Thrombocytopenia, an Important Interfering Factor of Antiviral Therapy and Hepatocellular Carcinoma Treatment for Chronic Liver Diseases


Departments of Digestive Disease Information & Research* and Medicine**, Kurume University School of Medicine, Kurume 830-0011, Japan

Received 12 June 2009, accepted 30 July 2009

Edited by KOICHI OSHIMA

Summary: In patients with chronic liver diseases, thrombocytopenia is a common manifestation which interferes with antiviral therapy for hepatitis C virus (HCV), and with hepatocellular carcinoma (HCC) treatment. While thrombopoietin-receptor agonist is expected to improve thrombocytopenia for patients with chronic liver diseases in 2-3 weeks, there is still a lack of fundamental data about short-term variations in the natural course of platelet count in cirrhotic patients, and the impact of thrombocytopenia on antiviral therapy for HCV-infected patients and patients being treated for HCC. The aims of this study are to investigate sequential changes in platelet count and the impact of thrombocytopenia on antiviral therapy and HCC treatment in patients with chronic liver diseases. A total of 726 chronic liver disease patients were enrolled in this study. Changes of platelet count were examined during a 4-week follow-up. Risk of discontinuation or reduction of peginterferon dosage was evaluated in HCV patients with moderate thrombocytopenia (5-10×10⁴/μL). Risk of platelet transfusion or splenectomy was evaluated in HCC patients with severe thrombocytopenia (<5×10⁴/μL). No significant changes of platelet count were observed in cirrhotic patients with thrombocytopenia during a 4-week follow-up. The rate of discontinuation or reduction in dosage of peginterferon was 85.2% (23/27) in patients with moderate thrombocytopenia. Risk of discontinuation or reduction of peginterferon dosage was 3.4-times higher in HCV patients with thrombocytopenia than in those without thrombocytopenia. In HCC patients with severe thrombocytopenia, the frequency of platelet transfusion or splenectomy during HCC treatment was 57.9% (22/38). Risk of platelet transfusion or splenectomy in HCC patients with thrombocytopenia was 57.9-times higher than in those without thrombocytopenia. In conclusion, we demonstrated no significant variation in the short-term natural course of platelet count in cirrhotic patients. In chronic liver disease patients with moderate and severe thrombocytopenia, about 85% of patients treated with peginterferon, and 60% of patients receiving HCC treatments suffered from thrombocytopenia-related limitations, respectively.

Key words eltrombopag, thrombocytopenia, peginterferon, hepatocellular carcinoma, liver cirrhosis

INTRODUCTION

Thrombocytopenia is a common manifestation in patients with chronic liver diseases [1,2]. Thrombocytopenia is also an exclusion criterion for antiviral therapy in patients with chronic hepatitis C virus (HCV) infection [3]. Development of thrombocytopenia during antiviral therapy also causes discontinuation or reduction of dosage [3,4]. In addition, severe thrombocytopenia interferes with hepatocellular...
carcinoma (HCC) treatment [5]. Severe thrombocytopenia has generally been considered a contraindication for heptatectomy [6]. Thus, thrombocytopenia is an important factor that can interfere with clinical treatments in patients with chronic liver diseases. Splenectomy or partial splenic embolization is generally considered an effective therapeutic approach for thrombocytopenia [7-10]. However, these therapeutic procedures are invasive and are not always an option for patients with advanced chronic liver diseases like liver cirrhosis.

There are various theories about thrombocytopenia in chronic liver diseases. Portal hypertension, hypersplenism and bone marrow suppression are factors associated with thrombocytopenia [11,12]. In patients with HCV infection, direct megakaryocyte suppression and antibody-mediated platelet destruction are also involved in the development of thrombocytopenia [12]. In addition, decreased production of thrombopoietin, a hematopoietic growth factor, was recently found to be a causative factor associated with thrombocytopenia in patients with chronic liver diseases [13,14]. Moreover, both interferon and chemotherapy induce decreased platelet count through down-regulation of thrombopoietin production [15,16] and capture of platelets by liver [17,18].

Several new drugs for thrombocytopenia are now under development [19]. A new orally administered thrombopoietin-receptor agonist named eltrombopag increases platelet production through induction of proliferation and differentiation of megakaryocytes in vivo [20]. In a phase 1 clinical study of eltrombopag, platelet number began rising at day 5 and peaked at day 15 in healthy male subjects [21]. Results from a randomized placebo-controlled clinical trial showed eltrombopag increased platelet counts in a dose-dependent manner in patients with chronic immune thrombocytopenic purpura [22]. The effectiveness and safety of eltrombopag on thrombocytopenia were demonstrated in a phase II clinical trial in 74 patients with liver cirrhosis [23]. After 4 weeks of treatment platelet counts significantly increased in a dose-dependent fashion. Thus, eltrombopag is the first thrombopoietic drug to safely and consistently increase platelet count in patients with liver cirrhosis. However, there are several issues which need to be considered and clarified before eltrombopag can be generally prescribed and administered to patients with chronic liver diseases. Among these is the question of short-term sequential changes in platelet count in cirrhotic patients. There is currently no available data on weekly changes in platelet count, and therefore it is important to clarify the natural course of platelet count in cirrhotic patients.

The aims of the present study were to investigate 1) short-term sequential changes of platelet count in cirrhotic patients and 2) the impact of thrombocytopenia on antiviral therapy and HCC treatment in patients with chronic liver diseases.

MATERIALS AND METHODS

Subjects

A total number of 726 patients with chronic liver diseases were enrolled in the study. For investigation of short-term sequential changes of platelet count, a case-series study was conducted. Twenty-four (24) patients with liver cirrhosis (age 67.4±10.7; male/female=12/12; HCV-related, n=17; hepatitis B virus-related, n=2; alcoholic, n=1; cryptogenic, n=4) were enrolled via consecutive entry. All patients were hospitalized for treatment of ascites, hepatic encephalopathy, or diabetes mellitus. In order to eliminate treatment effects on platelet count, patients who were treated with interferon, radiofrequency ablation, transarterial chemoembolization, chemotherapy, endoscopic variceal ligation, or endoscopic injection sclerotherapy were excluded. Patients with bacterial infection were also excluded, because inflammation affects platelet count [24].

For investigation of risks of thrombocytopenia-related limitations during antiviral treatment, 190 patients with chronic HCV infection were enrolled. Inclusion criteria were i) antiviral treatment aiming at elimination of HCV, ii) HCV genotype 1b, and iii) HCV viral load >2.0 log IU/mL or >100 KIU/mL. Patients who were receiving low-dose peginterferon for prevention of HCC development were excluded.

For investigation of risks of thrombocytopenia-related limitations during HCC treatment, 512 patients with HCC treated by radiofrequency ablation, transcatheter arterial chemoembolization or hepatic arterial infusion chemotherapy (Child-Pugh grade A, n=371; grade B, n=120; grade C, n=21) were enrolled. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, 1984, and the Declaration of Tokyo, 1975 as reflected in a prior approval by the institutional review committee.
Laboratory determinations

Venous blood samples were taken in the morning after a 12-hour overnight fast. Platelet count, hemoglobin levels, white blood cell count, prothrombin time, and serum levels of alanine aminotransferase (ALT), albumin, total bilirubin, and C-reactive protein (CRP) were measured using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital) as previously described [25].

Short-term sequential changes of platelet count

During a 4-week follow-up period in patients with liver cirrhosis, sequential changes of platelet count were examined in moderate (5 to 10×10^4/μL of platelet count) and severe thrombocytopenia groups (<5×10^4/μL of platelet count) as previously described [26,27]. Platelet count and biochemical parameters were examined once a week for 4 weeks.

Incidence and risk of thrombocytopenia-related limitations during antiviral treatment for HCV

Risk of discontinuation or reduction of peginterferon dosage (peginterferon alfa-2a or peginterferon alfa-2b combined administration with ribavirin) was evaluated retrospectively in HCV patients with moderate thrombocytopenia over their entire treatment period. None of the patients discontinued or reduced peginterferon dosage because of socioeconomic conditions.

Incidence and risk of thrombocytopenia-related limitations during HCC treatment

Inclusion criteria for platelet transfusion and splenectomy were a decrease in platelet count (<3×10^4/μL) or an aggravation of hemorrhagic status, such as subcutaneous hemorrhage and gingival hemorrhage. Risk of platelet transfusion or splenectomy during HCC treatment was evaluated retrospectively in patients with severe thrombocytopenia. Patients treated with splenectomy for interferon therapy after HCC treatment were also included.

Statistical analysis

All data are expressed as mean±SD for continuous variables. Statistical significance of changes in parameters during a 4-week period was analyzed by using the Friedman test. Statistical significance of risks of thrombocytopenia-related limitations during interferon and HCC treatments was analyzed by chi-squared test. P values <0.05 were considered significant.

RESULTS

Sequential changes in platelet count

During a 4-week natural course follow-up, there was no significant change in platelet count in any of the patients (Table 1). Serum levels of ALT, albumin, total bilirubin, CRP, and prothrombin time also showed no significant changes in any patients (Table 1). In a stratified analysis, no significant change of platelet count was observed in either the moderate thrombocytopenia group or the severe thrombocytopenia group during a 4-week natural course follow-up (Fig. 1). Levels of other biochemical parameters were not significantly different during a 4-week follow-up in either the moderate thrombocytopenia group or the severe thrombocytopenia group (data not shown).

Incidence and risk of thrombocytopenia-related limitations during interferon treatment

Moderate thrombocytopenia was observed in 14.2%...
(27/190) of HCV patients receiving combined peginterferon and ribavirin treatment in this study (Table 2). The incidence of discontinuation or reduction in dosage of peginterferon was 85.2% (23/27) due to progression to severe thrombocytopenia (<5×10⁴/μL). This rate was significantly higher than the incidence of 62.6% (102/163) observed in patients with minor thrombocytopenia or normal range (Table 2). The odds ratio was 3.4. Although the sustained virologic response (SVR) rate was 29.6% in patients with moderate thrombocytopenia, there was no significant difference in SVR rate between patients with and without moderate thrombocytopenia (Table 3).

Incidence and risk of thrombocytopenia-related limitations during HCC treatment

Severe thrombocytopenia was observed in 3.2% (12/371), 19.2% (23/120), 14.3% (3/21) of HCC patients in Child-Pugh grade A, B, and C groups, respectively. The incidence of platelet transfusion or splenectomy during HCC treatment was 57.9% (22/38) in patients with severe thrombocytopenia. This was significantly higher than the incidence of 2.4% (11/463) seen in HCC patients without severe thrombocytopenia (Table 4). The odds ratio was 57.9.

---

### Table 2.
**Effects of mild thrombocytopenia before treatment on discontinuation or reduction of peginterferon in patients with chronic hepatitis C**

<table>
<thead>
<tr>
<th>Platelet count</th>
<th>Discontinuation or reduction</th>
<th>Number of Patients</th>
<th>Odds Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10×10⁴/μL</td>
<td>Yes</td>
<td>23</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥10×10⁴/μL</td>
<td>Yes</td>
<td>102</td>
<td>61</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td></td>
<td>125</td>
<td>65</td>
<td>190</td>
</tr>
</tbody>
</table>

Note. Data are expressed as percentage of the total number of patients and number of patients of each category. Statistical significance was analyzed by chi-squared test.

### Table 3.
**Effects of mild thrombocytopenia on sustained viral response rate in patients with chronic hepatitis C**

<table>
<thead>
<tr>
<th>Platelet count</th>
<th>Sustained virologic response</th>
<th>Number of Patients</th>
<th>Odds Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10×10⁴/μL</td>
<td>Yes</td>
<td>8</td>
<td>19</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥10×10⁴/μL</td>
<td>Yes</td>
<td>75</td>
<td>88</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td></td>
<td>83</td>
<td>107</td>
<td>190</td>
</tr>
</tbody>
</table>

Note. Data are expressed as percentage of the total number of patients and number of patients of each category. Statistical significance was analyzed by chi-squared test.
DISCUSSION

This study demonstrated that there were no changes in platelet count during a 4-week natural course follow-up in patients with chronic liver diseases. About 85% of patients receiving thrombocytopenia-related antiviral treatments either had to discontinue or reduce peginterferon dosage due to clinical thrombocytopenia conditions, and 60% of patients receiving HCC treatment were similarly affected by thrombocytopenia-related limitations.

Although platelet count is unstable and is known to decrease with disease progression in cirrhotic patients [28], no data is available for short-term sequential changes in platelet count in cirrhotic patients. After treatment with eltrombopag, a thrombopoietin-receptor agonist, platelet number begins rising at day 5 and peaks at day 15 [21]. Thus, it is important to clarify the natural course of platelet count in cirrhotic patients before eltrombopag can be generally administrated to patients with chronic liver diseases. In this study, we first demonstrated that platelet count had no significant variation during a 4-week follow-up in patients with liver cirrhosis. Because invasive treatments may affect platelet count, as observed in a previous study [29], hospitalized cirrhotic patients receiving non-invasive treatments were selected for investigation of short-term sequential changes of platelet count. As hepatic inflammation and liver function are also known to affect platelet count [28], laboratory biochemical and liver function parameters were also examined and no significant changes in ALT, albumin, total bilirubin, CRP levels, and prothrombin time were found during a 4-week period. Excluding these factors, the absence of a significant change in platelet count among cirrhotic patients in this study likely reflects the natural course of this disease. Considering that one possible reason for thrombocytopenia is decreased thrombopoietin production in patients with chronic liver diseases [14], administration of eltrombopag, a thrombopoietin-receptor agonist, is expected to be helpful in treating patients with chronic liver diseases at various stages.

Thrombocytopenia can prevent antiviral treatment in patients with chronic HCV infection, however little information is available regarding this issue. In our study, the risk of discontinuation or reduction of peginterferon dosage was about 85% in patients with moderate thrombocytopenia. This was significantly higher than that in patients without moderate thrombocytopenia. Similarly, dose modifications of peginterferon are required in about 20% of patients with no thrombocytopenia [30]. Interferon decreases platelet count through suppression of differentiation in megakaryocytes [16] and capture of platelets by liver [17,18]. A novel thrombopoietin mimetic improves interferon alpha-induced thrombocytopenia in vivo [16]. In humans, eltrombopag increases platelet count in cirrhotic patients with HCV infection. Thus, eltrombopag may reduce the risk of discontinuation or reduction of peginterferon dosage in HCV patients.

Although no significant association between moderate thrombocytopenia and SVR rate, which is the rate of continued undetectable serum HCV-RNA 6 months after the completion of anti-viral treatment, was found in our study, Backus et al reported that decreased platelet count is an independent negative predictor for SVR rate in HCV patients treated with peginterferon and ribavirin [31]. The reason for this discrepancy is unclear. However, one possibility is the effect of confounding factors for continuation of

<table>
<thead>
<tr>
<th>Platelet transfusion or splenectomy</th>
<th>Number of Patients</th>
<th>Odds Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>22</td>
<td>38</td>
<td>57.875</td>
</tr>
<tr>
<td>No</td>
<td>16</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelet count &lt;5×10⁴/μL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count ≥5×10⁴/μL</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Patients treated with splenectomy for initiation of interferon therapy is included. Data are expressed as percentage of the total number of patients and number of patients of each category. Statistical significance was analyzed by chi-squared test.
This study was supported, in part, by a Grant-in-Aid for Young Scientists (B) (No. 19790643 to T.K.) and a Grant-in-Aid for Scientific Research (C) (No. 21590865 to M.S.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by Health and Labour Sciences Research Grants for Research on Hepatitis from the Ministry of Health, Labour and Welfare of Japan, and by a Grant for Cancer Research from the Fukuoka Cancer Society.

ACKNOWLEDGMENTS: This study was supported, in part, with SVR rate such as sex, age, hepatic fibrosis, and insulin resistance were not matched between patients with and without moderate thrombocytopenia. Thus, these factors may account for the discrepancy between our data and the previous report. Another possibility is differences in administration strategy for peginterferon and ribavirin. In the other study the treatment period was 48 weeks and overall SVR rate was 20.5%. On the other hand, our treatment period was up to 72 weeks and SVR rate was 29.6% even in patients with moderate thrombocytopenia. Thus, prolonged treatment period might have improved the SVR rate, thus eliminating the association between moderate thrombocytopenia and SVR rate.

HCC is now treated with invasive therapies such as resection and radiofrequency ablation, and non-invasive therapies such as chemotherapy [32]. In either case, severe thrombocytopenia seems to adversely affect patient tolerance of these HCC treatments. However, the real incidence of thrombocytopenia-related limitation for HCC treatments is unclear. In our study, about 60% of HCC patients with severe thrombocytopenia received platelet transfusion or splenectomy to improve their thrombocytopenia status. This proportion may be higher than that in other general institutions, because our hospital is a government designated oncology center specializing in HCC. Therefore, we have a relatively high proportion of severe end stage patients desiring intensive care. Although splenectomy and arterial splenic embolization are efficient methods for treating HCC patients with severe thrombocytopenia [7-10], these treatment options are not always available for patients with advanced liver cirrhosis. Thus, severe thrombocytopenia is still a major unresolved issue affecting HCC treatment, and eltrombopag, a thrombopoietin-receptor agonist, is expected to improve HCC treatment.

In conclusion, we demonstrated no change in platelet count during a 4-week natural course follow-up in patients with liver cirrhosis. In patients with moderate and severe thrombocytopenia, about 85% of patients treated with peginterferon and 60% of patients receiving HCC treatment suffered from thrombocytopenia-related limitations, respectively. Since little information is available about short-term sequential changes in platelet count and thrombocytopenia-related limitations on interferon and HCC treatment, this report will provide fundamental information useful when considering administration of eltrombopag to patients with chronic liver diseases.

REFERENCES


