original paper
Haematologica 1996; 81:3-7

INCREASED P-SELECTIN PLASMA LEVELS IN PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA

Nicola Vianelli, Lucia Catani, Luigi Gugliotta, Franco Nocentini, Stefano Baravelli, Giustina Lancellotti, Sante Tura

Hematology Institute "Seràgnoli", University of Bologna, Italy

ABSTRACT

Background. **Thrombotic thrombocytopenic purpura (TTP) is a rare vascular disorder of unknown etiology. There is evidence to support the hypothesis that platelets and endothelium play a pivotal pathogenetic role. Immunological assays for plasma thrombomodulin and P-selectin levels have recently been made available and they allow simple evaluation of endothelial damage and endothelial/platelet activation, respectively. In this study, we measured the plasma levels of thrombomodulin, P-selectin and von Willebrand factor in 9 TTP patients during active disease and at the time of complete remission (CR).**

Methods. **Thrombomodulin, P-selectin and von Willebrand factor were measured by enzyme immunoassay.**

Results. **Mean thrombomodulin and von Willebrand factor plasma values were always within the normal range. P-selectin plasma levels, both in the active phase of the disease and in CR (median 312 and 185 ng/mL, respectively), were significantly higher than in normal controls (mean 96±35 ng/mL, median 88 ng/mL; $p< 0.05$). However, the mean value of P-selectin in CR (median185 ng/mL) was significantly lower than that observed at diagnosis ($p < 0.05$). In addition, an inverse relationship between P-selectin plasma levels and platelet count ($r= –0.526$; $p= 0.03$) was observed.**

Conclusions. **These findings suggest that activation of platelets and/or endothelium may play a relevant role in the pathogenesis of TTP.**

Key words: thrombotic thrombocytopenic purpura, thrombomodulin, P-selectin

Thrombotic thrombocytopenic purpura (TTP) is a rare disease of unknown etiology characterized by microangiopathic hemolytic anemia, consumption thrombocytopenia, neurologic symptoms, renal impairment and fever.[1]

The formation of arteriolar microthrombi plays an important role in the pathogenesis of the disease. The microthrombi consist primarily of platelets with only small amounts of fibrin.[2] In this disease, in fact, the soluble coagulation system does not seem to be involved. A relevant pathogenetic role has been ascribed to the activation of platelets with consequent aggregation and/or agglutination;[3-7] however, the occurrence of concomitant endothelial damage cannot be excluded.[8]

Plasma manipulation, in particular plasma exchange (PE), is presently considered an effective procedure for reaching complete remission (CR) in TTP patients.[9]

Very recently two glycoproteins, thrombomodulin (TM) and P-selectin (P-S) were evaluated as markers of endothelial and platelet status. Thrombomodulin is an endothelial cell membrane glycoprotein involved in the protein C anticoagulant pathway. High plasma levels of TM are considered markers of endothelial dam-

Correspondence: Dott. Nicola Vianelli, Istituto di Ematologia "L. e A. Seràgnoli", Università di Bologna, Policlinico S.Orsola, via Massarenti 9, 40138 Bologna, Italy. Tel. international +39.51 390413, Fax. international +39.51.398973.

Acknowledgements: we wish to thank Dr. Roberto Massimo Lemoli for his helpful suggestions, and we gratefully acknowledge the expert technical assistance of Valeria Martelli.
Received June 12, 1995; accepted November 22, 1995.

age.[12] P-selectin is a glycoprotein contained in platelet α-granules and endothelial Weibel-Palade bodies. It belongs to the adhesive molecule family and is involved in leucocyte adhesion. P-selectin is expressed on the cell surface and is secreted as a consequence of cell activation. Because high plasma levels of P-selectin suggest endothelial/platelet activation, this glycoprotein has been proposed as a marker of thrombotic disease.[13-15] Assays for the evaluation of TM and P-S plasma levels have recently been developed. Von Willebrand factor (vWF) has also been long proposed as a marker of endothelial cell damage. This protein has been detected in platelets and endothelial cells; however, experimental data support the convinction that most, if not all, circulating vWF is likely to originate from endothelium.[16]

In this study we evaluated platelet and endothelial involvement in TTP patients with active disease and at the time of CR by testing TM, P-S and vWF plasma levels.

Patients and Methods

Patients

This study included nine patients with TTP (2 males and 7 females; median age 39 years, range 20-64). Eight were newly diagnosed and one patient with chronic relapsing TTP was in the acute phase of the disease. All patients were submitted to plasma exchange procedures and prednisone and/or ticlopidine and/or vincristine were administered in a few cases. One patient died early during induction therapy (day +4), whereas the 8 remaining ones reached complete remission (CR). None of these 8 patients in CR has relapsed at a median follow-up of 40 months (range 12-60 months).

Evaluation of TM, P-S and vWF plasma levels

Evaluation of TM, P-S and vWF plasma levels was performed before treatment and repeated two or more months after the achievement of CR. Briefly, venous blood samples were collected in 3.8% sodium citrate and the platelet poor plasma was stored at –20°C. Control samples were collected from 26 normal sex- and age-matched subjects.

Plasma thrombomodulin was evaluated by using a commercially available enzyme immunoassay (Diagnostica Stago, Asserachrom Thrombomodulin, France). P-selectin was measured using a sandwich enzyme-linked immunoadsorbent assay (P-Selectin EIA kit, Takara Biomedicals, Japan),[17] and von Willebrand factor was tested with an enzyme immunoassay (Asserachrom vWF, Boehringer Mannheim, Germany), according to the manufacturer's instructions.

Statistical analysis

Median and interquartile ranges (25th to 75th percentile) were reported in order to describe data.

The Mann-Whitney and Wilcoxon tests for matched pairs were utilized in order to evaluate differences among groups.

BMDP Statistical Software, 1991 ed. (BMDP Statistical Software, Cork, Ireland) was used. The level of significance was set at 0.05. All P-values in this report correspond to two-sided significance tests.

Comparisons among the groups were evaluated by the Student's t-test.

Results

Clinical and laboratory data are reported in Table 1 and Figure 1. Plasma TM levels in TTP patients with active disease and in CR (median = 33 and 24 ng/mL, respectively) were not significantly different from those obtained in normal controls (mean = 30±8 ng/mL, median = 30 ng/mL), and there was no significant difference between baseline and CR values (Table 1). Plasma P-S levels during active disease (median = 312 ng/mL) were significantly higher than normal controls (mean = 96±35 ng/mL; median = 88 ng/mL; $p < 0.01$), whereas CR values (median =185 ng/mL) were significantly lower than in the active phase of the disease ($p < 0.05$), although they still remained significantly ($p < 0.05$) higher than in normal controls. It should be pointed out that during the remission phase 4 out of the 8 patients reached normal values. In addition, we observed an inverse

Table 1. Clinical and laboratory data for the 9 TTP patients studied.

SEX-AGE	SEVERITY SCORE[1]	Hb (g/dL)		PLATELETS (10^9/L)		LDH (U/L)		THROMBOMODULIN[2] (ng/mL)		P-SELECTIN[3] (ng/mL)		vWF[4] (%)	
		D	CR	D	CR	D	CR	D	CR	D	CR	D	CR
F-42	5	6.4	12.9	20	205	2000	123	14	12	337	110	96	108
F-14	6	6.1	13.5	16	229	2800	148	33	36	162	116	83	92
M-32	7	8.0	15.0	12	191	884	160	38	29	362	286	69	82
M-40	5	7.6	12.0	4	289	1446	173	29	18	386	200	122	93
F-64	6	6.4	11.8	10	160	1700	213	54	42	305	171	105	102
F-52	6	7.2	14.8	14	178	2000	178	35	35	152	235	113	113
F-20	6	8.4	12.5	17	212	1474	273	32	5	313	293	93	119
F-22	5	5.4	13.7	26	205	1188	136	15	19	262	73	125	95
F-42	6	6.1	/	12	/	1900	/	45	/	534	/	109	/
MEDIAN		6.4	13.2	14.0	208	1700	166	33	24	312[5]	185[6]	105	98
75°		7.6	14.2	17	253	2000	195	38	35	362	260	113	110
25°		6.1	12.2	12	198	1446	142	29	15	262	113	93	92
TILE													

[1]According to Rose et al. (Am J Med 1987); [2]Normal value 30±8 ng/mL; [3]Normal value 96±35 ng/mL; [4]Normal value 108±10%. Thrombomodulin and von Willebrand factor plasma levels did not show significant differences between active disease phase (D) and complete remission (CR) and were not different with respect to the normal controls. [5]P-selectin in active phase of TTP was significantly higher (p=0.001) than in normal controls. [6]P-selectin in CR was significantly higher (p=0.05) than in normal controls but significantly lower (p=0.05) than in active phase.

correlation between P-S plasma levels and platelet counts (r= – 0.526; p = 0.03).

Von Willebrand factor plasma levels always remained in the normal range and there was no

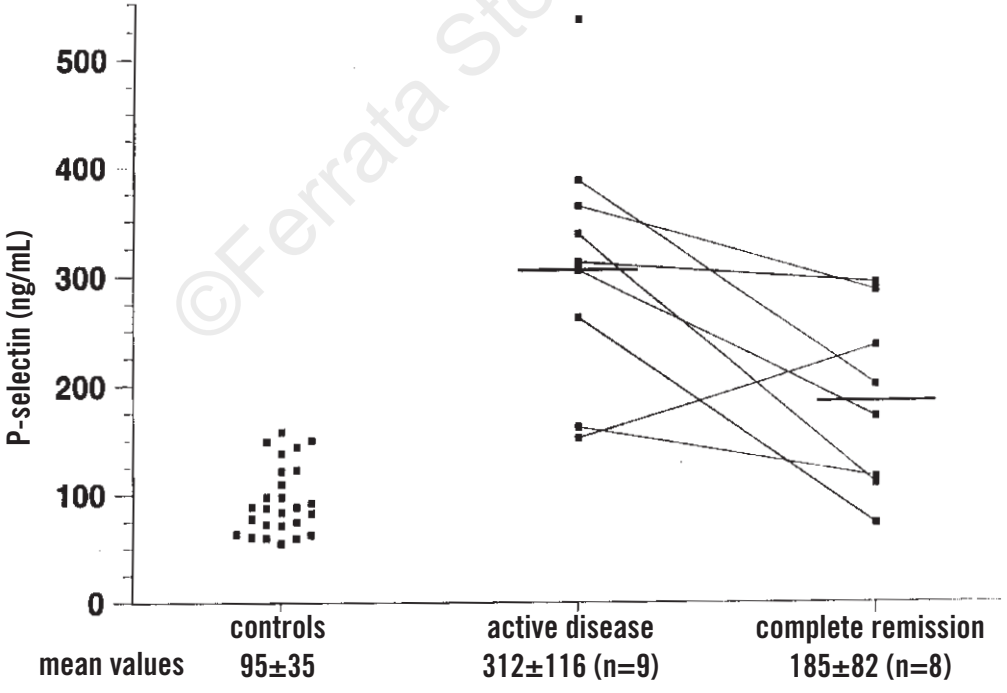

Figure 1. Plasma P-selectin levels in TTP patients during active disease phase and in complete remission.

significant difference between baseline and CR values. We did not find any significant correlation between either TM and P-S levels at diagnosis or P-S levels at diagnosis and the severity score.

Discussion

TTP is a disease in which platelets and endothelium play a relevant pathogenetic role; however, the exact mechanism of their involvement is poorly understood. The availability of laboratory tests for TM (marker of endothelial activation/damage) and P-S (marker of endothelial and/or platelet activation) have brought a better understanding of the pathogenesis of this disease. It has been reported that plasma TM in TTP is increased in patients with active disease and that its level significantly decreases when CR is achieved.[18] In our experience, mean plasma TM values were found to be within the normal range both at diagnosis and in CR. However, it should be underlined that conflicting results may be due to different methods employed for detection of TM plasma levels. The assay reported here had already been successfully used to monitor TM levels in patients with veno-occlusive disease following bone marrow transplantation.[17] Interestingly, plasma vWF levels in our patients were also in the normal range both during the active phase of the disease and in CR.

In our study, plasma P-S was always increased in patients with active TTP and significantly decreased when CR was obtained, reaching the normal range in 4 out of the 8 patients in CR. An inverse relationship (p=0.03) between plasma P-S level and platelet count was observed in our patients and, interestingly, the only patient with a rapidly fatal disease had the highest basal P-S level (534 ng/mL). In agreement with our data, Katayama *et al.* previously showed high P-S levels during the active phase of TTP and a normalization of the protein in the three patients studied in remission phase.

These results seem to support the hypothesis that hematological remission in TTP may be associated in some patients with the persistence of abnormal endothelial and/or platelet activation. This is also corroborated by the persistence of high TGF-β1 plasma levels in some patients in CR.[19] Our findings suggest that platelet and endothelial cell activation appears to be the main mechanism of platelet aggregation rather than damage to endothelial cells. These results are further confirmed by the normal vWF levels observed both in active disease and in CR.

The only patient in our study with a fulminant disease course did not have a particularly high severity score as compared to the other patients, but she did have the highest basal P-S plasma level. In a larger series of TTP patients it would be useful to evaluate the prognostic value of P-S level at diagnosis as well as the predictive value of the P-S level in CR on the probability of relapse.

In conclusion, our study suggests that activation of platelets and/or endothelium may play a relevant role in the pathogenesis of TTP.

References

1. Hau CK. Clinicopathologic features of thrombotic thrombocytopenic purpura. Semin Hematol 1987; 24:71-81.
2. Bukowsky RM. Thrombotic thrombocytopenic purpura: a review. Prog Hemost Thromb 1982; 6:287-337.
3. Murphy WG, Moore JC, Kelton JG. Calcium-dependent cysteine protease activity in the sera of patients with thrombotic thrombocytopenic purpura. Blood 1987; 70:1683-7.
4. Falanga A, Consonni R, Ruggenenti P, Barbui T. A cathepsin-like cysteine proteinase proaggregating activity in thrombotic thrombocytopenic purpura. Br J Haematol 1991; 79:474-80.
5. Kelton JG, Moore JC, Murphy WG. Studies investigating platelet aggregation and release initiated by sera from patients with thrombotic thrombocytopenic purpura. Blood 1987; 69: 924-8.
6. Moake JL, Mc Pherson PD. Von Willebrand factor in thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome. Transfusion Med Rev 1990; 4:163-6.
7. Moore JC, Murphy WG, Kelton JG. Calpain proteolysis of von Willebrand factor enhances its binding to platelet membrane glycoprotein IIb/IIIa: an explanation for platelet aggregation in thrombotic thrombocytopenic purpura. Br J Haematol 1990; 74:457-64.
8. Ruggenenti P, Remuzzi G. Thrombotic thrombocytopenic purpura and related disorders. Hematol Oncol Clin North Am 1990; 4:219-41.
9. Bukowski RM, Hewlett JS, Reimer RR, Groppe CW, Weick JK, Livingston RB. Therapy of thrombotic thrombocytopenic purpura: an overview. Semin Thromb Hemost 1981; 7: 1-8.
10. Centurioni R, Bobbio Pallavicini E, Porta C, et al. Treatment of thrombotic thrombocytopenic purpura with high-dose immunoglobulins. Results in 17 patients. Haematologica 1995; 80:325-31.
11. Bobbio Pallavicini E, Porta C, Tacconi F, et al. Intravenous prostacyclin (as epoprostenol) infusion in thrombotic

thrombocytopenic purpura. Four case reports and review of the literature. Haematologica 1995; 80:429-37.
12. Ishii H, Mayerus PW. Thrombomodulin is present in human plasma and urine. J Clin Invest 1985; 76:2178-81.
13. Hoffman M, Monroe DM, Roberts HR. Platelet activation in patients with thrombotic thrombocytopenic purpura. Am J Hematol 1993; 42:182-5.
14. Chong BH, Murray B, Berndt MC, Dunlop LC, Brighton T, Chesterman CN. Plasma P-Selectin is increased in thrombotic consumptive platelet disorders. Blood 1994; 83:1535-41.
15. Katayama M, Handa M, Araki Y, et al. Soluble P-Selectin is present in normal circulation and its plasma level is elevated in patients with thombotic thrombocytopenic purpura and haemolytic uraemic syndrome. Br J Haematol 1993; 84:702-10.
16. Blann AD, Taberner DA. A reliable marker of endothelial cell dysfuction: does it exist? Br J Haematol 1995; 90: 244-8.
17. Catani L, Gugliotta L,Vianelli N, et al. Endothelium and bone marrow transplantation Bone Marrow Transplant 1995 (in press).
18. Wada H, Ohiwa M, Kaneko T, et al. Plasma Thrombomodulin as a marker of vascular disorders in thrombotic thrombocytopenic purpura and disseminated intravascular coagulation. Am J Hematol 1992; 39:20-4.
19. Zauli G, Catani L, Gugliotta L, Vianelli N, Mattioli Belmonte M, Tura S. Increased serum levels of transforming growth factor beta-1 in patients affected by thrombotic thrombocytopenic purpura (TTP): its implications on bone marrow haematopoiesis. Br J Haematol 1993; 84: 381-6.