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Title: Clinical implications of serum autotoxin in regular follow up after pediatric living donor liver transplantation for biliary atresia

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Abstract

Background: Pediatric patients sometimes develop graft fibrosis after living donor liver transplant (LDLT). Autotaxin is a recently developed serum marker for hepatic fibrosis. We studied the relationship between serum autotaxin levels and histological findings in patients after LDLT for biliary atresia (BA). Methods: Information on patients aged <19 years who received LDLT for BA and were followed for at least 1 year after LDLT was gathered. Autotaxin levels were compared with pathological fibrosis scores. Results: The study included 52 patients, of whom 4 patients had no fibrosis (F0), 36 patients had F1 fibrosis, and 12 patients had F2. The median serum autotaxin level was 0.89 mg/L. In patients with portal vein (PV) complications such as stenosis or thrombosis (n=7), the mean autotoxin level was 1.25 mg/L compared with 0.95 mg/L in patients without PV complications (p=0.004). Among patients without PV complications, the mean autotaxin level was 0.90, 0.88, and 1.18 mg/L in F0, F1, and F2 fibrosis, respectively. The mean autotaxin was higher in F2 fibrosis than in F0 or F1 fibrosis (p<0.05). Autotoxin had a high area under the curve (0.86) with the cut-off level of 0.897 mg/L. Conclusion: Serum autotaxin is a novel marker for liver fibrosis in patients after pediatric LDLT for BA.

Keywords: liver fibrosis, cirrhosis, liver biopsy, liver transplantation, fibrosis marker

Type of study: Study of Diagnostic Test

Level of evidence: Level II

Abbreviations: AST: aspartate aminotransferase; ALT: alanine aminotransferase; APRI: aspartate aminotransferase to platelet ratio index; AUC: area under the curve; BA: biliary atresia; CT: computed tomography; DUS: doppler ultrasound; FIB-4: fibrosis-4; LDLT: living donor liver transplant; LPA: lysophosphatidic acid; LPC: lysophosphatidylcholine PCR: polymerase chain reaction; PV: portal vein ; ROC: receiver operating characteristic

Introduction

Liver transplantation has become the definitive treatment for end-stage liver disease in children. The major indication for liver transplantation among pediatric patients is biliary atresia (BA). We have previously shown that most patients with BA develop graft fibrosis after pediatric living donor liver transplant (LDLT) [1]. Advanced fibrosis is associated with decompensated cirrhosis, potential need for re-transplantation, and poor prognosis. Furthermore, advanced fibrosis is highly correlated with poor prognosis after liver transplant and graft liver fibrosis in adults [2, 3].

Therefore, liver biopsy is essential in order to identify pathological changes in patients after LDLT. Although liver biopsy is the gold standard for assessing the degree of liver fibrosis, it is an invasive technique requiring general anesthesia in children. Non-invasive assessment of graft liver fibrosis after transplantation is important.

Autotaxin is a 125-kDa glycoprotein, also known as ectonucleotide pyrophosphatase/ phosphodiesterase 2, that is secreted by mainly adipose tissue. Functioning as a phospholipase, autotaxin catalyzes the hydrolysis of lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA) [4]. Higher levels of autotaxin are considered to be the result of delayed degradation of circulating autotaxin due to sinusoidal endothelial cell dysfunction in liver fibrosis [5]. Serum autotaxin levels

were associated with hepatic dysfunction and native liver fibrosis in BA [6, 7]. Several studies have attempted to estimate histological severity in post-transplant patients using various serum biomarkers, but the accuracy of these techniques remains unsatisfactory [8]. Therefore, we focused on serum autotaxin, which might be used as a liquid biopsy to assess graft liver fibrosis after LDLT in BA. We performed this study to examine the relationship between serum autotaxin levels and liver histological findings in patients with BA who underwent LDLT.

Methods

Patients

This study included patients under 19 years of age at the time of LDLT for BA between 1999 and 2019 who were followed for at least 1 year after LDLT at our institutions. All patients received routine biopsies per protocol every 1 to 3 years. Portal vein (PV) flow after LDLT was followed with routine Doppler ultrasound (DUS). When PV stenosis was suspected, computed tomography (CT) and angiography were performed. We examined the relationship between serum autotaxin levels and last available histological findings of liver fibrosis. We compared them with other laboratory markers of liver fibrosis, including FIB-4 index, aspartate aminotransferase (AST) to platelet ratio

index (APRI), platelet count, AST to alanine aminotransferase (ALT) ratio (AST/ALT ratio), and levels of type IV collagen 7s domain and hyaluronic acid. All patients received steroids and standard tacrolimus-based immunosuppression per protocol.

Measurement of autotaxin levels

Serum levels of autotaxin were measured using a two-site enzyme immunoassay. The assay reagent, which is commercially available in Japan, was purchased from Tosho Corp. (Tokyo, Japan). It was used with the automated immunoassay analyzer AIA-2000 system (Tosho Corp.), which included automated dispensing of specimen (10 μL), incubation of the reaction cup, bound/free washing, dispensing of 4-methylumbelliferyl phosphate substrate, and fluorometric detection. The limit of detection of this assay was estimated to be 0.021 mg/L [9].

Histological assessment

Liver biopsy samples were assessed with hematoxylin–eosin and Masson's trichrome stains. Liver biopsies were performed percutaneously with a 16-gauge biopsy needle under ultrasound guidance. Patients were under either general anesthesia or intravenous sedation. After staining, fibrosis was staged based on the METAVIR score. No serious procedure-related complications occurred.

Statistical analysis

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

Results

Demographic characteristics of the study patients

Of the 52 patients were enrolled in this study, there were 16 males and 38 females. The median age at LDLT was 1.6 years (range, 0.5–18.7 years). Serum autotaxin levels were measured at a median of 11.2 years (range, 1.7–34.2 years) after LDLT. Immunosuppression therapy consisted of tacrolimus alone (n=35), tacrolimus and everolimus (n=6), tacrolimus and a steroid (n=6), and other (n=5). All patients remained on immunosuppressive therapy. Serum autotaxin levels ranged from 0.35 to 1.69 mg/L (median, 0.89 mg/L). Regarding the degree of liver fibrosis based on histological examination, F0 was observed in 4 patients (8%), F1 in 36 (69%) patients, and F2 in 12

(23%) patients. Type IV collagen 7s and hyaluronic acid in 4 patients were not measured. Therefore, analysis of these data was performed in 48 patients. The characteristics of the study patients are shown in Table 1.

Autotaxin and fibrosis

Stratified by fibrosis stage (F0–F2), mean serum autotaxin levels (SD) were as follows: F0, 1.06 mg/L (0.35 mg/L); F1, 0.91 mg/L (0.24 mg/L); and F2, 1.18 mg/L (0.24 mg/L). Mean serum autotaxin levels were significantly higher in patients with F2 fibrosis than those in patients with F1 fibrosis (p=0.0021) (Fig. 1).

Among other conventional fibrosis markers, mean type IV collagen 7s domain level was higher in patients with F2 fibrosis (7.2 ng/mL) versus F1 fibrosis (5.3 ng/mL) (p=0.014). There were no statistically significant differences for the other five conventional fibrosis markers: FIB-4 index, APRI, platelet count, AST/ALT ratio, and hyaluronic acid.

Autotaxin and PV complications

We compared serum autotaxin levels in patients with versus without PV complications (Fig. 2). There were 7 patients with PV complications, which included 2 patients with PV thrombosis and 5 patients with PV stenosis, and 45 patients without PV complications. Patients in each fibrosis stages with PV complications were one patient in

F0, 4 patients in F1 and 2 patients in F2. Mean serum autotaxin levels (SD) were 1.25 mg/L (0.22 mg/L) in patients with PV complications and 0.95 mg/L (0.25 mg/L) in patients without PV complications (p=0.004). Serum autotaxin levels were high even in patients with stage F0 fibrosis.

To avoid the effect of PV stenosis on serum autotaxin levels, we evaluated serum autotaxin levels by fibrosis stage in the subset of patients with no PV complications (n=45). Stratified by fibrosis stage (F0–F2), mean serum autotaxin levels (SD) were as follows: F0 (n=3), 0.90 mg/L (0.15 mg/L); F1, (n=32) 0.88 mg/L (0.22 mg/L); and F2 (n=10), 1.18 mg/L (0.26 mg/L). Mean autotaxin levels were significantly higher in patients with F2 fibrosis than in patients with F0 (p=0.06) or F1 fibrosis (p=0.0006) (Fig. 3).

ROC analysis

For predicting advanced fibrosis (F2) among patients without PV complications, an autotaxin level of 0.897 mg/L yielded the highest AUC (0.86). The AUC, optimal cutoff point, sensitivity, and specificity for each marker of fibrosis are summarized in Table 2. For predicting advanced liver fibrosis, autotaxin had higher AUC than the six conventional fibrosis markers evaluated in this study.

Discussion

Long-term follow-up after pediatric LDLT is challenging. Many centers are performing protocol-driven liver biopsy. There are many reports documenting graft fibrosis after pediatric liver transplantation. The prevalence ranges from 31% to 97% despite liver function test results that are almost normal [1, 10-12]. Patients with BA after LDLT can develop biopsy-proven fibrosis, even in the context of normal liver function as demonstrated by blood tests. In adults, these abnormalities are mainly related to recurrent primary disease. However, BA is not considered a recurrent disease.

Fibrosis in the liver is a wound healing response to chronic injury. Some mechanisms of chronic injury can cause graft fibrosis in the transplanted liver, such as alloimmune inflammation and biliary outflow obstruction [10, 13]. The development of donor-specific antibodies in allograft fibrosis after LDLT has been investigated [12, 14]. The mechanism of injury in graft is similar to autoimmune hepatitis.

Early detection of graft fibrosis is essential because it might result from chronic rejection and graft failure might develop. However, liver biopsy is an invasive procedure. Moreover, the evaluation of fibrosis can be uncertain due to sampling error and variations among observers. Therefore, alternative biomarkers have been investigated.

Autotaxin regulates a variety of cellular processes, including proliferation,

migration, angiogenesis, fibrogenesis, and cancer progression [15]. Autotaxin is synthesized by a variety of normal cells and tissues, secreted into the circulation as a glycoprotein, and later degraded by liver sinusoidal endothelial cells [16]. Liver fibrosis is initiated by the activation of hepatic stellate cells, which results in the production and accumulation of collagen and other extracellular matrix components in the liver parenchyma. Watanabe et al. demonstrated elevated serum autotaxin levels in patients with chronic hepatitis C. They also found that autotaxin is a key enzyme for converting LPC to LPA and that plasma LPA levels are correlated with serum autotaxin levels in patients with chronic liver disease [17]. Autotaxin levels could also be used to evaluate the status of native liver fibrosis in patients with BA [6].

We found that serum autotaxin levels in patients after LDLT for BA are correlated with the degree of fibrosis as evaluated by liver biopsy. Autotaxin levels increase in stages, as graft liver fibrosis progresses. Although the autotaxin level of F0 was higher than F1, there was no significant difference in autotaxin level between F2 and F0. This result is considered to be an error due to outliers because the number of patients with F0 disease was small (n = 4) and the difference in serum autotaxin levels between F0 and F1 was small. Therefore, such a result was obtained. For autotaxin, the AUC for diagnosis of F2 fibrosis was sufficiently high. Therefore, the diagnostic performance of

autotaxin was comparable to that of other non-invasive markers. These results suggest that autotaxin is a promising tool for identifying patients with significant graft fibrosis.

PV stenosis is sometimes seen in patients after pediatric LDLT. The rate of PV stenosis in patients after pediatric LDLT was reported to be approximately 10% [18, 19]. Because PV stenosis might result in graft failure, early detection is essential to achieving long-term graft survival. We showed elevation of serum autotaxin levels in this study. As autotaxin is cleared by liver sinusoidal endothelial cells [16], decreased blood flow into the liver due to PV stenosis might lead to higher blood concentrations of autotaxin. Porto-systemic shunting secondary to portal hypertension might cause decreased autotaxin clearance, resulting in higher autotaxin serum concentrations. PV stenosis is definitively diagnosed with transhepatic PV angiography. Serum autotaxin level might be useful in addition to DUS and CT for deciding whether to proceed with angiography, an invasive procedure.

Fibroscan is a modality to detect liver fibrosis that is in development. Fibroscan uses transient elastography, a technique that has been validated in adult and pediatric populations. Liver stiffness measurement is significantly correlated with histological liver fibrosis stage [20, 21]. Transient elastography measures liver stiffness in a tissue volume that is approximately a cylinder with a diameter of 1 cm and a length of 4 cm. Although

this volume is larger than the volume of a biopsy specimen, the area assessed by transient elastography can be still part of the liver. Autotaxin can show the fibrosis status of the entire liver. In LDLT, a partial liver graft is used. A previous study has shown that the usefulness of elastography varies by anatomic factors [22]. A report confirmed a discrepancy between fibrosis and elastography results for grafts on the left side because elastography was affected by physical limitations [23]. In addition, there are no reports demonstrating that elastography is useful for detecting portal veins.

We acknowledge several limitations of this study. One was that measurement of autotaxin was not the same time of liver biopsy. The reason was that liver biopsy had been performed a maximum every 3 years. In patients after LDLT, fibrosis did not progress rapidly due to the stable course with protocol biopsy. This study was a pilot study to show a tendency of autotaxin level. Therefore, further study is required to evaluate autotaxin level at the time of biopsy. And there were not enough patients to produce statistically significant results for no liver fibrosis (F0). We observed only mild to moderate fibrosis (F0–F2 disease). Therefore, longer follow-up is required to elucidate the relationship between graft fibrosis and graft outcome.

In conclusion, autotaxin is a novel serum marker for liver fibrosis in patients

after pediatric LDLT for BA. Further follow-up is required to determine the relationship between autotaxin and the progression of fibrosis.

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Figure Legends

Figure 1. Autotaxin and fibrosis stages: Serum autotaxin level in F2 fibrosis was higher than F1 fibrosis statistically. Data were expressed as mean with standard deviation (SD). Fibrosis stages were descried METVIR score.

Figure2. Relationship between autotaxin and portal vein complications: Serum autotaxin level in portal vein complications (thrombosis and stenosis) were higher than without complication statistically. Data were expressed as mean with standard deviation (SD).

Figure 3. Autotaxin and fibrosis stages without portal vein complications: Serum autotaxin level in F2 fibrosis was higher than F1 fibrosis statistically. Data were expressed as mean with standard deviation (SD). Fibrosis stages were descried METVIR score.

Figure 4. Receiver operating characteristic (ROC) curve analysis: ROC analysis of autotaxin and the stages of fibrosis without portal vein complications. F2 was regarded as positive. Fibrosis stages were described METVIR score.

Table 1. Patient demographics: Interval from LDLT indicates duration between serum autotaxin measurement and living donor liver transplant. Data were expressed as median with ranges. Histological findings were based on METAVIR score. Type IV collagen 7s and hyaluronic acid in 4 patients were not measured. Therefore, analysis of these data

was performed in 48 patients. APRI: the AST to Platelet Ratio Index; AST: aspartate aminotransferase; ALT: alanine transaminase; FIB-4: Fibrosis-4

Table 2. Receiver Operating Characteristic of fibrosis markers. Summary of AUC, optimal cutoff point, sensitivity, and specificity for each fibrotic marker: Histological findings were classified by METAVIR score. APRI: the AST to Platelet Ratio Index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; FIB-4: Fibrosis-4 index