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Elevated plasma neutrophil elastase concentration is associated with disease activity in patients with thrombotic thrombocytopenic purpura

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ABSTRACT

Introduction: Genetic and autoimmune risk factors contribute to the development of thrombotic thrombocytopenic purpura (TTP) but triggers are needed to bring about acute disease.
The aim of the study was to investigate the association of neutrophil activation with acute TTP, to assess whether neutrophil activation changes during plasma exchange therapy and to show if complement- and neutrophil activation are parallel, characteristic processes in acute TTP.
Materials and Methods: Altogether 49 EDTA-plasma samples of 21 TTP patients with acute disease and 17 in remission were investigated along with 20 healthy controls.
A stable complex of PMNE-proteinase-inhibitor was measured by ELISA (Calbiochem, Merck-Millipore, Darmstadt, Germany).
Results: Acute disease was associated with significantly increased PMNE levels, the group medians were similarly low in TTP patients in remission and in healthy controls. Increased PMNE levels were characteristic for hematologically active and ADAMTS13 deficient form of TTP. PMNE concentration inversely correlated to disease activity markers platelet count ($r = -0.349$, $p = 0.032$) and hemoglobin levels ($p = -0.382$ $p = 0.018$). Achievement of remission was associated with significant reduction of plasma PMNE levels ($p = 0.031$, Wilcoxon test). There was positive correlation between PMNE levels and complement activation markers C3a and Bb.
Conclusions: We report increased PMNE levels in acute TTP and showed its association to activity markers of acute TTP and complement activation. Effective treatment of an acute TTP episode resulted in marked decrease in PMNE levels. Our data support and extend previous observations that neutrophil extracellular traps may be released in acute TTP and potentially contribute to the pathophysiology of this disease.

Introduction

Various etiological factors represent increased risk for the development of thrombotic thrombocytopenic purpura (TTP), a life-threatening disorder. Characteristic clinical features of TTP are microangiopathic hemolytic anemia, thrombocytopenia and various involvements of other organs, for example the kidneys and the nervous system [1,2]. In most cases development of functional inhibitors (autoantibodies) against the von Willebrand factor (VWF) cleaving protease (A Disintegrin and Metalloproteinase with ThromboSpondin-1 like motifs-13, ADAMTS13) predispose to disease. Inherited variations of ADAMTS13 may also confer increased risk of TTP development, whereas a minor part of TTP patients presents with non-ADAMTS13 deficient disease form [3]. Severe deficiency of ADAMTS13 leads to the increased presence of unusually large multimers of VWF that promote the formation of platelet rich thrombi in the capillaries and small vessels leading to tissue ischemia and organ failure [4].

However, ADAMTS13 deficient patients may remain asymptomatic for several years [5,6]. Furthermore, several patients may go into sustained clinical remission despite deficient ADAMTS13 activity [7,8]. In addition, although patients with acute TTP often present without acute disease in history, in the majority of TTP patients infections or pregnancy precede the acute disease flare [9]. These well-known clinical observations collectively indicate that multiple hits may be necessary for the development of clinically active TTP, and ADAMTS13 deficiency, even though it is probably the most important predisposing risk factor, it is alone not sufficient to cause acute TTP.

Previously our group reported on the presence of complement activation in patients with acute TTP [10] an observation recently confirmed in an independent cohort [11]. Our data indicated that activation of the classical/lectin and alternative pathways that lead to the activation of the terminal pathway was present in TTP. It is known

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that activation of the complement system and of neutrophil granulocytes is characteristic for most infections and also in pregnancy, especially if complicated with preeclapmsia and/or fetal growth restriction [12,13]. Therefore, we hypothesized that neutrophil activation may associate with acute, ADAMTS13-deficient TTP and potentially contribute to the development of clinically active thrombotic microangiopathy. Supporting this hypothesis were results of a recent study showing increased circulating DNA and myeloperoxidase levels in patients suffering from thrombotic microangiopathies (TMAs) [14]. Hence, the aim of the current study was to formally investigate the association of neutrophil activation with acute TTP, to assess whether neutrophil activation changes during plasma exchange therapy and to show if complement- and neutrophil activation are parallel, characteristic processes in acute TTP.

Materials and Methods

Patients and Plasma Samples

Thirty-eight patients with TTP were enrolled in this single-research laboratory based investigation providing diagnostic services (ADAMTS13 and complement measurements) since August, 2007 for patients suspected to have HUS or TTP in Hungary. The patient enrolment for this study was closed in January, 2011. The criteria used to guide patient stratification, diagnosis and sample selection have been described in details [10]. Briefly, diagnosis of TTP was based on one or more episodes of Coombs-negative microangiopathic hemolytic anemia with thrombocytopenia defined as serum lactate dehydrogenase (LDH) >450 U/L; fragmented erythrocytes in the peripheral blood smear and platelet count <150 G/L; only patients with severely decreased ADAMTS13 activity levels (<5%), or history of it, were included in this study. Patients with acute oligo-anuric renal failure were excluded from the study. Hematological remission (HR) was determined when platelet counts were >150 G/L on two consecutive days without any sign of hemolysis even if there were any neurological, renal or other residual clinical symptoms, whereas complete remission (CR) was established when, platelet count remained above the lower limit continuously for at least 1 month.

Table 1 shows clinical and laboratory data of the cohort who had available plasma samples for the current study. Using samples of this TTP cohort we measured plasma polymorphonuclear cell elastase (PMNE) levels during acute disease flare, in remission, and for comparison in controls. Samples of 38 TTP patients have been investigated. Twenty-one patients with acute disease (mean age 40 years, SD 15, 17 women), 17 TTP patients in remission (mean age 42 years, SD 10, 14 women) and 20 healthy age- and sex matched controls (mean age 35 years, SD 17, 15 women) were enrolled. Eight patients with acute

Table 1
Clinical and laboratory data of the 38 patients with thrombotic thrombocytopenic purpura.

Registry code	Age at blood sampling (years)	Sex	Acute/ Remission	ADAMTS13 activity of patient's sample (FRETS-VWF73); reference range 67-147%	ADAMTS13 activity of mixed sample (patient's plasma with pooled plasma 1:1) (FRETS-VWF73); reference range >50% of the daily control sample	Hemoglobin; reference ranges, females 123–153 g/L, males 140–175 g/L	Platelets; reference range 150–400 G/L	White Blood Cells; reference range 4–10 G/L	Absolute neutrophil count; reference range 2.12-7.5 G/L	PMN elastase proteinase inhibitor complex (ng/mL)
HUN1	26	F	Acute	**0**	**0**	80	33	31,50	25,51	3,10
HUN15	55	M	Acute	**0**	**0**	108	22	12,06	10,61	0,82
HUN23	38	M	Acute	**0**	**30**	91	45	6,40	3,21	0,63
HUN43	62	F	Acute	**0**	**26**	72	28	6,53	5,68	0,53
HUN50	17	F	Acute	**0**	**9**	76	17	11,20	7,71	2,72
HUN56	44	F	Acute	**0**	**0**	83	44	6,81	4,44	0,57
HUN62	35	F	Acute	**0**	**11**	81	18	11,63	9,13	1,76
HUN68	43	M	Acute	**5**	**6**	64	9	6,30	4,38	1,15
HUN72	13	F	Acute	**0**	**0**	72	84	10,80	8,48	5,61
HUN76	57	F	Acute	**0**	**23**	85	30	7,11	5,02	2,46
HUN85	49	F	Acute	**0**	**0**	85	31	11,34	10,26	0,62
HUN86	42	F	Acute	**0**	**5**	78	12	5,37	3,17	0,91
HUN96	22	F	Acute	**5**	**23**	53	23	7,16	4,48	1,59
HUN97	51	F	Acute	**0**	**15**	126	43	12,19	5,88	1,01
HUN112	71	F	Acute	**0**	**ND**	103	44	3,69	2,14	0,72
HUN113	25	F	Acute	**0**	**0**	109	22	10,45	6,21	4,19
HUN123	45	F	Acute	**0**	**18**	97	76	32,82	12,61	2,92
HUN126	36	M	Acute	**0**	**0**	69	43	6,79	3,63	1,81
HUN127	30	F	Acute	**0**	**0**	75	12	8,93	8,09	1,90
HUN131	44	F	Acute	**0**	**0**	126	48	11,12	7,77	0,49
HUN134	44	F	Acute	**0**	**0**	80	19	7,99	5,84	1,08
HUN3	40	M	Remission	**0**	**30**	154	244	10,51	8,20	0,54
HUN28	36	F	Remission	**68**	**61**	NA	213	NA	NA	0,88
HUN31	33	F	Remission	**0**	**29**	153	275	6,82	3,41	0,47
HUN35	37	F	Remission	**0**	**0**	123	272	18,70	10,28	0,86
HUN53	51	F	Remission	**0**	**18**	135	174	6,85	4,67	0,57
HUN54	56	F	Remission	**104**	**ND**	140	115	6,11	3,92	12,00
HUN58	46	F	Remission	**74**	**ND**	136	158	5,71	3,31	0,49
HUN59	44	F	Remission	**112**	**ND**	119	184	5,85	3,35	0,81
HUN65	35	F	Remission	**0**	**44**	150	388	15,70	11,41	4,22
HUN70	47	F	Remission	**97**	**33**	NA	213	NA	NA	0,73
HUN78	47	F	Remission	**0**	**0**	134	210	7,94	4,64	0,55
HUN80	31	M	Remission	**109**	**87**	169	246	9,68	4,84	1,13
HUN81	21	F	Remission	**7**	**20**	124	308	22,93	19,42	1,05
HUN89	33	M	Remission	**0**	**23**	156	260	5,37	2,84	0,54
HUN100	40	F	Remission	**109**	**75**	144	213	5,88	3,65	0,47
HUN101	61	F	Remission	**79**	**65**	123	196	6,89	5,49	0,58
HUN115	51	F	Remission	**19**	**ND**	149	433	12,99	10,05	0,42

NA: not available; ND: not done.