: a mutation, predisposing haplotype and a triggering factor [10]. Around 50% of aHUS cases are preceded with a gastrointestinal or upper respiratory tract infection [5,11-13]. Other triggering events include pregnancy, cancer and use of medications [14]. No associated trigger was found in the presented case.

Diagnosis of aHUS must be differentiated with other thrombotic microangiopathies. Hemolytic anemia, thrombocytopenia and acute renal insufficiency, fever and a possible central nervous system involvement are associated with HUS in the course of E. coli O157 infection. No gastroenteritis was present in the described case and Shiga toxin in stool was negative. Neurological involvement accompanied by fever, thrombocytopenia, hemolytic anemia and renal insufficiency is characteristic for TTP caused by low activity of ADAMTS13. In this case ADAMTS13 activity was normal. Furthermore, thrombotic microangiopathies must be differentiated with systemic lupus erythromatosus (SLE), syndrome of hemolytic anemia, elevated liver enzymes, low platelet count (HELLP), disseminated intravascular coagulation (DIC) and thrombotic microangiopathies (TMA) secondary to drugs like quinine, simvastatin, interferon or calcineurin inhibitors. If plasmapheresis, is unavailable then FFP transfusion remains the first line of aHUS treatment. The treatment provides the lacking complement factors [15]. Exchange of 1-2 plasma volumes in each plasmapheresis is recommended as was the case in our patient [16]. Immunosuppressive therapy has a limited utility in the treatment of

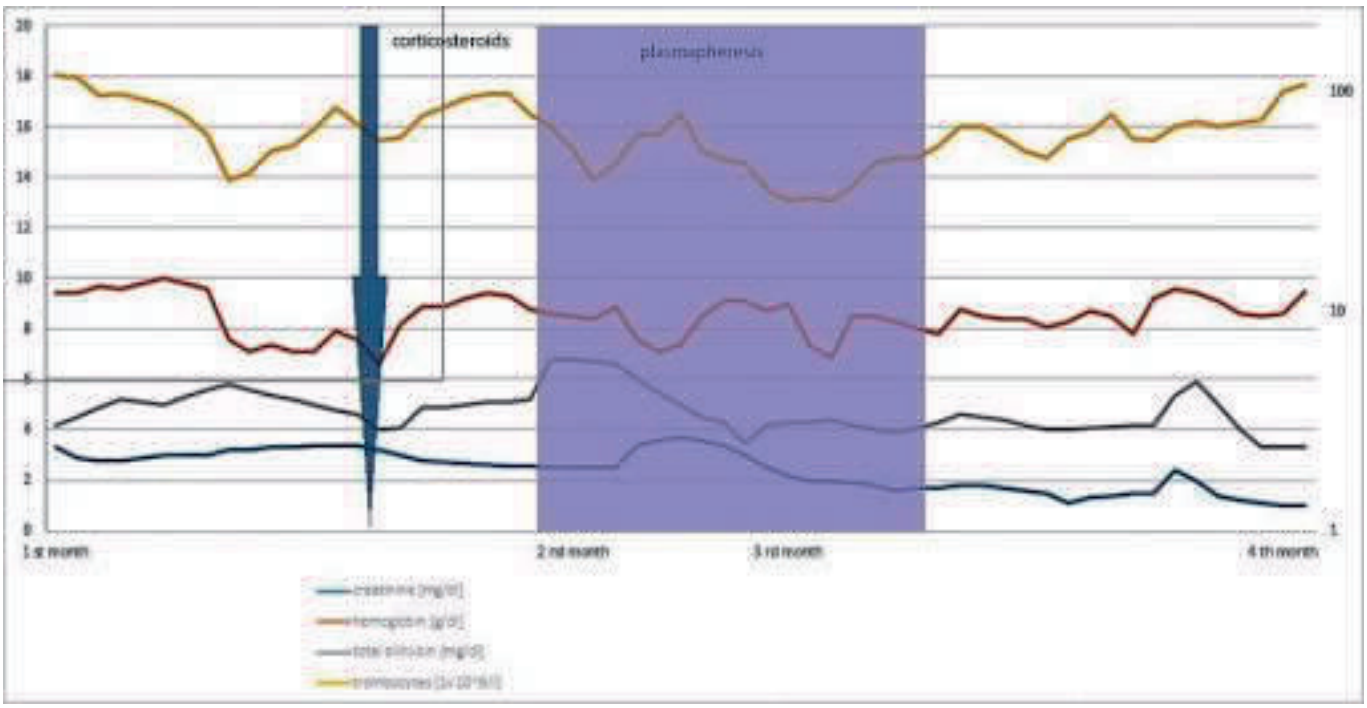

Figure 5
Patient's clinical course.
Przebieg leczenia.

aHUS showing some effect only in cases related to the development of autoantibodies against complement factor H anti-CFH [17]. However, FFP infusions, 3 therapeutic plasma exchanges and steroids provided only a temporary improvement.

In the 2009 European Medicine Agency (EMA) approved eculizumab for aHUS therapy. It is a monoclonal antibody against C5 complement factor. Its use is highly effective with 80% of the patients achieving long term remission, as opposed to 5% of patients treated with plasmaphereses [18]. Due to lack of approval eculizumab was unavailable in Poland at that time. That was the reason the patient started experimental therapy as a part of phase 2 clinical trial.

Liver manifestation of aHUS is rare. Final diagnosis was established after thorough, extensive diagnostics including genetic testing. Treatment was ineffective, thus other methods of treatment were sought. The main limitation of our manuscript was the inability to treat with eculizumab at that time.

Summary

The above described case presents the difficulty of diagnosing an atypical clinical course of aHUS. Treatment of aHUS presents an even greater challenge, especially if plasma exchanges are ineffective. It is advisable to search for new genes associated with aHUS/TTP. In justified cases, specific diagnosis is possible only after genetic testing, especially for a long period of illness when the symptoms are not spectacular. aHUS is rare syndrome with poor prognosis. approximately 70% of patients die, it requires dialysis or developing chronic kidney disease within a year after diagnosis [19-20]. It is necessary to search for new therapeutic possibilities.

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