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Soluble Plasma Thrombomodulin Levels in Patients with Chronic Myeloproliferative Disorder

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Summary: The plasma levels of soluble thrombomodulin (TM) were measured in 44 patients with chronic myeloproliferative disorder, 15 with polycythemia vera (PV), 29 with essential thrombocythemia (ET), and a group of 62 matched healthy controls. The younger patients had significantly lower TM levels (mean: 15.6 ± 4.8 ng/mL) than the older patients (mean: 28.6 ± 8.2 ng/mL, $p < .001$). Moreover, a significant negative correlation between platelet counts and plasma TM levels in healthy persons was noted ($r = 0.317$, $p < .05$). The only significant difference we found in plasma TM levels between patients and controls or among patients was between the young patients with ET (mean: 29.0 ± 19.2 ng/mL) and young healthy controls (mean: 15.6 ± 4.8 ng/mL). It is possible that younger ET patients with more active platelets are more susceptible to earlier vascular damage. The lack of any significant difference compared with the older patient population supports this hypothesis. **Key Words:** Thrombomodulin—Essential thrombocythemia—Polycythemia vera.

Thrombomodulin (TM) is an integral membrane protein expressed on the surface of vascular endothelial cells and endowed with a potent anticoagulant activity characterized by the inactivation of thrombin and, concomitantly, the activation of protein C (1,2). Soluble forms of TM have been found in blood and urine and appear to be derived from injured endothelial cells or are proteolytically cleaved from TM by proteases (3,4). Elevated plasma TM levels are a marker of endothelial cell damage as shown in diabetic microangiopathy, thrombotic thrombocytopenic purpura, or systemic vasculitis (5–10).

Although previous studies showed that essential thrombocythemia (ET) is not associated with vascular endothelial cell damage as judged by the levels of plasma TM (6,11), it recently has been suggested by van Genderen et al. (12) that an increased platelet count may lead to a rise of plasma TM levels. In the present study we evaluated TM plasma levels in 44 patients with chronic myeloproliferative disorder and in a group of matched healthy controls.

PATIENTS AND METHODS

We studied 62 normal controls (30 males, mean age 48.0 ± 20 years, range 21–85, mean platelets count 200 ± 55.9 × 10^9/L; 32 females mean age 45.0 ± 18.9 years, range: 20–87, mean platelets count 247 ± 66.8 × 10^9/L) and 44 patients (21 males, mean age 63.7 ± 13.5 years, range 41–84; 23 females, mean age 53.8 ± 18.3, range 20–83) with chronic myeloproliferative disease. Fifteen had polycythemia vera (PV) and 29 essential thrombocythemia (ET). The diagnosis was established in agreement with the polycythemia study group criteria (13,14). At the time of study the patients were in stable clinical states, none had thrombotic or hemorrhagic episodes or chronic renal or hepatic insufficiency. This is important because TM is cleared mainly by the kidney and liver (4,15). Some patients were undergoing antiplatelet therapy and/or were given cytotoxic medication, and 9 of them with PV had normal platelet counts. However, none of the patients were given heparin because it seems to reduce the soluble plasma TM levels (16). The characteristics of patients are shown in Table 1.

The healthy controls and patients, who were studied in a fasting state, were informed about the study and gave written consent.

Blood samples were obtained from an antecubital vein without the use of tourniquets and collected through a butterfly catheter (21 × ¾-inch tubing, LKDS, Milan, Italy) into a polypropylene syringe (Terumo, Leuven, Belgium) containing 3.8% sodium citrate (dilution 1:10). The blood was transferred into plastic tubes and centrifuged at 3,000 × g at 4°C (Varifuge RF, Heraeus, Hanau, Germany). The platelet-poor plasma samples were stored at −70°C until assayed.

Soluble TM was measured by a commercial 2-site

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TABLE 1. *Characteristics of patients with PV, ET, and controls**

	Controls			PV			ET		
	Total	Males	Females	Total	Males	Females	Total	Males	Females
Number	62	30	32	15	9	6	29	12	17
<45 years	21	10	11	–	–	–	10	2	8
>45 years	41	20	21	15	9	6	19	10	9
Platelets × 10^9/L ± SD	224 ± 65	200 ± 55	247 ± 66	446 ± 247	455 ± 259	432 ± 251	800 ± 318	718 ± 295	858 ± 329
Patients with NISAIDs**	–	–	–	8	5	3	18	8	10
Patients with vascular diseases†	–	–	–	7	4	3	8	3	5

* Nine patients with PV had platelet counts within normal limits.
** NSAIDs, nonsteroidal anti-inflammatory drugs.
† Patients with thromboembolic accidents (cerebrovascular accidents, deep vein thrombosis, coronary artery disease, erythromelalgia).

enzyme-linked immunosorbent assay (ELISA) using two mouse monoclonal antihuman antibodies reacting with two distinct and remote sites of the TM molecule (Asserachrom™ Thrombomodulin Enzyme Immunoassay, Diagnostica Stago Ltd., Asnières sur Seine, France) (17). A solid support (micro-ELISA plate) is coated with a specific mouse monoclonal antibody reacting with a TM epitope close to the fourth EGF-like structure. The bound TM was next revealed by the use of a second mouse anti-TM monoclonal antibody reacting with an epitope that is present on the amino-terminal lecitin-like and labeled with horseradish peroxidase. The bound enzymatic activity was demonstrated by this oxidative action on the substrate orthophenylenediamine in the presence of urea peroxide. This assay can be used to detect the various forms of soluble plasma TM (17). Plasma TM level was expressed as ng/mL.

STATISTICAL ANALYSIS

The results were calculated as a mean ± standard deviation (SD) of the mean. The analysis of differences was

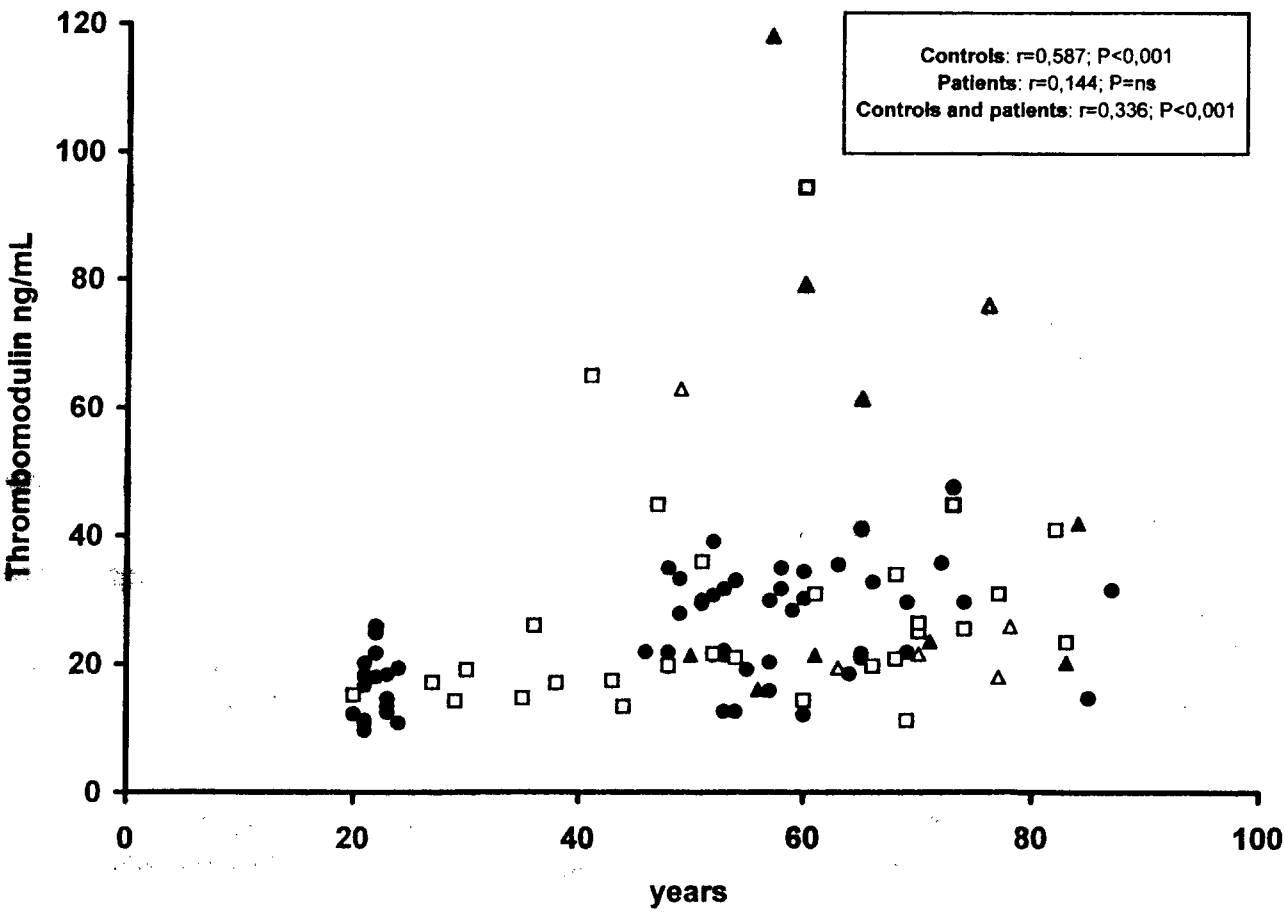

FIG. 1. Correlation between plasma thrombomodulin in ng/mL and age (years) in patients and controls considered separately or together. ●, controls; △, PW; □, ET; ▲, PV with normal PLTs.

performed by the Student's t test. A p value <.05 was taken to be significant. Correlation coefficients were determined by Person test and from linear correlation analysis.

RESULTS

In agreement with Loreth et al. (18), we found a significant correlation between age (years) and TM plasma level in healthy persons ($r = 0.587$, $p < .001$) (Fig. 1). Moreover, the young controls with a mean age of 21.9 ± 1.14 had a significantly lower plasma TM level than the control group with a mean age of 59.0 ± 9.6 (young: TM mean level 15.6 ± 4.8 ng/mL; older: 28.6 ± 8.2 ng/mL; $p < .001$). Although as shown by Amiral et al. (17) and Loreth et al. (18), females had a lower TM plasma level than males, no significant difference was noted.

Our patients were matched with controls for age and sex. The cut-off limit between young and older subjects was 45 years of age because the majority of our patients were >45 years. Moreover, among the Loreth healthy persons up to 50 years of age, plasma TM levels appeared to be similar (18). We found a significant difference in TM plasma levels between the young patients with ET and the young healthy controls (patients: mean TM 29.0 ± 19.2 ng/mL; controls: 15.6 ± 4.8 ng/mL, $p < .027$). In contrast, any significant difference between patients and normal controls with an age >45 years or between young and older patients was noted (Fig. 2). Although limited in size because of the number of patients, we could not find any significant difference of TM plasma levels between ET and PV or between patients that at the time of study had platelet counts within normal limits and those with high platelet counts (Fig. 2).

No correlation was found between platelet counts and plasma levels of TM when all patients were considered together ($r = 0.185$; p = NS) (Fig. 3). In contrast, a significant negative correlation was present in normal controls ($r = 0.317$, $p < .05$) (Fig. 3).

DISCUSSION

Plasma thrombomodulin (TM) has been considered a good marker of endothelial cell injury (19). High TM plasma levels had been found in pathologic conditions associated with vascular damage such as in systemic vasculitis or in diabetes with microangiopathy (5–10). Recently, in contrast to Wada et al. (6) and Bellucci et al. (11), van Genderen et al. (12) found high plasma TM levels in patients with essential thrombocythemia (ET) and suggested that an increased platelet count may lead to a rise of plasma TM because the patients, who suffered

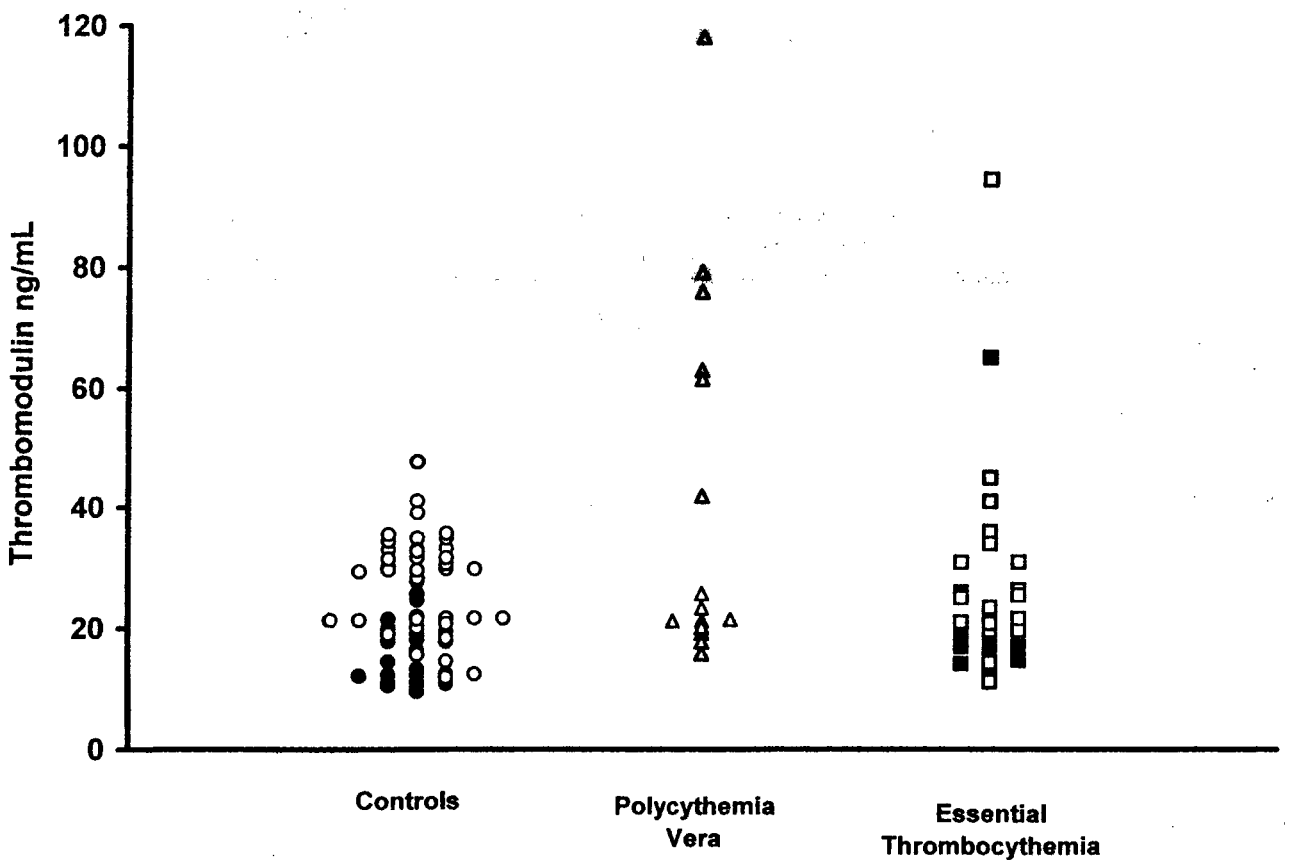

FIG. 2. Plasma thrombomodulin levels in ng/mL in patients and controls. Dots and black squares represent subjects <45 years.

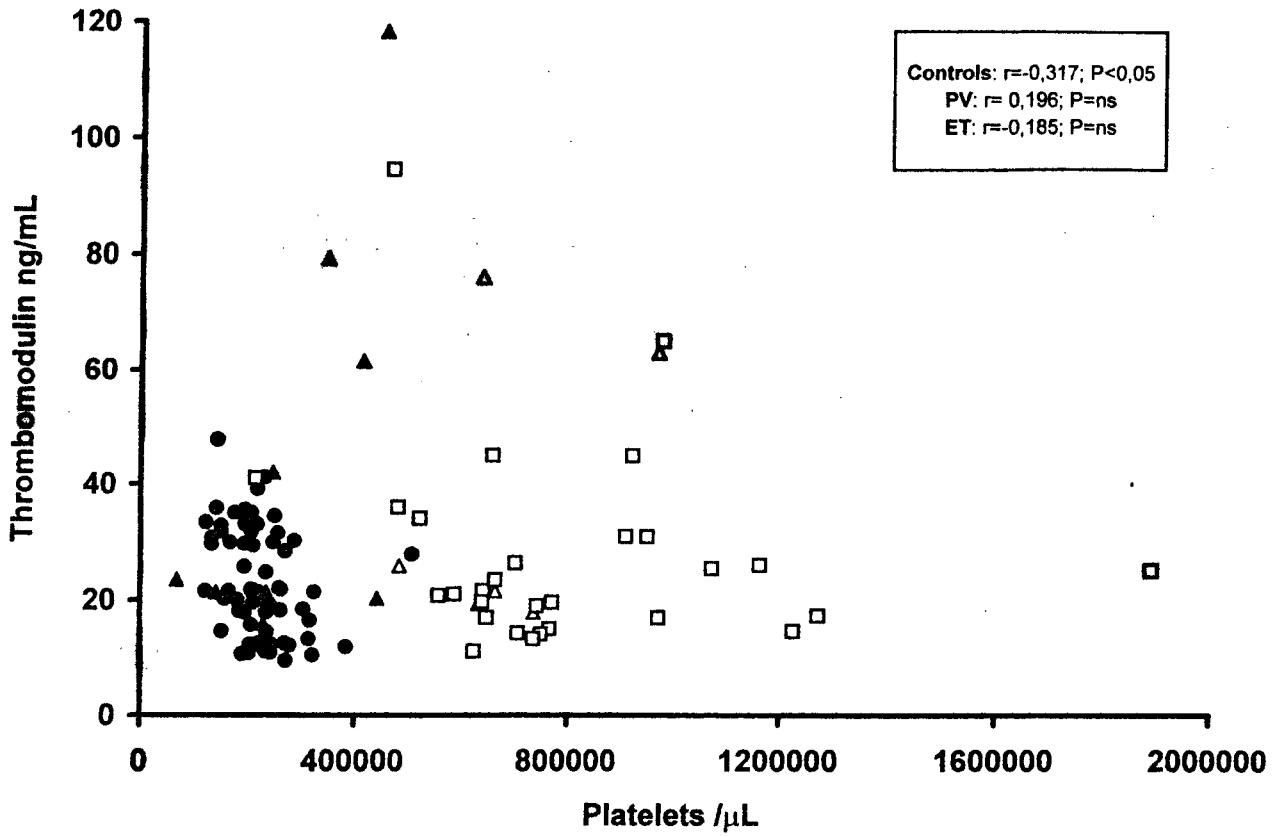

FIG. 3. Correlation between plasma thrombomodulin in ng/mL and platelet counts in patients and controls. ●, controls; △, PV; □, ET; ▲, PV with normal PLTs.

for erythromelalgia, did not reach a normal plasma TM range despite the disappearance of signs and symptoms resulting from aspirin treatment.

We studied 44 patients with chronic myeloproliferative disorder and the majority of them had an elevated platelet count. Plasma TM levels in patients >45 years were similar to that of healthy persons matched for age and sex. In addition, we found, in agreement with Loreth et al. (18), a positive correlation in the control group between age and TM. Patients who showed a platelet count within normal limits at the time of study did not have a significant variation of plasma TM levels when compared with patients with high platelet counts or with the control group. Even considering patients taking antithrombotic therapy or the different pathology of chronic myeloproliferative disorder, no significant differences with the control group were noted.

Although plasma TM concentration was similar and not statistically different between patients ±45 years, the young group with ET showed a significant increase as compared with the young healthy controls. Although thrombotic accidents seem to be less frequent in young than in older patients with ET, young age per se is not a favorable prognostic factor in this acquired condition (20–22). It is possible that younger patients with more active platelets are more susceptible to vascular damage earlier as judged by the increased level of TM. The failure to show any significant difference with the older patient and control population further supports this hypothesis.

It is interesting to note that in the control group we found a negative correlation between platelet count and TM. The lower platelet count could represent an increased platelet turnover with vascular involvement and plasma TM could be the expression of an endothelial cell damage.

The fact that among all patients no correlation was found between platelet counts and plasma TM further supports this hypothesis since a variety of qualitative platelet abnormalities have been described in chronic myeloproliferative disorder (23).

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