CASE REPORTS

Thrombotic microangiopathy with renal involvement: Case report and considerations on differential diagnosis and treatment

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Abstract

The first approach to a patient with thrombotic microangiopathy (TMA) involves differentiating thrombotic thrombocytopenic purpura (TTP) from hemolytic uremic syndrome (HUS), since both pathogenesis and treatment differ. Case-by-case decision making based on all the clinical, laboratory and response-to-therapy criteria can result in an accurate diagnosis, leading to the most appropriate therapy and a better chance of improving clinical outcome while preserving organ function. We report the case of a 21-year-old female admitted for TMA characterized by severe renal involvement and no neurological symptoms. Clinical onset occurred after a three-day long episode of diarrhea. First analysis showed no evidence of shiga toxin-producing *E. Coli* (STEC) and revealed severely deficient ADAMTS13 activity, which is common in TTP. After an initial response to steroids and plasma exchange therapy (PEX), the patient became PEX-dependent. A complete remission and PEX-independence was achieved by a B cell depletion therapy with Rituximab. Results of the subsequent genetic analysis showed a new heterozygous variant p.Arg448Leu, as an isolated mutation, in the complement factor I gene.

Keywords

Thrombotic microangiopathy, Acute kidney injury, Thrombotic thrombocytopenic purpura, Hemolytic uremic syndrome, Rituximab, Complement factor I gene mutation

1 Introduction

The first approach to a patient with thrombotic microangiopathy (TMA) imposes differentiating thrombotic thrombocytopenic purpura (TTP) from typical hemolytic uremic syndrome (tHUS) or atypical (aHUS) hemolytic uremic syndrome, as their pathogenesis and treatment differ [1, 2].

The thrombotic microangiopathy syndromes are extraordinarily heterogeneous. They may be hereditary or acquired and occur at any age. The onset can be sudden or gradual. Despite their diversity, TMA syndromes are united by common, defining clinical and pathological features. The clinical features include microangiopathic hemolytic anemia, thrombocytopenia, and organ injury [1-3]. However, differentiating between different TMAs is crucial in order to optimize the therapeutic approach and distinguishing between TTP and HUS might still represent a medical challenge [1].

Neurological injury is more frequent in patients with TTP, but has also been found in aHUS. Severe renal impairment is characteristic of HUS, but has also been seen in TTP; deficient ADAMTS13 activity has been reported in almost all TTP cases, but also in 13%-55% of aHUS cases; complement activation is usual in aHUS, but also plays a role in immune acquired TTP.

Nevertheless, the identification of pretreatment clinical and laboratory variables to predict the patient's response to therapy to include PEX is crucial to optimize the management, to choose the most appropriate therapy and to improve clinical outcomes while preserving organ function [3].

2 Case report

A 21-year-old female patient was admitted for abdominal pain, vomiting, and diarrhea that had been ongoing for 3 days. Laboratory tests showed thrombocytopenia with 17,000/mm^3 platelets (PTLs), hemolytic anemia (Hb 11 g/dl, total bilirubin 6 mg/dl, indirect bilirubin 5.5 mg/dl, LDH 3,895 U/L, haptoglobin 1 mg/dl, presence of schistocytosis), microscopic hematuria at the urinary sediment examination, proteinuria > 300 mg/dl, renal failure (sCr 1.7 mg/dl) and mild hypocomplementemia (C3 75 mg/dl; C4 7 mg/dl). She had neither hypertension nor neurological symptoms. First diagnostic hypothesis was TMA. However, differentiating between typical or atypical HUS or TTP was not possible at the time of admission. The patient had no family history of TMA, was not taking oral contraceptives, and had no vascular devices. Her previous medical history was characterized by episode of tonsillitis, asymptomatic double left renal excretory district with normal renal function, and two previous episodes of widespread normocomplementemic urticaria of unidentified origin (successfully treated with steroids and antihistamines).

Further laboratory tests showed negative viral markers (HBsAg, HCVAb, HIV) and no serological evidence of connective tissue disorders (negative antinuclear antibodies, anti DNA antibodies, anti-neutrophil cytoplasmic antibodies, lupus anticoagulant, anticardiolipin antibodies, anti-beta 2 glycoprotein 1 antibodies. Malignancies were also ruled out (negative chest X-ray, breast and abdominal ultrasonography, brain MRI, gastroscopy, gynecological examination) and negative disseminated intravascular coagulation (DIC) panel.

During admission, stool and blood samples were tested for STEC, for ADAMTS13 activity, anti-ADAMTS13 antibodies and complement gene mutations.

A first line therapy with steroids (methylprednisolone 0.8 mg/kg/day) and daily PEX (with one and a half plasma volume replacement in every session) were started (see Table 1). Nevertheless, few hours following the beginning of the treatment with steroids and PEX, further worsening of both hemolytic anemia (Hb 6.9 g/dl) and thrombocytopenia (PLTs 7,000/mm^3) occurred, diuresis decreased and peripheral edema developed, thus requiring hemodialysis to be started.

After some days of treatment, diuresis gradually increased and diarrhea ended. Meanwhile, a negative coproculture for STEC 0157 was reported. Baseline value of ADAMATS13 activity before PEX was 0% with no evidence of anti-ADAMTS13 antibodies. A gradual recovery of renal function (that allowed stopping dialysis after 6 sessions) was achieved after 15 days of treatment with steroids and daily PEX. PTLs count and LDH levels slowly improved, but no

amelioration was observed in haptoglobin levels. ADAMATS13 activity level increased during the follow-up as detailed in Table 1.

Table 1 shows clinical events and laboratory panels up to 166 days after admission. Baseline serum protein electrophoresis showed a minimal increased in the alpha-1 zone (5.9%, n.v. < 4.9%). Urine protein electrophoresis (at day 22) showed increased level of RPG (0.25 mg/dl, n. v. < 0.1), albumin (789 mg/L, n.v. < 30) and IgG (33.30 mg/L, n.v. < 8.5). Taking into the account the above, the case was collegially discussed and a second diagnostic hypothesis of TTP was formulated on the basis of the severely deficient ADAMTS13 activity, even despite the lack of neurological symptoms. One could speculate that a diagnosis of acquired TTP could not be ruled out, on the basis of complement consumption. However, no evidence of anti-ADAMTS13 antibodies was reported. Nevertheless, anti-ADAMTS13 antibodies can be occasionally undetected. Congenital TTP, was ruled out because of the late clinical onset (first manifestation at age 21), normal ADAMTS13 activity in both parents, and a prompt normalization of ADAMTS13 levels after 7 sessions of PEX.

When PTLs count increased up to 100,000/mm^3, the frequency of PEX sessions was reduced from daily to every other day. However, a new drop in PTLs count and complement levels was observed. As she developed a PEX-dependence, a B-cell depletion therapy with Rituximab (375 mg/m^2 weekly for 4 times) was added. Clinical condition gradually improved: PTLs normalized in 4 weeks, and remained stable even after discontinuing plasma and steroids. Hemoglobin, haptoglobin and complement gradually improved and a complete recovery of renal function was observed and ADAMTS13 activity remained at 100%.

Table 1. Therapy and laboratory biomarkers detailed from day 1 to end of the follow-up

	Day																
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Therapy																	
Hemodialysis session			X	X	X	X	X		X								
Plasma exchange session	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	
Plasma infusion																	
Blood transfusion			X	X			X		X		X			X		X	
Steroids	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Rituximab 375 mg/m^2																	
Laboratory Results																	
Creatinine (0.4-1.0 mg/dl)	**1.8**	**3.3**	**5.8**	**5.8**	**5.3**	**5.0**	**4.8**	**4.1**	**5.6**	**3.6**	**4.8**	**4.8**	**4.8**	**3.9**	**3.0**	**2.4**	**2.0**
Platelets (140-390 × 10^3)	**13**	**7**	**14**	**18**	**27**	**31**	**38**	**45**	**45**	**60**	**59**	**61**	**72**	**93**	**105**	**111**	**120**
Hemoglobin (12-18 g/dl)	**11.5**	**9.5**	**6.9**	**7.2**	**9.2**	**8.5**	**7.7**	**8.5**	**7.5**	**8.2**	**7.1**	**7.9**	**7.4**	**6.7**	**8.2**	**7.1**	**8.2**
LDH (230-500 U/l)	**3895**	**2641**		**1936**	**1487**	**1929**	**2196**	**1945**	**1588**	**1238**	**1004**	**813**	**650**	**655**	**632**	**561**	
Haptoglobin (40-200 mg/dl)	**1**	**1**	**1**	**1**	**3**	**2**	**2**	**2**				**1**		**1**	**1**	**1**	
C3 (88-201 mg/dl)							**76**										
C4 (16-47 mg/dl)							**8**										
ADAMTS13 activity (%)	**0***						**100**										

(Table 1 continued on page 30)

Table 1. (Continued).

	Day																
	18	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**29**	**36**	**43**	**74**	**105**	**135**	**166**
Therapy																	
Hemodialysis session																	
Plasma exchange session		X		X													
Plasma infusion					X	X	X		X	X		X					
Blood transfusion		X															
Steroids	X	X	X	X	X	X	X	X	X	X	X	X	X	X	stop		
Rituximab 375 mg/m^2					X						X	X	X				
Laboratory Results																	
Creatinine (0.4-1.0 mg/dl)	**2.1**	**1.5**	**1.4**	**1.2**	**1.1**	**1.1**	**1.2**	**1.1**	**1.2**	1.0	0.9	1.0	0.8	0.9	0.8	0.6	0.8
Platelets (140-390 × 10^3)	**130**	**118**	**115**	**108**	**106**	**114**	**108**	**112**	**117**	**98**	**112**	**124**	150	236	231	190	272
Hemoglobin (12-18 g/dl)	**7.7**	**7.7**	**9.5**	**9.0**	**8.6**	**9.3**	**8.7**	**9.5**	**9.6**	**9.4**	11.9	12.6	14.0	13.5	12.8	12.4	13.1
LDH (230-500 U/l)	**541**	**522**	442	**504**	388	436	464	496		**504**	**547**	425	462	380	369	335	336
Haptoglobin (40-200 mg/dl)	**1**	**1**	**1**					**1**			**1**	**1**	**3**	53	72	26	82
C3 (88-201 mg/dl)	**69**		**67**		**59**			**76**			90	110	91	97	112	83	119
C4 (16-47 mg/dl)	**7**		**8**		**4**			**9**			**11**	**14**	**15**	16	21	15	7
ADAMTS13 activity (%)												**104**				99	

*at baseline, before PEX. Out of normal range values are marked in bold.

On day 135, emergency surgery was required for phlegmonous appendicitis. There was no relapse of the disease. Currently, on day 180, the patient is asymptomatic, with normal blood pressure and a persistently normal laboratory panel: serum creatinine 0.61 mg/dl, eGFR (CKD-EPI) 130 ml/min/1.73m^2, proteinuria 0.12 g/L, platelets 202,000/mm^3, hemoglobin 12.9 g/dl, LDH 167 U/L, haptoglobin 53 mg/dl, C3 100 mg/dl, C4 16 mg/dl, ADAMTS13 activity 100%.

Some weeks after clinical remission, the genetic analysis had been available. No mutation were retrieved in the sequence analysis of complement factor H (CFH), membrane cofactor protein, C3, complement factor B, thrombomodulin gene coding regions and exon-intron junctions. Quantitative analysis by multiplex ligation-dependent probe amplification (MLPA) of the CFH gene and CFHR3, -R1, -R2, -R5 regions did not identify any deletions or duplications. Conversely, a new heterozygous variant p.Arg448Leu in the complement factor I (CFI) gene, that had never been described before in the Literature [4], was identified as an isolated mutation.

3 Discussion

The presented case highlights how differencing among thrombotic microangiopathy syndromes can be a challenge for treating physicians.

Our case should be defined as aHUS. However, although a moderate reduction of ADAMTS13 activity has been described in 50% of patients with aHUS [5], some open questions still remain. Those include the role of severe

ADAMTS13 deficiency in such a case, the mechanisms underlying the effects of Rituximab in inducing remission, the possibility of an overlap between acquired TTP and aHUS. Finally, when differencing between TTP and aHUS is challenging, the optimal management (to include Rituximab or Eculizumab) in case of relapse is debatable.

Although severe renal involvement is not common in TTP, and no neurological signs were observed in our case, the diagnosis of acquired TTP could be a working diagnosis. This hypothesis could explain both the severity of ADAMTS13 deficiency and the complete response to Rituximab, as previously discussed [6-8]. In this context, the identification of a new CFI gene mutation is of uncertain significance. The mother of the patient has been tested positive for the same mutation in heterozygous and she never developed any sign/symptoms of TMA.

In detail, the c.1343G > T substitution in exon 11 encodes the missense variant p.Arg448Leu, located in the serine protease domain where are sited most of the functional mutations of the CFI gene. Although this mutation is defined as benign by some Authors *in-silico* prediction models (such as Polyphen, http://genetics.bwh.harvard.edu/pph2/), one could not exclude that this variant might lead to FI instability and/or misfolding. Furthermore, despite the Arg448 residue is not conserved, p.Arg448Leu is not present in Website databases with over 60,000 genomes studied. Testing by Next Generation Sequencing for 13 genes (including CFH, CFI, CFB, C3, MCP, THBD, DGKE, CFH-Related genes 1-5 and ADAMTS13) did not provide any further evidence.

However, a pathogenic role for this abnormality is still debatable and further analysis would be warranted to properly address this issue.

The patient is currently monitored for the ADAMTS13 activity, and has been scheduled to receive a new course of Rituximab in case of relapse.

When reviewing the literature searching for an association between low level of ADAMTS13 activity and complement gene mutations, we found few studies addressing this topic [5, 9-10].

Feng *et al.* [5] investigating 29 patients with aHUS, showed that complement gene mutations were identified in over 50% of the patients. Albeit none of the patients showed a severe ADAMTS13 deficiency (less than 10%), reduced complement and reduced ADAMTS13 activity (< 60% of normal activity) were found in over 60% and 50% of patients, respectively. Szarvas *et al.* [9] identified a previously not reported mutation in CFH (Ser722Stop), supporting the diagnosis of complement-mediated HUS in a pediatric case with decreased, but not deficient ADAMTS13 activity. Finally, Remuzzi and co-workers [10] analyzed the genetic basis of phenotype heterogeneity in TTP in two sisters. The patients had ADAMTS13 deficiency as a result of two heterozygous mutations (causing V88M and G1239V changes). Besides, a heterozygous mutation (causing an S890I change) in factor H of complement was found in the patient with chronic renal failure but not in her sister, who presented with exclusive neurologic symptoms.

Taken the above together, the available evidences support the existence of a subgroup of patients with aHUS with reduced ADAMTS13 activity, albeit an association with a severe deficiency (*e.g.* less than 10%) has never been clearly demonstrated. Finally, we find only one reported case of complete ADAMTS13 deficiency and CFH mutation [10].

Take home messages based on our case are:

- Diarrhea in TMA is not a unequivocal sign of STEC-tHUS, and non STEC-related diarrhea may be the triggering event in a patient developing TTP or aHUS;
- Distinguishing HUS from TTP might be challenging, and a tailored management based on clinical, laboratory and response-to-therapy criteria is essential;

- The heterogeneity of the clinical picture observed in both HUS and TTP always requires a systematic search for STEC, for deficiency of ADAMTS13 activity and for complement gene mutations in all patients with TMA. Indeed, one could bear in mind that in very selected cases features can overlap.

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