Title	Clinical Implications of Serum Mac-2 Binding Protein in Patients After Living Donor Liver Transplantation for Biliary Atresia
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Note	

Introduction

Living donor liver transplantation (LDLT) has become the definitive treatment for children with end-stage liver disease in the setting of insufficient cadaveric donors. The major indication for LDLT among pediatric patients is biliary atresia (BA). BA is essentially a non-recurrent disease unlike other diseases that require LDLT such as viral hepatitis, autoimmune hepatitis, or primary sclerosing cholangitis. After liver transplantation, patients are generally followed with routine blood work, including liver function tests (LFTs) in the clinic. However, pathological changes are sometimes detected even if LFTs are within the normal range[1]. Therefore, liver biopsy is crucial for identifying pathological changes in patients after LDLT.

Liver biopsy, an invasive procedure, is the gold standard for assessing the degree of liver fibrosis. However, it might cause problems such as organ injury, hemorrhage, and pain. Pediatric patients need general anesthesia for liver biopsy. The evaluation of fibrosis might be subject to sampling error and interobserver variation[2]. Therefore, the development of non-invasive biomarkers is desirable.

Portal vein (PV) stenosis is sometimes seen in patients after pediatric LDLT. The rate of PV stenosis after adult deceased donor transplantation has been reported [3-5]. The rate of PV stenosis in patients who have undergone pediatric LDLT can be higher than in adult patients who underwent deceased donor liver transplantation [6, 7]. Ultrasonography is usually used to screen for PV stenosis. However, ultrasonography is dependent on technician performance and can be problematic because children can not stay still. Therefore, another supportive biomarker is needed.

Recently, Mac-2–binding protein glycosylation isomer (M2BPGi) was recognized as a glycol biomarker of liver fibrosis in patients with chronic hepatitis C who have a distinctive fibrosis-associated glycoalteration[8]. M2BPGi has been shown to be a useful fibrosis predictor in various chronic liver diseases. However, only some reports about M2BPGi in liver transplant recipients are available[9-12]. There have been no data available about the effect of serum M2BPGi concentration on routine follow-up for patients with BA after LDLT. We performed this study to examine the relationship between serum M2BPGi levels, liver histological findings, and other laboratory parameters in patients after LDLT for BA .

Methods

Patients

Patients aged <19 years at transplant who underwent LDLT for BA at our institution and followed for at least 1 year after LDLT were eligible for study participation. All patients received standard tacrolimus-based immunosuppression and steroids. No patients were withdrawn from immunosuppressive therapy.

Per-protocol biopsy was performed routinely every 1–5 years. Fibrosis was staged with the Metavir score system. Serum M2BPGi levels were compared with pathological fibrosis scores. We examined the effect of M2BPGi level on histological findings of liver fibrosis compared with other laboratory markers, including APRI, FIB-4 index, platelet count, AST to alanine aminotransferase (ALT) ratio, and type 4 collagen 7s as markers of liver fibrosis.

APRI was calculated using Wai's formula: (AST/upper limit of normal)/platelet count (expressed as platelets × 10^9/l) × 100.[13] The FIB-4 index was calculated using Sterling's formula: age (years) × AST (IU/l) / platelet count (×10^9//l) × √ALT (IU/ l)).[14]

PV flow after LDLT was followed with routine Doppler ultrasound (DUS) on a yearly basis. When PV stenosis was suspected, enhanced computed tomography (CT) was performed. When PV stenosis was suspected with enhanced CT, angiography was performed for definitive diagnosis. PV thrombosis was diagnosed with enhanced CT.

Measurement of M2BPGi

Serum M2BPGi levels were measured with automatic chemiluminescence enzyme immunoassay machine HISCL-800 (Sysmex, Kobe, Japan), which involved a two-step sandwich chemiluminescent enzyme immunoassay. M2BPGi measurements were indexed with values obtained using the following equation: cutoff index (COI) = ([M2BPGi] sample [M2BPGi] negative control (NC)) / ([M2BPGi] positive control (PC) + [M2BPGi] negative control (NC)). The PC was supplied as a calibration solution standardized to yield a COI value of 1.0. [8].

Histological assessment

Liver biopsy specimens were assessed with hematoxylin–eosin and Masson trichrome staining. Liver biopsy was performed percutaneously with a 16-gauge biopsy needle. No serious procedure-related complications occurred among any study participants. All biopsies were performed using an ultrasound-guided maneuver under either general anesthesia or intravenous sedation. Experienced pathologists at our hospital evaluated the samples. Fibrosis stages were staged as: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, liver cirrhosis [15].

Statistical analysis

Receiver operating characteristic (ROC) curve analysis was performed to calculate the area under the curve (AUC) for laboratory markers of liver fibrosis and the presence of advanced fibrosis (F2) on histological examination. For continuous variables, comparisons among groups were carried out using Student's t-test. Data are expressed as medians (range) or means (SD); $p<0.05$ was considered to be statistically significant. Statistical analyses were carried out with JMP 11 software (SAS Institute, Cary, NC, USA). This study was approved by our hospital institutional review board (approval number 17482).

Results

Patient characteristics

Of the 56 study participants, liver biopsy was performed at a median of 10.5 years (range, 1.7–32.2 years) after LDLT. According to the last available biopsy specimen, 7 patients (12%) had no fibrosis (F0), 36 patients (64%) had F1 fibrosis, and 13 patients (23%) had F2 fibrosis. None of the patients had F3 or F4 fibrosis. M2BPGi values in the current analysis ranged from 0.2 to 1.8 COI (median value, 0.8 COI). The characteristics of the study patients are shown in **Table 1.**

M2BPGi and liver fibrosis

Stratified by fibrosis stage, mean serum M2BPGi (SD) values were as follows: F0, 0.60 COI (0.28 COI); F1, 0.73 COI (0.39 COI); and F2, 1.07 COI (0.45 COI). Mean serum M2BPGi values were significantly higher in patients with F2 fibrosis than in patients with F0 and F1 fibrosis ($p<0.012$) (**Figure 1 a**).

Conventional laboratory markers and liver fibrosis

Conventional fibrosis markers including type 4 collagen 7s (n=51), hyaluronic acid (n=33), APRI (n=56), and FIB-4 index (n=56) were evaluated. The mean type 4 collagen 7s value (SD) by fibrosis stage (F0–F2) was 4.77 ng/ml (0.92 ng/ml) for F0 (n=7), 5.63 ng/ml (1.42 ng/ml) for F1 (n=34), and 6.12 ng/ml (1.78 ng/ml) for F2 (n=12). The mean hyaluronic acid

(SD) level by fibrosis stage was 28 ng/ml (33 ng/ml) for F0 (n=4), 25 ng/ml (27 ng/ml) for F1 (n=26), and 20 ng/ml (8 ng/ml) for F2 (n=3). The mean APRI (SD) value by fibrosis stage was 0.29 (0.10) for F0, 0.43 (0.26) for F1, and 0.53 (0.47) for F2. The mean FIB-4 (SD) value by fibrosis stage was 0.28 (0.12) for F0, 0.39 (0.24) for F1, and 0.53 (0.38) for F2.

Among other conventional fibrosis markers, the mean FIB-4 index was higher in patients with F2 fibrosis versus F0 fibrosis (p=0.045). There were no statistically significant differences for the other three conventional fibrosis markers (type 4 collagen 7s, hyaluronic acid, and APRI) by fibrosis stage.

M2BPGi and PV stenosis

We compared serum M2BPGi values in patients with and without PV complications (**Figure 1 b**). There were 5 patients with PV complications, which consisted of 2 patients with PV thrombosis and 3 patients with PV stenosis. There were 51 patients without PV complications. The mean serum M2BPGi value (SD) was 1.57 COI (0.29 COI) in patients with PV complications and 0.72 COI (0.35 COI) in patients without PV complications (p=0.0001). For predicting PV complications, the M2BPGi value of 1.07 COI yielded the highest AUC (0.96) (**Figure 2 a**).

M2BPGi and liver fibrosis without PV stenosis

To avoid the effect of PV stenosis on serum M2BPGi values, serum M2BPGi values was evaluated by fibrosis stage in patients without PV complication (n=51). Stratified by fibrosis stage, mean serum M2BPGi values (SD) were as follows: F0, 0.60 COI (0.28 COI); F1, 0.64 COI (0.26 COI); and F2, 1.02 COI (0.43 COI). Mean M2BPGi values were significantly higher in patients with F2 fibrosis than in patients with F0 (p=0.0077) or F1 fibrosis (p=0.0007).

ROC Analysis

For predicting advanced fibrosis (F2) among patients without PV complications, the M2BPGi level of 0.94 mg/L yielded the highest AUC (0.78) (**Figure 2 b**). The AUC, optimal cutoff point, sensitivity, and specificity for each marker of fibrosis are summarized in **Table 2**. For predicting advanced fibrosis, M2BPGi levels had a higher AUC than the six conventional fibrosis markers evaluated in this study.

Discussion

Long-term follow-up after pediatric LDLT is essential. Many transplant programs are performing per-protocol liver biopsy because patients with BA after LDLT can have a high prevalence of histopathological abnormalities such as biopsy-proven fibrosis despite having normal LFTs measured during regular clinical visits[1].

Kuno et al. [8] reported that M2BPGi is a predictor of liver fibrosis that performed better than other markers such as the FIB-4 index and hyaluronic acid. They suggested that

M2BPGi might be a reliable serum biomarker. M2BPGi is useful for assessing liver fibrosis in patients with BA[16]. Although M2BPGi is a new indicator of liver fibrosis, M2BPGi COI levels might differ by the etiology of liver fibrosis, even among patients with the same degree of fibrosis[17].

A few investigators have evaluated M2BPGi after liver transplantation. Kimura et al. reported that M2BPGi levels are strongly influenced by inflammation. They revealed that M2BPGi is a disease activity marker in transplant recipients. M2BPGi measurement might be useful for detecting early-stage liver inflammation in adult patients [9]. Sasaki et al. reported that M2BPGi is an accurate and non-invasive method for detecting significant fibrosis after LDLT, mostly among patients with viral hepatitis. M2BPGi was superior to Fibroscan findings and the Fib-4 index[12]. Uchiyama et al. reported that M2BPGi levels dramatically decrease after LDLT. A subsequent increase in M2BPGi levels predicted development of small-for-size syndrome[10]. These reports about M2BPGi after liver transplantation included adult patients and patients with non-BA disease. Therefore, we investigated the efficacy of M2BPGi in transplant recipients in order to determine how M2BPGi levels change in patients with BA who have undergone LDLT. Because BA is a non-recurrent disease, M2BPGi levels are purely affected by liver transplantation.

We found that serum M2BPGi levels in patients who have undergone LDLT for BA are correlated with the stage of fibrosis as assessed by liver biopsy. Serum M2BPGi levels increase in stages as graft liver fibrosis progresses. In patients who have undergone LDLT for BA, ROC analysis demonstrated that serum M2BPGi has better ability to detect fibrosis in grafts with grade F2 fibrosis than other conventional serum biomarkers such as the FIB-4 index, APRI, and type IV collagen 7s. M2BPGi had a high AUC when the cutoff level was defined as 0.97 COI. Therefore, M2BPGi had better diagnostic performance than other non-invasive markers. These results suggest that M2BPGi is a promising tool for identifying LDLT recipients with advanced graft fibrosis that cannot be detected by routine blood examination. Thus, our study results might be of clinical importance.

PV stenosis is sometimes seen in patients after pediatric LDLT. The rate of PV stenosis in patients who have undergone pediatric LDLT has been reported to be approximately 10% [6, 7]. Because PV stenosis might result in graft failure, early detection is important to achieving long-term graft survival for children. We showed that patients with PV complications had elevated serum M2BPGi levels in this study.

Kimura et al showed that Kupffer cells derived from macrophage in the liver expressed M2BPGi[9]. They suggested that M2BPGi levels reflect the activity of the fibrogenic process rather than the amount of accumulated extracellular matrix. Graft ischemia might affect M2BPGi levels. M2BPGi from hepatic stellate cells induces Mac-2 expression in

Kupffer cells, which in turn activates hepatic stellate cells to become fibrogenic[18]. The effects of hepatic stellate cells on the severity of ischemic injury in the liver affect their associated molecular mechanisms[19]. Mild ischemia with PV stenosis might induce hepatic stellate cells to produce M2BPGi. There are only a few biological markers in routine blood tests that have been reported to detect PV stenosis, other than LFTs[20]. Screening for PV stenosis is usually done with ultrasonography. However, ultrasonography depends on technician performance. PV stenosis is definitively diagnosed with transhepatic PV angiography. However, angiography is invasive and might cause complications. Serum M2BPGi levels might be useful in addition to DUS and CT for deciding whether to proceed with angiography, an invasive procedure.

We acknowledge several limitations to the present study. Liver biopsy to assess the degree of liver fibrosis is associated with sampling error. There were not enough patients to produce a statistically significant result for mild liver fibrosis. Ultrasonic transient elastography is another less invasive method for evaluating liver fibrosis that has been recently reported as highly useful[21]. We did not perform elastography and thus could not compare M2BPGi levels to elastography findings. Further analysis will be needed. Thus, caution should be exercised when interpreting our results. We suggest M2BPGi measurement once every 3 months for patients after liver transplantation. If M2BPGi levels are elevated with normal liver function, a per-protocol liver biopsy and ultrasound examination should be performed. Alternatively, if M2BPGi levels are normal, per-protocol liver biopsy can be skipped; if stricture is suspected based on DUS or CT, angiography can be deferred. The cutoff level for M2BPGi can be ≥1.0 COI.

In conclusion, M2BPGi is a novel serum marker for liver fibrosis in patients who have undergone pediatric LDLT for BA. Further study is required to determine the relationship between M2BPGi levels and the progression of fibrosis.

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