The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Atypical hemolytic uremic syndrome: genetic landscape challenge

Laura Valiña[1,2], Bernardo López Andrade[3], Josep Miquel Bauça[1,2]

[1] *Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma, Balearic Islands, Spain*

[2] *Institut d'Investigació Sanitària de les Illes Balears (IdISBa), Spain*

[3] *Department of Hematology and Hemotherapy, Hospital Universitari Son Espases, Palma, Balearic Islands, Spain*

ARTICLE INFO

Corresponding author:
Laura Valiña Amado
Hospital Universitari Son Espases
Ctra. de Valldemossa, 79, module J-0
07010 Palma, Balearic Islands
Spain
E-mail: laura_valinha@hotmail.es

Key words:
alternative complement, hemolytic syndrome, kidney injury

CASE REPORT

An obese and current smoker 23-year-old woman presented to the Emergency Department with abdominal pain, two episodes of vomiting and watery non-bloody diarrheic depositions. She indicated she had not urinated for ten hours. The patient had come the previous day complaining of intense headache, being prescribed dexketoprofen, diazepam and metoclopramide, as she had had previous episodes of whiplash. She was taking no medication and had no other remarkable medical records.

At this second presentation, a complete blood count and biochemical study were requested, revealing leukocytosis with neutrophilia, marked thrombocytopenia, increased creatinine, bilirubin and aminotransferases (Table1). The patient was afebrile. An abdominal echography was performed where normal size and morphology of liver, biliary ducts, gallbladder and kidneys were observed. She was admitted at Nephrology Department and kept under observation with a diagnostic workup of acute kidney injury.

On the following day, leukocyte count almost normalized, but a marked decrease on platelet count was seen. Creatinine increased to 7.87 mg/dL, haptoglobin was undetectable and LDH activity was 5,340 U/L. Four to five schistocytes/field were observed in peripheral blood smear. The patient remained anuric since the day of admission. All these findings prompted a work-up for thrombotic microangiopathy (TMA).

DISCUSSION

TMA is a set of pathologies characterized by microvasculature thrombosis and organic dysfunction, originated by different etiologies that can be categorized as congenital or acquired.

The endothelium is injured and the release of von Willebrand Factor (vWF) induces thrombogenesis. Thrombi usually occlude small-caliber blood

Table 1 Evolution of hematological and biochemical parameters

	Ref. interval	Day 1	Day 2	Day 3	Day 5	Day 10	Day 27	After 8 months
Leukocytes, 10^3/µL	3.5 – 11.5	**23.3***	**18.7***	15.3	12.4	14.3	19.3	9.82
Neutrophils, 10^3/µL	2.5 – 11.0	**20.3***	**15.9***	**12.1***	8.04	5.89	10	4.71
Erythrocytes, 10^6/µL	4.00 – 6.00	4.82	4.34	**3.92***	**3.15***	**2.65***	4.46	4.63
Platelets, 10^3/µL	120 - 400	**32.8***	**10.9***	**11.4***	**20.0***	122	179	292
Creatinine, mg/dL	0.57 – 1.11	**5.99***	**7.87***	**9.46***	**7.58***	**9.53***	**1.25***	0.74
Total bilirubin, mg/dL	0.2 – 1.2	**2.9***	**3.4***	**2.4***	**2.1***	0.3	-	-
Direct bilirubin, mg/dL	0.0 – 0.5	**1.3***	-	-	**0.7***	-	-	-
ALT, U/L	0 - 55	**443***	**325***	**121***	-	37	-	-
GGT, U/L	9 - 36	**204***	**159***	**66***	-	**62***	-	-
Haptoglobin, mg/dL	14 - 258	-	**<8***	-	**<8***	-	220	-

CRP, mg/dL	0.0 – 0.5	-	**17.98***	-	-	2.64	0.13	-
LDH, U/L	125 - 220	-	**5,340***	**4,027***	**1,718***	**463***	-	-
Procalcitonin, ng/mL	0.0 – 0.06	-	**> 100***	-	**37.41***	-	-	-

Abbreviations: ALT (alanine aminotransferase); GGT (gamma-glutamyltransferase); HAPT (haptoglobin); CRP (C-reactive protein); LDH (lactate dehydrogenase).
** Values out of the reference interval.*

vessels, especially in the kidney. Obstruction in other organs' vessels may also occur, such as brain, heart, gut, pancreas and lung, hence the observation of common extra-renal symptoms. Blood pressure is usually elevated in patients suffering from TMA, however the one here presented always had values within reference interval. Erythrocyte fragmentation takes place due to friction with the thrombi, thus generating a non-immune hemolytic anemia.

The consensus recommendations in 2016 [1] suggest that TMA must be considered in any patient presenting with microangiopathic hemolytic anemia, thrombocytopenia, schistocytes in peripheral blood (more than one percent) and biochemical signs of non-immune hemolysis (elevated LDH, indirect bilirubin and low haptoglobin levels, with negative Coombs assay). The presence of schistocytes only could be enough in case of clinical evidence.

Procalcitonin, a sepsis biomarker, was greatly increased in this patient despite having negative blood culture results, in accordance with the fact that 40-60% of patients with sepsis may yield a negative blood culture [2]. On day two, intravenous ciprofloxacin was started since enterotoxigenic *Escherichia coli* infection was suspected, however no clinical response was observed and the patient did get worse.

According to etiology, TMA can be classified in: (1) thrombotic thrombocytopenic purpura (TTP), caused by a decreased activity (lower than ten percent) of ADAMTS-13 (a disintegrin- like and metalloprotease with thrombospondin type one motif number 13), which can be of genetic or immune source (antibodies developed after treatment with ticlopidine or clopidogrel); (2) hemolytic uremic syndrome (HUS), as a result of bacterial infections such as the shiga toxin-producing *E coli* or *Streptococcus pneumoniae* (via neuraminidase); (3) atypical HUS (aHUS), associated with genetic or immune complement system alterations (mutations in MCP, CFH, THBD, CFB and C3; antibodies against CFH); and (4) secondary TMA (Table 2) [3].

The best way to start the differential diagnosis is to assess ADAMTS-13 activity. When higher than ten percent, TTP may be ruled out. The patient had an ADAMTS-13 activity of 82%. The second step would be testing for STEC or STEC-like infections. Stool and blood cultures were negative. Urine culture yielded 16,000 cfu/mL of *E coli.*

Although more commonly associated with intestinal STEC infections, HUS can also be induced by urinary tract STEC infections, however this isolated *E coli* strain was not tested for shiga toxin production [4]. Viral serologies (HIV, HAV, HBV, HCV, CMV, EBV and influenza virus) and assays for fecal *E coli* (ECEH, ECEP, ECET, ECEA), *S pneumoniae* and other bacteria were all negative. Bearing in mind these results, an infectious cause was not very likely.

Immunoglobulins were within the reference intervals. The complement study on day two displayed a slight increase in C3 and C4 without clinical relevance.

Rheumatoid factor, ANA, ANCA and anti-glomerular basement membrane were negative. Thus, an autoimmune disease was discarded. Direct and indirect Coombs tests were negative, hence ruling out an autoimmune hemolytic anemia. Pregnancy test was negative.

Methylmalonic aciduria with homocystinuria is produced by a mutation in the CblC gene, due to a deficiency in methylcobalamin and adenosylcobalamin associated with HUS. Although more commonly seen in neonates, two different cases have been reported in adults [5,6].

Table 2 Causes of secondary thrombotic microangiopathy

Causes of secondary TMA	
Pregnancy	HELLP syndrome
	Postpartum
Systemic diseases	Systemic lupus erythematosus
	Antiphospholipid syndrome
	Scleroderma
	Vasculitis
Treatments	C mitomycin, quinine, gemcitabine, cisplatin, ionizing radiation, interferon, VEGF and tyrosine kinase inhibitors (sunitinib, imatinib and dasatinib), ticlopidine, clopidogrel, calcineurin inhibitors (cyclosporine, tacrolimus), sirolimus, valaciclovir, oral contraceptives, etc.
Others	HIV infection
	Glomerulopathy
	Malignant arterial hypertension
	H1N1 infection (influenza A)
	Neoplasia
	Methylmalonic aciduria with homocystinuria
Solid organ or bone marrow transplantation	

Quantification of folate, vitamin B12 and homocysteine could not be performed, as all blood samples were significantly hemolyzed.

Once discarded all other causes in the differential diagnosis, aHUS was suspected. aHUS has a prevalence of one to two cases per million in the USA and 0.11 cases per million in Europe. In children, no gender-dependent incidence has been described, while in adults it is more commonly seen in women. aHUS may emerge at any age, being more frequent in childhood [9].

A screening for possible complement alternative pathway regulatory protein alterations was performed (suspecting of aHUS), including serum alternative pathway H factor (CHF), MCP (Membrane Cofactor Protein; CD46) and I factor concentrations; antibodies anti-H factor; CHF functional alteration assay; and a Western Blot of HF and FHRs. A comprehensive genetic study was also performed, assessing pathogenic variants in the following genes: CFH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, C3, CFI, MCP, CFB, THBD, DGKE, CFP and ADAMTS-13; none of them being found. Heterozygotic change in MCP (CD46) exon 6 (c.686>A, p.Arg229Gln, rs201380032) was detected as variant of unknown significance.

CD46 flow cytometry can be used to assess for genotype/phenotype correlation in unclear cases. Further testing of CFH (H3) risk haplotype polymorphism revealed a deletion in CFHR3-CFHR1 in heterozygosis as well, known to be a common polymorphism in Spanish population, only relevant in homozygosis [7,8]. Biochemical and immunological studies of the complement did not demonstrate any abnormalities.

Genetic variant effect prediction algorithms are used to determine the likely consequences of amino acid substitutions on protein function. The genetic variants prediction study indicated a possible benign effect on the functionality of the protein, as stated by the reference operator laboratory. Furthermore, MCP levels in peripheral blood leukocytes were optimal. The MCP variant detected is not pathogenic and thus not the causal agent of the disease. A few mutations of alternative complement pathway regulatory proteins were described that relate to this syndrome. However, those would only explain 60% of aHUS cases. Some polymorphisms predispose to the development of aHUS when other environmental factors are present.

After five sessions of plasmapheresis and methylprednisolone administration, no response to treatment was observed, so therapy with eculizumab [10] was started on day six. Eculizumab treatment must be initiated only after having confirmed *N meningitidis* vaccination, as the treatment increases the risk of infection by this microorganism due to its mechanism of action (C5 binding, precluding its cleavage into the effector molecules). If the patient is not vaccinated, vaccine must be applied at least 14 days prior to eculizumab initiation. If eculizumab treatment cannot be delayed, appropriate antibiotic prophylaxis must be added since the moment of the vaccination for 14 days. Simultaneous ceftriaxone prophylaxis was set. 48 hours after the first dose of eculizumab, the platelet count increased and LDH activity decreased.

By the day of the medical discharge, creatinine was almost normal. The patient was kept under eculizumab treatment every 14 days, having totally restored her kidney function. After 13 months of treatment and no relapse or complication, eculizumab suspension was decided by the Nephrologist. The patient has not suffered any relapse three months afterwards.

REFERENCES:

1. Go RS, Winters JL, Leung N, et al. Thrombotic Microangiopathy Care Pathway: A Consensus Statement for the Mayo Clinic Complement Alternative Pathway-Thrombotic Microangiopathy (CAP-TMA) Disease-Oriented Group. Mayo Clin Proc. 2016 Sep;91(9):1189-211.

2. de Prost N, Razazi K, Brun-Buisson C. Unrevealing culture-negative severe sepsis. Crit Care. 2013 2013 Sep 26;17(5):1001.

3. Afshar-Kharghan V. Atypical hemolytic uremic syndrome. Hematology Am Soc Hematol Educ Program. 2016 Dec 2;2016(1):217-225.

4. Chiurchiu C, Firrincieli A, Santostefano M, et al. Adult nondiarrhea hemolytic uremic syndrome associated with Shiga toxin Escherichia coli O157:H7 bacteremia and urinary tract infection. Am J Kidney Dis 2003 Jan;41(1):E4.

5. Masias C, Vasu S, Cataland SR. None of the above: thrombotic microangiopathy beyond TTP and HUS. Blood 2017 May 25;129(21):2857-2863.

6. Cornec-Le Gall E, Delmas Y, De Parscau L et al. Adult-Onset Eculizumab-Resistant Hemolytic Uremic Syndrome Associated With Cobalamin C Deficiency. Am J Kidney Dis. 2014 Jan;63(1):119-23.

7. Loirat C, Frémeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011 Sep 8;6:60.

8. Angioi A, Fervenza FC, Sethi S, et al. Diagnosis of complement alternative pathway disorders. Kidney Int 2016 Feb;89(2):278-88.

9. Campistol JM, Arias m, Ariceta G, et al. An update for atypical haemolytic uraemic syndrome: Diagnosis and treatment. A consensus document. Nefrología. 2015; 35(5):421-47.

10. Legendre CM, Licht C, Muus P, et al. Terminal Complement Inhibitor Eculizumab in Atypical Hemolytic–Uremic Syndrome. New Engl J Med 2013 Jun 6;368(23):2169-81.