Points to clarify seem to be the factors enhancing splenic sequestration and impaired thrombopoiesis.

Cirrhotic thrombocytopenia is a multi-factorial condition but our results suggest that accelerated platelet clearance in the periphery due to splenic sequestration seems to be the main factor for thrombocytopenia in patients with cirrhosis, rather than impaired thrombopoiesis due to TPO insufficiency.

Implication for health policy/practice/research/medical education:
Cirrhotic thrombocytopenia is a multi-factorial condition but our results suggest that accelerated platelet clearance in the periphery due to splenic sequestration seems to be the main factor for thrombocytopenia in liver cirrhosis, rather than impaired thrombopoiesis due to TPO insufficiency. Points to clarify seem to be the factors enhancing splenic sequestration and impaired thrombopoiesis.

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stimulates thrombopoiesis. TPO levels are correlated with platelet production. (4, 5). Data at the literature about TPO levels and its relation with thrombocytopenia for cirrhotic patients is conflicting. Although in most studies serum TPO levels are decreased in liver cirrhosis, thrombocytopenia is correlated positively with TPO levels and platelet counts are increased after recovery of liver synthesis function; orthotopic liver transplantation; some studies have been reported that serum TPO concentrations are elevated or normal in thrombocytopenic cirrhosis patients, no correlation was determined between TPO levels and thrombocytopenia, and rather than TPO levels, decreased platelet counts are associated with other factors like splenic sequestration (6-20).

2. Objectives

The aim of this study was to evaluate serum thrombopoietin levels and its relationship with thrombocytopenia in patients with cirrhosis.

3. Patients and Methods

3.1. Patients

Ninety-two cirrhotic patients among all cirrhotic patients who admitted to our clinic between 01.03.2005-01.02.2013 (mean age 58 ± 11 years, 42 females and 50 males) with or without thrombocytopenia and 45 healthy controls (23 females and 22 males, mean age 54 ± 10 years) were enrolled by simple random sampling to patient and control groups of this case control study performed at Eskisehir-Turkey. The number of patient and control groups were determined by two sample t-test power analysis with an expected power of 0.87. Eskisehir Osmangazi University local ethics committee approved the study (28.02.2005 ethics committee acceptance number: 9) and informed consents were obtained. Diagnosis of liver cirrhosis was made according to the clinical and laboratory parameters, while the diagnosis was confirmed by histological examination in 72 of the patients, diagnosis of cirrhosis was established by liver function tests (bilirubin, albumin, international normalized ratio), ultrasound findings (ascites and splenomegaly, dilated portal and splenic veins, presence of portal collaterals and irregular-nodular contours of the liver) at patients with contraindications to liver biopsy. Exclusion criteria were as follows: diagnosis of essential thrombocytocemia or history of familial essential thrombocytocemia, inherited or acquired bone marrow failure, previous or ongoing application of recombinant hematopoietic growth factors, autoimmune diseases rather than autoimmune hepatitis or history of immune suppressive treatment, diagnosis of a hematopoietic or solid malignancy, antiviral therapy application within last six months. The severity of liver cirrhosis was assessed according to the Child Pugh’s classification (21). The cause of liver cirrhosis was hepatitis B virus (HBV) infection in 22 cases, hepatitis C virus (HCV) infection in 38 cases, alcohol in four cases, autoimmune hepatitis in 10 cases and cryptogenic cirrhosis in 18 cases. Thirty of the patients were at Child A, 32 of the patients were at Child B and 30 of the patients were at Child C stage. Complete blood count, liver function tests including serum albumin levels and prothrombin time were determined at biochemistry and haematology laboratories of Eskisehir Osmangazi University, Medical Faculty. The size of the spleen was measured by ultrasound, and the spleen index was calculated by multiplication of the long and short axes. Characteristics of the patients are summarized at Table 1.

3.2. TPO Assay

Venous peripheral blood samples were collected and centrifuged at 2500 rpm for 10 minutes. The serum was separated and stored at -75°C. TPO levels were detected by ELISA (Quantikinine, RD Systems, Wiesbaden Nordenstand, Germany) at haematology laboratory.

3.3. Statistical Analysis

Statistical analysis was performed using a commercial statistical software package version 13.0 for Windows. Data are shown as the mean ± standard deviation (SD). Means between two groups were compared by using the one-way analysis of variance, (Tukey post-hoc test) used to evaluate the association between two variables. A value of P < 0.05 was accepted as statistically significant.

4. Results

Platelet counts were lower in patients with cirrhosis...
(97000 ± 8000/mm³) than in healthy subjects (240000 ± 51000/mm³, P < 0.001). Significant difference was found for platelet counts among child A, B and C stages (Child A vs. Child B P < 0.05 Child A vs. Child C P < 0.001–Child B vs. Child C P < 0.05). Serum TPO concentration was higher (69 ± 12 pg/mL) in cirrhotic group than healthy controls (49 ± 9 pg/mL) (P < 0.05). No significant difference in TPO levels were found among the Child A, B and C stages (64 ± 11 pg/mL, 75 ± 13 pg/mL and 68 ± 19 pg/mL, respectively). TPO levels were similar among the patient groups with different etiology. Spleen size and SVI was significantly higher in the cirrhotic patients than healthy controls (148 ± 14 mm vs. 98 ± 11 mm, P < 0.001-9167 ± 287 cm² vs. 4118 ± 123 cm²). Significant difference was determined for spleen size and spleen index among children A, B and C stages (64 ± 11 pg/mL, 75 ± 13 pg/mL and 68 ± 10 pg/mL, respectively).

### Table 2. TPO Levels, PLT Count, Spleen Size and Index in Cirrhosis and Healthy Subjects

<table>
<thead>
<tr>
<th></th>
<th>Child A, (n = 3)</th>
<th>Child B, (n = 32)</th>
<th>Child C, (n = 30)</th>
<th>Liver Cirrhosis, Total, (n = 92)</th>
<th>Healthy Subjects, (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPO, pg/mL</td>
<td>64 ± 11</td>
<td>75 ± 13</td>
<td>68 ± 10</td>
<td>69 ± 12</td>
<td>49 ± 9</td>
</tr>
<tr>
<td>PLT count, mm³</td>
<td>13358 ± 9208</td>
<td>92416 ± 7607</td>
<td>58316 ± 5476</td>
<td>97000 ± 8000</td>
<td>240000 ± 51000</td>
</tr>
<tr>
<td>Spleen size, cm²</td>
<td>119 ± 14</td>
<td>144 ± 13</td>
<td>175 ± 11</td>
<td>148 ± 14</td>
<td>98 ± 11</td>
</tr>
<tr>
<td>SVI, cm³</td>
<td>6616 ± 216</td>
<td>9363 ± 246</td>
<td>12529 ± 300</td>
<td>9167 ± 287</td>
<td>4118 ± 123</td>
</tr>
</tbody>
</table>

* Data are presented as Mean ± SD.
* Abbreviation: SVI, Spleen volum index.
* Liver Cirrhosis (Total) versus healthy subjects P < 0.05
* Liver Cirrhosis (Total) versus healthy subjects P < 0.001
* Child A vs. Child B P < 0.05 Child A vs. Child C P < 0.001–Child B vs. Child C P < 0.05.
* Liver Cirrhosis (Total) versus healthy subjects P < 0.001.
* Liver Cirrhosis (Total) versus healthy subjects P < 0.001.

As a result diminished thrombocyte production due to TPO; principal regulator of megakaryogenesis and thrombopoiesis, which is predominantly produced by the liver; insufficiency secondary to advanced liver failure is accused as the reason of thrombocytopenia in cirrhosis (26). While some of the studies determined low plasma levels of TPO (6, 11, 13, 15, 18), others evaluated normal or high plasma levels in contrast of decreased thrombocyte levels at cirrhotic patients (5, 9, 20, 26, 27). Bone marrow examinations of cirrhotic patients revealed absolutely normal findings (28, 29).

In our study, cirrhotic patients had higher TPO levels than healthy subjects and a statistically significant negative correlation was determined between platelet counts and serum TPO levels. Circulating TPO levels is regulated through its binding to the TPO receptor; which is mainly expressed on bone marrow megakaryocytes and circulating platelets; rather than the up-regulation or down-regulation of its production (4, 5). While TPO is produced constantly by the liver, kidney, and marrow stroma, its circulating levels depends on the total amount of TPO receptor (25). The observed increase in circulating TPO in our study might be explained by decreased consumption of TPO secondary to thrombocytopenia and contradictory data throughout the literature may be related with ignorance of the TPO receptors at bone marrow.

Proportion of reticulated platelets (RP) in total platelets (%RP) and glycopcalicin index (GCI) are indicators of platelet turnover (30). The accelerated platelet turnover in cirrhotic patients indicates an accelerated platelet clearance in the periphery through hypersplenism. Spleen volume index was significantly elevated and platelet counts were significantly reduced in cirrhotic patients than the healthy controls in our study. A strong inverse correlation was observed between platelet count and spleen size in the patients.

The identification of megakaryocytes based on morphological studies at the light microscopy is difficult and results are therefore often unreliable especially in cases of elevated platelet turnover. Dual-color immunofluorescence staining and flowcymetry seems to be a much reliable method (25). Therefore, either the analysis...
of marrow megakaryocytes by conventional light microscopy method or assuming the marrow megakaryocyte density as normal in liver cirrhosis will not be reliable.

In conclusion, cirrhotic thrombocytopenia is a multifactorial condition but our results suggest that accelerated platelet clearance in the periphery due to splenic sequestration seems to be the main factor for the thrombocytopenia in liver cirrhosis, rather than impaired thrombopoiesis due to TPO insufficiency. Points to clarify seems to be the factors enhancing splenic sequestration and impaired thrombopoiesis.

Authors’ Contribution
All authors contributed to this work equally.

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