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Clinical experience in treatment of thrombotic thrombocytopenic purpura - hemolytic uremic syndrome with 28 patients

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Background: Neither optimal treatment nor significance of ADAMTS13 (A Desintegrin And Metalloprotease with ThromboSpondin type 1 repeats) activity for diagnosis and therapy of thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS) have not been defined yet. The aim of the report is to analyze response to different volumes of plasma exchange (PE), and relationship to ADAMTS13. Design and methods: 28 patients clinically diagnosed with idiopathic TTP (n=18), secondary TTP (n=4), atypical HUS (n=3) and typical HUS (n=3) manifested 31 acute episodes. Patients were treated with PE in 26, and with plasma transfusion in 5 episodes with additional different therapies. Results: PE volumes were as follows: 1 in 7, 1.5 in 3, 2 in 14, and intensifying schedule (1 to 1.5) in 2 episodes. Procedure number was lower in patients treated with 2 and 1.5 (p=0.019) than in those treated with 1 volu-me exchange and PE intensifying, respectively (p=0.010). PE response rate was 25/26 (96.15%). Exacerbation frequency was higher in idiopathic TTP patients (3/19) treated with 1 compared with patients treated with >1 volume exchange (p=0.003). Survival rate was 25/28 (89.29%). ADAMTS13 activity was reduced in 22 with severe deficiency in 14 patients. Conclusion: Patients responded to different treatments regardless of ADAMTS13 activity, requiring less PEs with larger volume exchanges.

Key words: TTP-HUS syndrome, plasma exchange, ADAMTS13

INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) often classified as a thrombotic microangiopathies (TMA) still present syndromes difficult to recognize and treat with mortality rate of about 10% to 20%, even with rapid diagnosis and proper therapy[1]. In spite the fact that it is generally agreed that once diagnosis of TTP and atypical HUS is suspected plasma exchange (PE) therapy should be promptly started, the optimal schedule for PE with any exchange media nor the volume and duration of exchange therapy has not yet been determined[1-9]. Administration of fresh frozen plasma (FFP) at a rate of about 30mL/kg/day can be provided initially if delays with PE arrangement are unavoidable[1-7]. In the absence of well accepted protocol for PE, many different empirical practices exist depending on individual experience and personal conviction. It is typically ordered on a daily basis with 1.0 to 1.5 times the predicted patient's plasma volume exchanged with plasma replacement. Treatment response quides the number of PEs[2,7,8,10]. Since first line treatment doesn't work in some patients, multiple immunosuppressive therapies have been employed with varying success. Clinical trials are lacking to guide the use of immunosuppressive agents[1,4,7,11-15].

The short term prognostic usefulness of ADAMTS13 (a desintegrin and metalloproteinase with thrombospondin motif-13) testing at the time of acute TTP remains unsettled at the moment[16]. Currently, PE treatment is indicated for all adults patients with a clinical diagnosis of TTP or atypical HUS, which gives favorable results even in patients without severe ADAMTS13 deficiency suggesting that many factors contribute to acute episode[4,16,17].

This small retrospective study examines 7 years of data on TTP/HUS patients. We sought to analyze response to different volumes of PE, exacerbation/relapse rates and relationship to ADAMTS13.

PATIENTS AND METHODS

During the period spanning from August 2004 to February 2010, 28 patients clinically diagnosed with acquired TTP and HUS had been analyzed. Patients were admitted into the three hospitals in Belgrade, Serbia: Clinic of Hae-

matology - Clinical Centre of Serbia, Military Medi-cal Academy, and Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić". Three patients identified as having idiopathic TTP manifested two episodes each. Total number of episodes was 31.

At presentation, diagnosis was established based on the presence of classical diagnostic criteria[18], when at least two of the following criteria were met: direct Coombs negative microangiopathic hemolytic anemia-MAHA with elevated LDH levels as well as fragmented erythrocytes in peripheral blood smear, thrombocytopenia with no apparent alternative explanations, fever, and signs and symptoms compatible with central nervous system (CNS) and kidney ischemia. According to clinical presentation of acute episode, the patients have been assorted into four clinical categories: 1. idiopathic TTP: 18 patients/21 episodes; 2. secondary TTP: 4 patients; 3. diarrhea positive (D+) HUS - typical HUS: 3 patients; 4. diarrhea negative (D-HUS) - atypical HUS: 3 patients. Patients who predominantly had neurologic involvement were diagnosed with TTP whereas those who showed predominant severe renal impairment were assigned as HUS. Idiopathic TTP was defined as TMA with predominantly neurologic involvement occurring in patients with no apparent preexisting disease. TTP episode was classified as secondary when it was associated with known predisposing conditions: autoimmune disease, systemic disease, malignancy. Patients with previously normal renal function presented with predominant renal impairment/ acute kidney injury - AKI associated with a prodromal diarrhea was identified as having D+ HUS. Stool culture, detection of shiga-toxin in stools as well as serum antibodies to shiga toxin and serum antibodies to Escherichia coli 0157:H7 lipopolysaccharides have not been done due to lack of technique. D-HUS was defined as TMA with predominant clinical presentation of renal impairment/AKI in patients with previously normal renal function[7,12,17,19]. Diagnosis of AKI was made based on existing specific criteria introduced by the Acute Kidney Injury Network[20,21]. Diagnosis of the disease was made without knowledge of the results of ADAMTS13 assays. Presenting clinical and laboratory characteristics are described in Table I.

Clinical outcomes were defined as follows: 1. complete remission (CR) with no PE treatment within 1-30 days followed laboratory and clinical normalization (normal platelet count for 2 consecutive days, recovery of LDH and haptoglobin to normal, normalization of neurological status with a rising hemoglobin as well as gradually ameliorating of renal impairment with the exception of the patients with irreversible kidney dysfunction who underwent hemodialysis regular program); 2. delayed response to standard treatment(persistent/recurrent thrombocytopenia and hemolysis and new neurological abnormalities development during the course of treatment after the series of 7 PE); 3. therapeutic failure (no improvement at all in clinical symptoms and laboratory values or patient's death); 4. exacerbation (recurrent thrombocytopenia plus PE resumption after 1 day or more but less than 30 days after CR achievement); 5. relapse (clinical and laboratory deterioration after 30 days of CR achievement)[4,17,22]. All patients or their guardians gave informed, written consent.

TREATMENT

Treatment practice was institution of PE or/FFP transfusion using different therapeutic modalities, depending on the decision of the attending physician. PE was performed in the treatment of 26/31 TTP-HUS episodes whereas FFP transfusion was administered in the treatment of 5/31 episodes. In 23/26 episodes PE was performed using discontinuous centrifugation principle with Haemonetics model MCS3p equipment and using continuous flow centrifugation with Cobe Spectra Apheresis and Multifilter, Fresenius machines in 1 and 2 episodes respectively.

Treatment schedule with regard to plasma volume exchange (p.v.e) per procedure was as follows: 2 p.v.e. in 14 episodes; 1.5 p.v.e. in 3 episodes; 1 p.v.e. in 7 episo-des; in 2 episodes PE was intensified from 1 p.v.e. in ini-tial treatment to 1.5 p.v.e. thereafter. FFP was used as replacement fluid in 25 episodes and albumin in 1 episode. In addition to PE, patients received adjuvant steroid the-rapy. In 23 episodes pulse methylprednisolone (1g/day i.v.) was given for the first 3 days from the presentation, thereafter followed by per orally prednisone administrati-on in dose 1mg/kg/day for 20-30 days with gradually dose tapering during the next month. For the treatment of 6 epi-sodes, prednisone was given in dose 1-2mg/kg/day for 20-30 days with dose reduction until the remedy cessation. For the treatment of 2 episodes, corticosteroids have not been used. In 5 episodes patients received FFP and corticosteroids as standard therapy.

Second line treatment with additional immunosuppressive therapy was introduced in the treatment of 16 episodes. This treatment comprised of: vincristine in 8 episodes (among which in one case of disease activity exacerbation and in one case at the day of CR achievement), intravenous immunoglobulin-IVIg in 5 episodes (among which in one case of exacerbation), cyclophosphamide in 2 and azathioprine in 1 episode. Hemodyalisis was performed in 5 episodes. Consolidation treatment conducted with either PE or FFP using different practice (Table II). All patients received symptomatic and supportive therapy (red blood cells transfusion, antibiotics, folate supplementation). Hepatitis B vaccination was given using a platelet threshold of 50x109/L. None of the patients received platelet transfusion.

Measurement of ADAMTS 13 activity and anti-ADAMTS13 antibodies

Plasma samples have been collected at admission. For each patient, appropriate consent was obtained. The ADAMTS13 activity was measured at the Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre in Milan, Italy. Protease plasmatic activity was assayed using collagen binding method on citrated plasma as previously described[22]. ADAMTS13 activities were arbitrarily divided into four categories: 1. less than 6% which is the detection limit of the assay (severe deficiency); 2. between 6% and

TABLE 1

PRESENTING CLINICAL AND LABORATORY FEATURES OF TTP AND HUS ACUTE EPISODES (N=31)

Age/ sex	Clinical type	Pl $x10^{9/l}$	Hbg/L	LDH U/L	Re %	Cr/BNUmmol/L/mmol/L	Haemorrhage	CNS abnormalities	Renal symptoms/signs	Fever
55f	Idiopat.	36	79	602	8.5	50/4.6	haematuria	headache hemiparresis	hemoglobinuria proteinuria	yes
27f	Idiopat.	14	89	4559	5	136/12	purpura haematomas	headache giddnies	hemoglobinuria proteinuria	yes
27m	Idiopat.	20	93	1372	17	102/7.6	purpura	no	hemoglobinuria proteinuria lumbal pain	no
45m	Idiopat.	50	93	634	15	83/4.1	purpura vaginal bleeding	headache dizziness	hemoglobinuria erythrocyturia proteinuria	no
49f	Idiopat.	14	112	1272	5	78/6.4	haematomsa	headache	hemoglobinuria erythrocyturia proteinuria	yes
45f	Idiopat.	28	92	2929	7	183/84	purpura	no	hemoglobinuria erythrocyturia proteinuria	yes
33f	Idiopat	31	85	2089	5	87/3	no	headache parestesias	hemoglobinuria proteinuria	yes
51m	Idiopat	16	65	1726	5	144/7	no	headache	hemoglobinuria erythrocyturia	yes
53m	Idiopat	63	129	710	3	72/7.7	no	no	no	no
39f	Idiopat	5	73	1246	9	70/3.2	no	headache paresis facialis parestesias, aphasia disorientation hallucination	pproteinuria erythrocyturia	yes
25f	Idiopat	23	65	3909	12	41/6	no	headache parestesias	no	no
21m	Idiopath.	5	117	1394	4.6	13779	no	headache modified behavior somnolence	no	yes
30m	Idiopat	19	81	1319	12	91/7	purpura haematomas	no	no	no
34f	Idiopat	22	70	3347	14	61/5.1	purpura haematomas	headache	proteinuria erythrocyturia	yes
48f	Idiopathic	19	73	2797	16.6	96/9.3	prupura vaginal bleeding	epileptic attak	hemoglobinuria proteinuria lumbal pain	no
17f	Idiopat	12	103	2795	9.3	67/10.1	no	headahe,aphasia unconsciousness	hemoglobinuria erythrocyturia proteinuria	no
17f	Idiopat	78	113	517	1.88	63/4.5	no	no	no	no
41f	Idiopat	28	72	1740	8.3	83/7.8	purpura	dysphasia, headache, parestesias, numbness	proteinuria erythrocyturia	no
33f	Idiopat	80	115	783	5.6	62/3.8	haematomas	no	no	yes
66f	Idiopat	3	86	4789	9	80/20.2	haematomas	stupor	not done	yes
30f	Idiopat	11	78	2709	19	49/4.9	no	headache, disorientation hallucination, stupor	proteinuria erythrocyturia	yes
23f	Second	11	91	2750	17	107/29.9	purpura haematomas	headache polyneuropathia sensomotoria	hemoglobinuria proteinuria	no
64f	Second.	41	56	1213	10	94/9.2	no	convulsion stupor/coma	no	no
68f	Second.	54	109	1187	4	104/15.9	no	no	hemoglobinuria	yes
36f	Second	10	52	4199	20	77/10.3	haematomas vaginal bleeding	slurred speach, bihemiparesis, parestesias disorientation somnolence	hemoglobinuria, erythrocyturia, proteinuria	no
52f	D+HUS	95	96	925	2	813/45.6	no	no	AKI	yes
62f	D+HUS	36	81	1551	6	429/43.8	no	no	AKI	yes
40n	D+HUS	21	106	2057	10	378/33	no	no	hemoglobinuria, erythrocyturia, proteinuria	no
28f	D-HUS	121	71	994	1	635/25.7	no	headache, blurred vision	AKI	no
20f	D-HUS	47	86	1088	7	258/50.9		no	AKI	yes
32m	D-HUS	101	98	790	3.7	1221/45	no	no	AKI	yes

TABLE 2

TREATMENT OF TTP AND HUS EPISODES (n=31)

Age/sex	Clinical type	Plasma exchange No/p.v.e	Consolidation treatment PE/FFP	Steroid therapy	Additional immune therapy	Dialysis	CR	E	Death
55f	Idiopathic	3/2	1/5 days	yes	no	no	yes	no	no
27f	Idiopathic	5/2	2/3 days	yes	no	no	yes	no	no
27m	Idiopathic	8/2	2/3 days	yes	vincristine	no	yes	no	no
45f	Idiopathic	5/2	-/3days	yes	no	no	yes	no	no
49f	Idiopathic	5/1.5	-/3 days	yes	no	no	yes	no	no
45f	Idiopathic	3/2	1/3 days	yes	no	no	yes	no	no
33f	Idiopathic	10/2	-/5 days	yes	vincristine	no	yes	no	no
51m	Idiopathic	5/2	-/4 days	yes	no	no	yes	no	no
53m	Idiopathic	5/2	1/6 days	no	vincristine	no	yes	no	no
39f	Idiopathic	6/1	-/9 days	?	no		yes	yes	no
25f	Idiopathic	9/1	-/3 days	yes	vincristine	no	yes	no	no
21m	Idiopathic	5/1	-/3days	yes	vincristine	no	yes	yes	no
30m	Idiopathic	4/1.5	-/3 days	yes	no	no	yes	no	no
34f	Idiopathic	12/1	-/4 days	yes	vincristine IVIg	no	yes	no	no
48f	Idiopathic	10/1+5/1.5	no	yes	vincristine IVIg	no	yes	no	no
17f	Idiopathic	5/1+6/1.5	2/4 days	yes	IVIg	no	yes	no	no
17f	Idiopathic	4/2	3/-	no	no	no	yes	no	no
41f	Idiopathic	7/1	2/-	yes	IVIg	no	yes	yes	no
33f	Idiopathic	FFP	no	yes	no	no	yes	no	no
66f	Idiopathic	FFP		yes	no	no	no		yes
30f	Idiopathic	5/1.5	1/5 days	yes	no	no	yes	no	no
23f	Secondary	13/2	no	yes	cyclophosphamide	no	yes	no	no
64f	Secondary	3/2		yes	no	no	no		yes
68f	Secondary	5/2	1/2 days	yes	azathioprine	no	yes	no	no
36f	Secondary	FFP	no	yes	no	no	yes	no	no
52f	D+HUS	3/2	no	yes	no	10	yes	no	no
62f	D+HUS	10/2	3/-	yes	vincristine	3	yes	no	no
40m	D+HUS	FFP		yes	no	no	no		yes
28f	D-HUS	33/1		yes	IVIg	3x/week	dialysis	no	no
20f	D-HUS	22/1		yes	cyclophosphamide	3x/week	dialysis	no	no
32m	D-HUS	FFP		yes	no	3x/week	dialysis	no	no

25% (moderate deficiency); 3. between 26% and 46% (mild deficiency); 4. greater than 46% (normal). Anti-ADAMTS13 antibodies were detected by western blot technique, as previously reported22.

STATISTICS

Statistical analyses were done in program SPSS for Windows ver. 18 and Statistica ver. 6.0. Data were evaluated by applying Chi-square test, Student t-test, Pearson's correlation coefficient test, and Spearman's rank correla-

TABLE 3

PLASMA EXCHANGE MODALITIES IN THE TREATMENT OF TTP AND HUS EPISODES (n=26)

PE modalities	Episodes N^0	Mean	SD	Min	Max	Total PE number
1 p.v.e.	7	13.43	10.374	5	33	94
1.5 p.v.e.	3	4.67	0.577	4	5	14
2 p.v.e.	14	5.86	3.159	3	13	82
1 p.v.e. + 1.5 p.v.e.	2	18.00	4.243	15	21	36

PE: plasma exchange; p.v.e.: plasma volume exchange

TABLE 4

RESPONSE TO PE TREATMENT IN TERMS OF ADAMTS13 ACTIVITY AND ANTIBODIES PRESENCE (N=26)

clinical type	Episode number	ADAMTS13< 6%	ADAMTS13 6-25%	ADAMTS13 25-46%	ADAMTS13 >46%	Antibodies positive	Antibodies negative	PE≤ 7	PE >7	Death	R
Idiopathic	14	14				14		8	6		4
Idiopathic	2		2				2	2			
Idiopathic	3			3		2	1	2	1		1
Secondary	3		3			2	1	1	1		
D+HUS	2				2		2	1	1		
D-HUS	2			2			2		2		

PE: plasma exchange; R: relapse

tion test. Statistical significance was set at $p<0.05$ and $p<0.01$.

RESULTS

CLINICAL PRESENTATION

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. The time between the onset of the presenting symptoms and clinical diagnosis ranged from 1 to 30 days. The mean duration of symptoms prior to diagnosis was 10.4 days.

Three patients (n016,13,5) with idiopathic TTP presented with their first relapse which has been manifested 6, 7 and 11 years after initial episode. The others manifested their initial acute episode of TTP or HUS. Two episodes have been manifested in three patients with idiopathic TTP each (cases n04a,7a,14a). Patient n07a manifested his second relapse with acute myocardial infarction which was diagnosed based on the presence of sudden chest pain, electrocardiographic alterations (ST elevation in D1, D2, AVL, V2-V5, negative T in V2-V4), and elevated cardiac troponin 8.57μg/L (normal <0.1μg/L), according to recommendation of European Society of Cardiology guidelines[23].

Secondary TTP was related with connective tissue systemic disease (patient n019), cancer (patient n020), syndrome sicca (patient n021) and systemic lupus erythematosus - SLE (patient n022). One of three patients defined as having D+HUS was on tacrolimus regular intake due to allogenous stem cell transplantation 3 years ago (patient n025). One of three patients defined as having D- HUS exhibited onset of disease in the context of SLE (patient n027).

ADAMTS13 activity and anti-ADAMTS13 antibodies

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 activity was 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 (all patients with D+ HUS and 1/3 patients with D- HUS).

Anti-ADAMTS13 antibodies were demonstrable in a total of 17/28 (60.71%) patients. The antibodies were detected in 15/18 (83.3%) patients diagnosed with idiopathic TTP. In plasma of all patients with idiopathic TTP and severe deficiency of ADAMTS13 (13/18;72.2%) 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 one with syndrome sicca related form with moderately reduced protease activity. In patients with TTP secondary to connective tissue disease and malignancy, antibodies were not present. In samples of 2 patients with D- HUS and mildly reduced ADAMTS13 activity antibodies were absent. In the category of normal protease activity antibodies were not present.

RESPONSE TO TREATMENT AND OUTCOME

PE was the principal treatment in 26/31 episodes: idiopathic TTP 19, secondary TTP 3, D+HUS2, and D-HUS2. In the remaining 5 episodes patients received FFP instead of PE. Results of ADAMTS13 activity measurements were not known at the time of the patient's treatment.

Table II gives data pertaining the treatment course and outcome for our patients with TTP and HUS. Table III presents TTP and HUS episodes being treated with different PE modalities, total number of PE until CR achievement, mean values, and standard deviations.

PE was carried out by 1 p.v.e. in the treatment of 7 episodes (patients n08,9,10,12,15,26,27): idiopathic TTP 5, and D- HUS 2 with either FFP replacement fluid in 6 cases, and using albumin in 1 D- HUS epoisode (patient n026). PE was performed once a day in the cases n08,9,10,12,26 whereas in the cases n015 and n027 the regimen was on alternate day. The number of procedures per episode ranged 5-33 (average=13.43). Total of 94 procedures have been performed for the treatment of 7 episodes. All patients received adjuvant corticosteroid therapy. Additional immune therapies were administered in 5 episodes: vincristine (cases n09,12), IVIg (cases n012,26), and cylophosphamide (case n027). Patient n010 received vincristine during disease exacerbation. Also, patient n015 was given with IVIg during exacerbation.

In 14 episodes (13 patients) PE regimen was double volume exchange performed once daily: idiopathic TTP 9, secondary TTP 3, and D+HUS 2 episodes (patients n01,2,3,4,5,6,7,7a,14a,19,20,21,23,24). In addition to PE, corticosteroids were administered in 12 of 14 episodes. The patients underwent a median of 5.86 sessions (range=3-13). Total number of procedures required for remission of 14 episodes was 82. In addition to PE, corticosteroids were administered in 12 out of 14 episodes. Corticosteroids were considered to be contraindicated in the treatment of episode 7a because of acute myocardial infarction that was the initial presentation of the patient's second relapse. According to decision of the treating pediatric team, corticosteroids were not given in 14a episode (patient's first relapse) due to the presence of only mild thrombocytopenia and hemolysis as well as taking into account patient's age. Additionally, hemodyalisis was performed in patients n023 and n024 who presented with AKI. Second line treatment was considered in 5 episodes, and included vincristine (patients n03,6,21), cyclophosphamide (patient n019), and azathioprine (patient n021). In the case n021, during the course of PE treatment azathioprine was introduced instead of corticosteroids following the attack of acute myocardial infarction. Due to presentation with serious clinical picture as well as relapsing disease, vincristine was given at the start of remission in n07a episode. Patient n020 manifested her initial TTP episode secondary to disseminated breast cancer. She had no response and died of progressive disease during the course of PE/corticosteroid treatment.

Daily PE regimen by 1.5 p.v.e. has been used in the treatment of 3 episodes with idiopathic TTP form (patients n04a,11,18). Total number of procedures until achievement of complete remission was 14. The average number of procedures was 4.67 (range=4-5). All patients were on corticosteroid therapy. For the treatment of 2 episodes with idiopathic TTP form (patients n0 13,14) PE was commenced by 1 p.v.e. once a day but afterwards augmented by daily 1.5 p.v.e. regimen. The mean duration of PE sessions ranged 15-21 (average=18). Total number of performed procedures was 36. Patients received corticosteroid therapy in conjunction with PE. In addition to first line treatment, patient n013 was given with vincristine and IVIg whereas patient n014 received IVIg.

Patients with delayed clinical response to standard treatment who required >7 procedures were treated by either single and double volume exchanging as well as PE intensifying from 1 to 1.5 volume.There were 11 episodes with delayed response: idiopathic TTP 7, secondary TTP 1, D+ HUS 1, and D- HUS 2 (Table IV). In plasma of late responders, ADAMTS13 activity was heterogeneous with predominantly severe deficiency (23.07%). All patients with prolonged courses of PE treatment were on second line immune therapy.

Overall PE response rate was 25/26 (96.15%).A mean number of PE procedures required for CR achievement was significantly lower in episodes treated by 2 p.v.e. compared to episodes treated by 1 p.v.e. ($p=0.019$) as well as in episodes treated by 1.5 p.v.e. compared to those treated by intensifying modality ($p=0.010$). Patients in all categories of protease activity levels attained favorable response. Among patients with idiopathic TTP, it was found that those with detectable antibodies needed more exchange sessions to achieve remission than those without antibodies ($p<0.01$).

Exacerbation of disease activity occurred in 3/19 idiopathic TTP episodes (patients n08,10,15). Exacerbations have been manifested in a group of patients being treated with one plasma volume exchanging. In disease activity exacerbation, patient n08 treated with three PE sessions by daily 1 p.v.e. regimen, in treatment of patient n010 five daily PE procedures were performed (2 by 2 p.v.e. followed by 3 by 1 p.v.e.) with adding vincristine, and patient n0 15 was treated with five daily PE procedures by 1 p.v.e. with adding IVIg. All patients received corticosteroids. Exacerbation frequency was significantly higher in idiopathic TTP patients treated with 1 compared with those treated with >1 plasma volume exchange ($p=0.003$).

The other five patients (cases n016,17,22,25,28) received FFP. Patient n016 manifested her first relapse six years after idiopathic TTP initial episode. Her initial episode was associated with pregnancy. PE was withheld because

of mild clinical presentation as well as an initial response to plasma transfusion and cocorticosteroid therapy. Patient n017 with idiopathic TTP died several hours after admission before PE could be initiated. Patient n022 with underlying SLE manifested her first TTP episode 2 years after own initiative cessation of specific therapy for main disease. It couldn't be possible to arrange PE service, so patient treated with FFP with adding pulse methylprednisolone (1g/day i.v.) for the first 3 days followed by 1mg/kg pronisone. It has been shown that patients n016,17 and n022 had severely reduced ADAMTS13 activity with detectable antibodies. Patients n016 and n022 achieved CR. Patient n025 underwent allogenous stem cell transplantation 3 years ago, and has been immunosupressed with cyclosporine. Thereafter he was given with tacrolimus instead of cyclosporine due to its neurotoxic effect. Patient died on 3th hospitalization day. Initially, patient n028 was misdiagnosed with glomerulonephritis. Rapid kidney hematoma development/progression occured after renal biopsy has been done on 1th hospitalization day. Patient underwent urgent nephrectomy. Patient was diagnosed with HUS on the basis of pathohistological finding. Patient is on regular hemodyalisis program 3 times a week.

On the whole, 25 out of 28 patients survived. Survival rate was 89.29%. So far, none of the 25 survived patients of this cohort died, but relapse occurred in 5 patients during the follow up lasting 1-7.5 years to the present time (cases n03,4,7,13,14). ADAMTS13 activity was severely reduced (<6%) in the presence of antibodies at the time of their first acute episode in the cases n0 3,7,14 as well as at the time of the first relapse in the case n0 13 when the patient was tested for the first time. Protease activity was mildly reduced (37%) with detectable antibodies in the serum sample of patient n0 4. Patients n03 and n04 manifested one relapse 3 and 4 years respectively after initial episode. Patient n07 had two relapses within the first year following the initial episode. During above mentioned follow up period, patient n013 manifested 3 relapses. First episode occurred 7 years after initial one, second relapse 8 months after first relapse, and third relapse 6 months after second one. In stable remission after third relapse, patient underwent splenectomy.Patient n014 had two relapses within a year after initial episode.

DISCUSSION

In patients included in this study, the mean time delay from the onset of disease and diagnosis lasted for 10.4 days with a total of 25.8% and 48.4% patients have been presented with pentad and tetrad, respectively suggesting poor disease recognition and postponed treatment institution.

Of 13 patients with idiopathic TTP and undetectable ADAMTS13 activity, 12 of them survived. Survivors were treated with PE. One death occurred on a day of admission before PE institution. Mortality rate among these patients is 7.7%. Fatalities in some others studies are 3/18 (16.6%)[17], 3/16 (18.7%)[12], 2/12 (16.6%)[24], 3/35 (8.6%)[19]. Including all idiopathic TTP patients, mortality rate is 5.5% (1/18) which is lower than rate of 15% (3/20) reported in Zheng study[12]. Undetectable ADAMTS13 in the presence of specific antibodies was demonstrated in one patient with secondary TTP with underlying SLE, who was treated with FFP and high dose corticosteroids with success. This is in agreement with opinion that good responses to FFP transfusion alone may be expected in some patients with acquired TTP similar to hereditary TTP25. Among 3 remaining patients with secondary TTP, 2 of them with partially reduced ADAMTS13 level in the presence of antibodies who were underwent double volume PE therapy responded with CR. These results are in contrast to prevailing opinion that patients with secondary TTP almost never have severe ADAMTS13 deficiency and usually do not respond to PE9.

D+HUS is not considered a disease indication for PE. In D+HUS, Shiga toxins may attach to glomerular capillary endothelial cells and stimulate cells to release "unusually large" von Willebrand factor (UL-vWf) multimers. In addition, Shiga toxin may activate platelets and promote adhesion and aggregation of platelets onto UL-vWf multimers. The category III indication for D+ adult HUS was assigned by ASFA because of the limited and/or conflicting data available in the literature[26]. In our patients defined with D+ HUS, no stool cultures, Shiga toxin, serum antibodies to shiga toxin and serum antibodies to Escherichia coli O157:H7 lypopolysaccharides, were perforemed due to lack of /technique. These diagnostic limitations and the inability to accurately differentiate between the two syndromes was the rationale for PE. In this cohort, two patients with D+HUS responded with CR to PE supported with dialysis. In two patients with D-HUS, poor response may be, in some degree, attributable to PE regimen at alternate day as well as the use of albumin as replacement fluid in one episode.

We could not demonstrate any significant association between PE response and ADAMTS13 activities. Delayed responses to PE treatment requiring >7 sessions were observed paramountly in patients with idiopathic TTP and undetectable ADAMTS13 (6/11) (Table IV). With the exception of one death in patient with TTP secondary to underlying malignant medical disorder as well as two patients with D-HUS with irreversible renal impairment and need for dialysis support, the others attained durable CR. For idiopathic TTP, our results are similar to those founded that patients with and without severe ADAMTS13 deficiency have had similar response rate and short-term survival[12,17,27,28]. None of our patients with idiopathic TTP treated with PE died. In all patients with idiopathic TTP and delayed response CR was achieved with adding immune therapy comprised of vincristine and IVIg. In terms of antibodies presence, most patients (8/11) with poor response to PE treatment had antibodies. It was found that idiopathic TTP patients with detectable antibodies needed more exchange sessions to achieve remission than those without antibodies ($p<0.01$). This result is in accordance with some others findings[17,24,27,29].

Responses to larger volume exchanges (double and 1.5 respectively) have been more favorable compared to 1 p.v.e. and intensifying regimen, respectively, in the light of needed procedures for attainment/maintenance of CR as well as with regard to disease activity exacerbation. Total number of procedures for the treatment of 7 episodes using 1 p.v.e. was 94 whereas for the therapy of 14 episodes by 2 p.v.e. was 82. The mean numbers were 5.9 and 13.4 respectively (p=0.019). The mean number of procedures for the treatment of 3 episodes by 1.5 p.v.e. was 4.7.

In this study, exacerbation occurred in a total of 3/26 (11.5%) episodes treated with PE. All exacerbations referred to idiopathic TTP episodes (3/19) with exacerbation rate of 15.7% among this group of patients. Exacerbations were significantly associated with episodes treated with single volume exchange (p=0.003). Without additional post-remission exchanges, discontinuation of PE has been shown to be associated with prompt TTP exacerbation, with reported frequency of 18-86%[1,2,3,30,31]. This frequency is higher compared with the exacerbation rate in our study. Early rebound of antibodies against ADAMTS13 manifested with laboratory/clinical amelioration and requiring the resumption of PE have been observed in some patients[30,31]. Our results concerning the more favorable response with no exacerbations obtained by 1.5 and 2 p.v.e. respectively may be, in part, explained by the fact that anti-ADAMTS13 antibodies of IgG isotype are present in more than 80% of acquired TTP. These antibodies are partitioned 45% intravascularly and 55% extravascularly . Calculating the volume of plasma as 5% of body weight in kg, 1 p.v.e. will reduce intravascular IgG concentration by a mean of 54%. 2 p.v.e. will produce a mean reduction of 88%. After each reduction, extravascular antibodies equilibrates between the vascular and tissue compartment. This is complete at approximately 18 hours (disease redistribution which with new antibodies synthesis is responsible for rebound). After a series of PE, the net reduction which can be sustained is a function of volume and time[32].

The limitations of this study are: retrospective nature, small number of patients, plasma volumes were different between patients and at times, the use of steroids was not universal, the immunosuppression varied greatly.

CONCLUSION

TTP-HUS syndrome may vary in severity which may pose dilemmas concerning multiple therapies generally employed. Our results suggest that some patients may respond to FFP. Also, patients with immune secondary TTP may have benefit with PE. In some autoimmune TTP episodes larger volume exchanges may be more effective compared to single volume exchange, requiring fewer exchanges in a shorter period of time to achieve stable remission. Measurement of ADAMTS13 activity and specific antibodies on diagnosis may help in real time administration of second line treatment, and in consideration of consolidation treatment schedule.

SUMMARY

KLINIČKO ISKUSTVO U LEČENJU 28 BOLESNIKA SA TROMBOZNOM TROMBOCITOPENIJSKOM PURPUROM - HEMOLIZNO UREMIJSKIM SINDROMOM

Optimalan protokol lečenja i značaj aktivnosti ADAMTS13 (A Desintegrin And Metalloprotease with ThromboSpondin type 1 repeats) u dijagnozi i terapiji trombozne trombocitopenijske purpure (TTP) i hemolizno uremijskog sindroma (HUS) nisu definisani. Cilj rada je da se ustanovi korelacija izmedju odgovora na izmenu plazme (IP) i aktivnosti ADAMTS13. Prikazujemo rezultate studije koja je obuhvatila 28 novodijagnostikovanih bolesnika sa kliničkom dijagnozom idiopatskog TTP (n=18), sekundarnog TTP (n=4), atipicnog HUS (n=3) i tipičnog HUS (N=3). Tri bolesnika su ispoljila 2 akutne episode. TTP. Standardan protokol lečenja sastojao se od sprovodjenja IP u 26 epizoda i transfuzije zamrznute sveže plazme u 5 epizoda, uz dodatnu primenu različitih imunosupresivnih terapija.

Zapremina izmenjene plazme (cirkulatorni volumen plazme - c.v.p.) pri proceduri iznosila je: 1 c.v.p. u 7 epizoda; 1.5 c.v.p. u 3 epizode; 2 c.v.p. u 14 epizoda; u 2 epizode u inicijalnom lečenju volumen izmenjene plazme iznosio je 1 c.v.p. sa intenzifikacijom izmene plazme na 1.5 c.v.p. u nastavku lečenja. Bolesnicima lečenim izmenom 2 odnosno 1.5 c.v.p. izvršen je značajno manji broj IP (p=0.019) u odnosu na bolesnike lečene izmenom 1 c.v.p. odnosno intenzifikacijom izmene plazme (p = 0.010). U ovoj studiji, stopa odgovora na IP iznosi 25/26 (96.15%). Učestalost egzacerbacija je značajno veća kod bolesnika sa idiopatskim TTP (3/19) lečenih izmenom 1 c.v.p u odnosu na bolesnike lečene izmenom > 1c.v.p. (p = 0.003). Stopa preživljavanja iznosi 25/28 (89.29%).

Aktivnost ADAMTS13 je snižena kod 22 bolesnika, medju kojima je kod 14 utvrdjen težak deficit proteaze.

Bolesnici sa svim kliničkim tipovima TTP-HUS i sa svim kategorijama aktivnosti ADAMTS13 su odgovorili na IP. IP sa izmenom > 1 c.v.p. je efikasnija u odnosu na IP sa izmenom 1 c.v.p..

Ključne reči: TTP-HUS sindrom, izmena plazme, ADAMTS13

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