Platelets Activation and Liver Transplantation

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Abstract

Transient thrombocytopenia is a common phenomenon after living donor liver transplantation (LDLT), and severe thrombocytopenia after LDLT is associated with graft loss and poor patient outcomes. The various causes of thrombocytopenia include bone marrow hematopoiesis failure due to decreased thrombopoietin (TPO) production in the injured liver, platelet destruction associated with splenomegaly, and the activation and consumption of platelets due to various forms of thrombosis, including disseminated intravascular coagulation (DIC), thrombotic microangiopathy (TMA), and venous thromboembolism (VTE).

The observation of biomarkers such as soluble platelet glycoprotein VI (sGPVI), TPO, von Willebrand factor (VWF), VWF propeptide (VWFpp), and disintegrin-like and metalloproteinase with thrombospondin type-1 motifs member 13 (ADAMTS13) is useful in the evaluation of the mechanisms of thrombocytopenia in patients who undergo LDLT. The presence of these biomarkers, including sGPVI, ADAMTS13, VWF and VWFpp, suggests that platelet activation occurs in the early phase of LDLT and that vascular endothelial cell injury occurs on post-operative days 7-14.

Keywords: sGPVI; Living donor liver transplantation; Thrombocytopenia; Mortality; ADAMTS13

Introduction

Living donor liver transplantation (LDLT) was first performed in Japan in 1989 [1]. Unique technical, physiological, and logistical innovations in LDLT [2,3] have since developed. Over the last two decades, LDLT operations have been markedly improved by innovations that now achieve results comparable to those obtained with deceased donor liver transplantation (DDLT). Technical improvements in living donor surgery have led to the generalization of pediatric LDLT with excellent patient and graft survival outcomes [4]. There is no evidence to support a higher incidence of hepatocellular carcinoma (HCC) recurrence after LDLT than after DDLT [5].

However, room for further innovation remains, particularly with adult LDLT. The 5-year graft survival rate is less than 70% when ABO-incompatible LDLT is performed in children [2,6], and specific diseases and preoperative patient conditions are associated with different transplantation outcomes [3,7,8]. In the registry of the Japanese Liver Transplantation Society from November 1989 to December 2010, the 1-, 5-, 10-, and 20-year patient survival rates were 88.3%, 85.4%, 82.8%, and 79.6%, respectively [9]. The 1-, 5-, and 10-year overall survival and disease-free survival rates after LDLT for patients with combined hepatocellular-cholangiocarcinoma were 87.5%, 72.9%, and 48.6% and 85.7%, 85.7%, and 85.7%, respectively [10]. Nationwide surveys of acute liver failure (ALF) are conducted annually in Japan, and 20% of patients with ALF undergo liver transplantation (LT) [11]. In LDLT for patients with ALF, the cumulative patient survival rate at 1 year after LT was 79% [11]. The causes of a poor outcomes after LDLT are said to include graft dysfunction and various complications such as infection, thrombosis, bleeding, graft-versus-host disease [12], thrombotic microangiopathy (TMA) [13,14], and disseminated intravascular coagulation (DIC) (Table 1) [15].

Table 1: The causes of thrombocytopenia in LDLT.

Role of Platelets

Platelets, which are anucleate blood cells, are derived from megakaryocytes, which play important roles in hemostasis. Platelets contain not only the proteins needed for hemostasis such as serotonin, adenosine 5′-diphosphate, adenosine 5′-triphosphate, and sphingosine 1-phosphate, but also many growth factors such as hepatocyte growth factor, insulin-like growth factor, vascular endothelial growth factor, epidermal growth factor, platelet-derived growth factor, and transforming growth factor-β, which are required for tissue regeneration or repair [16-18]. Platelets play a crucial role in...
promoting liver regeneration [19,20] as well as both preventive and promoting effects on the progression of liver fibrosis and both protective and harmful effects concerning acute liver injury in vitro and in vivo. In the clinical setting, the increase in the number of platelets induced by platelet transfusion improves the liver function in patients with chronic liver diseases and cirrhosis [18]. In addition, it has been reported that splenectomy, which increases the platelet count, contributes to the improvement of the liver function [20]. However, increased platelets have been conversely reported to exert harmful effects on liver fibrosis and acute liver injury including thrombosis, viral hepatitis, and ischemia-reperfusion [18,21].

**Thrombocytopenia and Patient Outcomes**

Transient thrombocytopenia is a common phenomenon after LT, and the recovery of platelet counts is clinically significant. In 1992, McCaughan et al. [11] were the first to report that thrombocytopenia on post-operative day (POD) 14 after LT was associated with the patient survival: the platelet counts in non-survivors were significantly lower than those in survivors \( (88 \times 10^3 \text{ vs. } 174 \times 10^3/\mu\text{l}; p<0.01). \) Furthermore, they reported that graft liver dysfunction was the most useful independent predictor of a nadir of platelet counts after LT, although various mechanisms of thrombocytopenia were proposed. Following this report, two additional studies [22,23] confirmed that severe thrombocytopenia after LT was associated with graft loss or a poor patient outcome. However, the precise mechanisms by which post-transplant thrombocytopenia occurs and its relationship with graft dysfunction remain unclear. The mechanisms contributing to graft dysfunction are multifactorial and include small-for-size graft [5], old age [24], ischemic reperfusion injury, sinusoidal endothelial cells injury, platelet aggregation, immunological reactions, and inflammatory responses [25,26]. The platelet counts in patients with successful LT usually increase by POD14.

Although the patients in our study gradually recovered from early thrombocytopenia after LDLT within 14 days, there were several patients who showed a delayed recovery of their platelet count, exhibiting prolonged thrombocytopenia [27]. Adult LDLT patients were divided into 2 groups based on their platelet counts \( (100 \times 10^3 / \mu\text{l}) \) on POD14: high- and low-platelet (HP and LP, respectively) groups. The 6-month survival rate in the LP group was significantly lower than that in the HP group \( (61.1\% \text{ vs. } 93.5\%) \) [27], suggesting that a platelet count of \( <100 \times 10^3 / \mu\text{l} \) on POD14 is a strong predictor of the patient survival after LDLT (Figure 1).

It was recently reported in DDLT that a low platelet count on POD-5 was associated with graft loss and mortality after LT, thus suggesting that thrombocytopenia can be a poor prognostic marker [28].

**Splenectomy and Thrombocytopenia**

Splenectomy increases the platelet count almost without exception, as it removes the major site of platelet destruction and reduces antibody production, resulting in prolonged platelet survival times [29]. Post-splenectomy transient thrombocytosis occurs and unusually reaches a peak on POD14. However, it has been reported that the peak platelet count following splenectomy after LDLT occurs on POD28 [30-32]. Although splenectomy is not usually performed for DDLT patients, several transplant centers performing LDLT have introduced simultaneous splenectomy to control portal pressure in small-for-size graft recipients, preventing thrombocytopenia in HCV-positive recipients for whom postoperative direct antiviral agents instead of interferon treatment is planned, and for patients undergoing ABO-incompatible LDLT [30-32]. Marubashi et al. [31] found that seven patients who underwent simultaneous splenectomy showed a remarkable increase in their platelet counts after LDLT on POD14, with the peak in the platelet count seen at POD28. We encountered several LDLT patients who had suffered from prolonged thrombocytopenia even after splenectomy [27]. Our study also showed that the platelet counts after splenectomy in operations other than LDLT significantly increased on POD7 and peaked on POD14. In LDLT, however, the platelet counts remained low until POD7 and significantly increased on POD14 but did not peak on POD14, even after splenectomy.

**Biomarkers of Thrombocytopenia**

**Soluble platelet glycoprotein VI (sGPVI)**

sGPVI is a type I transmembrane glycoprotein of the immunoreceptor family that is constitutively associated and expressed with the Fc receptor γ-chain, an immunoreceptor tyrosine-based activation motif-bearing receptor [33]. Upon platelet activation, the platelet surface GPVI is cleaved by proteases, such as ADAM10, releasing sGPVI [34,35]. sGPVI has recently received attention as a platelet activation marker, as described below. Several groups have reported that sGPVI is a useful biomarker of diseases caused by platelet activation, such as acute coronary syndrome and stroke [36,37]. The plasma sGPVI levels have been reported to be significantly increased in patients with thrombosis during the postoperative period [38] and in patients with DIC or TMA [39], suggesting that the plasma sGPVI levels increase in a thrombotic state, which activates platelets (Tables 2 and 3).
Thrombopoietin (TPO) is produced at a constant rate mainly in the normal hepatocytes [41], is known as the primary platelet regulator [42], and the steady-state amount of TPO is regulated by the thrombopoietin receptor, which is present on platelets [43]. In patients with a normal liver function, when the platelet counts significantly decrease under circumstances such as massive bleeding, there is a significant increase in the TPO levels, resulting in the production of platelets by megakaryocytes.

**Von Willebrand factor (VWF) and VWF propeptide (VWFpp)**

Pre-pro VWF is synthesized in endothelial cells and megakaryocytes, the VWFpp is cleaved but remains stored together with mature VWF in alpha-granules (megakaryocytes) and Weibel-Palade bodies (endothelial cells). After the secretion of VWFpp and VWF into the plasma from endothelial cells (after induction by physiological or pathological stimuli), VWFpp dissociates from VWF [44]. VWF mediates the adhesion of platelets to sites of vascular damage by binding to specific platelet membrane glycoproteins.

**TPO-Thrombopoietin; sGPVI- Soluble platelet glycoprotein VI; VWF-Von Willebrand factor; VWFpp- VWF propeptide; ADAMTS13-Disintegrin-like and metalloproteinase with thrombospondin type-1 motifs member 13; UL-VWFM- Unusual large VWF multimers.**

### Table 2: The biomarkers for thrombocytopenia in LDLT.

<table>
<thead>
<tr>
<th>Before operation</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y=14.14+0.07X R=0.359 (p&lt;0.01)</td>
<td>Y= 21.24+0.05X R=0.201 (p&lt;0.05)</td>
<td>Y=33.38-0.10X R=-0.331 (p&lt;0.01)</td>
<td>Y=18.68+0.08X R=0.253 (p&lt;0.028)</td>
<td>Y=19.42+0.05X R=0.566 (p&lt;0.001)</td>
</tr>
<tr>
<td>X- Platelet count; Y- sGPVI.</td>
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### Table 3: Relationship between the platelet count and sGPVI levels.

**Thrombopoietin (TPO)**

Ichikawa et al. [40] described out the role of TPO, which is catabolized by platelets within the spleen: the serum TPO levels after splenectomy peaked on POD3–5 and were then significantly reduced, which caused transient thrombocytosis on POD14. TPO, which is produced at a constant rate mainly in the normal hepatocytes [41], is known as the primary platelet regulator [42], and the steady-state amount of TPO is regulated by the thrombopoietin receptor, which is present on platelets [43]. In patients with a normal liver function, when the platelet counts significantly decrease under circumstances such as massive bleeding, there is a significant increase in the TPO levels, resulting in the production of platelets by megakaryocytes.

### Von Willebrand factor (VWF) and VWF propeptide (VWFpp)

Elevated levels of VWF and VWFpp levels have been reported in cases of thrombotic thrombocytopenic purpura (TTP) [45] and DIC [46].

Disintegrin-like and metalloproteinase with thrombospondin type-1 motifs member 13 (ADAMTS13)

ADAMTS13 which is almost entirely produced by stellate cells in the hepatic sinusoid, specifically cleaves multimeric VWF [47,48]. In vascular endothelial cells, if plasma ADAMTS13 activity decreases, the number of unusually large VWF multimers (UL-VWFMs) significantly increases. Since UL-VWFMs show strong platelet aggregation activity, an increase in the level of UL-VWFMs leads to platelet clumping and/or thrombus formation [49]. Markedly decreased ADAMTS13 levels have been reported in cases of TTP [50].

**Mechanism of Thrombocytopenia in LDLT**

Thrombocytopenia is a common complication in liver diseases such as LDLT and occurs due to various causes, including bone marrow hematopoiesis failure due to the decreased production of TPO in the injured liver, increased platelet destruction with splenomegaly, the activation and consumption of platelets due to thrombosis, such as in cases of DIC [51], TMA [13], and venous thromboembolism (VTE) [52].

**Platelet production**

The TPO levels on POD14 were significantly higher in the LP group than in the HP group, while those on POD28 in the LP group were significantly decreased from those on POD14, being instead similar to those in the HP group despite the low platelet levels. This suggested that the constant production of TPO in the hepatocytes was preserved in the LP group on POD14, while on POD28 its production was significantly impaired, suggesting graft dysfunction. Although the reasons why the preoperative TPO levels were significantly higher in the LP group than in the HP group remained unclear, the low preoperative platelet counts in the LP group may be associated with the high TPO levels [27].

**Increase in platelet destruction with splenomegaly**

Thirty-eight (23.9%) of 159 adult patients who underwent LDLT were reported to have splenomegaly at 6 months after LDLT [53]. The spleen volume and the platelet levels at one month after LDLT may predict persistent splenomegaly at six months after LDLT. The predictive factors for hypersplenism at six months after LDLT may be the platelet levels at one week and at one month after LDLT. In a study to investigate the differences in the portal hemodynamics between DDLT and LDLT, although the portal venous pressure decreased after graft implantation, it was higher in LDLT patients with a smaller graft size than in DDLT patients [54].

**Activation and consumption of platelets due to thrombosis**

TMA: TMA is an infrequent but severe life-threatening disorder in solid organ transplant recipients. A small number of studies on TMA in LDLT recipients have been reported [13,14]. Decreased ADAMTS13 after LDLT might be associated with prolonged thrombocytopenia [55]. It has been reported that low ADAMTS13 activity may result from its low production or from increased consumption in the injured liver. In liver cirrhosis patients, the production of ADAMTS13 in hepatic stellate cells was reported to be decreased [56]. The behaviors...
of ADAMTS13, VWF, and VWFpp have previously been reported in LDLT patients with TMA [13,53].

DIC: Decreased ADAMTS13 and elevated VWFpp have been reported in patients with DIC [57] and TMA [13], suggesting that ADAMTS13 is also consumed through the continuing cleavage of VWF or from the low production of ADAMTS13 in DIC patients. Although DIC and TMA are different diseases, they display similar hemostatic abnormalities to patients with LDLT. It is considered that local DIC may occur in cases of ABO incompatible LDLT [58].

VTE: Forty-eight (17%) of the 282 consecutive adult LDLTs recipients between April 2006 and December 2011 had pre-existing portal vein thrombosis (PVT) [59]. Although a fatal outcome occurred in a severe PVT patient who received an LDLT [60], excellent survival rates were reported in patients with PVT who underwent LDLT [59]. In another study, 68 (2.9%) of 2402 patients who underwent LDLT had PVT and those patients with PVT were found to have a worse prognosis than those without PVT [61].

Medication

Antibiotics, immunosuppressive agents such as mycophenolate, tacrolimus, and cyclosporin after LDLT, heparin, and many drugs can cause thrombocytopenia. Although the mechanisms differ by drug, monitoring the platelet count is important in at-risk patients, and the suspected drugs should be decreased or stopped when adverse event occurs.

Behavior of Biomarkers in LDLT

Markedly decreased platelet counts and elevated sGPVI levels were observed, with the lowest platelet counts and the highest sGPVI levels seen on POD3. The sGPVI levels were positively correlated with the platelet counts before LDLT and negatively correlated with those on POD3, suggesting that the activation of platelets might be the highest on POD3. The ADAMTS13 levels in the patients with LDLT were the lowest on POD1, but the peaks differed between survivors and non-survivors [13,27,55]. The VWFpp levels were markedly elevated on POD7, but the peaks also differed between survivors and non-survivors [13,27,55]. The VWFpp and ADAMTS13 levels were considered to reflect vascular endothelial cell injury or liver graft dysfunction. The differences in hemostatic markers such as antithrombin (AT) and prothrombin time (PT), between the survivors and non-survivors were significant after POD14, suggesting that the liver function may still have been stable at POD14. ADAMTS13, VWF, and VWFpp have previously been studied as biomarkers for complications and poor outcomes after LDLT [13,27,55]. The high incidence of complications in non-survivors suggested that several complications might have contributed to deaths observed within 90 days after LDLT.

Conclusions

Severe thrombocytopenia after LDLT is associated with both graft loss and poor patient outcomes, thus underscoring the importance of monitoring the platelet count in patients after LDLT. Although the causes of thrombocytopenia vary, the mechanism should be clarified in each case. Therefore, the observation of biomarkers, such as sGPVI, TPO, VWF, VWFpp and ADAMTS13, is useful in the evaluation of the mechanisms of thrombocytopenia in patients who undergo LDLT.

Conflicts of Interest Statement

All of the authors declare no conflicts of interest.

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