Srp Arh Celok Lek. 2013 Jul-Aug;141(7-8):466-474
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1308466V
UDC: 616.155.2-005.6

Diagnostic Relevance of ADAMTS13 Activity: Evaluation of 28 Patients with Thrombotic Thrombocytopenic Purpura – Hemolytic Uremic Syndrome Clinical Diagnosis

Dragica Vučelić[1], Danijela Miković[2], Zoran Rajić[3], Nebojša Savić[4], Živko Budišin[5], Nebojša M. Antonijević[6,7], Slobodan Obradović[8], Dragana Jevtić[7,9], Roberta Palla[10], Carla Valsecchi[10], Flora Peyvandi[10]

[1]Clinic of Digestive Surgery, Clinical Center of Serbia, Belgrade, Serbia;
[2]Blood Transfusion Institute of Serbia, Belgrade, Serbia;
[3]Clinic of Hematology, Clinical Center of Serbia, Belgrade, Serbia;
[4]Clinic of Vascular and Endovascular Surgery, Clinical Center of Serbia, Belgrade, Serbia;
[5]University Children's Hospital, Belgrade, Serbia;
[6]Clinic of Cardiology, Clinical Center of Serbia, Belgrade, Serbia;
[7]School of Medicine, University of Belgrade, Belgrade, Serbia;
[8]Clinic of Emergency Internal Medicine, Military Medical Academy, Belgrade, Serbia
[9]Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić", Belgrade, Serbia;
[10]U.O.S. Dipartimentale per la Diagnosi e la Terapia delle Coagulopatie, A. Bianchi Bonomi Haemophilia and Thrombosis Center, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Università degli Studi di Milano and Luigi Villa Foundation, Milan, Italy

SUMMARY

Introduction The significance of ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin motif-13) activity for diagnosis and therapy of thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) is still a controversial issue.

Objective The aim of this report was to analyze the value of ADAMTS13 measurements in the diagnosis of TTP and HUS.

Methods At presentation, we analyzed patients with idiopathic TTP (n=18), secondary TTP (n=4), diarrhea positive HUS (n=3) and diarrhea negative HUS (n=3) treated in Belgrade, Serbia from 2004 to 2010. ADAMTS13 activity from acute phase samples was measured using the residual collagen binding activity assay at the Haemophilia and Thrombosis Centre, Milan, Italy.

Results There was a significant correlation between reduced ADAMTS13 activity and idiopathic TTP diagnosis (p=0.000) as well as between lower ADAMTS13 activities and higher reticulocytes (p=0.017) and lactate dehydrogenase levels (p=0.027). Significant correlation was also found between higher protease activity and diagnosis of HUS (p=0.000). There was a statistically significant correlation between higher ADAMTS13 activities and higher platelets count (p=0.002), blood urea nitrogen (p=0.000), and creatinine level (p=0.000).

Conclusion Severe ADAMTS13 deficiency points at the diagnosis of idiopathic TTP and it is present in the secondary TTP but not in HUS.

Keywords: thrombotic thrombocytopenic purpura; hemolytic uremic syndrome; ADAMTS13; diagnosis

INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are manifestations of excessive microvascular platelet adhesion/aggregation, and present dangerous thrombotic microangiopathies (TMA) with dramatic clinical presentation and sometimes fatal outcome, even with rapid diagnosis and proper therapy. Most frequently involved organs are the brain, kidneys, heart, spleen, pancreas, and adrenals [1, 2, 3].

The pathophysiology of TTP appears to involve the largest of the unusually large von Willebrand factor (ULvWF) polymers. Upon expression by endothelial cells, vWF is secreted into the circulation as ULvWF. Normally, these multimers are processed into smaller regular sized multimers in the high-shear environment of the arterial circulation which will not spontaneously react with platelets [1, 4]. The vWF processing activity is now known to be a specific vWF-cleaving metalloprotease in normal plasma that prevents persistence in the circulation of ULvWF multimers [5, 6]. Specific vWF-cleaving metalloprotease is characterized as a 13th member of a large array of structurally related enzymes known as the ADAMTS (disintegrin and metalloprotease with thrombospondin type 1 repeats) family, and is denoted as ADAMTS13. In patients with TTP, processing of the ULvWF multimers is impaired due to the deficiency of the ADAMTS13. This eventually results in formation of the characteristic blood clots found in the microcirculation [1, 4].

Correspondence to:
Dragica VUČELIĆ
Clinic of Digestive Surgery
Clinical Center of Serbia
Dr Koste Todorovića 6
11000 Belgrade
Serbia
vucelicd@beotel.net;
vucelicd@gmail.com

There are several types of TTP [7, 8]. Congenital chronic relapsing TTP is associated with a severe constitutional deficiency of ADAMTS-13 due to inherited ADAMTS13 gene defects (2% to 3% of patients) [9, 10]. Chronic relapsing TTP usually manifests in childhood. If recognized early enough and treated successfully, it then recurs at approximately three-week intervals indefinitely. Recurrences are predictable enough that treatment with plasma infusion will prevent them [1, 7, 8].

A much more common are acquired forms of TTP. Pathophysiology of most acquired forms of TTP is related to autoantibodies against ADAMTS13 [1, 4, 5]. Acute idiopathic TTP is the most common type of TTP seen in clinical practice. Single episode TTP occurs without a known cause and has no recurrence (about two thirds of patients). In the remainder, the disease recurs at intermittent and unpredictable intervals (intermittent or relapsing TTP) [1, 8, 10]. Many patients with acquired idiopathic TTP have absent or severely reduced plasma ADAMTS13 activity during an initial episode as well as during later recurrences. In these patients, ADAMTS13 activity usually increases toward normal on recovery from a single or recurrent episode [1]. If an identifiable cause (infections, autoimmune conditions, lymphomas, cancer, chemotherapeutics, immunosuppressants, platelet aggregation inhibitors allogeneic hematopoietic stem cell transplantation, pregnancy) precipitates the illness it is classified as secondary [7, 8]. It has been considered that patients with secondary TTP almost never have severe ADAMTS13 deficiency [4].

TTP is one of the most difficult diagnoses for the clinical hematologist to make, due to the rarity of the disease and the poor specificity of clinical and laboratory signs and symptoms. The incidence of idiopathic TTP is estimated to be 4.5/1 million person/year, being higher in blacks [10, 11]. Severe thrombocytopenia and microangiopathic hemolytic anemia – MAHA (without an alternative cause) along with neurologic symptoms and signs due to predominantly ischemia or infarction of brain (in 50% to 71% of episodes), constitute the characteristic TTP clinical triad [1, 4]. Fever and/or renal dysfunction occur in a minority of these patients. Consumptive thrombocytopenia and MAHA, together with fragmented red cells in blood smear and elevated serum levels of lactate dehydrogenase, (LDH) provide the clinician with the most diagnostically useful laboratory signs [11].

In closely related HUS, ischemia is predominantly renal. HUS is clinically recognized by simultaneous occurrence of thrombocytopenia and MAHA along with acute renal failure [1, 12]. Approximately 50% of cases of acquired HUS appear after gastrointestinal infection caused by Shiga toxin–producing Escherichia coli 0157:H7 (diarrhea associated hemolytic uremic syndrome – D+HUS or typical HUS or epidemic form) [4, 8]. HUS without antecedent diarrhea is referred to as "atypical HUS" (diarrhea negative hemolytic uremic syndrome – D-HUS or sporadic form). ADAMTS13 deficiency rarely if ever occurs in D+HUS. Patients with atypical HUS seldom have ADAMTS13 deficiency and frequently turn out to have abnormalities in the regulation of the alternate complement pathway due to mutations in complement factor H, factor I, factor B, or membrane cofactor protein [1, 4, 8].

Although traditionally considered separate entities, the distinction between TTP and HUS remains a matter of controversy because both syndromes often overlap [2]. Severe renal involvement in a patient with TTP or extrarenal manifestations in a patient with HUS can obfuscate clinical boundaries between the two syndromes [1].

TTP and HUS are clinical diagnosis [1]. As a practical matter, very few laboratories can perform ADAMTS13 assays rapidly enough, and the clinician must make a diagnosis and initiate therapy without this information. Three features of the classic pentad: renal involvement, neurologic symptoms, and fever are not necessary in establishing the diagnosis. Thus, the presence of thrombocytopenia and MAHA is sufficient for the diagnosis as not all other features are always present from the outset [4, 12, 13].

Although it has been proposed [14] that severe ADAMTS13 deficiency specifically defines TTP, the current prevailing opinion is that not all patients appropriately diagnosed with idiopathic TTP on the basis of clinical and laboratory symptoms and signs have severe protease activity [11]. On the other hand, total deficiencies of ADAMTS13 were observed in TTP forms secondary to other diseases or conditions [3]. In contrast to another largely accepted concept that patients with HUS have normal or moderately reduced ADAMTS13 activities, it has been demonstrated that reduced or undetectable levels of protease activity were present in plasma of some patients clinically diagnosed as having HUS [3, 15]. These provocative findings supported the views that these two syndromes should be identified clinically as TTP-HUS [2, 16].

It is well documented in TTP patients that early recognition and diagnosis as well as timely treatment with plasma exchange (PE) as the first-line therapy reduce mortality rate with long-term remission of 80-90%. The presence of antibodies of ADAMTS13 and the accumulation of ULvWF multimers in the patient's plasma of acquired disease types suggest that the removal of such pathologic entities with PE would result in improvement of patients. The use of fresh frozen plasma (FFP) as the replacement fluid has the added benefit of infusing normal donor ADAMT13. In patients without antibodies to the cleaving protease (congenital TTP), only 10 to 15 mL/kg of FFP infused every 2 to 3 weeks can achieve remission [1, 2, 7, 8, 12, 17, 18, 19]. Alternative therapeutic approaches, mainly based on immunosuppressive agents, have been used for resistant patients, but there is still no consensus as to the best approach [13]. Furthermore, some patients with HUS improved in association with PE. It is therefore appropriate to urgently commence daily PE procedures for all adult patients and older children with a clinical diagnosis of TTP or HUS [1].

OBJECTIVE

In 28 patients with TTP and HUS we evaluated the relationship between diagnostic laboratory parameters and

clinical diagnosis, the value of ADAMTS13 measurements in establishing the diagnosis of TTP and HUS, and correlation between ADAMTS13 activities and anti-ADAMTS13 autoantibodies with standard diagnostic parameters.

METHODS

Patients

In a 6-year period (between August 2004 and February 2010) 28 patients clinically diagnosed with TTP and HUS were admitted into three hospitals in Belgrade, Serbia: Clinic of Hematology - Clinical Centre of Serbia, Military Medical Academy, Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić". There were 22 female (78.6%) and 6 male (21.4%) patients included in this study. The median age was 39 years with a range of 17 to 68 years.

Clinical diagnosis

Clinical diagnosis was established based on the presence of MAHA (hemoglobin level bellow 120 g/L, elevated LDH, undetectable serum haptoglobin, evidence of erythrocytes fragmentation: at least 2 schistocytes per high-power field in peripheral blood smear, reticulocytosis, negative direct antiglobulin test), thrombocytopenia (platelet count less than 150×10^9/L), presence/absence of fever, presence/absence of neurological signs and symptoms, and presence/absence of renal dysfunction/acute kidney injury (AKI) [20]. The measurements of laboratory parameters were performed according to the conventional methods using blood samples obtained on admittance. Table 1 presents the main patient's clinical and laboratory characteristics at the time of diagnosis.

Idiopathic TTP was defined as TMA with predominantly neurologic involvement occurring in patients with no apparent preexisting disease. Secondary TTP was defined as TMA with predominantly neurologic abnormalities in the presence of known predisposing conditions/precipitants for disease onset: autoimmune disease, systemic disease, malignancy, bone marrow transplant. D+ HUS was defined as TMA with predominant clinical presentation of renal impairment/AKI associated with a prodromal diarrhea in patients with previously normal renal function. D- HUS in patients without prodromal diarrhea was defined as TMA with predominant clinical presentation of renal impairment/AKI in those with previously normal renal function [4, 21]. Diagnosis of AKI was made based on existing specific criteria introduced by the Acute Kidney Injury Network: rapid time course (less than 48 hours), reduction in kidney function defined with absolute increase in serum creatinine of ≥0.3 mg/dl (≥26.4 μmol/l) or percentage rise in serum creatinine of ≥50% respectively as well as reduction in urine output defined as <0.5 ml/kg/hr for more than 6 hours [22, 23]. In all patients with AKI diagnosis, renal function was previously normal. The diagnosis of the disease was made on clinical grounds without knowledge of the results of ADAMTS13 assays.

Clinical presentation was identified as idiopathic TTP in 18, secondary TTP in 4, D+HUS in 3, and D- HUS in 3 patients. Secondary TTP was associated with systemic autoimmune diseases (patients N° 19, 21, 22) and cancer (patient N° 20). Patient N° 19, 21, 22 were diagnosed with connective tissue systemic disease, syndrome sicca and systemic lupus erythematosus (SLE). One patient (patient N° 25) defined as having D+HUS was on tacrolimus regular intake due to allogenous stem cell transplantation 3 years ago. One patient (patient N° 27) with D-HUS exhibited onset of disease in the context of SLE.

ADAMTS13 activity measurement

Plasma samples were collected on admission. The ADAMTS13 activity was measured at the Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre in Milan, Italy.

ADAMTS13 activity was assayed using collagen binding enzyme immunoassay. The source of VWF used as protease substrate was a purified VWF concentrate (Facteur Willebrand Humain Tres Haute Purité) provided by Laboratoire Francais du Fractionnement et des Biotechnologies (Lille, France). The product, which lacks endogenous ADAMTS13 activity, was reconstituted to a VWF concentration of 100 U/mL, aliquoted and stored at -30°C. The concentrate was then thawed, diluted 1 in 33 with 5 M urea in 5 mM Tris-HCl, pH 8, and incubated for 10 min at room temperature. Subsequently, 50 μL of the dilution of the VWF source were added to 100 μL of serial dilutions (1/10, 1/20, 1/40) of test plasma as a source of ADAMTS13. Test plasmas were diluted in 5 mM Tris-HCl, pH 8 containing 12.5 mM BaCl2 and 1 mM Pefabloc SC (Roche, Mannheim, Germany). Values were expressed as percentages of pooled normal plasma. The most recent laboratory evaluation of assay reproducibility yielded an intra-assay coefficient of variation of 9% and an inter-assay coefficient of variation of 12%. The lower limit of sensitivity was 6% of protease levels in pooled normal plasma taken as the reference standard. The lower value of the reference interval (46%) was set at the 5th percentile of the distribution of the values obtained in 200 healthy individuals [24].

Anti-ADAMTS13 antibodies testing

Anti-ADAMTS13 antibodies were detected by western blot analysis, as previously reported [24].

In this study, ADAMTS13 activities were arbitrarily divided into four categories, similar to the practice of some others authors [16]. The categories were as follows: 1. less than 6% which is the detection limit of the assay (severe deficiency); 2. between 6% and 25% (moderate deficiency); 3. between 26% and 46% (mild deficiency); 4. greater than 46% (normal).

doi: 10.2298/SARH1308466V

Table 1. Clinical features (N=28)

Patient No	Year of onset	Age (years)	Sex	Clinical type	Episode	Plt ($\times 10^9$/L)	Hb (g/L)	LDH (U/L)	Ret (%)	Cr (μmol/L)	BUN (mmol/L)	Neurological abnormalities	Fever
1	2004	55	F	Idiopathic	Initial	36	79	602	8.5	50	4.6	Headache, hemiparesis	Yes
2	2005	27	F	Idiopathic	Initial	14	89	4559	5	136	12	Headache, giddiness	Yes
3	2005	27	M	Idiopathic	Initial	20	93	1372	17	102	7.6	/	No
4	2005	45	F	Idiopathic	Initial	50	93	634	15	83	4.1	Headache, dizziness	No
5	2007	45	F	Idiopathic	First R after 11 years	28	92	2929	7	183	24	/	Yes
6	2007	33	F	Idiopathic	Initial	31	85	2089	5	87	3	Headache, paresthesias	Yes
7	2007	51	M	Idiopathic	Initial	16	65	1726	5	144	7	Headache	Yes
8	2008	39	F	Idiopathic	Initial	5	73	1246	9	70	3.2	Headache, paresis n. facialis, paresthesias, aphasia, disorientation, hallucination	Yes
9	2008	25	F	Idiopathic	Initial	23	65	3909	12	41	6	Headache, paresthesias	No
10	2009	21	M	Idiopathic	Initial	5	117	1394	4.6	137	9	Headache, modified behavior, somnolence	Yes
11	2009	30	M	Idiopathic	Initial	19	81	1319	12	91	7	/	No
12	2009	34	F	Idiopathic	Initial	22	70	3347	14	61	5.1	Headache	Yes
13	2009	48	F	Idiopathic	First R after 7 years	19	73	2797	16.6	96	9.3	Seizures	No
14	2009	17	F	Idiopathic	Initial	12	103	2795	9.3	67	10.1	Headache, aphasia, coma	No
15	2009	41	F	Idiopathic	Initial	28	72	1740	8.3	83	7.8	Dysphasia, headache, paresthesias, numbness	No
16	2009	33	F	Idiopathic	First R after 6 years	80	115	783	5.6	62	3.8	/	Yes
17	2009	66	F	Idiopathic	Initial	3	86	4789	9	80	20.2	Stupor	Yes
18	2010	30	F	Idiopathic	Initial	11	78	3709	19	49	4.9	Headache, disorientation, hallucination, stupor	Yes
19	2004	23	F	Secondary	Initial	11	91	2750	17	107	29.9	Headache, sensomotor polyneuropathy	No
20	2005	64	F	Secondary	Initial	41	56	1213	10	94	9.2	Seizures, stupor/coma	No
21	2007	68	F	Secondary	Initial	54	109	1187	4	104	15.9	/	Yes
22	2008	36	F	Secondary	Initial	10	52	4199	20	77	10.3	Slurred speech, bihemiparesis, paresthesias disorientation, somnolence	No
23	2005	52	F	D+HUS	Initial	95	96	925	2	813	45.6	/	Yes
24	2006	62	F	D+HUS	Initial	36	81	1551	6	429	43.8	/	Yes
25	2008	40	M	D+HUS	Initial	21	106	2057	10	378	33	/	No
26	2008	28	F	D-HUS	Initial	121	71	994	1	635	25.7	Headache, blurred vision	No
27	2008	20	F	D-HUS	Initial	47	86	1088	7	258	50.9	/	Yes
28	2008	32	M	D-HUS	Initial	101	98	790	3.7	1221	45	/	Yes

N – number of patients; F – female; M – male; R – relapse; Plt – platelets; Hb – hemoglobin; LDH – lactate dehydrogenase; Ret – reticulocytes; Cr – creatinine; BUN – blood urea nitrogen

Statistical analysis

Data were entered into a program SPSS for Windows version 18 and Statistica version 6.0. Variables were analyzed by using Chi-square test, Student t-test, Pearson's correlation coefficient test, and Spearman's rank correlation test. P values <0.05 and <0.01 were considered to be significant.

RESULTS

Clinical presentation

Among 28 acute episodes, 25/28 patients presented with their initial TTP or HUS episode, whereas 3/28 patients (cases Nº 5, 13, 16) with idiopathic TTP experienced their first relapse after 11, 7, and 6 years from initial episode.

On diagnosis of 28 acute episodes, patients had multiple presenting symptoms and signs. Thrombocytopenia and MAHA were present in all patients. Of 18 idiopathic TTP acute episodes, heterogeneous neurological abnormalities were the paramount presenting symptoms occurring in 14 (77.7%) patients. The structure of central nervous system (CNS) abnormalities included headache, parestesias/paresis/numbness, disorientation/modified behavior, dysphasia/aphasia, hallucination, seizures, and stupor/coma. Headache was the most frequent symptom occurring in 12 patients (66.6%). Fever was present in 11 (61.1%) cases. Renal dysfunction, in the absence of AKI, presented with alterations of urinanalysis, such as micro-/macroscopic hematuria, proteinuria as well as moderately elevated blood urea nitrogen (BUN) and creatinine levels was manifested in 13 (72.2%) patients; some of them (2/13; 15.4%) experienced lumbal pain.

Among 4 patients with secondary TTP, CNS abnormalities (headache, slurred speech, disorientation, paresthesias, bihemiparesis, sensomotor polyneuropathy, seizures, somnolence/stupor/coma) were present in 3 (75%), fever in 1 (25%), and mild renal impairment in 3 (75%) patients.

In the subgroup of 3 patients classified as having D+HUS, CNS abnormalities were absent in all of them while fever was present in 2 (66.6%) patients. AKI was manifested in 2 patients.

In the subgroup of 3 patients classified as having atypical HUS, all of them exhibited AKI. Headache and blurred vision were present in 1 patient. Also, one of them manifested fever.

Laboratory parameters

Laboratory parameters, median values, and standard deviations among the subgroups of patients classified as having idiopathic TTP (N=18), secondary TTP (N=4), D+HUS (N=3) and D- HUS (N=3) are listed in Table 2. Haptoglobin was undetectable in all patients (data not shown).

Relation of standard laboratory diagnostic parameters to TTP and HUS clinical type

The relationship between diagnostic laboratory parameters and the clinical diagnosis showed statistically significant differences ($p<0.01$) for the mean values of platelets, reticulocytes, BUN and creatinine. These results indicate that observed average values for platelets, BUN and creatinine were significantly higher ($p<0.00001$, $p<0.00001$, $p<0.00001$) whereas reticulocytosis was found to be significantly lower ($p=0.007$) among the patients clinically diagnosed with HUS compared to patients diagnosed with TTP.

Relationship between ADAMTS13 activity and TTP and HUS clinical diagnosis

ADAMTS13 activities being measured in samples collected from all patients on presentation are described in Table 3. The samples were tested in acute phase of the disease.

Severe ADAMTS13 deficiency was detected in patients with idiopathic (13/18) and secondary TTP (1/4). In the category of moderate protease deficiency there were 2/18 patients with idiopathic and 3/4 patients with secondary TTP. A mildly reduced ADAMTS13 activities were found in 3/18 patients with idiopathic TTP and 2/3 patients with D-HUS. Normal protease levels were measured only in the cases with HUS. In all patients with D+HUS protease levels were normal.

Thus, none of the patients with idiopathic and secondary TTP had normal and none of those with HUS had severe/moderate ADAMTS13 activity.

There was a significant correlation between reduced ADAMTS13 activities and idiopathic TTP clinical diagnosis ($r=0.631$; $p<0.00001$). A significant correlation was obtained in a relationship of higher protease activities with HUS clinical diagnosis ($r=0.701$; $p<0.00001$).

Table 2. Laboratory results on diagnosis (N=28)

Laboratory tests	Clinical type			
	Idiopathic TTP	Secondary TTP	D+ HUS	D- HUS
Plt ($\times 10^9$/L)	3-80 (23.3±18.4)	10-54 (29±22)	21-95 (50.6±31.9)	47-121 (89.6±31.2)
Hb (g/L)	65-117 (84.9±15.3)	52-109 (77±27.6)	81-106 (94.3±10.3)	71-98 (85±11)
Ret (%)	4.6-19 (10.1±4.6)	4-20 (12.7±7.1)	2-10 (6± 3.2)	1-7 (3.9±2.45)
LDH (U/L)	602-4789 (2316±1333)	1187-4199 (2337.2±1440.3)	925-2057 (1511±463)	790-1088 (957.3±124.4)
Cr (μmol/L)	41-183 (90.1±37.9)	77-107 (95.5±13.5)	378-813 (540±194.1)	258-1221 (704.6±396.2)
BUN (mmol/L)	3-24.3 (8.3±5.7)	9.2-29.9 (16.3±9.5)	33-65.6 (47.4±13.5)	25.7-50.9 (40.5±10.7)

TTP – thrombotic thrombocytopenic purpura; D+ HUS – diarrhea positive hemolytic uremic syndrome (HUS); D- HUS – diarrhea negative HUS

doi: 10.2298/SARH1308466V

Table 3. ADAMTS13 functional activity in acute TTP and HUS episodes (N=28)

Clinical type	ADAMTS13							
	<6%		6–25%		26–46%		>46%	
	N	%	N	%	N	%	N	%
Idiopathic TTP	13	72.2	2	11.1	3	16.7	0	0
Secondary TTP	1	25.0	3	75.0	0	0	0	0
D+HUS	0	0	0	0	0	0	3	100.0
D-HUS	0	0	0	0	2	66.7	1	33.3

Table 4. Anti-ADAMTS13 antibodies in the frame of clinical diagnosis and different categories of protease level (N=28)

Disease	Anti-ADAMTS13 antibodies	ADAMTS13							
		<6%		6–25%		26–46%		>46%	
		N	%	N	%	N	%	N	%
Idiopathic TTP	Absent			2		1			
	Present	13	72.2			2	11.1		
Idiopathic TTP	Absent			2					
	Present	1	25.0	1	25.0				

Relationship between anti-ADAMTS13 antibodies and TTP or HUS clinical diagnosis

Anti-ADAMTS13 antibodies were demonstrable in a total of 17/28 (60.7%) patients. The antibodies were identified among the patients that were categorized as having reduced protease activities. In the category of normal protease activity antibodies were not present (Table 4).

The antibodies were detected in 15/18 (83.3%) patients diagnosed with idiopathic TTP. In plasma of all patients (13/18; 72.2%) with idiopathic TTP and severe deficiency of ADAMTS13 antibodies were present. Among the remaining patients with idiopathic TTP, antibodies were present in 2/3 (11.1%) patients in category of mild protease activity. The patients with idiopathic TTP and moderate protease activity (2/18) had no demonstrable antibodies.

Among the patients with secondary TTP forms antibodies were detected in plasma of patient with SLE-associated disease with severe ADAMTS13 activity and in plasma of patient with syndrome sicca-related form with moderately reduced protease activity. Patients with underlying cancer and connective tissue systemic disease had no detectable antibodies.

In acute episode there was a significant link (r=0.533; p=0.003) of anti-ADAMTS13 antibodies presence with idiopathic TTP. Presence of antibodies was more frequent in idiopathic compared with other clinical forms. None of the patients with HUS had antibodies.

Analysis of laboratory diagnostic parameters in relation to protease activities and antibodies presence

Statistically significant correlations were obtained between higher ADAMTS13 activities and greater platelets number (r=0.568; p=0.002), BUN (r=0.717; p<0.00001), and creatinine levels (r=0.701; p<0.00001).

On the other hand, a significant correlations were demonstrated between lower ADAMTS13 activities and higher reticulocytes (r=0.448; p=0.017) and LDH levels (r=0.417; p=0.027).

In terms of antibodies presence/absence, statistically significant correlations were obtained between antibodies presence and lower platelets number (r=0.491; p=0.008) as well as higher reticulocytes (r=0.485; p=0.009).

There was significant link between the absence of antibodies and higher BUN (r=0.666; p<0.00001) and creatinine (r=0.602; p=0.001) levels.

DISCUSSION

In our patients, searching for statistical relationship between standard laboratory diagnostic parameters and TTP and HUS clinical diagnosis revealed differences across clinical type. There was a significant link between severity of renal function impairment i.e. clinical diagnosis with HUS and higher average values for platelets, BUN and creatinine as well as a relationship between higher reticulocytosis and TTP clinical diagnosis. These findings are in agreement with defining diagnostic features of TTP and HUS considering HUS description as a syndrome characterized by moderate thrombocytopenia (mean platelets number of $36x10^9$/L) and hemolysis with predominant renal abnormalities, and TTP as a syndrome with severe thrombocytopenia and excessive hemolysis, respectively [8, 12, 21].

In this study, we found that decreased levels of ADAMTS13 were present in all patients (N=22) given diagnosis TTP, either primary or secondary forms. The prevalence of reduced ADAMTS13 activities was significantly higher in patients clinically diagnosed with idiopathic TTP. The great majority of the patients clinically diagnosed with idiopathic TTP (72.2%), had undetectable protease activity**.** On the whole, the results of protease activities in acute idiopathic TTP obtained in this small cohort are partially in concur with original findings [4, 5] but are in agreement with some others [24, 29, 35, 43]. Publications of Tsai and Lian [5] and Furlan et al. [6] independently found that the levels of plasma vWF cleaving protease were greatly reduced or absent during acute TTP episodes, with a return to a baseline values after recovery. They have detected autoantibody directed against the protease. The lock of cleaving protease was also determined in congenital TTP form, but in the absence of specific autoantibody. On the other hand, in patients with HUS, vWF cleaving protease activities were normal or only moderately reduced. Veyrader et al. [3] investigated multicenter prospective cohort of 111 patients (66 manifesting as TTP and 45 as HUS). They found normal ADAMTS13 level in some patients with idiopathic TTP, and decreased protease level in some patients with HUS. Among patients with idiopathic TTP and decreased protease level, in around one third of them partial deficit was observed. In Vesely et al. [16] prospective study of 142 TTP-HUS patients, among 48 patients within the clinical category of idiopathic TTP, only 16 (33.3%) of them had severe protease deficiency.

Also, severe protease deficiency occurred among 2/10 women in immediate postpartum period of their first pregnancy. None of the patients with HUS and secondary drug associated TTP-HUS had severe protease deficiency. Peyvandi et al. [25] prospectively analyzed 100 patients with TTP. Nearly one third of the patients (28/100, 28%) had normal ADAMTS13 activities. Only approximately half of the patients (48/100, 48%) had very low levels (<10%) of ADAMTS13 activity. Moderately reduced protease levels were detected in 24/100 (24%) patients.

In our patients, severe ADAMTS13 deficiency was associated with the presence of specific antibodies in all patients suggesting the principal involvement of immune pathomechanism. Overall frequency of specific antibodies in our idiopathic TTP patients was 83.3% (15/18) with the presence of antibodies in all patients in severe ADAMTS13 deficiency category. Veyrader et al. [3] detected inhibitors in 31/65 (48%) patients with reduced ADAMTS13 activities. Inhibitors were observed only in patients with severe ADAMTS13 deficiency. In Vesely et al. [16] study, anti-ADAMTS13 antibodies were measured in 17/18 (94%) patients with severe protease deficiency. In Peyvandi et al. [25] study, in more than half of patients ADMTS13 deficiency was not associated with the presence of inhibitors.

Of the remaining our patients, two had moderately and three mildly decreased ADAMTS13 activity associated with the presence of antibodies in two patients with mildly protease deficiency. The finding of antibodies in the plasma of patients with incomplete inactivated antigen is consistent with previous report that postulated incomplete inactivation of antigen as possible explanation for the partially reduced protease level with specific antibodies [25]. Severe ADAMTS13 activity secondary to the presence of antibodies was also found in one of our patients with secondary TTP and underlying SLE which is in correspondence with previous report [3]. Among the secondary forms partial protease deficiency without antibodies were demonstrated in two cases suggesting the influence of others possible mechanisms formerly proposed. These include: the presence of platelet aggregating factors, antibodies to platelets, antibodies to endothelial cells, endothelial injury, increased level of plasminogen activator inhibitor type 1 (PAI-1) and genetic factors [26-30].

Unlike TTP, in our patients normal ADAMTS13 values were predominant in patients diagnosed with HUS: in samples of all patients with D+HUS, and one patient with D-HUS (33.3%). Protease activities in the absence of antibodies were only slightly decreased in 2/3 (66.6%) patients with D-HUS. A significant correlation was obtained in a relationship of higher protease activities with HUS clinical diagnosis. The findings in our HUS patients are in accordance with generally accepted opinion relying on the majority of literature data [3, 5, 6, 16, 25].

Concerning the correlation between ADAMTS13 activity and laboratory characteristics we found significance referred to the correlation of higher ADAMTS13 activities and greater platelets number, BUN, and creatinine levels. Moderate thrombocytopenia as well as significant increment of BUN and creatinine values are criteria for HUS, and actually in this entity normal/mildly reduced activities of ADAMTS13 are registered in our study. In contrast, significant correlations were demonstrated between lower ADAMTS13 activities and higher reticulocytes and LDH levels. Severe thrombocytopenia/hemolysis with high elevation of LDH and substantial bone marrow response define TTP in our study. These findings indicate that in the majority of patients two syndromes may be discriminated based on either the values of laboratory parameters and ADAMTS13 activity.

CONCLUSION

In this study, we found that reduced ADAMTS13 activity characterizes both primary and secondary TTP with significantly high association of severe protease deficiency with idiopathic TTP. Conversely, normal ADAMTS13 activity points to the diagnosis of typical HUS but is not present in idiopathic and secondary TTP. Either normal or slightly reduced protease activity in the absence of specific antibodies defined atypical HUS. ADAMTS13 deficiency was associated with the severity of hemolysis and TTP clinical diagnosis with predominantly neurological abnormalities whereas patients diagnosed as HUS with normal/mildly reduced protease level manifested more impaired kidney function.

REFERENCES

1. Moake JL. Thrombotic Thrombocytopenic Purpura. In: Kitchen CS, Alving BM, Kessler CM, editors. Consultative Hemostasis and Thrombosis. Philadelphia: Saunders; 2007. p.405-20.
2. Mannucci MP. Thrombotic thrombocytopenic purpura and the hemolytic uremic syndrome: much progress and many remaining issues. Haematologica. 2007; 92:878-80.
3. Veyraider A, Obert B, Houllier A, Meyer D, Girma JP. Specific von Willebrand factor-cleaving protease in thrombotic microangiopathies: a study of 111 cases. Blood. 2001; 98:1765-72.
4. Sadler JE. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. Blood. 2008; 112:11-8.
5. Tsai HM, Lian EC-Y. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med. 1998; 339:1585-94.
6. Furlan M, Robles R, Galbusera M, Remuzzi G, Kyrle PA, Brenner B, et al. von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. N Engl J Med. 1998; 339:1578-84.
7. Suvajdžić-Vuković N. The role of von Willebrand protease in aetiopathogenesis of thrombotic thrombocytopenic purpura. Srp Arh Celok Lek. 2007; 135:360-6.
8. Myers L. Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: pathophysiology and management. Nephrol Nurs J. 2002; 29:171-82.
9. Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, et al. Mutations in a member of the ADAMTS13 gene family cause thrombotic thrombocytopenic purpura. Nature. 2001; 413:488-94.
10. Peyvandi F, Palla R, Lotta LA. Pathogenesis and treatment of acquired idiopathic thrombotic thrombocytopenic purpura. Haematologica. 2010; 95:1444-7.

doi: 10.2298/SARH1308466V

11. Mannucci MP, Peyvandi F. TTP and ADAMTS13: When Is Testing Appropriate? Hematology Am Soc Hematol Educ Program. 2007:121-6.
12. George JN. How I treat patients with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Blood. 2000; 96:1223-9.
13. Suvajdžić-Vuković N, Budišin Ž, Elezović E. The successful treatment of refractory thrombotic thrombocytopenic purpura with vincristine: a case report. Haema. 2005; 8:300-3.
14. Tsai HM. Is severe deficiency of ADAMTS-13 specific for thrombotic thrombocytopenic purpura? Yes. J Thromb Haemost. 2003; 1:625-31.
15. Loof AH, van Vliet HH, Kappers-Klunne MC. Low activity of von Willebrand factor- cleaving protease is not restricted to patients suffering from thrombotic thrombocytopenic purpura. Br J Haematol. 2001; 112:1087-8.
16. Vesely SK, George JN, Lämmle B, Studt JD, Alberio L, El Hanake MA, et al. ADAMTS13 activity in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: relation to presenting features and clinical outcomes in a prospective cohort of 142 patients. Blood. 2003; 102:60-8.
17. Rock GA, Shumak KH, Buskard NA, Blanchete VS, Kelton JG, Nair RC, et al. Canadian Apheresis Study Group. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. N Engl J Med. 1991; 325:393-7.
18. Bell WR, Braine HG, Ness PM, Kickler TS. Improved survival in thrombotic thrombocytopenic purpura-hemolytic-uremic syndrome. Clinical experience in 108 patients. N Engl J Med. 1991; 325:398-403.
19. Suvajdžić-Vuković N, Pandurović R, Rajić Z, Milosević R, Bogdanović A, Lazarević V, et al. Treatment of 36 cases of thrombotic thrombocytopenic purpura.Vojnosanit Pregl. 2004; 61:621-7.
20. Amorosi EL, Ultmann JE. Thrombotic thrombocytopenic purpura: report of 16 cases and review of the literature. Medicine (Baltimore). 1966; 45:139-59.
21. Alford SL, Hunt BJ, Rose P, Machin SJ on behalf of Hemostasis and Thrombosis Task Force of the British Committee for Standards in Haemathology. Guidelines on the diagnosis and management of the thrombotic microangiopathic hemolytic anaemias. Br J Haematol. 2003; 120:556-73.
22. Webb S, Dobb G. ARF, ATN or AKI? It's now acute kidney injury. Anaesth Intensive Care. 2007; 35:843-4.
23. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. for the Acute Kidney Injury Network. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Critical Care. 2007; 11:R31.
24. Peyvandi F, Lavoretano S, Palla R, Feys HB, Vanhoorelbeke K, Battaglioli T, et al. ADAMTS13 and anti-ADAMTS13 antibodies as markers for recurrence of acquired thrombotic thrombocytopenic purpura during remission. Haematologica. 2008; 93:232-9.
25. Peyvandi F, Ferrari S, Lavoretano S, Canciani MT, Mannucci PM. Von Willebrand factor cleaving protease (ADAMTS13)and ADAMTS13 neutralizing autoantibodies in 100 patients with trombotic thrombocytopenic purpura. Br J Haematol. 2004; 127:433-9.
26. Murphy WG, Moore JC, Kelton JG. Calcium-dependent cysteine protease activity in sera of patients with thrombotic thrombocytopenic purpura. Blood. 1987; 70:1683-7.
27. Moore JC, Murphy WG, Kelton JG. Calpain proteolysis of von Willebrand factor enhances its binding to platelet membrane glicoprotein Iib/IIIa: an explanation for platelet aggregation in thrombotic thrombocytopenic purpura. Br J Haematol. 1990; 74:457-64.
28. Burns ER, Zucker-Franklin D. Pathologic effects of plasma from patients with thrombotic thrombocytopenic purpura on platelets and cultured vascular endothelial cells. Blood. 1982; 60:1030-7.
29. Leung DY, Moake JL, Havens P, Kim M, Pober JS. Lytic anti-endothelial cell antibodies in hemolytic uraemic syndrome. Lancet. 1988; 2:183-6.
30. Rock GA, Kelton JG, Kenneth H, Shumak KH, Noel A, Buskard NA, et al. Laboratory abnormalities in thrombotic thrombocytopenic purpura. Canadian Apheresis Group. Br J Haematol. 1998; 103:1031-6.

Дијагностичка применљивост активности *ADAMTS13*: процена код 28 болесника са тромбозном тромбоцитопенијском пурпуром и хемолизно-уремијским синдромом

Драгица Вучелић[1], Данијела Миковић[2], Зоран Рајић[3], Небојша Савић[4], Живко Будишин[5], Небојша М. Антонијевић[6,7], Слободан Обрадовић[8], Драгана Јевтић[7,9], Роберта Пала[10], Карла Валзеки[10], Флора Пејванди[10]

[1]Клиника за дигестивну хирургију, Клинички центар Србије, Београд, Србија;
[2]Институт за трансфузију крви Србије, Београд, Србија;
[3]Клиника за хематологију, Клинички центар Србије, Београд, Србија;
[4]Клиника за васкуларну и ендоваскуларну хирургију, Клинички центар Србије, Београд, Србија;
[5]Универзитетска дечја клиника, Београд, Србија;
[6]Клиника за кардиологију, Клинички центар Србије, Београд, Србија;
[7]Медицински факултет, Универзитет у Београду, Београд, Србија;
[8]Клиника за ургентну медицину, Војномедицинска академија, Београд, Србија;
[9]Институт за здравствену заштиту мајке и детета Србије „Др Вукан Чупић", Београд, Србија;
[10]Одељење за дијагностику и лечење коагулопатија, Центар за хемофилију и тромбозу „А. Бјанки Бономи", *Fondazione IRCCS Cà Granda – Ospedale Maggiore Policlinico*, Универзитет у Милану и Фондација „Луиђи Вила", Милано, Италија

КРАТАК САДРЖАЈ

Увод Значај дијагностичке специфичности активности *ADAMTS13* (енгл. *A Disintegrin And Metalloproteinase with ThromboSpondin motif-13*) у тромбозној тромбоцитопенијској пурпури (ТТП) и хемолизно-уремијском синдрому (ХУС) и даље је предмет расправа.

Циљ рада Циљ истраживања је био да се испита значај активности *ADAMTS13* у дијагнози акутног ТТП-ХУС.

Методе рада Испитани су болесници са идиопатским ТТП (18 испитаника), секундарним ТТП (4), дијареја-позитивним (Д+) ХУС (3) и дијареја-негативним (Д-) ХУС (3) који су лечени у Београду у периоду 2004–2010. године. Активност *ADAMTS13* мерена је у акутној фази болести методом резидуалног колаген-везујућег имуноесеја у Центру за хемофилију и тромбозу у Милану.

Резултати Добијена је статистички значајна удруженост између снижене активности *ADAMTS13* и идиопатског ТТП (p=0,000), као и између снижене активности *ADAMTS13* с једне стране и виших вредности броја ретикулоцита (p=0,017) и лактат-дехидрогеназе (p=0,027) са друге. Уочена је и значајна корелација између виших активности *ADAMTS13* и дијагнозе ХУС (p=0,000). Статистички значајна корелација је забележена и између више активности *ADAMTS13* с једне стране и виших вредности броја тромбоцита (p=0,002), уреје (p=0,000) и креатинина (p=0,000) са друге.

Закључак Недостатак *ADAMTS13* тешког степена говори у прилог дијагнози идиопатског ТТП и налази се и у секундарном ТТП, али није одлика ХУС.

Кључне речи: тромбозна тромбоцитопенијска пурпура; хемолизно-уремијски синдром; *ADAMTS13*; дијагноза

Примљен • Received: 15/03/2012 **Прихваћен • Accepted:** 22/05/2013

doi: 10.2298/SARH1308466V