Usefulness of thrombopoietin in the diagnosis of peripheral thrombocytopenias

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ABSTRACT

Background and Objective. Thrombocytopenia of peripheral origin is basically due to platelet destruction or splenic sequestration. Thrombopoietin (TPO) regulates platelet production stimulating megakaryocyte proliferation and maturation. The evaluation of TPO levels may be a useful tool in the diagnosis of thrombocytopenias of unknown origin. We tried to determine the value of TPO levels in some thrombocytopenias classically considered as peripheral.

Design and Methods. Serum TPO levels and platelet counts were measured in 32 thrombocytopenic patients with liver cirrhosis (LC) and 23 with chronic hepatitis C (CHC) viral infection, in 54 patients with a clinical and serological diagnosis of autoimmune thrombocytopenic purpura (AITP), and in 88 patients infected with the human immunodeficiency virus (HIV).

Results. Patients with LC, AITP and HIV had lower platelet counts than patients with CHC. The degree of thrombocytopenia did not, however, correlate with the TPO levels. HIV infected patients (246 ± 304 pg/mL) and AITP patients (155 ± 76 pg/mL) had higher TPO levels than controls (121 ± 58 pg/mL). TPO levels in patients with CHC (125 ± 40 pg/mL) did not differ from those in control subjects, but were slightly decreased in patients with LC (104 ± 56 pg/mL).

Interpretation and Conclusions. Reduced TPO production could be involved in the development of thrombocytopenia in LC patients, but not in patients with early stages of CHC viral infection. HIV and AITP patients had slightly raised levels of TPO. As TPO levels are normal or slightly increased in most peripheral thrombocytopenias, these data alone are not sufficient to distinguish the different types of peripheral thrombocytopenias, these data alone are not sufficient to distinguish the different types of peripheral thrombocytopenias. However, new insight into thrombopoiesis may be achieved with measurements of thrombopoietin (TPO) levels and other reticulocytes.

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Key words: thrombopoietin, thrombocytopenia, liver diseases, autoimmune thrombocytopenic purpura, human immunodeficiency virus

Thrombocytopenias may be classified into two main groups, central and peripheral, according to their etiology. Central thrombocytopenia occurs in bone marrow disorders in which there is impaired platelet production, whereas peripheral thrombocytopenia is basically due to platelet destruction (i.e. immune or microangiopathic mechanisms) or splenic sequestration (hypersplenism). Several diagnostic tools may discriminate the etiology of an isolated thrombocytopenia. However, new insights into thrombopoiesis may be achieved with measurements of thrombopoietin (TPO) levels and other reticulocytes.

TPO is a recently purified cytokine which is mainly produced in the liver and cleared through binding to its specific ligand, c-Mpl, located on the surface of megakaryocytes and platelets. The evaluation of TPO concentrations in several diseases has shown that TPO levels are increased when thrombocytopenia results from defective platelet production due to shortage of megakaryocytes. Patients with similar platelet counts but different megakaryocytic masses thus have different TPO levels: patients with aplastic anemia have few bone marrow megakaryocytes and high levels of circulating TPO, in contrast to patients with autoimmune thrombocytopenic purpura (AITP) who have a large number of megakaryocytes and normal or slightly increased TPO concentrations. The evaluation of TPO levels may, therefore, be a useful diagnostic tool in the initial evaluation of thrombocytopenias of unknown origin.

We measured circulating serum TPO levels in 3 groups of patients with thrombocytopenia due, completely or at least in part, to a peripheral mechanism. As TPO levels are high in patients with a low megakaryocytic mass, we tried to explain the TPO levels recorded and determine whether or not the measurement of circulating TPO could be useful in the diagnosis of a thrombocytopenia of unknown origin or in the evaluation of a putative peripheral thrombocytopenia.

Design and Methods

Groups of subjects

The first group was made up of thrombocytopenic patients with chronic liver diseases. Two subgroups
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were analyzed: 32 patients with liver cirrhosis (LC), and 23 with chronic hepatitis C (CHC) viral infection but without evidence of LC. Liver cirrhosis was diagnosed on the basis of clinical, laboratory, ultrasound and histologic findings. CHC viral infection was confirmed by a MEIA test (Axsym™ HCV version 3.0 Abbott Laboratories, Abbott Park, IL, USA) and a SIA test (Chiron™ Riba™ HCV 3.0 Emeryville, CA, USA).

The second group was formed by 54 consecutive patients with AITP diagnosed clinically (acquired thrombocytopenia and exclusion of circumstances that may cause thrombocytopenia) and serologically (evidence of platelet autoantibodies, positive direct test and eluate, using the immunofluorescence test).7 Of them, 49 patients had primary AITP and 15 were considered to have chronic AITP. Twenty-three out of the 54 patients were receiving steroid treatment at the time of evaluation.

The third group comprised 88 patients infected with human immunodeficiency virus (HIV) type 1. Specific antibodies against HIV were detected using a MEIA test (Axsym™ HIV-1/HIV-2, Abbott Laboratories, Abbott Park, IL, USA). When this test was positive a Western blot was performed to validate the results. Of this group, 20 patients (NT-HIV) had normal platelet counts whereas 68 were thrombocytopenic (T-HIV), 15 of whom tested positive for platelet antibodies.

A fourth group of 43 healthy subjects was used as controls.

**TPO measurements**

Serum samples were separated from peripheral blood, divided into aliquots and stored frozen until used. TPO levels were measured in duplicate using an ELISA kit (Quantikine™ Human TPO Immunoassay, R&D Systems, Minneapolis, MN, USA). Briefly, samples were incubated on a microtiter plate coated with a murine monoclonal antibody against TPO. After washing, TPO was labeled with a second monoclonal antibody conjugated to horseradish peroxidase. After a further washing, substrate solution was added and optical density was determined at 450 nm by an automated reader.

**Statistics**

Results are reported as mean ± standard deviation. The one-way analysis of variance was used to compare means between pairs of groups. p values lower than 0.05 were considered statistically significant.

**Results**

**Platelet counts**

Patients with LC and AITP had a similar degree of thrombocytopenia, 73±29 and 74±48·10⁹/L platelets, respectively. Analyzing AITP patients, those receiving steroid treatment had significantly higher platelet counts (90±61·10⁹/L) (p = 0.03) than non-treated patients (62±32·10⁹/L). Thrombocytopenic HIV-1 infected patients also had moderate thrombocytopenia (81±36·10⁹/L), although slightly less severe than the aforementioned groups. Thrombocytopenic patients infected with hepatitis C virus showed the highest platelet counts (110±23·10⁹/L). However, we found no correlation between TPO levels and peripheral platelet counts in any group.

**Circulating serum TPO levels**

Thrombocytopenic patients with liver cirrhosis had slightly, although not statistically significant, lower serum TPO levels (104±56 pg/mL) than the control group (121±58 pg/mL). Circulating TPO concentrations in thrombocytopenic patients with chronic hepatitis C viral infection (125±40 pg/mL) did not differ from those of healthy subjects (Figure 1).

Patients with AITP had significantly higher serum TPO levels (155±76 pg/mL) (p=0.01) than healthy controls (Figure 1). However, only 13 patients had TPO levels above the range of controls (mean±SD). We also found that AITP patients under steroid treatment had lower TPO levels (127±44 pg/mL) (p = 0.01) than patients who did not require any treatment (177±88 pg/mL) (Figure 2). We could not find
any statistical difference in serum TPO levels between
patients with primary and secondary AITP (data not
shown).

Both thrombocytopenic and non-thrombocytopenic HIV-1 infected patients had significantly higher serum TPO levels (262±342 and 191±86 pg/mL, \( p = 0.008 \) and \( p < 0.001 \) respectively) than healthy subjects (Figure 3). The whole group of HIV-1 infected patients thus had a higher circulating TPO concentration (246±204 pg/mL) than that of the control subjects (Figure 3). Mildly thrombocytopenic patients and the subgroup with platelet antibodies exhibited serum TPO levels (192±117 and 182±76 pg/ml, respectively) similar to those of non-thrombocytopenic HIV-1 seropositive patients. Patients with pancytopenia showed the highest TPO levels (756±714 pg/mL) of this group.

Discussion

Although a humoral factor regulating megakaryocytopoiesis and thrombopoiesis was suspected many years ago,\(^9\) there has been renewed interest in this field since the recent recognition of the receptor c-Mpl and its specific ligand, TPO.\(^9,14\) In vitro and in vivo studies have shown an increase in the number and ploidy of megakaryocytes and in platelet counts following the administration of recombinant TPO.\(^14,17\) TPO is, therefore, considered the principal regulator of both megakaryocytopoiesis and thrombopoiesis.\(^18,19\)

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Although TPO mRNA has been detected in several organs including bone marrow stroma,\(^20\) TPO is mainly produced in the liver.\(^2\) Circulating TPO is then cleared through binding to c-Mpl, internalization and degradation.\(^4,21\) As c-Mpl is located on the surface of progenitor cells, megakaryocytes and platelets,\(^22\) both megakaryocytic\(^1\) and platelet mass\(^23\) are involved in the maintenance of TPO levels. A small megakaryocytic mass, such as occurs in aplastic anemia, is associated with high TPO concentrations,\(^23\) whereas a large megakaryocytic mass, such as occurs in AITP, is usually associated with normal or slightly increased TPO levels.\(^24,25\) The megakaryocytic mass therefore seems to be more important than the platelet mass in the regulation of TPO levels.\(^26\) Recent data indicate that stromal TPO mRNA correlates with megakaryocyte counts in healthy subjects and in patients with AITP. TPO produced by bone marrow stromal cells may, therefore, be the key regulator of megakaryocytopoiesis.\(^27\)

We measured serum TPO levels in three main groups
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of thrombocytopenic patients: 1) patients with liver damage due to LC or CHC viral infection; 2) patients suffering from AITP; and 3) patients infected with HIV-1. In all these groups, thrombocytopenia may be explained, at least in part, by a peripheral mechanism, i.e. immune platelet destruction or hypersplenism.

We found that thrombocytopenic patients with LC had slightly lower serum TPO concentrations than control subjects. The mechanism that explains the thrombocytopenia in these patients is basically platelet pooling and hypersplenism. However, Koike et al. recently showed a reduced absolute number of reticulated platelets using flow cytometry, indicating that an impaired platelet production, as was initially suspected from kinetic studies, may also play an important role in the etiology of this thrombocytopenia. Moreover, Qian et al. generated tissue-specific knockout mice by transplanting the liver of TPO-deficient mice into wild-type recipients and observed a 60% reduction in circulating platelet counts. This illustrates the importance of hepatic TPO in the maintenance of platelet counts. We consider that the finding of slightly reduced TPO levels in LC patients could therefore be explained by reduced TPO production due to hepatocyte necrosis and is a mechanism to bear in mind when evaluating the origin of thrombocytopenia in such patients. Moreover, TPO could be a therapeutic option for thrombocytopenic LC patients. Concerning patients with CHC viral infection, TPO levels in this subgroup did not differ from those of healthy subjects, excluding a defect in TPO production at the early stages of the disease. An immune mechanism is therefore a possible etiology of thrombocytopenia in these patients.

Patients with AITP had slightly higher serum TPO concentrations than controls. AITP is characterized by thrombocytopenia due to platelet autoantibodies, leading to peripheral platelet destruction in the reticuloendothelial system. As the number of bone marrow megakaryocytes is normal or increased in AITP, normal or reduced TPO levels would be expected. We determined serum TPO levels in a clinically and serologically well-defined AITP population and found a slightly increased mean TPO concentration, but most patients had TPO levels similar to those of controls. Apart from the peripheral platelet destruction, megakaryocytic damage may be responsible for the high TPO levels found in a minority of patients. We also found that patients receiving steroid treatment had lower TPO concentrations than those who did not require treatment. One possible explanation for this finding is that we did not collect serum samples at the onset of the disease but rather at the time of evaluation of platelet antibodies, when treated patients had higher platelet counts and probably a greater megakaryocytic mass than non-treated patients. Our observation is in concordance with platelet kinetic studies which show that untreated patients exhibit an inappropriately low platelet turnover and also with the decrease in stromal TPO mRNA expression following steroid treatment. Interestingly, Sakane et al. recently reported increased TPO levels in AITP patients with a poor response to steroid treatment, suggesting that TPO levels might be important for predicting the response to steroids.

Both thrombocytopenic and non-thrombocytopenic patients with HIV-1 infection had significantly higher serum TPO levels than healthy controls. Thrombocytopenia in HIV-1 seropositive patients was initially considered secondary to immune peripheral platelet destruction but is now regarded as multifactorial; a direct role of HIV-1 infecting stem cells and megakaryocytes, hypersplenism, myelosuppressive drugs or bone marrow infiltration may also be involved. As TPO levels in HIV-1 seropositive patients with evidence of platelet antibodies were only slightly raised, other mechanisms should explain the higher TPO levels obtained in the rest of HIV-1 infected patients. The finding of high TPO levels and reduced platelet counts may suggest impaired platelet production, probably due to the HIV-1 infection. Previous platelet kinetic studies in these patients demonstrated reduced platelet survival, thus supporting the hypothesis of ineffective platelet production. Cole et al. also reported an increase in TPO levels and in marrow megakaryocyte mass in a recent well-designed study of six thrombocytopenic HIV-infected patients. Interestingly, they also found a markedly reduced number of megakaryocyte progenitors and a threefold increase in the number of TPO receptors on the platelet surface. All these findings may indicate ineffective platelet production, a main component in the development of HIV-1 related thrombocytopenia. We also observed that pancytopenic patients had the highest TPO levels of this group.

Although further studies analyzing the number of TPO receptors on platelets and megakaryocytes, stromal TPO levels, and TPO mRNA expression are needed to understand megakaryopoiesis and thrombopoiesis better, the measurement of TPO levels in thrombocytopenic patients further suggests that bone marrow involvement may also play a role in the origin of some classically considered peripheral thrombocytopenias. A reduced TPO production may be partially responsible for the development of thrombocytopenia in LC patients. In HIV-1 seropositive patients, we hypothesize that the impaired platelet production occurs probably secondary to the HIV infection of megakaryocytes or megakaryocyte precursors, while in AITP patients impaired platelet production could also be due to platelet antibodies directed against the same type of cells. In conclusion, we consider that TPO levels alone are not sufficient to distinguish different types of peripheral thrombocytopenia but may be useful to differentiate when a central mechanism is involved in the development of a thrombocytopenia of unknown origin. As high TPO levels suggest low megakaryocytic masses, we recom-
mend a bone marrow examination when very high TPO levels are found.

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IE designed the study, wrote the article and managed the statistical data. AH helped to perform the ELISA tests. EMD was responsible for the platelet antibody studies. RA provided the data concerning liver and HIV-1 patients. NPM helped with the data analysis and corrected the manuscript. All authors contributed to the interpretation of the results.

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References
5. Emmons RVB, Reid DM, Cohen RL, et al. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. Blood 1996; 87:4068-71.
27. Hirayama Y, Sakamaki S, Matsunaga T, et al. Con-


