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Significance of complement system diagnostics in thrombotic microangiopathies

Znaczenie diagnostyki układu dopełniacza w przebiegu mikroangiopatii zakrzepowych

Thrombotic microangiopathies (TMA) are rare life threatening diseases of various etiologies, making the identification of the specific forms and appropriate treatment difficult. The pathophysiology-based classification of various TMAs is important in making clinical decisions and planning the therapy outline. Detailed diagnostic management of the complement system, occasionally extended to include genetic workup is generally recommended in all TMA cases, as it allows for an individualized mode of treatment planning, estimates the prognosis and risk of disease recurrence, which is demonstrated by new HUS case reports cited in the current survey. In the paper, there are presented six cases of non-Shiga-toxin HUS including: two cases of aHUS caused by autoantibody against factor H (DEAP-HUS), aHUS caused by DGKE mutation, aHUS caused by MCP mutation, HUS caused by Streptococcus pneumoniae, and HUS secondary to Escherichia coli sepsis. In all the presented cases, the detailed complement system diagnostic and in five - also genetic workup were performed. The complexity of TMA, and the importance of complete differential diagnostic workup of all non-Shiga-toxin HUS cases due to the risk of developing CKD or ESKD, the risk of aHUS relapse both in native or transplanted kidneys, and to potential prolonged eculizumab treatment is highlighted by the current case reports.

Mikroangiopatie zakrzepowe (thro-mbotic microangiopathies - TMA) są to rzadkie zagrażające życiu choroby o zróżnicowanej etiologii, co powoduje, że identyfikacja poszczególnych postaci oraz właściwe ich leczenie jest trudne. Szczegółowa diagnostyka układu dopełniacza czasami wzbogacona o diagnostykę genetyczną jest zasadniczo zalecana we wszystkich przypadkach TMA, gdyż umożliwia zindywidualizowanie planu leczenia, oszacowanie rokowania oraz ryzyka nawrotu choroby, co zostało zobrazowane na przykładzie zamieszczonych opisów przypadków TMA u naszych pacjentów. W pracy przedstawiono sześć przypadków zespołu hemolityczno – mocznicowego (hemolytic uremic syndrome - HUS) nie związanego z zakażeniem bakteriami produkującymi Shigatoksynę - w tym: dwa przypadki atypowego HUS (aHUS) wywołanego obecnością przeciwciał przeciwko czynnikowi H (DEAP-HUS); przypadek aHUS wywołanego mutacją w genie diacyloglicerolu epsilon (DGKE); przypadek aHUS wywołanego mutacją w genie błonowego kofaktora białkowego (membrane cofactor protein – MCP); przypadek HUS wywołanego zakażeniem Streptococcus pneumoniae (Strep-HUS) oraz przypadek HUS wtórnego do posocznicy wywołanej przez Escherichia coli. U wszystkich dzieci została przeprowadzona szczegółowa diagnostyka układu dopełniacza; natomiast u piątki z nich – również diagnostyka genetyczna. Na przykładzie zamieszczonych opisów przypadków przedstawiono złożoność patogenezy TMA oraz znaczenie przeprowadzenia diagnostyki różnicowej we wszystkich przypadkach HUS nie związanych z Shigatoksyną, głównie z uwagi na istotne ryzyko wystąpienia przewlekłej lub schyłkowej niewydolności nerek, nawrotu aHUS w nerce własnej lub przeszczepionej jak i do potencjalnej przewlekłej terapii eculizumabem.

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Background

Thrombotic microangiopathies (TMA) are rare life threatening diseases of various etiologies, making the identification of the specific forms and appropriate treatment difficult. The etiology and pathophysiology-based classification of various TMAs is important to making clinical decisions and planning the therapy outline.

Clinical features that define hemolytic uremic syndrome (HUS) include the presence of acute kidney injury (AKI), signs of microangiopathic hemolytic anemia (elevation of lactate dehydrogenase - LDH, decreased haptoglobin and presence of fragmented red cells in the blood smear) and acute thrombocytopenia [1].

The majority of cases of pediatric HUS (90%) are associated with diarrhea and bacterial infections (such as enteropathogenic Escherichia coli – EPEC or Shigella dysenteriae) secreting Shiga-like toxins that provoke significant endothelial damage (Shiga toxin - HUS). Less frequently, HUS may also occur as a complication of various other infectious etiologies (e.g.: Streptococcus pneumoniae HUS / Strep-HUS, Influenza A / H1N1-HUS) or be secondary to coexisting diseases (e.g.: bone-marrow and solid organ transplantation, malignancies, autoimmune disorders, antiphospholipid syndrome, some drugs, malignant hypertension, HIV infection) [2].

According to the international consensus, the term of atypical HUS (aHUS) should be nowadays reserved for patients with HUS without any coexisting disease [2]. This means that in clinical practice, aHUS cases are limited to dysregulation of the alternative complement pathway (AP) HUS, mutations in the complement genes encoding membrane cofactor protein (MCP, CD46), C3, factor I (CFI), factor B (CFB), factor H (CFH), factor H related proteins 1-5 (CFHR 1-5) and thrombomodulin (THBD) or to the presence of auto-antibodies against factor H [2]. Excessive complement activation may be caused by gain-of-function in complement factors (due to genetic mutations) or loss-of-function in complement regulatory proteins (caused by genetic mutations or auto-antibodies). Approximately 60% of aHUS cases are diagnosed with a molecular AP abnormalities [3,4,5]. Other more rare factors, such as diacylglycerol kinase-epsilon (DGKE) mutations, and plasminogen (PLG) mutations have been also recently described as risk factors for the development of aHUS [6].

Thrombotic thrombocytopenic purpura (TTP) caused either by a severe congenital deficiency in metalloproteinase ADAMTS13 or by the acquired presence of anti-ADAMTS13 antibodies, should not be classified as aHUS, but has to be included in the differential diagnosis as an entity that has to be ruled out in patients suspected to have aHUS in the first sequence.

Summing up, the preceding data prove that the exact diagnosis of aHUS is particularly challenging, and mainly based on the exclusion of various clinical conditions and diseases, especially if aHUS triggering factors, such as various infections, are also simultaneously present. The following cases presentation exemplifies this difficult topic, underlying the necessity and importance of the requirement for full differential diagnostic workup, including genetic investigations of all aHUS cases, mainly after taking under consideration the fact that treatment modalities in particular cases may be significantly different.

Detailed diagnostic management of the complement system occasionally extended to include genetic workup is generally recommended in all non-Shiga toxin-caused HUS cases, as it allows for individualized mode of treatment planning, estimates the prognosis and risk of disease recurrence, which may be demonstrated based on the new case reports cited in the current survey.

In the first large series of patients with anti-complement factor H (CFH) antibodies-associated aHUS and deficiency of CFHR proteins antibody positive HUS (DEAP-HUS), the authors reported a poor outcome including death (9%), frequent relapses (58%), chronic kidney disease (CKD) in 39% and end-stage kidney disease (ESKD) in 27% after a mean follow up of 39 months [2]. However, in a recent cohort of children with this form of aHUS who were treated early with therapeutic plasma exchange (TPE), immunosuppressive medications and glucocorticosteroids (GCC), the outcome was much more favorable [7].

Material and Methods

In the current article we presented six cases of non-Shiga-toxin HUS including: two cases of aHUS caused by autoantibody against factor H (DEAP-HUS), aHUS caused by DGKE mutation, aHUS caused by MCP mutation, HUS caused by Streptococcus pneumoniae, and HUS secondary to Escherichia coli sepsis.

In our center there were three cases of DEAP-HUS that resulted in entirely different epilogues.

The first one from 2008 (a 7-year-old girl) ended up with ESKD within seven weeks from the onset of the disease. After two years of peritoneal dialysis (PD), prior to renal transplant (RT) preparation, the detailed diagnostic management confirmed DEAP-HUS and a proper treatment (TPE, mycophenolate mofetil - MMF, intravenous immunoglobulin-IVIG) made the whole transplantation procedure successful. The detailed information concerning this case is presented in publication by Grenda et al. [8].

In the current report, we present two new cases of DEAP-HUS that were managed in our center eight years later. The first one was a ten-year-old previously healthy boy (PP) who was admitted to our Department with signs and symptoms of nephritic-nephrotic syndrome (blood urea nitrogen – BUN: 8.3 mmol/l, serum creatinine – SCr: 76.3 umol/l, Cystatin C: 1.27 mg/l; estimated glomerular filtration rate - eGFR (Schwartz 2009): 58.8 mL/min/1.73 m^2, proteinuria: 2.14 g/l, erythrocyturia: 10-25 RBC/hpf); his detailed immunological diagnostics was within normal ranges, blood pressure (BP) was elevated, renal USG was normal. During the following three months, the renal parameters returned to normal ranges and proteinuria receded without any treatment. Six months after the first episode, during viral respiratory tract infection, the patient was admitted to hospital with signs and symptoms of HUS (BUN: 52.1 mmol/l, SCr: 390.3 umol/l, LDH: 3611 U/L, hemoglobin - Hb: 63 g/l, platelet number: 17.000/ul, proteinuria: 11.9 g/l, erythrocyturia: 80-120 RBC/hpf, direct antiglobulin test: negative), with oliguria (which lasted three days) and elevated BP. In initial complement evaluation only C3c was slightly decreased (0.7 g/l, ref. 0.9-1.8 g/L), whereas AP components and regulators were within the reference ranges. The patient was treated with a single hemodialysis session (HD) and (in total) with 7 TPE sessions, with a very good response within nine days, namely, his renal, hemolysis and complete blood count (CBC) parameters returned to the normal ranges. However, there was still nephrotic range proteinuria (12-17 g/l) persisting. In kidney biopsy performed during the second week of hospitalization - in light microscopy, the picture looked more like membranoproliferative glomerulonephritis - MPGN (glomerular lobulation, thickened capillary walls with double contouring), however, in electron microscopy, it looked more like TMA (rareness of lamina densa, podocytes foot processes effacement, endothelium edema). In detailed complement assessments, it was demonstrated that the patient had a positive titer of anti-CFH IgG auto-antibodies (218 AU/mL, ref.<110), a low level of factor H (71, ref. 250-880 mg/L), and signs of AP activation (a low level of C3c: 0.64 g/L), total AP complement activity: 55 % (ref. 70-105%). Genetic assessment showed homozygous deletion of the CFHR1 gene (DEAP-HUS), and heterozygous deletion of the CFHR3 gene. Due to suspicion of MPGN, the treatment with high doses of methylprednisolone - MP (15 mg/kg) and an intravenous cyclophosphamide (CP) course (500 mg/m^2) were initiated, and due to high BP - amlodipine was administered. The control complement results obtained two weeks afterwards confirmed the efficacy of the employed therapy, proving the disappearance of anti-CFH auto-antibodies and an increase of factor H level (287 mg/l). The renal parameters were normalized within two weeks, the proteinuria completely receded within four weeks. The patient is currently in a very good clinical condition, with negative anti-CFH auto-antibodies (102 AU/ml). The planned treatment includes six CP pulses and then under the anti-CFH auto-antibodies titer guidance – conversion into mycophenolate mofetil and gradual reduction of GCC.

The third DEAP-HUS case was an eight-year-old previously healthy boy (JS), who was admitted to our Department from a local hospital with signs and symptoms of HUS (oliguric, severely dehydrated, with yellow skin color, covered with numerous petechiae, BUN: 37.5 mmol/l, SCr: 238.5 umol/l, Cystatin C: 3.61 mg/l, LDH: 6482 U/L, Hb: 68 g/l, platelet count: 23.000/ul,

proteinuria 3.29 g/l, erythrocyturia: 50-80 RBC/hpf). His renal USG showed hyperechogenic, enlarged kidneys. In his medical history there were numerous severe episodes of vomiting after eating a hamburger in a local restaurant, but without diarrhea. The patient was treated with 3 HD sessions and (in total) with 9 TPE sessions, with a good response after the second TPE session – his renal, hemolysis and CBC parameters returned to the normal ranges. In detailed complement assessments, it was demonstrated that the patient had a positive titer of anti-CFH IgG auto-antibodies (1161 AU/mL, ref.<110), a low level of factor H (78, ref. 250-880 mg/L) and signs of AP activation (a low level of C3c: 0.86 g/L). Genetic assessment showed homozygous deletion of the CFHR1 gene, heterozygous deletion in exons 1-3 in both the CFHR3 and CFHR4 genes, and CD46 variations that are constituents of the MCPggaac risk haplotype. In kidney biopsy performed during the third week of hospitalization - in light microscopy, the picture looked more like MPGN (thickened capillary walls with double contouring, mesangial proliferation), however in electron microscopy, the picture was non-characteristic. Due to anti-CFH auto-antibodies aHUS - MP (15 mg/kg) and intravenous CP courses (750 mg/m^2) were initiated. Six weeks after the first symptoms of HUS, the renal parameters were within the normal limits; however there is still nephrotic range proteinuria (around 1 g/l) and hypertension requiring high doses of angiotensin converting enzyme inhibitor (ACEI), but the patient is currently in a very good clinical condition. The planned treatment includes six CP pulses and then under the anti-CFH auto-antibodies titer guidance – conversion into MMF and gradual reduction of GCC.

In the 2nd and 3rd case, the patients' native kidneys were saved and their function is so far absolutely normal. This vast difference is a result of not only better understanding the pathology of the disease, but also of the better access to specific diagnostics.

In approximately 10-15% of patients with aHUS, there are mutations of transmembrane complement alternative pathway regulator – membrane cofactor protein (MCP, CD46). MCP is expressed on the surface of most nucleated cells, including the kidney and endothelial cells, and protects cells from complement damage. In addition, MCP fulfills important roles in regulating various immune functions and acts as a receptor for various pathogens. MCP binds C3b through its extracellular domains, and acts as a cofactor of factor I in the proteolytic inactivation of C3b into iC3b [1,2]. Age at onset of aHUS in case of MCP mutations is usually above the 1st year of life [7].

In our center, there were two cases of MCP mutation, which finales were totally diverse.

The first one was a five-year-old girl (MJ) admitted to our Department with signs and symptoms of HUS (BUN: 20 mmol/l, SCr: 577 umol/l, LDH –2974 U/L, proteinuria: 2.6 g/l, erythrocyturia: field covered with red blood cells), with no preceding diarrhea, but with a positive history of viral respiratory tract infection. One TPE and 5 HD sessions were performed. The complement (C3c, C4) and other immunological tests were normal. Due to the severe hypertension and cardiac failure, the antihypertensive medications were initiated. The first kidney biopsy did not confirm HUS. The kidney function improved, but the hypertension persisted. Two years later, hypertension emergency with neurological symptoms was diagnosed and she required treatment in the pediatric intensive care unit (PICU). eGFR decreased to 50 ml/min/1.73m^2. The second kidney biopsy revealed vascular changes of thickened capillary walls and 50% of sclerotic glomeruli. The patient's nephrological follow-up visits were subsequently very irregular. Seven years later, she was again admitted to PICU with ESKD, cardiac failure and chorioretinitis. The HD sessions and pleural drainage were necessary. In the third kidney biopsy, vascular changes of thickened capillary walls and narrowed blood vessels were found again. In the immunological tests, the p-ANCA titer was positive, but the complement levels were within normal ranges. The GCC therapy did not improve the kidney function and that is why chronic HD therapy was initiated, during which she presented with severe hypertension despite intensive antihypertensive therapy. Due to refractory hypertension, she had bilateral nephrectomy performed. The genetic investigation revealed a heterozygous mutation (p.Pro155Leu) in exon 4 of the CD46 gene. Neither mutation in factor H nor anti CFH antibody presence was noted. After 14 months on HD, she was successfully transplanted from a cadaveric donor. There were no more HUS recurrences observed later in her follow up.

On the other hand, in the second case (MB) that was treated fifteen years later (the detailed description by Miklaszewska et al. [1]), where a new mutation in the CD46 gene was demonstrated and the aHUS case was triggered by Mycoplasma pneumoniae infection, the patient remains in a very good clinical condition with no renal sequelae.

Although complement abnormalities are typical in aHUS, the normal complement profile does not exclude the possibility of aHUS. Lemaire et al. reported that recessive mutations in the gene encoding for diacylglycerol kinase epsilon (DGKE) cause aHUS [9]. DGKE is a protein of the lipid kinase family expressed in the endothelium, platelets and podocytes, which has been identified in an autosomal recessive form of aHUS occurring in the first year of life [9,10,11]. The mechanism of HUS in DGKE mutation is most likely related to the activation of protein kinase C due to the loss of function of DGKE, leading to an upregulation of prothrombotic factors and platelet activation [12].

Below, we present a case of DGKE gene mutation which is considered functionally relevant as most probably no active DGKE protein is produced by the cells of the patient. A five-month-old previously healthy girl (MP) born of healthy unrelated parents, was admitted to PICU with signs of TMA and AKI requiring PD after an episode of non-bloody diarrhea. In the medical history, there had been loss of appetite, somnolence, fever and a few episodes of vomiting continued for three days. On admission to PICU, she was in a severe general condition, anuric, with very high blood pressure values and numerous petechiae. Chest X-ray demonstrated severe pneumonia; in the tracheal-bronchial aspirate, Klebsiella pneumoniae was cultured. In the laboratory tests there were signs suggestive of HUS (SCr: 336 umol/l, Hb: 63 g/l, platelet number: 20.000 / ul, LDH: 11994 U/L, direct antiglobulin test – negative, proteinuria: 25.2 g/l, erythrocyturia: 100 RBC/hpf). In the initial complement evaluation, both C3c and C4 were slightly decreased (C3c: 0.62 g/l, C4: 0.1 g/l). The following treatment was implemented: PD (16 days), synchronized intermittent mandatory ventilation - SIMV (30 days), total parenteral nutrition, wide spectrum of antibiotics, antihypertensive treatment, numerous transfusions of red blood cells (RBC), fresh frozen plasma (FFP) and due to a decreased level of IgG – IVIG infusions. After 42 days spent in PICU, there was a gradual recovery in the general patient condition and kidney function, but the girl remained severely hypertensive, requiring multiple antihypertensive regimens. The patient was treated with ACEI with good resolution of proteinuria and mild persisting hematuria. At the age of one year, during an upper respiratory tract infection, there occurred a relapse of aHUS (SCr: 150 umol/l, LDH: 2976 U/L, platelet number: 53.000 /ul, Hb: 62 g/l, nephrotic range proteinuria up to 14.0 g/l), which was treated with FFP infusions with a very moderate response lasting four weeks. Since then, during viral infections of the respiratory tract, the patient presented twice with transient proteinuria up to 2.5 g/l that lasted for a few days and decreased spontaneously to the opalescence level after the infection terminated. At the last follow-up, the patient who was at the time 2 years old had normal kidney function and no proteinuria, but mild erythrocyturia and hypertension requiring four antihypertensive medications in maximum doses. A detailed complement diagnostic workup proved the normal levels of the complement activation products, but also identified a homozygous variation causing early stop codon in the DGKE gene (c.966G>A (exon 6), p.W322*).

There are also forms of HUS that include cases triggered by neuraminidase-producing pathogens, such as Streptococcus pneumoniae (Strep-HUS). This is not an atypical form of HUS, but due to a high mortality rate and complicated clinical course it is worth mentioning. The data in the literature suggest that severe complement dysregulation and consumption accompany the progress of invasive pneumococcal disease-associated Strep-HUS and genetic variations of complement genes may

contribute to the development of this complication in some proportion of the affected patients [13].

Here, we present a case (BB) of a 4 – year-old previously healthy boy born of healthy unrelated parents that was admitted to pediatric pulmonology ward in a good general condition with lobar pneumonia. After a few days of antibiotic treatment, his condition deteriorated and he needed intubation and SIMV for 17 days. During his PICU stay, he developed HUS (SCr: 259 umol/l, BUN: 44.2 mmol/l, LDH: 20832 U/L, platelet count: 21.000/ul, Hb: 65 g/l, proteinuria: 14.0 g/l). The inflammatory markers were positive (C-reactive protein - CRP: 229.6 mg/l, procalcitonin – PCT: 45.6 mg/l). As the direct antiglobulin test assessed six days after the introduction of antibiotics was slightly positive and the blood culture and tracheal-bronchial aspirate were both positive for Streptococcus pneumoniae, the patient did not receive any FFP infusions. For 11 days, there was oligo-anuria and for six days, the patient required continuous renal replacement therapy (CRRT). On the 12-th day of the PICU stay, he developed pneumothorax that required drainage. In the initial complement evaluation, there was only slightly decreased C4 (0.09 g/l), whereas AP components were within the reference ranges. A detailed complement diagnostic workup proved the normal levels of the complement activation products, except the elevation of CFB (204%), terminal complement complex (sC5b-9: 482 ng/ml, ref. 110-252 ng/ml) and C3a anaphylatoxin (467 ng/mL, ref. 70-270 ng/ml). Of note was the elevation of complement factor B antigen that was also observed in both parents of the patient. At the last follow-up, four months after hospitalization, the patient is in a very good condition with normal kidney function, without proteinuria, erythrocyturia and hypertension.

Despite these advances in our understanding of the molecular basis of aHUS, no genetic defect has yet been found in one-half of aHUS patients, and incomplete penetrance of the disease in individuals carrying factor H, factor I or MCP mutations is relatively frequent, suggesting the existence of additional genetic factors that predispose to aHUS [14]. An example of such a patient may be our case presented below, where no abnormalities were found either in the complement system or in genetic tests, although this patient might be considered as presenting with secondary form of HUS due to concomitant infection. Nevertheless, due to the severe clinical course of the disease, the authors decided to present this case in this survey. A 10-month-old girl (HR) presented in our Department with high fever, urinary tract infection (UTI), without preceding diarrhea. In a local hospital, sepsis and AKI were diagnosed. In our center, the child was in a severe clinical condition, apathetic, hypovolemic, oliguric with normal BP and no petechiae. Laboratory tests confirmed HUS (platelet number: 17 000/ul, BUN: 35.4 mmol/l, SCr: 283 umol/l, LDH: 3521 U/L, proteinuria: 12 g/l, erythrocyturia: 20-30 RBC/hpf), elevated inflammatory markers (CRP: 74 mg/l, PCT: 12.1 ng/ml) and abnormalities in the coagulation system (elevated D-dimer and fibrinogen levels). The blood culture was positive for Escherichia coli (non EPEC), none of stool cultures was positive, direct antiglobulin test was negative. The broad spectrum of antibiotics, RBC, albumin, FFP, and IVIG infusions together with diuretics were introduced, but without satisfactory urine output response. In the face of failure of the conservative treatment, the PD for 4 days was initiated, which was complicated with massive leakage and malfunction of the catheter with necessity of its exchange. After two weeks, the platelet number was normalized, and renal function improved after three weeks. Hypertension, malnutrition and massive proteinuria were observed as complications of the HUS episode. After one year of follow-up, the child presented with one severe episode of UTI, eGFR reached about 70 ml/min/1.73m^2, with urine protein to urine creatinine ratio of 0.56 [mg%/mg%] on a small dose of ACEI treatment. The detailed complement research indicated secondary (to infection) activation of the classic (decreased total complement activity of the classic pathway: 28 CH50/ml (ref. 48-103 CH50/ml) and C1q Ag: 35 mg/l (ref. 60-180 mg/l)) and alternative (decreased total complement activity of the alternative pathway: 60 % (ref. 70-105%) and C3c: 0.85 g/l) complement pathway. Nevertheless, no mutations potentially responsible for aHUS were detected. Other immunological tests were also within the normal limits.

Discussion

The risk of death or ESKD during aHUS episodes is variable, depending on the basic causes. In a case of DGKE, it is 7% in the first month up to five years; while CKD grade 4–5 occurs at 20–25 years [9]. On the other hand, in case of MCP it is 17% and 0 %, respectively, although in patients with MCP mutation, the presence of mutations in other genes increases the risk of progression to ESKD as compared to patients with isolated MCP mutation [15].

The knowledge of the underlining genetic defect and its functional consequences is of utmost importance for patients' management, especially when the decision for transplantation must be made, as the highest risk factor for aHUS recurrence is the presence of CFH, C3 or CFB mutations (approximately 80%) and 50% in patients with CFI mutation, as compared to approximately 20% in patients with no identified complement mutation [2,16]. The recurrence risk is low in anti - CFH antibody aHUS if the antibody titer is low at the time of transplantation, while it is substantial if the titer is elevated [17, 18, 19, 20, 21]. The risk of post-transplant recurrence in patients with isolated MCP mutation has been shown to be lower than 10 % [15], while no post--transplant recurrence has been observed in patients with DGKE mutation [9]. According to the literature, the complement regulatory treatment is in this specific mutation (DGKE) ineffective; however, renal transplantation may be successful without the risk of relapse [12]. The screening for DGKE mutation should be performed in all children with onset of aHUS before the age of two years [9,10,11].

According to the European Pediatric Study Group for HUS guidelines from 2009, the first line therapy in aHUS is to initiate TPE within 24 hours of the diagnosis. However, in 2012, the French Study Group for aHUS/C3G [22], and in 2015 - Campistol el al. [23] in a consensus document recommend eculizumab as the first line therapy in aHUS in children.

It is of utmost importance to collect and store blood samples (for EDTA, EDTA plasma, citrate plasma and serum) from a patient before any treatment implementation, with purpose of specialized diagnostic workup.

Eculizumab is a monoclonal humanized antibody inhibiting C5 activation and the formation of the membrane attack complex, responsible for damage to self--structures in aHUS. In many prospective studies with aHUS patients, eculizumab effectively prevented the TMA process and was associated with long-term significant hematological and renal function improvements [23,24].

According to the literature, eculizumab is recommended as the early-use agent for aHUS in pediatric and adult patients with clinically suspected aHUS in native and transplanted kidneys (including aHUS recurrence treatment and aHUS prophylaxis). Furthermore, eculizumab should be considered in patients with secondary TMA refractory to regular treatment [23]. According to Walle et al. [24], patients with aHUS derive greater and more sustained eGFR recovery benefit from treatment with eculizumab when the therapy is started earlier. However, while planning chronic treatment in a TMA patient, one should remember that there are two situations when eculizumab is definitely not the first line therapy: the TTP cases [25, 26] and the cases caused by DGKE mutation(s) [9].

Currently, there are no trustworthy recommendations in the literature concerning the right treatment duration with eculizumab and the terms of its safe withdrawal, though increasing experience with the drug use will help better define this issue as well as other treatment strategies in the future [23]. Moreover, making the decision about eculizumab treatment one must keep in mind the necessity of antibiotic prophylaxis implementation against encapsulated bacteria (among others: Neisseria meningitidis and Streptococcus pneumoniae) as well as realization of targeted vaccinations at earliest convenience.

Conclusion

The diagnosis of TMA is challenging and is based mainly on the exclusion of ADAMTS13 deficient TTP and HUS caused by Shiga toxin. In case of thrombocytopenia and concurrent pneumonia in differential diagnosis apart from disseminated intravascular coagulation one always sho-

uld acknowledge Strep-HUS. Furthermore, at present, definitive exclusion or confirmation of a complement-related genetic defect is time-consuming, very expensive and not universally possible. Nevertheless, the complexity of TMA, and the importance of the complete differential diagnostic workup in all non-Shiga toxin-associated HUS cases due to the high risk of developing CKD or ESKD, the risk of aHUS relapse (both in native or transplanted kidneys) and potential prolonged eculizumab treatment is highlighted by the present case histories.

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