Thrombopoietin concentrations are low in patients with cirrhosis and thrombocytopenia and are restored after orthotopic liver transplantation

J Goulis, T N Chau, S Jordan, A B Mehta, A Watkinson, K Rolles, A K Burroughs

Abstract

Background—Thrombocytopenia in cirrhotic patients may be due to deficient production of thrombopoietin.

Aims—To determine the relation between thrombopoietin and thrombocytopenia in cirrhotic patients before and after orthotopic liver transplantation.

Methods—Thrombopoietin concentrations and platelet counts were measured in 43 cirrhotic patients and 21 normal controls and serially for 14 days after transplantation in 23/43 patients.

Results—27 of the 43 patients had thrombocytopenia (platelet count less than 120 × 10^9/l; group 1) whereas 16 patients had normal platelet count (group 2). Thrombopoietin concentrations were lower in group 1 than in group 2 (92.5 (20.3–286.3) v 226.6 (30.1–848.3) pg/ml, p=0.003) and normal controls (92.5 (20.3–286.3) v 158.3 (22.5–232.9) pg/ml, p=0.028). Post-transplantation thrombopoietin concentrations increased with a peak at day 5. The rise was significant in patients with low pretransplantation platelet count (89.1 (21.29–247.6) to 545.1 (66.2–2569) pg/ml; n=16, p=0.001) but not in those with normal platelet count (262.8 (30.1–848.3) to 315.1 (114–954.6) pg/ml; n=7, p=0.47). No correlation was found pretransplantation between spleen volume and platelet count (r=−0.11, p=0.6) or thrombopoietin concentrations (r=−0.04, p=0.8). However, pretransplantation thrombopoietin concentrations correlated with platelet count (r=0.47, p=0.015), whereas an inverse correlation was found between peak thrombopoietin concentrations and nadir platelet count (r=−0.41 p=0.049) post-transplantation.

Conclusions—Inadequate thrombopoietin production may contribute to cirrhotic thrombocytopenia. Thrombopoietin production is restored after liver transplantation leading to the resolution of thrombocytopenia.

(Out 1999;44:754–758)

Keywords: platelets; thrombocytopenia; thrombopoietin; cirrhosis; orthotopic liver transplantation

Thrombocytopenia is one of the most frequent haematological abnormalities in patients with cirrhosis and portal hypertension. It is generally considered to be due to the increased sequestration and destruction of platelets in the enlarged spleen which was defined as “hypersplenism”. However, it has been shown that only a few patients with advanced liver disease and thrombocytopenia respond with an increase in bone marrow production of platelets. Moreover portal decompression procedures, either by surgical shunts or transjugular intrahepatic portosystemic shunts (TIPS), have not led to a consistent rise in platelet count. The only procedure which definitively resolves the thrombocytopenia of liver disease is orthotopic liver transplantation (OLT).

Thrombopoietin (TPO) was recently cloned and identified as the primary cytokine involved in the maturation of megakaryocytes and formation of platelets. Serum TPO concentrations are inversely related to platelet concentrations in patients with haematopoietic disorders characterised by decreased megakaryocytes in bone marrow. The level of expression of mRNA for TPO is high in the liver indicating that this is the main source of its synthesis. Thus it has been proposed that deficiency of TPO production could be responsible for the thrombocytopenia in cirrhosis.

Serum TPO concentrations have been measured previously in patients with liver disease with different results due mainly to a varied study population (cirrhotic or non-cirrhotic patients, cirrhotic patients with or without thrombocytopenia, different severity of liver disease, etc.) and in the limitations of the assays used. Although two previous studies have reported that TPO rises after OLT, the TPO concentrations before OLT were undetectable because of the poor sensitivity of the assay, so that the relation to platelet number could not be established.

The aim of our study was to determine the relation between TPO and thrombocytopenia in consecutive cirrhotic patients with end stage liver disease, some of whom subsequently had OLT. Our hypothesis was that inadequate TPO production contributes to the pathogenesis of thrombocytopenia of severe liver disease, and that there would be a correlation between low platelet count and low TPO concentrations.

Abbreviations used in this paper: DIC, disseminated intravascular coagulation; HBV, hepatitis B virus; HCV, hepatitis C virus; OLT, orthotopic liver transplantation; TPO, thrombopoietin; TIPS, transjugular intrahepatic portosystemic shunts; CT, computed tomography.
Table 1  Demographic and laboratory characteristics of patients with (group 1) and without (group 2) thrombocytopenia

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=27)</th>
<th>Group 2 (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)*</td>
<td>53 (13)</td>
<td>46 (13)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>15/12</td>
<td>11/5</td>
</tr>
<tr>
<td>Cause of cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral hepatitis (B and C) (%)</td>
<td>10 (37)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Viral hepatitis and alcohol (%)</td>
<td>2 (7.5)</td>
<td></td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>7 (28)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>PBC/PSC (%)</td>
<td>5 (18.5)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Other (%)</td>
<td>3 (11)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Albumin (g/l)*</td>
<td>31 (7)</td>
<td>33 (8)</td>
</tr>
<tr>
<td>Bilirubin (µmol/l)*</td>
<td>155 (37)</td>
<td>85 (37)</td>
</tr>
<tr>
<td>Prothrombin time (s)*</td>
<td>26 (14)</td>
<td>20 (7)</td>
</tr>
<tr>
<td>WBC count (×10^9/mm^3)*</td>
<td>6.2 (0.7)</td>
<td>8.5 (1.0)</td>
</tr>
<tr>
<td>Platelet count (×10^9/mm^3)*</td>
<td>67 (30)</td>
<td>230 (57)</td>
</tr>
</tbody>
</table>

*Mean (SD).

THROMBOPOIETIN ASSAY

TPO was measured in serum samples with an enzyme linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, Minnesota, USA), which uses the quantitative sandwich type technique. ELISA plates were precoated with murine monoclonal antibody against TPO. Standards (recombinant human TPO in a buffered protein base with preservative, lyophilised) and samples were incubated on the plates for three hours. Bound TPO was detected using monoclonal antibody against TPO conjugated to horseradish peroxidase. Plates were washed between steps. The optical density of each well was determined using a microplate reader set to 450 nm within 30 minutes. Samples were stored at −70°C until analysed. All samples were assayed in duplicate. The lower detection limit of the assay was 20 pg/ml. The intra-assay variation was 7.5% and the interassay variation 10.5%.

STATISTICAL ANALYSIS

Results are presented as mean (SD) if quantitative variables were normally distributed and as median (range) if they were not. Serum TPO concentrations are not normally distributed, therefore the significance of the differences between group means of TPO was assessed by the Mann-Whitney U test. The Wilcoxon signed rank test was used for the analysis of paired samples. The Spearman’s rank correlation coefficient was used to examine the association between the parameters. A p value less than 0.05 was considered statistically significant.

Results

The study population comprised 43 patients. The cause of cirrhosis was alcoholic in 11 cases, viral hepatitis (due to hepatitis B virus (HBV) or HCV) in 12, viral hepatitis and alcohol in two, primary biliary cirrhosis in seven, primary sclerosing cholangitis in four, Wilson’s disease in one, Budd-Chiari syndrome in one, cryptogenic in two, and other causes in three. Table 1 shows the demographic and laboratory characteristics of the patients.

There were 27 patients in group 1 and 16 in group 2. Baseline platelet counts (mean (SD)) were 66 (30) × 10^9/l in patients from group 1 and 230 (87) × 10^9/l in those from group 2 (p<0.0001; table 1). Patients who had OLT had an uneventful post-transplantation period without serious complications. The protocol biopsies done on the seventh day after transplantation showed no features of acute

Patients and methods

PATIENT POPULATION

Platelet counts and serum TPO concentrations were measured in 43 patients with cirrhosis of the liver diagnosed by histological evaluation of hepatic biopsy specimens. Patients with hepatocellular carcinoma and disseminated intravascular coagulation (DIC) were excluded from the study.

Twenty three of the 43 patients underwent OLT because of end stage liver disease. These patients received an ABO matched cadaver liver and the standard immunosuppressive regimen consisted of tacrolimus or cyclosporine, azathioprine, and corticosteroids. All 23 patients who underwent OLT had a generally uneventful post-transplantation period without serious complications. Liver biopsies were performed by protocol seven days after transplantation.

Serum samples for TPO measurement were obtained from the 23 patients who had OLT, on the day before OLT, and on days 1, 3, 5, 7, 10, and 14 after OLT. One random serum sample was also taken from the 20 cirrhotic patients who did not undergo OLT. Platelet counts were measured on the day of TPO measurement for the non-transplanted patients who did not undergo OLT. Platelet counts and serum TPO concentrations were performed by protocol seven days after transplantation.

Serum samples for TPO measurement were obtained from the 23 patients who had OLT, on the day before OLT, and on days 1, 3, 5, 7, 10, and 14 after OLT. One random serum sample was also taken from the 20 cirrhotic patients who did not undergo OLT. Platelet counts were measured on the day of TPO measurement for the non-transplanted patients who did not undergo OLT. Platelet counts and serum TPO concentrations were performed by protocol seven days after transplantation.

According to baseline platelet count all the cirrhotic patients were divided into two groups: group 1 consisted of patients with thrombocytopenia (platelet count not more than 120 × 10^9/l) and group 2 patients without thrombocytopenia (platelet count greater than 120 × 10^9/l). The cut off point of 120 × 10^9/l was used in accordance with recent literature. In addition serum TPO concentrations and platelet counts were measured in 21 normal individuals (group 3).

Figure 1  Baseline TPO concentrations in cirrhotic patients with or without thrombocytopenia (TCP) and normal controls. Boxes represent the 50% percentile of the values; the inside line represents the median value.

<table>
<thead>
<tr>
<th>Patients with TCP</th>
<th>Patients without TCP</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>250</td>
<td>750</td>
</tr>
<tr>
<td>750</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>
cellular rejection in eight patients, mild rejection in 10, and moderate rejection in five.

TPO concentrations (median (range)) before OLT were significantly lower in patients with low platelet count (n=27) compared with those with normal platelet count (n=16) (92.5 (20.3–286.3) versus 226.6 (30.1–848.3) pg/ml, p=0.003) and to normal controls (92.5 (20.3–286.3) versus 158.3 (22.5–232.9) pg/ml, p=0.025). TPO concentrations in patients with normal platelet count did not differ from those in normal controls (p=0.11; fig 1).

Thrombopoietin concentrations increased from day 1 after OLT and reached a peak on day 5. The rise in TPO concentrations was significant in patients with low baseline platelet count (89.1 (21.3–247.6) to 545.1 (66.2–2569) pg/ml, p=0.001), but not in transplanted patients with normal baseline platelet count (262.8 (30.1–848.3) to 315.1 (114–954.6) pg/ml, p=0.47; table 2). Platelet counts decreased further from baseline in all the transplanted patients in the first days after OLT and reached a nadir concentration on day 5 post-OLT, which was the day of the peak TPO concentrations. Platelet counts subsequently increased and returned to normal concentrations four to six days after peak TPO (table 2), while TPO concentrations returned to normal values.

**RELATION BETWEEN PLATELET COUNT AND TPO CONCENTRATIONS**

Pre-OLT there was no correlation between spleen volume (as determined by spleen index) and platelet count ($r=−0.11$, $p=0.6$; fig 2) or TPO concentrations ($r=−0.04$, $p=0.8$; fig 3). However, there was a significant correlation between TPO concentrations before OLT and platelet count ($r=0.47$, $p=0.001$; fig 4). After transplantation there was an inverse correlation between peak TPO concentrations and nadir platelet count ($r=−0.41$, $p=0.049$; fig 5).

There was no statistical correlation between peak TPO concentrations after OLT and cold ischaemia time ($r=0.1$, $p=0.62$), prothrombin time ($r=−0.04$, $p=0.9$), or other parameters of liver function: maximum serum aspartate aminotransferase ($r=−0.001$, $p=0.98$), maximum serum alanine aminotransferase ($r=−0.04$, $p=0.85$), and maximum bilirubin ($r=−0.13$, $p=0.6$). Finally, there was no difference in the TPO concentrations between patients with (n=15) or without histological rejection (n=8) (412.9 (66.2–2569) versus 496.2 (85.1–1746) pg/ml, $p=0.97$).

**Discussion**

Serum TPO concentrations were measured in patients with end stage cirrhosis who were on the waiting list for liver transplantation. TPO concentrations were significantly lower in cirrhotic patients with thrombocytopenia (platelet count not more than $120 \times 10^9/l$) compared with those with normal platelet count (greater than $120 \times 10^9/l$). There was also
Thrombopoietin before and after orthotopic liver transplantation

757

inconsistent results, due mainly to di

in patients with liver cirrhosis have shown

Previous studies in which TPO was measured

thrombocytopenia of end stage liver disease.

in thrombocytopenia.18 The identification of

not new. Several years ago, it was reported that

tors capable of stimulating thrombopoiesis is

trations in the serum of all cirrhotic patients

limitations of the assays in previous studies16 17

a peak on the fifth day. The ELISA used in this

study (R&D Systems, Minneapolis, Minne-

ics with thrombocytopenia starting from the

first day after liver transplantation and reaching a

peak on the fifth day. The ELISA used in this

study (R&D Systems, Minneapolis, Minne-

sota, USA) was highly sensitive, having a lower
detection limit of 20 pg/ml. This overcame the

limitations of the assays in previous studies16 17

and permitted measurement of TPO concent-

rations in the serum of all cirrhotic patients

and normal subjects.

The hypothesis that liver could produce fac-
tors capable of stimulating thrombopoiesis is

not new. Several years ago, it was reported that

in rodent models, partial hepatectomy results in

thrombocytopenia.29 The identification of

the liver as the main source of TPO produc-
tion10 11 has led to the suggestion that deficien-
cy of this cytokine contributes to the

thrombocytopenia of end stage liver disease.

Previous studies in which TPO was measured

in patients with liver cirrhosis have shown

inconsistent results, due mainly to differences

in the study populations.13–15 19 The main prob-

lem with the interpretation of these studies is

that classification of patients in the different

study groups was performed independently of

the platelet count. Although cirrhotics had

generally lower platelet counts than their

control populations used as comparison, there

were patients with normal platelet counts in the

cirrhotic groups.9 10 12 Hence the concentra-
tions of TPO in cirrhotic patients with thrombocyto-

penia, which is the subgroup of interest, was

never clear. Furthermore, the differences in the

severity of liver disease are not generally taken

into account.

Our population consisted exclusively of

patients with end stage cirrhosis (with no Child

class A patients) who were on the waiting list

for liver transplantation. Over half of them

subsequently underwent liver transplantation, and

the rest were still waiting. Moreover we

separated patients with cirrhosis into those with

and without thrombocytopenia. We found that

patients without thrombocytopenia had

TPO concentrations comparable with the nor-

mal controls. In contrast, patients with throm-

bocytopenia had significantly lower TPO con-

centrations. We also found a significant corre-

lation between TPO concentrations and platelet
count in the whole group of cirrhotic patients. We

assume that cirrhotic patients, in whom production of TPO in the liver is main-
tained despite the severe liver disease, could counteract the sequestration of platelets in the

spleen and maintain normal platelet count. In

contrast, patients with inadequate production

do the cytokine inevitably develop thrombocyto-

penia. The recent findings of Schimodaira et al

that serum TPO concentrations may de-

crease with deterioration of protein producing

ability of the liver in cirrhotic patients are in

keeping with our data.11 More recently Sezai et

al have reported that serum TPO concentra-
tions are associated with portal haemodynamics.19 They performed colour

Doppler ultrasonography of the splenic vein

and showed that patients with hepatofugal flow
direction, which usually indicates progressive

liver disease, had lower serum TPO concentra-
tions.

The inability of cirrhotic patients to increase

platelet production by the bone marrow in

response to increased consumption has been

reported previously as the main determinant of

thrombocytopenia in a seminal study by Stein

and Harker.7 We suggest that this is caused by

inadequate TPO production. Another hypoth-
esis that has been proposed to explain throm-

bocytopenia together with low TPO concentra-
tions in patients with cirrhosis was through an

ill defined consequence of hypersplenism—

that is, the sequestration of platelets bound

with TPO in the spleen. However, we found no

correlation between the spleen volume and

platelet count before OLT. This finding is con-

sistent with previous reports that alleviation of

portal hypertension either by portocaval anas-
tomoses or TIPS has not had significant effects

in reducing thrombocytopenia as it usually
does not lead to a persistent increase in platelet

count.6 7 In addition the spleen volume does

not appear to affect serum TPO, as we found

no correlation between spleen index and TPO

concentrations. This is in keeping with a recent

study reporting that splenectomy does not sig-

ificantly alter the serum TPO concentration.11

In patients who underwent OLT, platelet

count decreased further in the first days after

transplantation as has been reported in previ-

ous studies.20 21 At the same time TPO concen-

trations increased and reached a peak on day 5

after transplantation. The peak in TPO con-

centrations was reached just after the nadir in

platelet count after transplantation. The in-

crease in TPO concentrations was significant

for the patients with pretransplantation throm-

bocytopenia. We also found an inverse corre-

lation between peak TPO concentrations and

nadir platelet count after OLT similar to that

observed in states of myelosuppression accom-

panying chemotherapy or radiation.11 22 The

increase in TPO concentrations was followed

by a subsequent rise in numbers of platelets

and return to normal counts within four to six

days after peak TPO. The time course between

peak TPO and subsequent normal platelet

count is the same as that reported in previous

experimental and clinical studies of myelosup-

pression induced thrombocytopenia.11 21 23 All

these data provide strong evidence that re-

stored TPO production accounts for the rapid

normalisation of platelet counts after OLT. It is

important to mention that this rapid resolution

of thrombocytopenia after transplantation oc-

curs in the context of increased activation and

sequestration of thrombocytes in the graft dur-

ing the first days after liver transplantation as

has been shown recently.25 26 This underlines

the importance of the restitution of adequate

TPO production.

Three previous series have reported a similar

increase in TPO after orthotopic liver

transplantation.14 17 27 However, neither of these

studies evaluated the volume of the spleen in

order to clarify its role in the thrombocytopenia

of liver cirrhosis. We believe that our study pro-

vides firm data to substantiate the association
of low TPO concentrations with low platelet counts in cirrhosis, but high TPO concentrations with low platelet counts after OLT when normal synthesis is restored.

Finally, we found that the restored TPO production after liver transplantation was independent of the allograft function as we found no correlation between peak TPO and cold ischaemia time, peak aminotransferase and bilirubin concentrations after transplantation, and the presence of histological rejection.

In conclusion, our findings provide evidence that inadequate TPO production seems to be the major cause for thrombocytopenia in liver cirrhosis, although increased splenic sequestration and the presence of histological rejection. Orthotopic liver transplantation can readily restore TPO production and lead to a rapid resolution of thrombocytopenia, and shows the putative normal feedback mechanism for maintenance of platelet numbers.

14 Akriviadis EA, Cohen SM, Chen DCP, et al. Thrombopoietin (TPO) levels are increased in patients with cirrhosis and do not appear to play a role in the pathogenesis of thrombocytopenia of cirrhosis [abstract]. Hepatology 1997;26:184A.
Thrombopoietin concentrations are low in patients with cirrhosis and thrombocytopenia and are restored after orthotopic liver transplantation

J Goulis, T N Chau, S Jordan, A B Mehta, A Watkinson, K Rolles and A K Burroughs

Gut 1999 44: 754-758
doi: 10.1136/gut.44.5.754

Updated information and services can be found at:
http://gut.bmj.com/content/44/5/754

These include:

References
This article cites 24 articles, 0 of which you can access for free at:
http://gut.bmj.com/content/44/5/754#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/