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Patients with thrombocytosis have normal or slightly elevated thrombopoietin levels

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ABSTRACT

***Background and Objective*. The distinction between clonal and reactive thrombocytoses is a frequent problem and implies different therapeutic options. As thrombopoietin (TPO) is the main regulator of megakaryocytopoiesis and thrombopoiesis, we measured TPO levels in patients with thrombocytosis in an attempt to understand the regulation and potential utility of distinguishing thrombocytoses.**

***Design and Methods.* Serum TPO levels, platelet counts, mean platelet volume, hemoglobin, erythrocyte sedimentation rate and age were evaluated in 25 patients with clonal thrombocytosis (15 with essential thrombocythemia, 6 with polycythemia vera and 4 with chronic myeloid leukemia) and in 50 patients with reactive thrombocytosis distributed in three groups: 1) patients in post-surgical states; 2) patients with solid tumors; and 3) patients with inflammatory diseases.**

***Results.* TPO levels were slightly increased in patients with clonal (135±50 pg/mL) and reactive (147±58 pg/mL) thrombocytosis compared with controls (121±58 pg/mL). Analyzing the different groups, patients with essential thrombocythemia had the lowest TPO levels (120±28 pg/mL) and patients with solid tumors the highest levels (162± 59 pg/mL). Patients with clonal thrombocytosis were older, had higher platelet counts, mean platelet volume and hemoglobin, and lower erythrocyte sedimentation rate than patients with reactive thrombocytosis.**

***Interpretation and Conclusions.* Minor differences were observed in TPO levels between patients with primary and secondary thrombocytoses. Erythrocyte sedimentation rate, but not TPO levels, may be a useful tool for discriminating both types of thrombocytoses.**

Key words: clonal thrombocytosis, reactive thrombocytosis, essential thrombocythemia, thrombopoietin

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A raised platelet count is usually a reflection of increased megakaryocyte and platelet production. It is thus important to determine whether or not it is associated with a myeloproliferative disorder. Thrombocytosis may appear in clonal myeloproliferative diseases such as chronic myeloid leukemia (CML), polycythemia vera (PV), agnogenic myeloid metaplasia and essential thrombocythemia (ET), a disorder with very high platelet counts frequently associated with an elevated prevalence of hemorrhagic and thrombotic complications.[1] Thrombocytosis is, however, more commonly associated with solid tumors, post-surgical, chronic inflammatory or iron deficiency states, and usually lacks clinical complications.[2] In these cases it is termed secondary or reactive thrombocytosis, in opposition to primary or clonal thrombocytosis which is associated with myeloproliferative disorders.

Different therapeutic options may be selected depending on the origin of the thrombocytosis.[3] It therefore seems essential to differentiate the exact cause of thrombocytosis. To date, there is no definitive diagnostic test to distinguish primary from secondary thrombocytosis, nor to discriminate among primary thrombocytoses.[4] Therefore, complete blood counts (platelet count, hemoglobin level and platelet distribution width), an abdominal ultrasound or scan, bone marrow examination (megakaryocyte evaluation, collagen fibrosis, iron), karyotype, erythrocyte sedimentation rate (ESR), fibrinogen and interleukin-6 levels, unstimulated growth of BFU-E and/or CFU-Meg or even the presence of fever may be required to correctly diagnose a thrombocytosis.[5-7] This wide variety of parameters led to the definition of diagnostic criteria for diseases such as ET, as reported by the *Polycythemia Vera Study Group*.[8]

Thrombopoietin (TPO) is a recently identified hematopoietic growth factor considered to be the main regulator of megakaryocyte proliferation and maturation and, subsequently, of platelet production.[9] TPO is constitutively produced in liver but also, to a lesser degree, in other organs.[10] It is cleared through a binding mechanism on the surface of megakaryocytes[11] and platelets.[12] Previous studies in thrombocytopenic patients suggest that megakaryocyte mass is more relevant than platelet mass in the

maintenance of circulating TPO levels; diseases with a lower megakaryocyte mass, such as aplastic anemia, therefore, exhibit high TPO levels.[13] The exact mechanisms regulating TPO levels in thrombocytosis are not, however, yet clear.[14] To investigate this and to evaluate whether TPO is a putatively useful parameter for discriminating thrombocytoses, we examined the circulating serum TPO concentrations in different groups of patients with primary or secondary thrombocytosis.

Design and Methods

Patients

We studied 75 patients with a platelet count higher than 600×10^9/L. Of them, 25 patients had myeloproliferative disorders (15 ET, 6 PV and 4 Ph1-positive CML). Diagnoses had previously been established according to the *Polycythemia Vera Study Group* criteria. There were 15 males and 10 females, with a median age of 62 years, the range being from 17 to 89 years. The other group consisted of 50 patients (33 males and 17 females; median age of 49 years) with reactive thrombocytoses. These patients were distributed in three subgroups: 1) thrombocytosis detected in post-surgical periods (n=15); 2) thrombocytosis accompanying solid tumors (n=20); and 3) thrombocytosis associated with inflammatory diseases (n=15). Patients who could be placed in more than one group were not included. The parameters analyzed in all patients were: serum TPO levels, platelet count, mean platelet volume (MPV), hemoglobin, ESR, age and sex. A group of 43 healthy subjects, with normal platelet counts, was used as a control.

TPO measurements

Peripheral blood samples were collected and allowed to clot before centrifugation at 1500 rpm for 15 min. The sera were separated, aliquoted and stored at –80ºC until assays were performed. Circulating serum TPO levels were quantified by an enzyme-linked immunosorbant assay (ELISA)(Quantikine™ Human TPO Immunoassay, R&D Systems, Minneapolis, MN, USA). Briefly, the samples and the recombinant human TPO used as standard were placed per duplicate into a 96 well microplate coated with a murine monoclonal antibody against TPO. After incubating for 3 h, a monoclonal antibody against TPO conjugated to horseradish peroxidase was added. A tetramethylbenzidine/hydrogen peroxide solution was finally required to develop color. Absorbances were measured at 450 nm. The minimum detectable dose of the assay was less than 15 pg/mL.

Statistics

Results are reported as the mean ± one standard deviation. The one-way analysis of variance was used to evaluate differences in serum TPO levels between groups. The correlation between two variables was determined using Spearman's rank correlation test. *p* values <0.05 were considered statistically significant.

Results

TPO levels

Circulating serum TPO levels were slightly increased in patients with primary or clonal thrombocytosis (135±50 pg/mL) and with secondary or reactive thrombocytosis (149±58 pg/mL) compared with normal subjects (121±58 pg/mL)(Figure 1); however, no statistical significance was found.

Examining the group with primary thrombocytosis, we found that TPO levels in patients with ET (120±28 pg/mL) did not differ from those of healthy subjects. We found no significant differences in TPO levels between ET patients receiving or not receiving cytoreductive treatment (data not shown). Patients with CML and PV had higher TPO concentrations (157±68 pg/mL), but this did not reach statistical significance. Among patients with secondary thrombocytosis, those with solid tumors (161±59 pg/mL) and inflammatory diseases (158±46 pg/mL) tended to have higher TPO levels than post-surgical patients (141±55 pg/mL) (Figure 2).

Searching for differences in TPO levels between ET and the other groups, we found that serum TPO concentrations were significantly lower in patients with

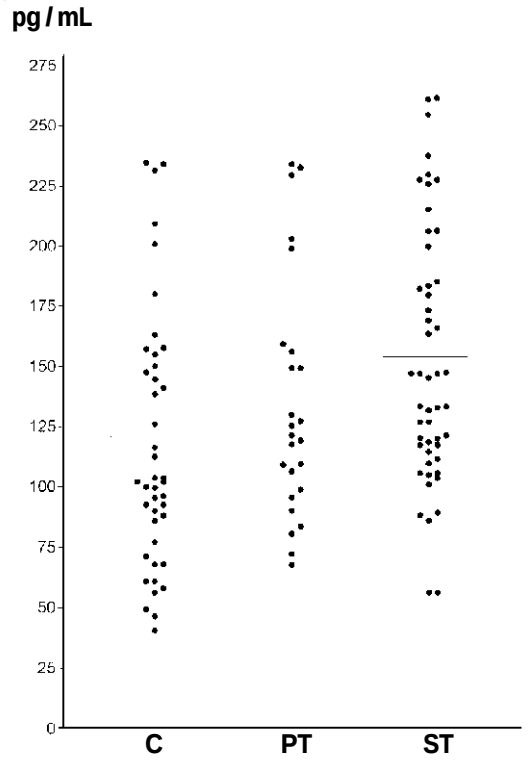

Figure 1. Serum TPO levels in healthy controls (C), patients with primary thrombocytosis (PT) and secondary thrombocytosis (ST). Horizontal lines represent mean values.

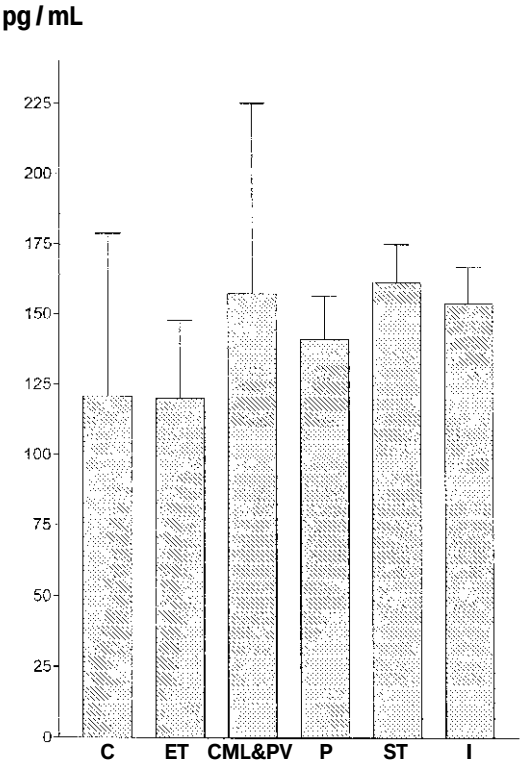

Figure 2. Mean TPO levels and standard deviation (bars) in 43 controls (C), 15 patients with essential thrombocythemia (ET), 10 with chronic myeloid leukemia (CML) and polycythemia vera (PV), 15 with thrombocytosis in a post-surgical state (P), 20 with thrombocytosis and solid tumors (ST) and 15 with thrombocytosis associated with inflammatory diseases (I).

ET than in patients with thrombocytosis associated with malignant (p=0.01) or inflammatory diseases (p= 0.04), or in the whole group of secondary thrombocytoses (p=0.02). However, no differences were observed on comparison with patients who had undergone surgery.

Table 1. Parameters evaluated in patients with primary and secondary thrombocytoses.

	ET	*CML & PV*	*P*	*ST*	*I*
Plt (x 10^9/L)	902±243	762±139	685±84	728±141	704±107
MPV (fL)	8.1±0.6	8.4±0.6	7.7±0.4	8.1±0.6	7.6±0.8
Hb (g/L)	130±19	132±29	107±16	100±15	104±15
ESR (mm/h)	6±5	2±1	60±36	85±43	72±40
Age (years)	63±21	60±19	44±22	62±12	52±25

Abbreviations: ET, essential thrombocythemia; CML, chronic myeloid leukemia; PV, polycythemia vera; P, post-surgical state; ST, solid tumours; I, inflammatory diseases; Plt, platelets; MPV, mean platelet volume; Hb, hemoglobin; ESR, erythrocyte sedimentation rate.

Evaluation of the parameters analyzed in the different groups

Comparing patients with clonal and reactive thrombocytoses, the former had significantly higher platelet counts (846±216×10^9/L) (p <0.001), MPV (8.2±0.6 fL) (p = 0.01), and hemoglobin (131±23 g/L) (p < 0.01), were significantly older (62±20 years) (p = 0.04), but had a lower ESR (5±5 mm/h) (p < 0.01). Differences in ESR seemed to be the most striking. We also found that patients with ET had higher platelet counts (p = 0.001) and hemoglobin (p < 0.001), and lower ESR (p < 0.001) than patients with secondary thrombocytosis (Table 1). None of these parameters was, however, useful in distinguishing between patients with ET and other myeloproliferative disorders (Table 1).

Correlation studies

Analyzing the groups of patients with primary and secondary thrombocytoses, no positive or negative correlations were detected between TPO levels and the following parameters: age, platelet counts, mean platelet volume, hemoglobin and ESR.

Discussion

Thrombocytoses can be divided into two main groups according to their etiology: primary or clonal thrombocytosis, which is present in myeloproliferative disorders, and secondary or reactive thrombocytosis, which may be a manifestation of many different non-clonal disorders. There is, however, no specific diagnostic test to differentiate these. The ultimate origin of both types of thrombocytosis is, to date, not well understood. In order to assess the value of TPO, the principal cytokine regulating platelet production, in distinguishing and clarifying the origin of thrombocytoses, we measured circulating serum TPO levels in patients with primary and secondary thrombocytoses.

We found that TPO levels in patients with thrombocytosis were, in general, slightly increased when compared with those obtained from healthy individuals. Previous reports have demonstrated that TPO production is constitutive[15,16] and that circulating TPO concentration is regulated via binding to the c-Mpl receptor, located on the surface of megakaryocytes[11] and platelets.[12] Thrombocytopenic patients with a low bone marrow megakaryocyte mass have high TPO levels, i.e. aplastic anemia, while patients with an increased megakaryocyte mass, i.e. idiopathic thrombocytopenic purpura, had normal or slightly raised TPO levels.[13,17,18] Following this model, TPO levels should be clearly reduced in both types of thrombocytosis due to the presence of large numbers of megakaryocytes and platelets. However, our results showed normal or slightly raised serum TPO levels.

Analyzing the results of the group with primary thrombocytosis, we observed that patients with ET had the highest mean platelet count, probably as a

consequence of a larger megakaryocytic mass, and serum TPO levels were similar to those in healthy controls. Knowing that there are no relevant mutations in the c-Mpl receptor gene in ET,[19] if the megakaryocyte and platelet TPO binding mechanism is valid, we would expect very low TPO levels. However, as the expression of c-Mpl receptor is markedly reduced in ET,[20,21] there seems to be an impaired uptake and catabolism of TPO and circulating TPO levels were, therefore, normal. Serum TPO levels in patients with clonal thrombocytosis (CML and PV), were similar to or slightly higher than those in ET patients. A recent study also demonstrated a markedly reduced or absent expression of the TPO receptor on platelets of patients with PV and idiopathic myelofibrosis.[22] According to this model, the lower platelet and/or megakaryocytic mass in patients with CML and PV would be responsible for the higher TPO levels found.

Reactive thrombocytosis is often a transitory finding associated with numerous conditions.[2] We examined three groups of patients with thrombocytosis secondary to neoplastic processes, inflammatory conditions and post-surgical states. They had significantly lower platelet counts, MPV, and hemoglobin and were significantly younger, but had a higher ESR than patients with clonal thrombocytosis. Serum TPO levels in the three groups analyzed were slightly increased compared to those of normal controls. As the conditions associated with reactive thrombocytosis are different from those associated with clonal myeloproliferative diseases, so might the origin of the thrombocytosis be different. In this sense, other cytokines involved in megakaryocytopoiesis such as interleukin-6[5,6,23] are increased in most patients with reactive thrombocytosis. Although further studies are needed in secondary thrombocytosis concerning the expression of the TPO receptor or the production of TPO mRNA, we speculate on possible explanations for the serum TPO levels observed. The first hypothesis could be that there is a reduced expression of the c-Mpl receptor as in ET patients. The second, more likely, was recently suggested by Cerutti *et al*.[24] as they observed that TPO behaves like an acute phase reactant peaking on the third day in post-surgery reactive conditions. Our results confirm their previous observation that ESR is a good marker for discriminating thrombocytoses and that no overlap was detected between clonal and reactive thrombocytoses. We did not find any correlation between ESR and TPO levels, but reactive thrombocytosis was associated with a high ESR and also high TPO levels.

As normal or slightly increased circulating serum TPO levels were detected in patients with both primary and reactive thrombocytosis, we consider that TPO levels are not a good marker for distinguishing thrombocytoses. Our results differ from a previous report[25] but are in concordance with another.[14] Despite the finding of similar TPO levels in both types of thrombocytosis, we think that the origin of the thrombocytoses may differ and other cytokines could be responsible for the high platelet counts in reactive thrombocytosis. Serial measurements of TPO concentrations and evaluations of the expression of TPO receptors on platelets and megakaryocytes should be performed to determine the real origin of the slightly raised TPO levels in secondary thrombocytosis.

Contributions and Acknowledgments

IE formulated the design of the study and was responsible for data analysis and writing the article. AH helped to perform all the ELISA tests. MC provided the serum samples. JM helped with the figures. NPM contributed to the data analysis and corrected the paper. All authors contributed to the interpretation of the results.

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Disclosures

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