Hematology

ISSN: 1024-5332 (Print) 1607-8454 (Online) Journal homepage: http://www.tandfonline.com/loi/yhem20

Markers of Endothelial and Platelet Status in Patients with Essential Thrombocythemia and Polycythemia Vera

C. Musolino, A. Alonci, G. Bellomo, O. Tringali, G. Spatari, C. Quartarone, V. Rizzo, L. Calabrò, G. Bagnato & N. Frisina

To cite this article: C. Musolino, A. Alonci, G. Bellomo, O. Tringali, G. Spatari, C. Quartarone, V. Rizzo, L. Calabrò, G. Bagnato & N. Frisina (1999) Markers of Endothelial and Platelet Status in Patients with Essential Thrombocythemia and Polycythemia Vera, Hematology, 4:5, 397-402, DOI: 10.1080/10245332.1999.11746464

To link to this article: https://doi.org/10.1080/10245332.1999.11746464

Hematology, 2000, Vol. 4, pp. 397–402
Reprints available directly from the publisher
Photocopying permitted by license only

Myeloproliferative Disease

Markers of Endothelial and Platelet Status in Patients with Essential Thrombocythemia and Polycythemia Vera

C. MUSOLINO*, A. ALONCI, G. BELLOMO, O. TRINGALI, G. SPATARI, C. QUARTARONE, V. RIZZO, L. CALABRÒ, G. BAGNATO and N. FRISINA

Division of Hematology and Department of Internal Medicine, University of Messina, Italy

(Received 7 April 1999; In final form 10 June 1999)

Vascular complications are the main cause of morbidity in polycythemia vera (PV) and essential thrombocythemia (ET). To investigate plasma concentrations of soluble P-selectin (sP-Sel.), soluble E-selectin (sE-Sel.) and soluble thrombomodulin (sTM) in relation to the presence of thromboembolic events 38 patients with Chronic Myeloproliferative Disorders (CMD) (14 PV pts and 24 ET pts), 15 age — matched controls and 15 patients with secondary thrombocytosis were studied. Plasma levels of P-Sel., E-Sel. and TM were significantly increased in the group of patients as compared with control subjects (respectively $p < 0.001$, $p < 0.04$ and $p < 0.01$). sP-Sel. levels showed no significant difference between the patients and those with secondary thrombocytosis. No difference in sP-sel levels were also observed between subgroups of CMD patients with and without vascular complications. However, among patients with ET, those with thrombosis had higher sP-Sel levels than those without thrombosis (1.177 ± 110.48 ng/ml vs 816.25 ± 99.27 ng/ml). High levels of sE-Sel and sTM were found in CMD patients (71.93 ± 39.08 ng/ml and 35.81 ± 20.79 ng/ml, respectively). Plasma sE-Sel. concentration was significantly higher in CMD patients with thrombosis than that in CMD patients without thrombosis ($p < 0.001$). There was no difference in sTM concentration between two groups. These findings indicate that sustained endothelium and platelet activation is present in patients with ET and PV and it might contribute to the pathogenesis of thromboembolic events in these patients.

Keywords: Soluble P-selectin, E-selectin, thrombomodulin, essential thrombocythemia, polycythemia vera, thrombosis

INTRODUCTION

Thromboembolic events frequently characterize chronic myeloproliferative disorders (CMD) [1]. Blood hyperviscosity, platelet count or other risk factors certainly play an important role in the development and progression of these complications, but no clear correlation between hematological parameters and the incidence of thrombosis has been established [2–5]. To investigate whether the presence of endothelial and platelet markers might be associated with an increased

*Corresponding author. Tel.: 090-2212364. Fax: 090-2935162.

risk for thromboembolic events in patients with essential thrombocythemia (ET) and polycythemia vera (PV) we assayed the plasma levels of soluble P-selectin (sP-Sel), soluble E-selectin (sE-Sel) and soluble thrombomodulin (sTM).

P-selectin, an adhesion molecule, is present in platelet α-granules and endothelial Weibel-Palade bodies and is involved in leukocyte-platelet-endothelium adhesion. Increased soluble levels have been found in cardiovascular disease, in diabetes mellitus and in atherosclerosis. As raised soluble levels are released after stimulation, this molecule could therefore be considered a new marker of platelet activation in vivo [6–10]. Cytokine-mediated endothelial cell activation results in increased surface expression of other leukocyte adhesion molecules, such as E-selectin. Soluble E-selectin is present in the supernatants of activated endothelial cells and raised plasma levels have been found in cancer, hypertension, diabetes and in coronary artery disease [11–12].

Thrombomodulin is a proteoglycan and increased levels of soluble thrombomodulin have been reported in chronic myelogenous leukemia [13], thrombotic thrombocytopenic purpura DIC [14] and collagen vascular diseases [15]. It Has been suggested that the levels of sTM are useful indicators of endothelial damage [16,17].

MATERIALS AND METHODS

We studied 38 patients (12 M, 26F, mean age 57.3 ± 11.8 years), 24 with ET and 14 with PV. The diagnosis was defined according to the criteria of the Polycythemia Vera Group [18]. Seventeen patients (10 with ET, 7 with PV) presented thrombotic manifestations in their past history. They included complete stroke, deep venous thrombosis, transient ischemic attack, cerebrovascular accident and digital gangrene.

During this study, 15 patients were considered before any chemotherapy, 23 patients were treated with hydroxyurea and/or phlebotomies when necessary. Fifteen patients received platelet antiaggregants (13 aspirin, 2 ticlopidine). No significant differences in age or duration of follow-up were found in the disease groups when they were divided into complication groups. Patients with hypercholesterolemia, diabetes, liver and kidney diseases were excluded from the study.

Fifteen healthy control subjects and 15 patients with secondary thrombocytosis (ST) were also studied. The diagnosis of secondary thrombocytosis (platelet count $\geq 600 \times 10^9$/l) was applied to patients without CMD who had a disease known to be associated with secondary thrombocytosis ($n°9$ after post-surgical, $n°5$ iron deficiency, $n°1$ splenectomy). They were with similar age and sex distributions of the patients.

All blood collections were carried out between 8.00 and 9.30 am after an overnight fast without venous stasis. Blood samples were obtained through a 21-gange needle into Vacutainer tubes containing 1/9 volume of 0.129 mol trisodium citrate. All plasma samples were iced for 10 min, there centrifuged at 2000 g for 15 min at 4 °C, and stored in small aliquots at −20 °C. Plasma samples from each individual were run in the same assay in duplicate.

Soluble P-selectin and E- selectin were measured by an enzyme-linked immunosorbent assay (Elisa) method (Bender-Med, Vienna, Austria). Plasma thrombomodulin was quantitated with commercial ELISA (Stago, France).

Platelet count were determined by Coulter counter analyzer.

Data were expressed as mean ± SD. Statistical analysis was performed by using the ANOVA one-way test and Pearson's coefficient of correlation. The significance level was set to $p < 0.05$.

RESULTS

sP-Selectin

In all patients the plasma sP-Sel was significantly increased to the healthy control group (798.45 ±

TABLE I Endothelium markers and soluble adhesion molecules levels in controls, in patients with ET., PV and ST

Parameter	Controls $n = 15$	ST $n = 15$	ET $n = 24$	PV $n = 14$
sP-sel (ng/ml)	152 ± 30	$515.7 \pm 317^{\ddagger}$	$996.8 \pm 390^{\ddagger}$	$509.7 \pm 417.3^{*}$
sE-sel (ng/ml)	44.9 ± 14.3	50.9 ± 8.8	$65.3 \pm 29.1^{*}$	$83.2 \pm 21^{*}$
sTM (ng/ml)	11.3 ± 4.7	$41.2 \pm 18^{\ddagger}$	$41.5 \pm 22^{\ddagger}$	$25.9 \pm 13.6^{\ddagger}$

$^{*}p < 0.04$;
$^{\dagger}p < 0.01$;
$^{\ddagger}p < 0.005$ (compared to controls).

457 ng/ml vs 152 ± 30 ng/ml; $p < 0.001$). The patients with ET had higher sP-S levels (996.87 ± 390 ng/ml) than those with PV (509.71 ± 417.31 ng/ml) ($p < 0.002$) (Table I). A significant difference was also found between the ET patients with thrombotic complications and those without thrombosis (1.177 ± 110.48 ng/ml vs 816.25 ± 99.27; $p < 0.02$), while no difference was recorded in the PV group between those with and those without thrombosis (573.07 ± 166.10 ng/ml vs 446.35 ± 170.64 ng/ml; p NS) (Figure 1). Moreover, no difference was observed between the CMD patients and the patients with secondary thrombocytosis (515.77 ± 317 ng/ml $p = 0.06$) but these patients showed significantly higher sP-S levels than the control group ($p < 0.005$).

sE-Selectin

The plasma levels of sE-selectin were higher in patients with ET (65.34 ± 29.15 ng/ml) and PV (83.21 ± 21 ng/ml) than in controls (44.96 ± 14.32 ng/ml).

Although the plasma level of sE-sel tended to be higher in patients with secondary thrombocytosis (50.98 ± 8.84 ng/ml) than in control patients, the difference was not significant (Table I). The plasma level of sE-sel was significantly higher in patients with thrombosis (103.15 ± 7.4 ng/ml)

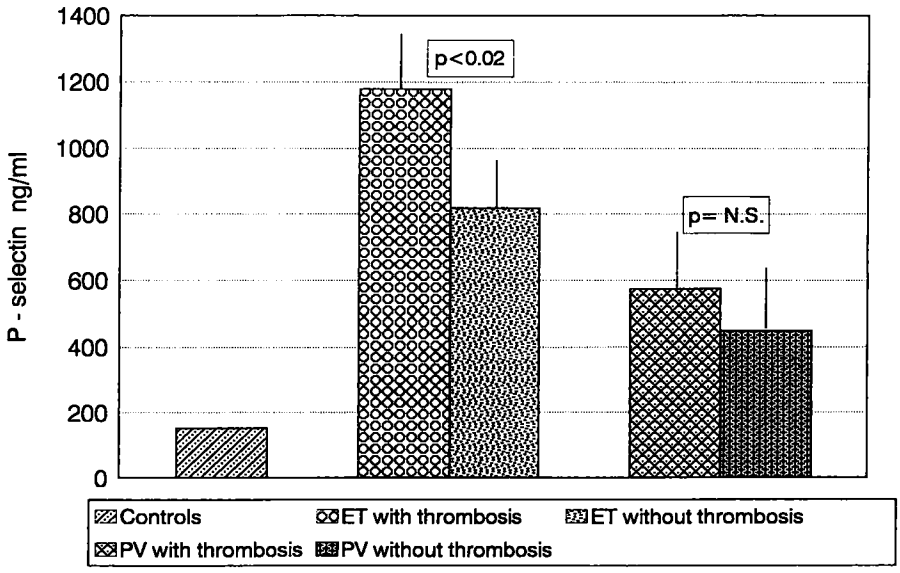

FIGURE 1 Plasma P-selectin levels of ET and PV patients with and without thrombosis.

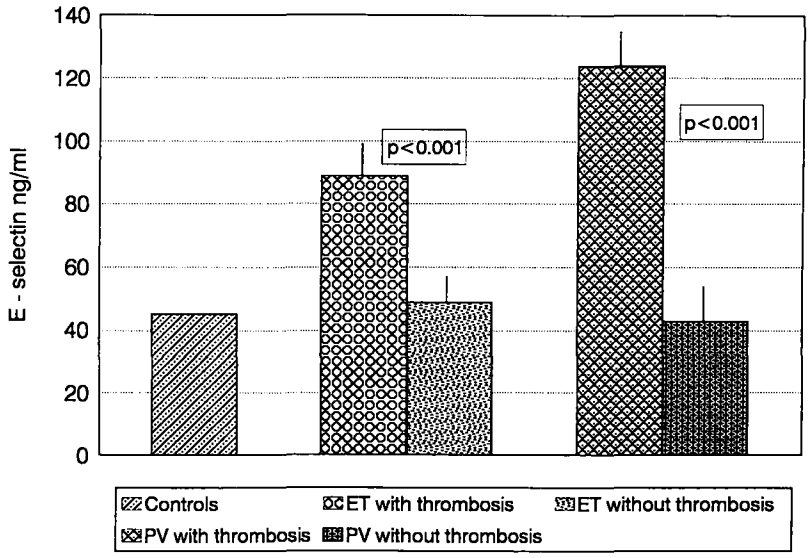

FIGURE 2 Plasma E-selectin levels of ET and PV patients with and without thrombosis.

than in patients without thrombosis (46.65 ± 5.5 ng/ml) ($p < 0.001$) in the overall study population (Figure 2).

Thrombomodulin

The ET patients had higher sTM levels than the healthy controls (41.56 ± 22.05 ng/ml vs 11.35 ± 4.7 ng/ml) ($p < 0.005$). Increased values were also found in patients with PV (25.96 ± 13.6 ng/ml) and secondary thrombocytosis (41.2 ± 18.8 ng/ml), but there was no significant difference between CMD patients and patients with secondary thrombocytosis (Table I). When the data were analyzed for subgroup of patients classified according to thrombotic complications, only patients with ET with thrombosis (53.65 ± 6.8 ng/ml) showed a significant difference in plasma levels of sTM compared to those without thrombosis (32.93 ± 5.2 ng/ml) (Figure 3).

Correlations

A positive correlation was found between platelet count, sP-selectin and sTM in all CMD patients ($r = 0.55$, $r = 0.44$, respectively).

There were no significant correlations between sP-Sel, sE-Sel and sTM.

DISCUSSION

The present study confirms the existence of endothelial and platelet activation in patients with ET and PV. Plasma levels of sP-sel, sE-sel and sTM were significantly elevated in all patients compared with the control group and there was a significant difference in relation with thrombotic complications.

Several studies have shown that patients with CMD have chronic activation of the coagulation system, such as a reduction of natural anticoagulants, an increase of plasma prothrombin fragment$_{1+2}$ and a hyperactivation of platelets [19,20,21]. However, these reports did not find any correlation between these abnormalities and the development of thrombosis.

We previously reported that the plasma sE-sel levels may reflect the activation of endothelial cells in CMD patients [22].

In this study, there were significant elevations of sE-sel in both ET patients and PV patients. We

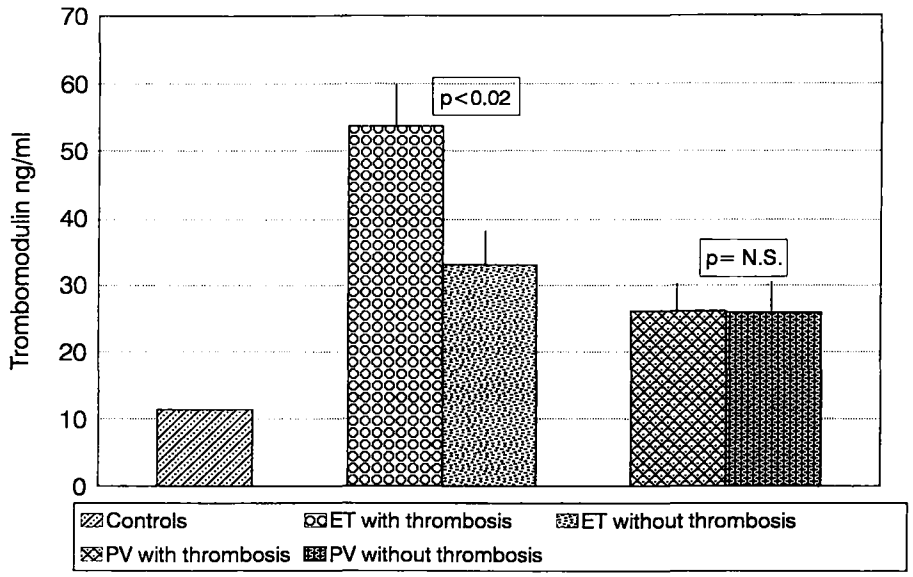

FIGURE 3 Plasma Thrombomodulin levels of ET and PV patients with and without thrombosis.

also found that the plasma concentration of sE-sel was further increased in CMD patients with thrombotic events.

Moreover, to assess the degree of endothelium damage, which might contribute to the development of thrombosis, plasma levels of sTM were also investigated.

Increased sTM concentration has been observed in disorders with vascular endothelium damage such as Wegener's granulomatosis, disseminated intravascular coagulation, collagen vascular diseases, diabetic microangiopathy [23,24,25]. Although there were elevated thrombomodulin levels in our patients, we did not find any correlation with the occurrence of thrombosis, except for the patients with ET. Moreover, levels of sTM did not correlate with plasma levels of sE-sel. The mechanism responsible for the production of circulating TM is different from that responsible for the production of soluble E-sel and soluble TM does not seem directly related to endothelium activation. Therefore, we suggest that sTM may reflect only the degree of endothelial damage, whereas sE-sel may be a specific marker of endothelial cell injury and activation. In addition, the increased plasma P-selectin levels in our patients also indicate likely in vivo activation/damage of platelets and endothelial cells. Elevated levels of sP-sel have been found in various diseases, characterized by damage of endothelial cells and thrombus formation [6,7,10]. In these disease states higher plasma levels of sP-sel might contribute to determine the overt thrombotic event.

In conclusion, our data suggest that excess platelet activity with different pattern of endothelial activation or damage seem to be associated with a greater risk of thrombosis and may play a role in the vascular complications of CMD.

However, follow-up studies are needed to verify the possible role of these molecules as prognostic markers of thrombotic risk.

References

[1] Schaefer, A. (1984). Bleeding and thrombosis in the myeloproliferative disorders. *Blood*, **64**, 1–12.

[2] Barbui, C., Cortelazzo, S., Viero, P., Bassan, R., Dini, E. and Semeraro, N. (1983). Thrombohemorragic complications in 101 cases of myeloproloferative disorders: relationship to platelet number and function. *Eur J Cancer Clin Oncol.*, **19**, 1593–99.

[3] Bellucci, S., Janvier, M., Tobelem, G., Flandrin, G., Charpak, Y., Berger, R. and Boiron, M. (1986). Essential thrombocytemias. Clinical evolutionary and biological data. *Cancer*, **58**, 2440–7.

[4] Landolfi, R., Rocca, B. and Patrono, C. (1995). Bleending and thrombosis in myeloproliferative disorders: mechanism and treatment. *Critical Reviews Oncol Haematol.*, **20**, 203–22.

[5] Regev, A., Stark, P., Blickstein, D. and Lahav, M. (1997). Thrombotic complications in essential thrombocythemia with relatively low platelet counts. *Am J Hematol.*, **56**, 168–72.

[6] Chong, B. H., Murray, B., Berndt, M. C., Dunlop, L. C., Brighton, T. and Chesterman, C. N. (1994). Plasma P-selectin is increased in thrombotic consumptive platelet disorders. *Blood*, **83**, 1535–41.

[7] Katayama, H., Handa, M. and Araki, Y. (1993). Soluble P selectin is present in normal circulation and its plasma levels is elevated in patients with thrombotic thrombocytopenic purpura and haemolitic uremic syndrome. *Br J Haematol*, **84**, 702–10.

[8] Blann, Ad, Amiral, J. and Mc Collum, Cn. (1996). Endothelial cell markers and the prediction of cardiovascular events. *Br J Haematol*, **93**, A692.

[9] Jilma, B., Fasching, P., Ruthuer, C., Rumplmayr, A., Ruzicka, S., Kapiotis, S., Wagner, O. F. and Eichler, H. G. (1996). Elevated circulating P-selectin in insulin dependent diabetes mellitus. *Thromb Haemost*, **76**, 328–32.

[10] Blann, A. D., Faragher, E. B. and Mc Collum, C. N. (1997). Increased soluble p-selectin following myocardial infarction: a new marker for the progression of atherosclerosis. *Blood coagulation and Fibrinolysis*, **8**, 383–90.

[11] Gearing, A. J. H., Haemingway, I., Piggot, R., Hughes, J., Rees, A. J. and Cashman, S. J. (1992). Soluble forms of vascular adhesion molecules E-selectin, ICAM-1 and VCAM-1: pathological significance. *ANN NY Acad Sci*, **667**, 324–31.

[12] Blann, A. D., Tse, W., Maxwell, S. R. J. and Waite, M. A. (1994). Increased levels of the soluble adhesion molecule E-selectin in essential hypertension. *j Hypert*, **12**, 925–8.

[13] Morishita, E., Masanari, S. and Asakura, H. (1992). Increased levels of plasma thrombomodulin in chronic myelogenous leukemia. *Am J Hematol*, **39**, 183–7.

[14] Wada, H., Ohiwa, M., Kaneko, T., Tamaki, S., Tanigawa, M., Shirakawa, S., Koyama, M., Hayeshi, T. and Suzuki, K. (1992). Plasma thrombomodulin as a marker of vascular disorders in thrombotic thrombocytopenic purpura and disseminated intravascular coagulation. *Am J Hematol*, **39**, 20–4.

[15] Ohdama, S. and Aoki, N. (1997). Increase of plasma thrombomodulin in systemic vasculitis. *Thromb Haemost*, **77**, 609.

[16] Ishii, H. and Majerua, P. W. (1985). Thrombomodulin is present in human plasma and urine. *J Clin Invest*, **76**, 2178–84.

[17] Ishii, H., Uchiyama, H. and Kazama, M. (1991). Soluble thrombomodulin antigen in conditionated medium is increased by damage of endothelial cells. *Thromb Haemost*, **65**, 618–23.

[18] Berlin, N. (1975). Diagnosis and classification of the polycythemias. *Semin Hematol.*, **12**, 339–51.

[19] Bucalossi, A., Marotta, G., Bigazzi, G., Galieni, P. and Dispensa, E. (1996). Reduction of antithrombin III., protein C and protein S levels and activated protein C resistance in polycythemia vera and essential thrombocythemia patients with thrombosis. *Am J Hematol.*, **52**, 14–20.

[20] Kornberg, A., Rahimi-Levene, N., Yoma, R., Mor, A. and Rachmilewitz, E. A. (1997). Enhanced generation of monocyte tissue factor and increased plasma prothrombin fragment$_{1+2}$ levels in patients with polycythemia vera: mechanism of activation of blood coagulation. *Am J Hematol.*, **56**, 5–11.

[21] Bellucci, S., Ignatova, E., Jaillet, N. and Boffa, M. C. (1993). Platelet hyperactivation in patients with essential thrombocythemia is not associated with vascular endothelial cell damage as judged by the level of plasma thrombomodulin, protein S., PAI-1, t-PA and vWF. *Thromb Haemost.*, **70**, 736–42.

[22] Musolino, C., Alonci, A., Allegra, A., Spatari, G., Bellomo, G., Tringali, O., Quartarone, C., Squadrito, G. and Quartarone, M. (1998). Increased levels of soluble adhesion molecule E-selectin in patients with chronic myeloproliferative disorders and thromboembolic complications. *Am J Hematol.*, **57**, 109–12.

[23] Boeme, M. W., Schmitt, W. H., Youinou, P., Stremmel, W. R. and Gross, W. L. (1996). Clinical relevance of elevated serum thrombomodulin and soluble E-selectin in patients with Wegener's granulomatosis and other systemic vasculitides. *Am J Med.*, **101**, 387–94.

[24] Wada, H., Ohiwa, M. and Kaneko, T. (1992). Plasma thrombomodulin as a marker of vascular disorders in thrombotic thrombocytopenic purpura and disseminated intravascular coagulation. *Am J Hematol.*, **39**, 20–4.

[25] Tamaka, A., Ishii, H., Hiraishi, S., Kamaja, M. and Meezawa, H. (1991). Increased thrombomodulin values in plasma of diabetic men with microangiopathy. *Clin Chem.*, **37**, 269–72.