Is the Thrombopoietin Assay Useful for Differential Diagnosis of Thrombocytopenia? Analysis of a Cohort of 160 Patients with Thrombocytopenia and Defined Platelet Life Span

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Background: Thrombopoietin (TPO), the major hormone controlling platelet production, has been measured in thrombocytopenias with discordant results. The aim of our work was to assess the value of the TPO assay for differential diagnosis of thrombocytopenias in a large cohort of patients classified according to the results of their platelet isotopic study.

Methods: We measured TPO (R&D Systems) in serum of 160 thrombocytopenic patients referred to our department for platelet life span isotopic studies. We classified patients as follows: (a) idiopathic or autoimmune thrombocytopenia group (ITP; patients with increased platelet destruction and shortened platelet life span; n = 67); (b) pure genetic thrombocytopenia group (patients with decreased platelet production, normal platelet life span, and without bone marrow aplasia; n = 55); (c) bone marrow aplasia group (BM; patients with decreased platelet production, normal platelet life span, and bone marrow aplasia; n = 13).

Results: In patients with pure genetic thrombocytopenia, TPO (median, 55 ng/L) was not different from TPO in patients with ITP (median, 58 ng/L) or controls (n = 54; median, 51 ng/L). Only in patients with bone marrow aplasia was TPO significantly higher (median, 155 ng/L) and negatively correlated to the platelet count (r² = 0.5014).

Conclusions: Although the median serum TPO is increased in thrombocytopenia with decreased platelet production from bone marrow aplasia, it does not differentiate patients with pure genetic thrombocytopenia from those with ITP.

Thrombopoietin (TPO)3 is the major hormone controlling megakaryocyte development and platelet production. Independent research groups identified it as the ligand of c-mpl protein expressed on the surface of megakaryocytes (1–4). This receptor is the cellular homolog of the viral oncogene v-mpl identified in murine myeloproliferative leukemia.

Mature human TPO, a 332-amino acid polypeptide with a predicted molecular mass of 35 kDa, is highly glycosylated, with carbohydrates increasing the molecular mass of the protein. The N-terminal region has general structural homologies with erythropoietin (erythropoietin-like domain) and is sufficient to activate mpl (5). The C-terminal portion of TPO, which has no homology to any other known protein, is required for efficient secretion and glycosylation and increases the stability and potency of the molecule in vivo (5). It is generally accepted that regulation of circulating TPO occurs mainly through TPO binding to megakaryocyte mpl (5, 6).

Thrombocytopenias are schematically classified according to two criteria: increased platelet destruction or decreased platelet production (Table 1) (7). Increased platelet destruction involves several mechanisms, including immunologic processes such as in autoimmune idiopathic thrombocytopenia (ITP), iso- or alloimmune thrombocytopenia; or nonimmunologic processes. Among the thrombocytopenias attributable to decreased platelet production are megakaryocyte hypoplasia; ineffective thrombopoiesis of various origins, such as chemotherapy or radiotherapy; and hereditary thrombocytopenias, in-

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1 Nonstandard abbreviations: TPO, thrombopoietin; ITP, idiopathic thrombocytopenia; and BM, bone marrow.
Alloimmune Platelet damage Hereditary

(8 patients with central thrombocytopenia)

Increased platelet destruction or decreased platelet production. Studies are performed, allowing identification of the mechanism of thrombocytopenia is unclear, isotopic life span studies are performed, allowing identification of decreased platelet production or increased platelet production.

In 1990, we defined a novel homogeneous group of patients with central thrombocytopenia (8,9), characterized by the following features: chronic thrombocytopenia, increased platelet volume or presence of macrocytic platelets, presence of megakaryocytes, normal autologous platelet life span, no evidence of splenic or hepatic destruction, and decreased platelet production. Although the mechanism of this thrombocytopenia has not been elucidated, an inherited trait with autosomal dominant transmission has been demonstrated in most cases. This now well-defined entity is called pure genetic thrombocytopenia.

The cloning of TPO has prompted the development of immunoassays, allowing the study of TPO concentrations in patients with either low or high platelet counts. To date, four different methods for detecting circulating TPO have been developed (10–13). Marsh et al. (10) used the combination of a monoclonal antibody against the ligand domain of TPO and a polyclonal antibody against full-length TPO. Meng et al. (11) used the combination of mpl coupled to immunoglobulin fragments and a polyclonal antibody against full-length recombinant TPO. Tahara et al. (12) measured TPO with a rabbit polyclonal antibody and a monoclonal antibody (TN1), both raised against recombinant TPO. The ELISA from R&D Systems uses two monoclonal antibodies raised against recombinant TPO (13). Table 2 summarizes TPO results reported in thrombocytopenia according to these four methods.

Table 2 summarizes TPO results reported in thrombocytopenia according to these four methods. It is worth noting that even with the same method, TPO values vary from one study to another. These discrepancies may be explained by small cohorts of heterogeneous thrombocytopenias, difficulties in clearly characterizing platelet disorders in some cases, and the different techniques used for TPO measurement. Different limits of detection for TPO have been reported, highlighting the various sensitivities of the assays. Reference to the quality of the sample (i.e., serum or plasma without residual platelets), which is an important point for the measurement of TPO, is not always available. Variations in TPO concentrations between EDTA-plasma and serum up to 50% have been reported (14).

In thrombocytopenias associated with megakaryocyte hypoplasia, such as in aplastic anemia or acute leukemias, high TPO concentrations have always been observed (6,10,11,15–22). On the other hand, in thrombocytopenia caused by increased platelet destruction, with bone marrow (BM) megakaryocytes present, discordant concentrations have been reported, the TPO concentration being either low or within the reference interval (6,20,23) or slightly higher but not significantly different from the concentration in healthy individuals and always lower than the concentrations found in aplastic anemia (17–20,24). No data about isotopic platelet life span were available in these studies.

In patients with essential thrombocytemia characterized by thrombocytosis associated with impaired mpl expression in both platelets and megakaryocytes, TPO was within the reference interval or increased (25–27), confirming that the TPO concentration is regulated mainly by mpl in megakaryocytes.

Taking advantage of a cohort of 160 thrombocytopenic patients explored by platelet life span studies, which allowed identification of decreased platelet production or increased platelet destruction, we measured TPO with a new commercially available technique based on the use of two monoclonal antibodies and, therefore, expected to be highly sensitive. The aim of this study, therefore, was to further analyze the usefulness of TPO measurements in the differential diagnosis of thrombocytopenia as a less invasive tool than isotopic life span studies.

Materials and Methods

Platelet Life Span

Isotopic platelet studies were conducted as described previously by labeling autologous platelets with indium-111 (9). Patients with a platelet life span ≥4 days, a high renewal rate (>25%), and increased platelet destruction (splenic or hepatic or both) were classified as ITP. Patients with a platelet life span ≥4 days, a low renewal rate (<25%), and no increased destruction were classified as...
having central thrombocytopenia attributable to decreased production. In this latter group, the patients with BM failure were classified into the BM aplasia group, and the other patients were classified into the pure genetic thrombocytopenia group. The production rate was calculated according to the formula: 
\[
\text{production rate} = \frac{\text{patient's platelet count}}{\text{patient's platelet life span}} \times \frac{\text{normal platelet count} \times 10^9/L}{\text{normal platelet life span (9 days)}}
\]
A decreased production rate was defined as a value <0.5.

**TPO ASSAY**

Serum TPO was determined in serum stored at −30 °C by a commercially available ELISA (Quantikine Human TPO Immunoassay; R&D Systems), according to the manufacturer's instructions. We chose to measure TPO in serum to avoid potential interference of residual platelets in the TPO assay.

Briefly, 200 μL of TPO calibrator, serum sample, or blank was added in duplicate to the wells of a microtiter plate precoated with an anti-TPO monoclonal antibody raised against purified recombinant TPO. The TPO calibrator consisted of recombinant full-length human TPO. After the plate was incubated for 3 h at 4 °C and washed, 200 μL of horseradish peroxidase-conjugated anti-TPO antibody was added to each well and incubated for 1 h at 4 °C. The color was developed with tetramethylbenzidine as substrate. TPO concentrations >100 ng/L were checked on a different sample. This assay recognized recombinant and human TPO without cross-reactivity with up to 50 μg/L erythropoietin (13).

**PATIENTS**

A total of 160 consecutive thrombocytopenic patients referred to the Nuclear Medicine Department between 1994 and 1998 for an isotopic platelet study were included in the present study. Oral informed consent to participate to the study was obtained from all patients, who were classified into homogeneous and well-defined groups based on the results of the isotopic study (Table 3). Patients with thrombocytopenia of multiple origin (decreased production and increased destruction, hyper-

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### Table 2. TPO results in the literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Controls</th>
<th>CTP</th>
<th>ITP</th>
<th>BM aplasia</th>
<th>TPO assay used in reference</th>
</tr>
</thead>
</table>
| (10)      | Median = 78  
(n = 117) | Median = 102  
(n = 77) | Median = 234 (n = 5) | Median = 1419  
(n = 13) | Technique from Marsh et al. (10)  
(TPO range, 1–1000 ng/L) |
| (15)      | Mean = 95.3  
(n = 98) | Mean = 195  
(n = 29) | Mean = 345  
(n = 23) | Mean = 2308  
(n = 54) | |
| (17)      | Median = 102  
(n = 77) | Median = 234 (n = 5) | Median = 656  
(n = 29) | Median = 656  
(n = 29) | |
| (11)      | Median = 0.5  
(n = 20) | Mean = 1.68  
(n = 71) | Mean = 12.7  
(n = 10) | Median = 1370  
(n = 18) | Technique from Meng et al. (11)  
(TPO range, 160–10 000 ng/L) |
| (12)      | Mean = 0.79  
(n = 50) | Mean = 1.76  
(n = 12) | Mean = 12.35  
(n = 12) | Median = 1149  
(n = 19) | Technique from Tahara et al. (12)  
(TPO range, 0.045–1.72 pmol/L) |
| (18)      | Mean = 0.79  
(n = 29) | Mean = 1.7  
(n = 27) | Mean = 12.7  
(n = 10) | Median = 1514  
(n = 13) | Technique from R&D Systems (13)  
(TPO range, 35–2000 ng/L) |
| (19)      | Mean = 0.79  
(n = 49) | Mean = 1.87  
(n = 43) | Mean = 12.35  
(n = 12) | Median = 1067  
(n = 10) | |
| (20)      | Mean = 0.76  
(n = 21) | Mean = 258.5  
(n = 17) | Mean = 1067  
(n = 10) | Median = 0  
(n = 24) | |
| (22)      | Mean = 54.5  
(n = 10) | | | | |
| (23)      | Median = 100  
(n = 15) | Median = 55  
(n = 55) | Median = 57.5  
(n = 67) | Median = 155  
(n = 13) | |
| Present study | Median = 51  
(n = 54) | | | | |

* The median TPO concentration, when available, is reported as it is not influenced by the limit of detection; otherwise the mean TPO concentration is reported.

* CTP, chronic central thrombocytopenia without megakaryocytopenia; MK, megakaryocytes in bone marrow.

* Either aplastic anemia or cancer.
spleenism, or myelodysplastic syndromes), who presented with both decreased production rates and increased platelet destruction, were not included in this study (n = 25).

Increased splenic destruction was observed in 10 cases, increased hepatic destruction in 3 cases, and mixed (hepatic and splenic) destruction in 3 cases; in 9 cases no site of sequestration was seen.

The mean age (± SD) of the 135 remaining patients was 43 ± 17 years. Platelet counts were 9–149 × 10^9/L (mean, 68.1 ± 10^9/L; median, 76 ± 10^9/L). These 135 patients were divided into three groups whose characteristics are displayed in Table 3:

Group A consisted of 13 patients with BM aplasia (hypoplasia of megakaryocytes), attributable to malignant disease with impaired BM. In these patients, platelet counts were 30–109 × 10^9/L, and the mean (± SD) platelet life span was 5.33 ± 2.64 days; a decreased platelet production rate (0.2–0.6 of the expected value) was observed in all but two cases.

Group B consisted of 67 patients with ITP. Platelet counts were 9–149 × 10^9/L. The mean platelet life span was 1.68 ± 0.84 days (range, 0.5–4 days). The renewal rate was increased: 78.4 ± 43.2%. The production rate was normal. The destruction site was most often splenic (47 of 67; 70%), sometimes hepatic (11 of 67; 16.4%), or both (5 of 67, 7.5%). In four cases, no destruction site was evident.

Group C consisted of 55 patients with pure genetic thrombocytopenia, according to the criteria mentioned above (8, 9). Platelet counts were 16–134 × 10^9/L. The mean platelet life span was 7.7 ± 1.44 days (range, 4–10 days). The renewal rate was normal: 15.3% ± 5.3%. The production rate was always decreased, and less than one-half of the expected value (mean, 0.3 of the expected value). No peripheric destruction site of platelets was found (in either the spleen or the liver). BM aspiration was performed in 24 cases (43.6%), and megakaryocytes were present in all cases.

The degree of thrombocytopenia was similar in all three patient groups: A, B, and C. Mean platelet volume was higher in group C than in group B, which was expected because increased platelet volume is a specific feature of pure genetic thrombocytopenia (9). The presence of macroplatelets was noted in 8 members of group B (11.9%) and in 30 members of group C (54.5%).

**Statistics**

Statistical analysis was performed using Prophet 5.0 software. The distribution-free Kruskal–Wallis test was used to compare the TPO concentration between the three patient groups and the control group. P < 0.05 was considered significant.

**Results**

**TPO Assay**

We first checked that the TPO concentration was stable in serum kept at −30 or −80 °C for at least 1 year (data not shown). The interassay CV was 8.9% (mean TPO, 360 ng/L). No bias in serum TPO measurements was detected by dilution experiments (recovery, 94–108%; data not shown). The lower limit of detection was 35 ng/L (as assessed by the mean + 2 SD of the zero calibrator
measured in duplicate in eight different experiments. Control values were determined by TPO concentrations measured in a control group of 55 healthy regular blood donors. Ten of the 55 controls had no detectable serum TPO (<35 ng/L). The median TPO concentration was 51 ng/L, and the highest TPO observed was 95 ng/L.

TPO in thrombocytopenia

The TPO concentrations measured in each group of patients are shown on Fig. 1 and Table 3. In patients in group A, with thrombocytopenia attributable to BM aplasia, TPO was always detectable but with a large dispersion of the results (mean, 402 ng/L; median, 155 ng/L). Nevertheless, TPO concentrations were significantly higher in this group than in the control group (P = 0.0006) or in groups B and C (P = 0.0024 and 0.0036, respectively). In 85.1% (57 of 67) of patients with thrombocytopenia caused by increased platelet destruction (group B) and 81.8% (45 of 55) of patients with pure genetic thrombocytopenia (group C), the TPO concentration was <100 ng/L. Although 20 patients (10 in group B and 10 in group C) had TPO values of 104–508 ng/L (in group B) and 105-1000 ng/L (in group C), there was no significant difference in TPO concentrations between groups B and C and the control group (P > 0.05).

A significant negative correlation between TPO concentration and platelet count was found only in members of group A with thrombocytopenia with BM aplasia (r² = 0.5014; Fig. 1, inset).

Discussion

Isotopic measurement of platelet life span allowed us to study TPO concentrations in homogeneous populations of thrombocytopenias. The TPO concentration was within reference values for 85% and 82% of patients with ITP or pure genetic thrombocytopenia, respectively, whereas patients with thrombocytopenias with megakaryocytic hypoplasia usually had high TPO concentrations (61% of the cases); in this latter group, we found a significant negative correlation between TPO concentration and platelet count. We found no specific biological or clinical features (such as platelet count or origin of the thrombocytopenia) to explain the high TPO concentrations in 10 ITP patients and 10 pure genetic thrombocytopenia patients or the normal TPO concentrations in 5 patients with megakaryocytic hypoplasia.

These results are in accordance with the majority of the results for TPO concentrations in ITP and thrombocytopenias with megakaryocytic hypoplasia (6, 10, 11, 15, 16, 21, 23). In pure genetic thrombocytopenia, only two studies have been reported to date. One study of 29 patients (24) showed high TPO concentrations in these patients, but in this study, ITP patients had also high TPO concentrations, suggesting that the basal TPO concentrations were different from those in our study. In the second study (22), the control cases were not random healthy donors but unaffected relatives of one single family. The authors reported higher TPO concentration (258 ± 150 ng/L) in members with autosomal dominant thrombocytopenia than in unaffected relatives (62 ± 29 ng/L).

Our study on a well-defined cohort of patients with thrombocytopenia clarifies the usefulness of TPO measurements in thrombocytopenias. Indeed, in cases with low megakaryocytes in BM and a high TPO concentration in serum, isotopic platelet life span measurements may be avoided because thrombocytopenia would be attributable to BM failure. In cases with low megakaryocytes in BM and TPO concentration within reference values, isotopic life span studies may elucidate the mechanism of thrombocytopenia. Finally, in cases with normal or high megakaryocytes in BM, the TPO assay is not useful and only the isotopic life span study should be performed.

In conclusion, our study presents the advantage of comparing TPO concentrations in well-defined homogeneous groups of patients according to their platelet life span, and platelet turnover and production rate. Although the TPO assay may be a useful additional noninvasive tool for identification of the mechanism of thrombocytopenia, it did not allow us to differentiate patients with pure genetic thrombocytopenia from patients with ITP, in whom isotope studies may still be requested. Although mutations in the c-mpl gene have been identified in congenital amegakaryocytic thrombocytopenia (28, 29), involvement of both the c-mpl and TPO genes in the pathogenesis of hereditary thrombocytopenia in two families has recently been excluded (30). However, analysis of the TPO/c-mpl system would certainly provide more insight into the mechanism of thrombocytopenia.
in the presence of a normal platelet life span in our patients.

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References


