Serum thrombopoietin levels in thrombocytopenic patients with liver cirrhosis

Thrombocytopenia is a common hematologic defect among patients with liver cirrhosis. The role of thrombopoietin (TPO), which is mainly produced by the liver, is under investigation in cirrhosis. The purpose of the present study was to evaluate the role of TPO in thrombocytopenia of cirrhotic patients and to reveal possible correlations with their clinical condition.

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Forty patients with liver cirrhosis (LC), (27 males/13 females, mean age 53.3±1.7 years, range 17-65) were included. Cirrhosis etiology was hepatitis B (HBV) and C (HCV) viruses (22 patients), alcohol (8 patients) and miscellaneous causes (10 patients). The patients were classified according to the Child score into three groups of clinical severity: A, B, and C. (Child A: n=26, Child B and C: n=14). Twenty healthy subjects formed the control group (10 males/10 females, mean age 48.6±2.1 years, range 36-71).

Serum TPO levels were measured by a commercial quantitative sandwich enzyme immunoassay (Quantikine, Human TPO Immunoassay, R&D Systems) and platelets were counted by means of a Coulter ONYX and peripheral blood smears. Albumin, total bilirubin, and prothrombin time (PT) were also determined for the Child score classification of the clinical condition. Spleen size was measured by clinical examination (mm below the left subcostal margin).

All results are expressed as mean ± SEM. Statistical significance was estimated by the non-parametric Mann-Whitney U test. Spearman's rank correlation or Pearson's correlation was used for the estimation of relationships, respectively, on the non-normal or normal distribution of values. Differences were statistically significant when p values were ≤ 0.01 and possibly significant between 0.01 and 0.05. Platelet counts in the patients with cirrhosis were 79±5.3×10^9/L vs 248±12.4×10^9/L in healthy individuals (p=0.0001) (Table 1). TPO serum concentrations were 71.8±10 pg/mL in cirrhotic patients vs 88.6±23.3 pg/mL in the control group) (p=0.81). Spleen enlargement in cirrhotic patients was 10±2 mm. TPO levels were not correlated with age (r=-0.13, p=0.31). In the patients with cirrhosis the TPO levels were not correlated with spleen enlargement (r=0.13, p=0.41) but were weakly correlated with platelet counts (r=0.36, p=0.023) (Figure 1). Spleen enlargement was inversely correlated with platelet count (r=-0.4, p=0.01). Fourteen patients classified as having Child B and C disease showed significantly lower platelet counts than the 26 patients with Child A classification (56±7.9×10^9/L vs 91±5.7×10^9/L, p=0.0009) and very significantly enlarged spleens (22.1±3.9 mm vs 3.5±1.1 mm, p=0.000001). The TPO levels in patients with Child B/C disease were =68.7±18.2 pg/mL vs 73.5±12.0 pg/mL in those with child A disease: p=0.38). We also evaluated the results in cirrhotic patients with similar clinical severity (Child A) and spleen enlargement but of different origin (viral origin n=12 vs alcoholic and primary biliary n=12). Post-viral cirrhotic patients had significantly lower platelet counts than the group of patients with alcoholic and primary biliary cirrhosis (75±8.2 ×10^9/L vs 103±5.9 ×10^9/L, p=0.01). TPO levels were 87.5±25.4 pg/mL vs 65.3±5.0 pg/mL, p=0.23. Thrombocytopenia is a common hematologic defect among patients with cirrhosis. The concept of hypersplenism proved to be unable to explain why splenectomy[1] or portosystemic shunt[2,3] failed to augment platelet counts in contrast to liver transplantation.[4,5] The major sites of TPO production are hepatocytes and proximal convoluted tubule cells in the kidney.[6,7] In thrombocytopenic subjects TPO m-RNA expression in the liver and kidney is not increased, but TPO m-RNA expression in bone marrow stromal cells is upregulated.[7] In cirrhosis though, the role of TPO is under investigation.

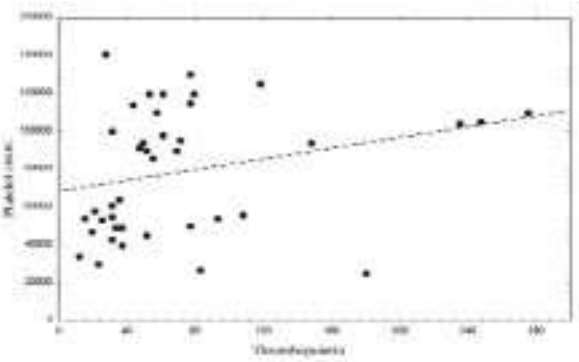

Figure 1. Correlation between platelet count and thrombopoietin in cirrhotic patients (r=0.36, p=0.023).

Table 1. Characteristics and results of cirrhotic patients and control group.

	Cirrhotics	*Controls*	
Subjects	40	20	
Sex	27M/13F	10M/10F	
Age (years)	53.3±1.7	48.6±2.1	p = 0.1
Platelets	79±5.3×10^9/L	248±12.4×10^9/L	p = 0.0001
Spleen enlargement	10.0±2.0 mm		
TPO	71.8±10.0 pg/mL	88.6±23.3 pg/mL	p = 0.81

Abbreviation: m±SEM (standard error of the mean).

The data of our study show that TPO concentrations in cirrhotic patients are slightly lower than those in the control group. As liver function deteriorates, serum TPO levels are slightly reduced, in accordance with previous reported data.[8]

We observed a positive slight correlation of TPO with platelet count in these patients, indicating that recombinant TPO could possibly be an effective drug to treat patients with cirrhosis and severe thrombocytopenia during bleeding episodes or when undergoing surgical procedures.

Splenomegaly is implicated in thrombocytopenia of cirrhosis; spleen size is inversely correlated with platelet count but is not correlated with TPO levels. Our data indicate that the etiology of cirrhosis is implicated in thrombocytopenia; patients with viral-induced cirrhosis had lower platelet counts and higher TPO levels than cirrhotic patients in the same clinical condition, with an equal spleen size but cirrhosis of different origin.

The significantly lower platelet counts in patients with cirrhosis of viral origin indicate that other factors may contribute to the thrombocytopenia, e.g. a direct viral effect on megakaryocytes[9] and anti-platelet antibodies. In our study these patients had TPO levels similar to those of the healthy individuals

(p=0.57) and this is in accordance with data concerning TPO levels in fulminant hepatitis.[10] Thus, we speculate that probably other factors are of primary importance in the thrombocytopenia of these patients. In conclusion, liver cirrhosis thrombocytopenia seems to be multifactorial and reduced TPO levels may contribute to this condition.

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Platelet aggregation is stimulated by L-asparginase in children with acute lymphoblastic leukemia and in normal individuals

L-asparginase treatment of childhood acute lymphoblastic leukemia (ALL) can be associated with severe thromboembolic complications. A decrease in anticoagulant proteins C, S and antithrombin III (ATIII) has been attributed to the enzyme. We tested the hypothesis that L-asparginase can also have a platelet agonist effect.

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Intensive use of L-asparginase in ALL is credited with producing a definite improvement in response and cure rates.[1] Due to a decreased biosynthesis of proteins C, S, and anti-thrombin III (ATIII),[2,3] its administration can be complicated by severe thrombotic events, which occur with an incidence of between 2.4% to 11.5%.[4,5] Different mutations in these anticoagulant proteins have been identified in ALL patients,[4] but their prevalences are those expected for the study population and, therefore, do not explain the increased incidence of thrombotic events. Indeed, this increased incidence implies additional disease or treatment-related procoagulant factors.[3,4] We postulated that the platelet phase of the coagulation mechanism could be involved in this complex hypercoagulable state through a platelet agonist effect exerted by L-asparginase. Twenty children (≤ 13 years old) newly diagnosed with ALL were included in the study. Informed consent was obtained. The induction of remission protocol included *E. coli* L-asparginase, 6000 IU/m^2/i.m., twice a week for 6 weeks. The control group consisted of nineteen healthy volunteers, with normal platelet counts and bleeding time.

Blood samples were obtained from the ALL children after administration of the third dose of L-asparginase, or once the platelet count had reached at least 150×10^9/L. The 20 mL samples of whole blood were taken into plastic tubes containing 3.8% sodium citrate, maintaining a 9:1 v/v relationship.

Platelet aggregation studies were carried out employing a previously described optical method.[6] Platelet-rich plasma (PRP) was obtained from whole blood by centrifuging the tube at 135g ×15 min at room temperature. Platelet-poor plasma (PPP), for adjusting the platelet count to 250×10^9/L and for setting the aggregometer reading to 0%, was obtained after centrifugation at 1,500g ×15 min.

Platelet responses were studied within the two hours after drawing the blood, maintaining the assay temperature at 37°C and the agitation speed at 1,000 rpm. The following agonists were employed:[6] 10 μM ADP, collagen 2.5 μg/mL, ristocetin 1.5 μg/mL, and 10 μM epinephrine. Saline dilutions of *E. coli* L-asparginase (Kyowa Hakko Kogyo Co. Ltd, Tokyo, Japan) to concentrations of 2,500, 5,000 and 10,000 IU/mL in 0.9% physiologic solution, pH 7.4, were employed as agonists in independent assays. For each assay, 50 μL of agonist were added to a plastic cuvette containing 450 μL of the PRP to be tested. The response was quantified as the maximum percentage of aggregation reached.

No difference in aggregation with standard agonists was found between ALL patients and controls (*not shown*). Optical aggregometry did, however, demonstrate a clear agonist effect of L-asparginase on platelets obtained from both leukemic patients and normal individuals, manifested as a potent primary wave of aggregation. Figures 1 and 2 display typical results obtained when platelets from ALL patients were stimulated with L-asparginase dilutions of 5,000 and 10,000 IU/mL, respectively. In these figures,