Studies on megakaryopoiesis in patients with myelodysplasia and idiopathic thrombocytopenic purpura
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CHAPTER V

Increased glycocalcin-index and normal thrombopoietin levels in patients with idiopathic thrombocytopenic purpura with a decreased rate of platelet production

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ABSTRACT

Thrombokinetic studies in idiopathic thrombocytopenic purpura (ITP) have shown that platelet turnover, also called platelet production rate, can be increased, normal or decreased. The present study focused on the question whether the reduced platelet production rate in a subgroup of patients with ITP as estimated with platelet kinetic studies, is caused by an inappropriate bone marrow platelet production, reflected by elevated thrombopoietin (TPO) levels or by augmented destruction of platelets in the bone marrow translated in an elevated glycocalcin (GC)-index. Thirty-five ITP patients were studied. The median TPO level did not differ significantly from normal controls [110 pg/ml (68-171) vs 114 pg/ml (93-146), $P = 0.7$]. A decreased platelet production rate was not associated with elevated TPO levels. In contrast, the reduced platelet production rate was associated with an elevated GC index ($P = 0.03$), suggesting an increased release of glycoprotein (GP) Ib from the membrane of platelets and/or megakaryocytes into the circulation. The correlation between the decreased release of platelets into the circulation (reflected by the reduced platelet production rate) and the increased shedding of the receptor complex GPIb (reflected by an increased GC index), suggests that in a subgroup of ITP patients intramedullary destruction of platelets and/or megakaryocytes contributes to thrombocytopenia in ITP.
INTRODUCTION

Idiopathic thrombocytopenic purpura (ITP) is characterized by thrombocytopenia in an otherwise healthy person. Platelet kinetic studies have shown that in classical ITP a reduced mean platelet life is observed in conjunction with a normal or increased platelet turnover (platelet production rate), whereas in a subgroup of ITP patients a shortened mean platelet life is demonstrated in conjunction with a decreased platelet production rate.1-5

Other methods to study aspects of thrombopoiesis are measurement of the plasma concentration of thrombopoietin (TPO) and glycocalcin. TPO is the major regulator of thrombopoiesis and induces differentiation, proliferation and endomitosis of megakaryocytes. Elevated TPO plasma levels are present in thrombocytopenia due to megakaryocyte deficiency, whereas in ITP normal to slightly increased TPO levels have been found.6-8 While in aplastic anemia and thrombocytopenia secondary to myelosuppressive therapy inverse correlations between TPO levels and the platelet count have been observed9,10, in ITP no such correlations have been described, indicating that the megakaryocyte mass contributes to TPO regulation.11 Glycocalcin (GC) represents the soluble, external part of the platelet and megakaryocyte membrane glycoprotein Ib (GP-Ib). The GC index, the GC normalized for the individual platelet count12, has been introduced as a parameter for platelet destruction.12,13

So far, little is known about whether a correlation exists between thrombokinetic parameters and plasma TPO and GC index levels, and whether the latter can replace platelet kinetic parameters in general or in specific disorders, such as ITP. It is also unclear whether the subgroup of ITP patients with a low platelet production rate is characterized by a decreased platelet production in the bone marrow and/or elevated TPO levels. The results of the present study demonstrate that the decreased platelet production rate in patients with ITP is not associated with an elevated TPO concentration. It appears that in this subgroup of patients, thrombocytopenia is not only due to increased peripheral platelet destruction but also the result of an increased destruction of platelets or megakaryocytes in the bone marrow, which was reflected by an elevated GC index.
PATIENTS, MATERIALS AND METHODS

Patients
Thirty-five adult patients with ITP were investigated. The diagnosis required that an otherwise healthy person, after history, physical examination, complete blood count, and examination of the peripheral blood smear, had an isolated thrombocytopenia (platelets less than $100 \times 10^9/L$) of undetermined etiology. Patients with associated systemic disease, such as human immunodeficiency virus infection or systemic lupus erythematosus, were excluded. The study protocol was approved by the institutional ethical committee; patients entered after informed consent was obtained.

Platelet kinetic studies
Autologous platelets were labeled with Indium-111 ($^{111}$In) tropolonate according to the recommendations of the International Committee for Standardization in Hematology (ICSH). After injection of the $^{111}$In-labelled platelets 2 ml venous blood samples were collected at regular intervals. Mean platelet life was calculated by the gamma function model proposed by the ICSH. Initial platelet recovery of $^{111}$In-labelled platelets in the circulation was calculated by extrapolating the blood disappearance curve to time zero. Platelet counts were stable during the study.

Platelet production rate was calculated following the description of Harker and Finch: platelet count ($\times 10^9/L$) $\times 0.9 \times$ blood volume (L) divided by mean platelet life (days) $\times$ initial platelet recovery (fraction). The formula is based on the assumption that at stable platelet counts, platelet removal from the circulation equals platelet release from the bone marrow. Thus, platelet production rate is defined as the number of platelets entering the circulation to maintain platelet count, and is not necessarily the same as the total platelet production in the body, because production in the bone marrow without release of platelets in the circulation may also occur.

Normal values (median, 25th-75th) were 60 (50-71) for initial platelet recovery, 223 $\times 10^9$/day (158-268) for platelet production rate and 9.2 (8.9-9.4) days for mean platelet life.

Plasma thrombopoietin and glycocalicin concentrations
Plasma thrombopoietin (TPO) concentrations were determined by enzyme-linked immunosorbent assay (ELISA; Quantikine, R&D systems, Minneapolis, USA) which
uses a monoclonal antibody directed against recombinant human TPO, according to the manufacturer’s instructions. The normal value is 114 pg/ml (93-146).

Plasma glycocalicin (GC) concentrations were measured by enzyme immunoassay (EIA; Takara Shuzo Co, Ltd). Blood was collected in citrate as anticoagulant and processed within 2 hours of collection. The glycocalicin index (GCI) is derived from [GC (µg/ml) × (250 × 10⁹/L)] divided by the individual platelet count. The normal value of the GCI is 0.7 (0.6-0.9).

**Statistical analysis**

Data are presented as median with 25th and 75th percentiles. Statistical analysis was performed using Kruskal-Wallis nonparametric analysis of variances and the Wilcoxon’s two sample test. Correlation was assessed with the Spearman’s rank correlation procedure. A *P* value of < 0.05 was considered statistically significant, and all tests were two sided.

**RESULTS**

Thirty-five patients with ITP were studied. Patient characteristics are depicted in Table 1. The platelet production rate was reduced in 9 patients, whereas in 26 patients it was normal (n = 17) or increased (n = 9) [median 395 × 10⁹/day, ranging between 300-950 × 10⁹/day]. Plasma TPO levels in all patients were not significantly different, compared to healthy controls [110 pg/ml (68-171) vs 114 pg/ml (93-146), *P* = 0.7]. There was no significant difference in plasma TPO levels in patients with a normal or increased platelet production rate, vs reduced platelet production rate [111 pg/ml (64-171) vs 109 (71-172), *P* = 0.8]. A significant correlation was found between the GCI and the platelet production rate (Fig. 1; *P* = 0.03). In patients with a normal or increased platelet production rate, the median GCI was 5 (3-10), compared to 12 (7-25) in patients with a decreased platelet production rate (*P* = 0.03). No significant correlation between GCI and mean platelet life was observed (*P* = 0.08). Patients with a mean platelet life ≤ 2 days had a GCI of 7 (3-26) compared to 5 (4-10) in patients with a mean platelet life > 2 days (*P* = 0.3).
Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Platelet production rate</th>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>decreased</td>
<td>normal/increased</td>
<td>all</td>
<td></td>
</tr>
<tr>
<td>No. patients</td>
<td>9</td>
<td>26</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>No. male/female</td>
<td>5/4</td>
<td>10/16</td>
<td>15/20</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>62 (30-68)</td>
<td>44 (32-67)</td>
<td>45 (32-66)</td>
<td>0.9</td>
</tr>
<tr>
<td>Platelets, × 10^9/L</td>
<td>22 (13-46)</td>
<td>63 (43-89)</td>
<td>58 (22-85)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean platelet life, days</td>
<td>2.6 (1.4-3.7)</td>
<td>1.9 (1.1-3)</td>
<td>2 (1.1-3)</td>
<td>0.5</td>
</tr>
<tr>
<td>Platelet production rate, ×10^9/d</td>
<td>100 (88-145)</td>
<td>255 (188-325)</td>
<td>195 (150-300)</td>
<td>0.004</td>
</tr>
<tr>
<td>Thrombopoietin</td>
<td>109 (71-172)</td>
<td>111 (64-171)</td>
<td>110 (68-171)</td>
<td>0.8</td>
</tr>
<tr>
<td>Glycocalcin index</td>
<td>12 (7-25)</td>
<td>5 (3-10)</td>
<td>5 (4-13)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Results are expressed as median (25th/75th percentile)

DISCUSSION

Most studies point to a constant rate of TPO production in the liver\textsuperscript{17}, and the plasma TPO level is regulated by the uptake of TPO by c-Mpl (TPO receptor) bearing cells, predominantly platelets and megakaryocytes, resulting in elevated TPO levels in case of hypomegakaryocytic thrombocytopenia\textsuperscript{7,8,10}. In contrast to previous findings\textsuperscript{18} we did not find a significant correlation between the platelet production rate and plasma TPO levels. In particular, the reduced platelet production rate was not associated with elevated TPO levels. These data suggest that the reduced release of platelets in the circulation is not due to a reduced mass of c-Mpl bearing cells in the bone marrow in these ITP patients.

The finding of a significant inverse correlation between the GCI and the platelet production rate indicates that the reduced platelet production rate is associated with an increased release of (components of the) GPIb complex in the circulation, probably as a result of shedding of the receptor complex from platelets and/or megakaryocytes upon destruction in the bone marrow. This is also in concordance with the finding of no significant correlation between GCI and mean platelet life.

The present findings suggesting intramedullary destruction of megakaryocytes are consistent with previous studies demonstrating that antiplatelet autoantibodies in patients with ITP can not only affect platelets but also megakaryocytes\textsuperscript{19,20}. 
The intramedullary destruction of platelets and/or mature megakaryocytes might consequently lead to a reduced release of platelets into the circulation, and an increase in the production in immature megakaryocytic precursors. Several studies have indeed shown that there is a relative increase in immature megakaryocytes in ITP patients. The normal TPO levels in our patients with a reduced platelet production rate are consistent with these findings. The finding of a lower platelet production rate in patients with more pronounced thrombocytopenia (see Table 1) suggests a more prominent intramedullary destruction in the more severe ITP in this study.

In conclusion, the present study indicates that (i) plasma TPO and GCI levels are related to the dynamics of megakaryocyte and platelet kinetics in the bone marrow and peripheral blood, and give complementary information to the results of the platelet kinetic parameters including mean platelet life and platelet production rate in ITP; (ii) in ITP patients with a decreased platelet production rate, an increased platelet and/or megakaryocyte destruction might occur in the bone marrow, as was recently demonstrated by ultrastructural studies. Further research is needed to evaluate the usefulness of determining the GCI in ITP patients in clinical practice.
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