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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Serum Mac-2 binding protein glycosylation isomer predicts grade F4 liver fibrosis in patients with biliary atresia

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Abstract

Background and Aim Mac-2 Binding Protein Glycosylation Isomer (M2BPGi) is a novel fibrosis marker. We examined the ability of M2BPGi to predict liver fibrosis in patients with biliary atresia.

Methods Sixty-four patients who underwent living donor liver transplantation (LDLT) were included [median age, 1.1 years (range 0.4–16.0), male 16 patients (25.0 %)]. We examined M2BPGi levels in serum obtained the day before LDLT, and we compared the value of the preoperative M2BPGi levels with the histological evaluation of fibrosis using the METAVIR fibrosis score. Subsequently, we assessed the ability of M2BPGi levels to predict fibrosis.

Results The median M2BPGi level in patients with BA was 6.02 (range, 0.36–20.0), and 0, 1, 1, 11, and 51 patients had METAVIR fibrosis scores of F0, F1, F2, F3, and F4, respectively. In patients with F4 fibrosis, the median M2BPGi level was 6.88 (quartile; 5.235, 12.10), significantly higher than that in patients with F3 fibrosis who had a median level of 2.42 (quartile; 1.93, 2.895, $p < 0.01$). Area under the curve analysis for the ability of M2BPGi level to predict grade fibrosis was 0.917, with a specificity and sensitivity of 0.923 and 0.941, respectively. In comparison with other fibrosis markers such as hyaluronic acid, procollagen-III-peptide, type IV collagen 7 s, and aspartate aminotransferase platelet ratio index, M2BPGi showed the strongest ability to predict grade F4 fibrosis.

Conclusion M2BPGi is a novel fibrosis marker for evaluating the status of the liver in patients with BA, especially when predicting grade F4 fibrosis.

Keywords Liver fibrosis · Mac-2 binding protein glycosylation isomer · Biliary atresia

Abbreviations

APRI	Aspartate aminotransferase platelet ratio index
AUC	Area under the curve
BA	Biliary atresia
LB	Liver biopsy
LDLT	Living donor liver transplantation
P-III-P	Procollagen-3-peptide
ROC	Receiver operating characteristics
M2BPGi	Mac-2 binding protein glycosylation isomer

Introduction

Cholestatic disease represented by biliary atresia (BA) is the leading cause of liver failure in children, and the most common indication for pediatric liver

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transplantation (LT) [1] Currently, 1- and 10-year native liver survival rates are approximately 60 and 50 %, respectively [2]. Because of the progressive nature of liver disease, long-term survival is achieved in only approximately 20 % of BA patients, and other patients need LT for end-stage liver failure in order to save their lives [3]. However, there are many various variations in the clinical course of each patient with BA, and the treatment strategy is different, particularly after Kasai portoenterostomy [4]. The most common indication for LT in patients with BA is cholestatic liver cirrhosis with jaundice, which is accompanied by an elevation in pediatric end-stage liver disease score [5]. In addition, recurrent cholangitis or portal hypertension accompanied by gastrointestinal bleeding, hypersplenism, or hepatopulmonary syndrome are common indications for LT, whether or not the patient has jaundice [6]. In such cases, it is especially difficult to evaluate the status of the native liver and to determine whether the patient can live with his or her native liver or needs LT.

Evaluating fibrosis of the native liver in patients with BA is an important factor in predicting prognosis, whether or not the patient shows jaundice [7]. However, the gold standard for evaluating fibrosis of the liver accompanied by BA is still liver biopsy (LB), which is an invasive method with a high risk of complications, including hemorrhage, abdominal pain, pneumothorax, hemothorax, cholangitis, bile leakage, sedation-related complications, and death [8]. Moreover, sampling errors [9] and observer variability [10] are often seen with LB, and it is difficult to perform repeatedly.

Recently, Mac-2 Binding Protein Glycosylation Isomer (M2BPGi), which is also known as '*Wisteria floribunda* Agglutinin-Positive Mac-2 Binding Protein (WFA^+-M2BP)', has been reported as a novel marker that reflects the progression of liver fibrosis in patients with chronic hepatitis C, non-alcoholic fatty liver disease (NAFLD), and primary biliary cirrhosis. [11–14] To our knowledge, this is the first report to examine the value of M2BPGi in patients with BA.

Patients and methods

Patients

We performed 272 pediatric living donor liver transplantations (LDLTs) between May 2001 and August 2015 at Jichi Medical University, in Tochigi, Japan. Among the most common indications for transplantation was BA, which was diagnosed in 185 patients (68.0 %). This study included 64 patients with BA who underwent pediatric LDLT at our institute for whom we could collect the frozen plasma during LDLT procedures performed between 2009 and 2015. Another patients who underwent LDLT before 2008 were excluded because the preserved methods of frozen plasma were different or difficult to obtain. BA diagnosis was confirmed with the histological examination.

Serum biomarker measurement

For patients who underwent LDLT, blood examinations were routinely performed and the remainder of the plasma was stored at −85 °C. M2BPGi was measured from the frozen plasma. Other conventional liver fibrosis markers, such as hyaluronic acid (HA), procollagen-3-peptide (P-III-P), type IV collagen 7 s, or aspartate aminotransferase

Table 1 Characteristics of the patients

	BA ($n = 64$)
Age (years)	1.1 (0.4–16.0)
Gender male	16 (25.0 %)
Disease	BA; 64
PELD score	9 (−14 to 33)
Indication for LT	Cholestatic cirrhosis; 48 (75.0 %)
	Cholangitis; 8 (15.0 %)
	Portal hypertension; 8(15.0 %)
METAVIR Fibrosis score	
F0	0 (0 %)
F1	1 (1.6 %)
F2	1 (1.6 %)
F3	11 (17.2 %)
F4	51 (79.7 %)
M2BPGi	6.02 (0.36 to 20.0)
HA (ng/mL)	238 (9 to 26,500)
P-III-P (U/mL)	3.1 (0.1 to 9.3)
Type III collagen 7 s (ng/mL)	14.5 (2.6 to 83.0)
Plt ($\times 10^4/\mu L$)	15.4 (3.3 to 110.0)
AST (U/L)	158 (20 to 167)
ALT (U/L)	100 (9 to 83)
T-Bil (mg/dL)	4.68 (0.32 to 54.36)
PT-INR	1.22 (0.97 to 3.50)
Alb (g/dL)	3.5 (2.4 to 5.5)
APRI (AST/plt)	10.3 (0.2 to 94.4)
Graft survival	62 (96.9 %)
Patient survival	62 (96.9 %)

The data are expressed as median (range)

BA biliary atresia, *PELD* pediatric end-stage liver disease, *M2BPGi* mac-2 binding protein glycosylation isomer, *HA* hyaluronic acid, *P-III-P* type III procollagen-*N*-peptide, *plt* platelet count, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *T-bil* total bilirubin, *PT-INR* prothrombin time international normalized ratio, *Alb* albumin

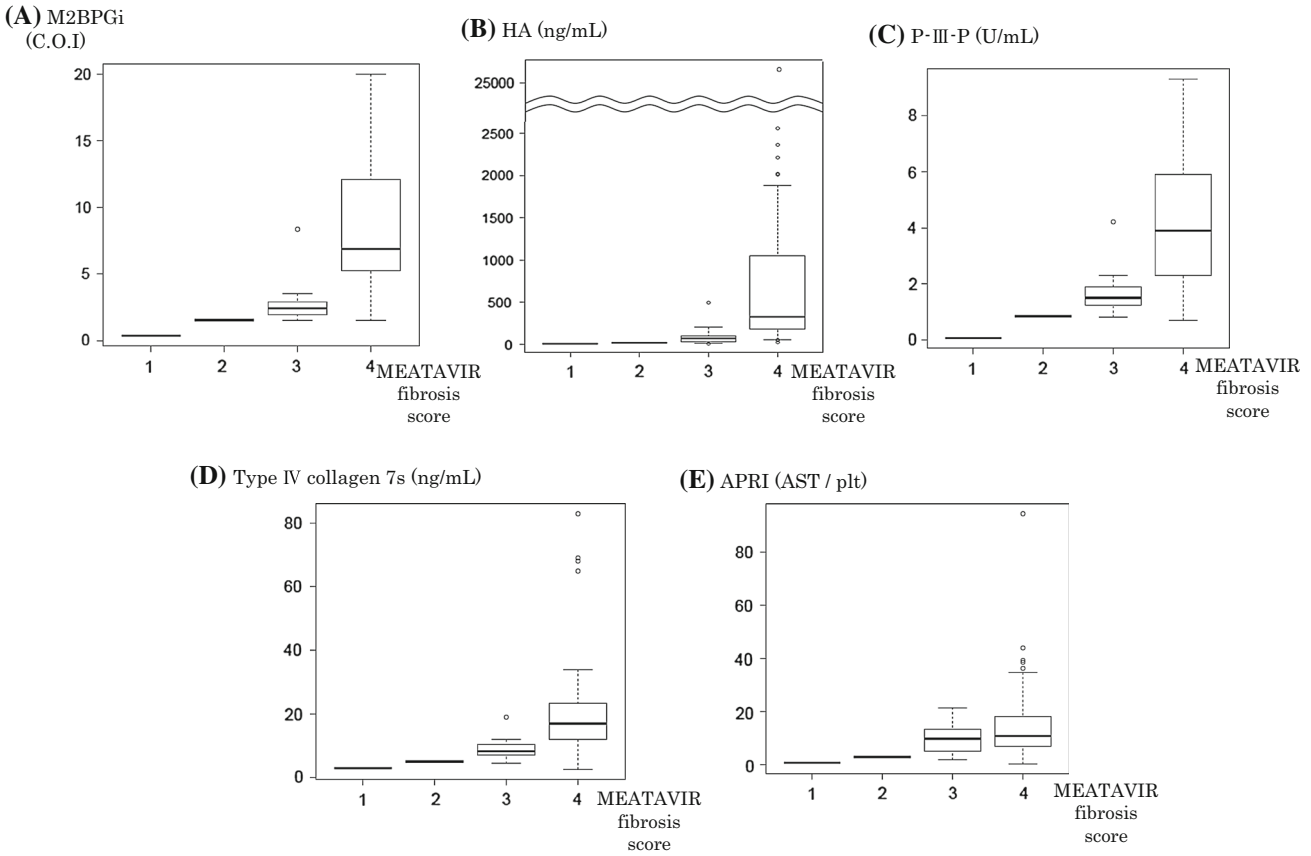

Fig. 1 **a–e** Comparison of each fibrosis marker with the METAVIR fibrosis score in patients with BA. M2BPGi, HA, P-III-P, and type IV collagen 7 s were significantly higher ($p < 0.01$) in patients with grade F4 fibrosis than F3. APRI was not statistically different in patients with F4 versus F3. (Mann–Whitney *U* test)

Table 2 Comparison of each parameters between in patients with Metavir F3 (n = 11) and Metavir F4 (n =51)

	Metavir F3 ($n = 11$)	Metavir F4 ($n = 51$)	p value*
PELD score	3 (−4, 21)	9 (−5, 17)	0.839
M2BPGi	2.42 (1.93, 2.895)	6.88 (5.235, 12.10)	<0.01
HA (ng/mL)	73 (32.5, 104)	329 (182.5, 1060)	<0.01
P-III-P (U/mL)	1.5 (1.25, 1.9)	3.9 (2.30, 5.9)	<0.01
Type IV collagen 7 s (ng/mL)	8.3 (7.1, 10.5)	17.0 (12.0, 23.5)	<0.01
APRI (AST/plt)	9.7 (4.95, 13.40)	10.8 (6.775,18.13)	0.136
Graft survival	11 (100.0 %)	49 (96.1 %)	1.000
Patient survival	11 (100.0 %)	49 (96.1 %)	1.000

* Mann–Whitney *U*-test and Fisher's exact test

$p < 0.05$ was defined as having statistically significant difference. The data are expressed median (25 %quartile, 75 %quartile) and number (percent)

platelet ratio index (APRI), were obtained from blood examinations performed the same day as a routine clinical examination.

M2BPGi was quantified on the basis of a lectin-antibody sandwich immunoassay using a fully automatic HISCL-5000 immunoanalyzer (Sysmex Co., Hyogo, Japan) [15]. The measured values of M2BP conjugated to WFA were indexed with obtained values using the following equation:

$$\text{cutoff index} = ([\text{WFA}^+ - \text{M2BP}]\,\text{sample} - [\text{WFA}^+ - \text{M2BP}]\text{NC})/([\text{WFA}^+\text{M2BP}]\,\text{PC} - [\text{WFA}^+ - \text{M2BP}]\text{NC})$$

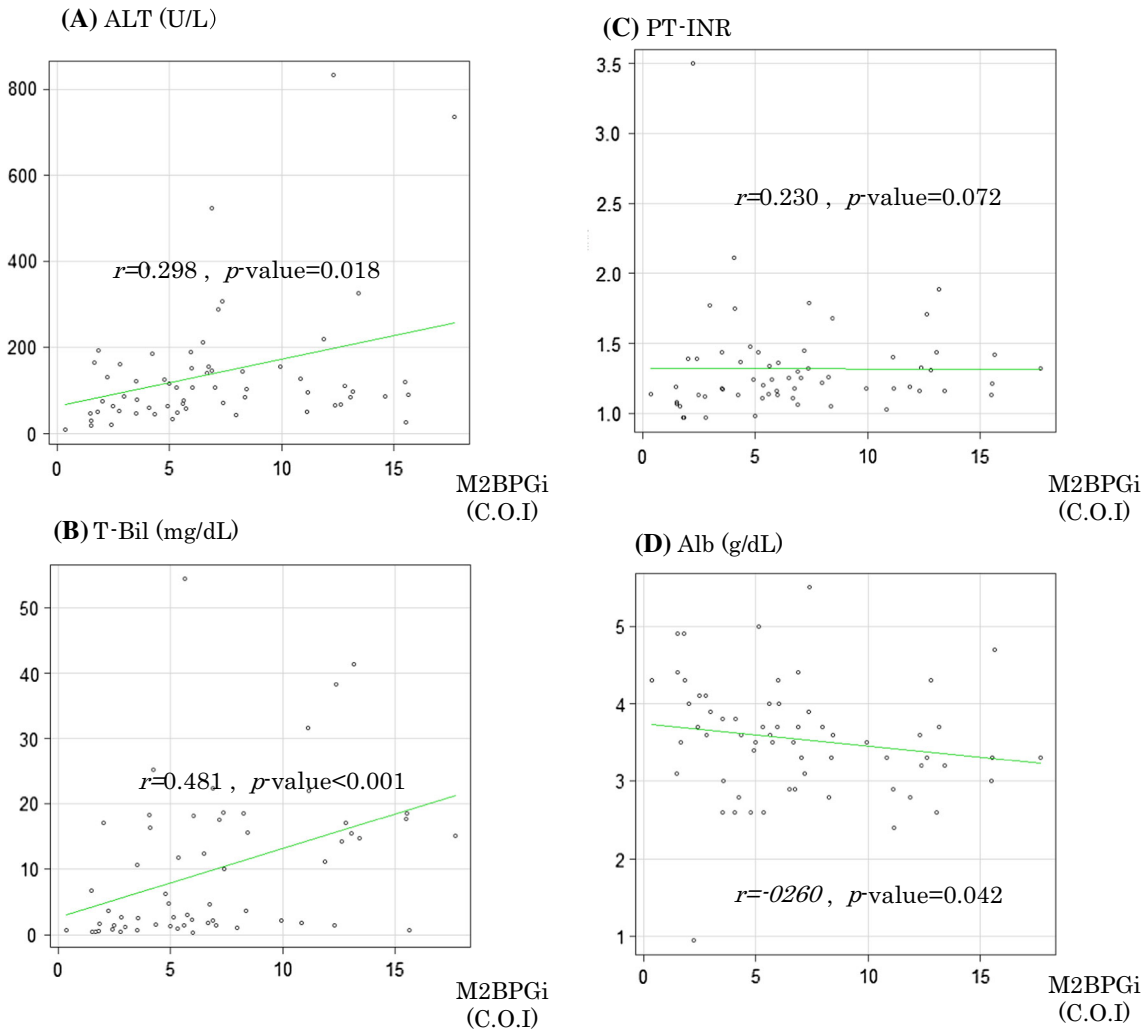

Fig. 2 **a–d** Spearman's rank correlation between M2BPGi and liver function parameters. T-Bil and ALT showed significant positive correlations with M2BPGi. (ALT, $r = 0.298$, p value $= 0.018$, T-Bil; $r = 0.481$, p value <0.001, respectively). Alb showed negative correlation with M2BPGi ($r = -0.260$, p value $= 0.042$)

where [$WFA^+ - M2BP$] sample is the $WFA^+ - M2BP$ level in the serum sample, PC is positive control, and NC is negative control. The positive control was supplied as a calibration solution preliminarily standardized to yield a cutoff index value of 1.0.

Histological evaluation of liver fibrosis

Histological evaluation of fibrosis was performed from the whole liver, which was excised during LDLT. The evaluation was performed on more than two different pieces of the liver that included the left lobe and right lobe, respectively. All liver specimens were stained with both of hematoxylin and eosin as well as azan. Fibrosis was assessed by the METAVIR scoring system, and all data were evaluated by two experienced pathologists and transplant surgeons.

Statistical analysis

Data are expressed as the median (range) or median (quartile). We performed Mann–Whitney U test to evaluate whether or not M2BPGi was useful to judge the METAVIR fibrosis score. Afterward, we examined the correlation of M2BPGi with liver function parameters and other conventional fibrosis markers by Spearman's rank correlation. To evaluate the ability to detect METAVIR fibrosis score F4, receiver operating characteristics (ROC) curve analysis was performed. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria) [16]. Differences with a value of $p < 0.05$ were considered significant.

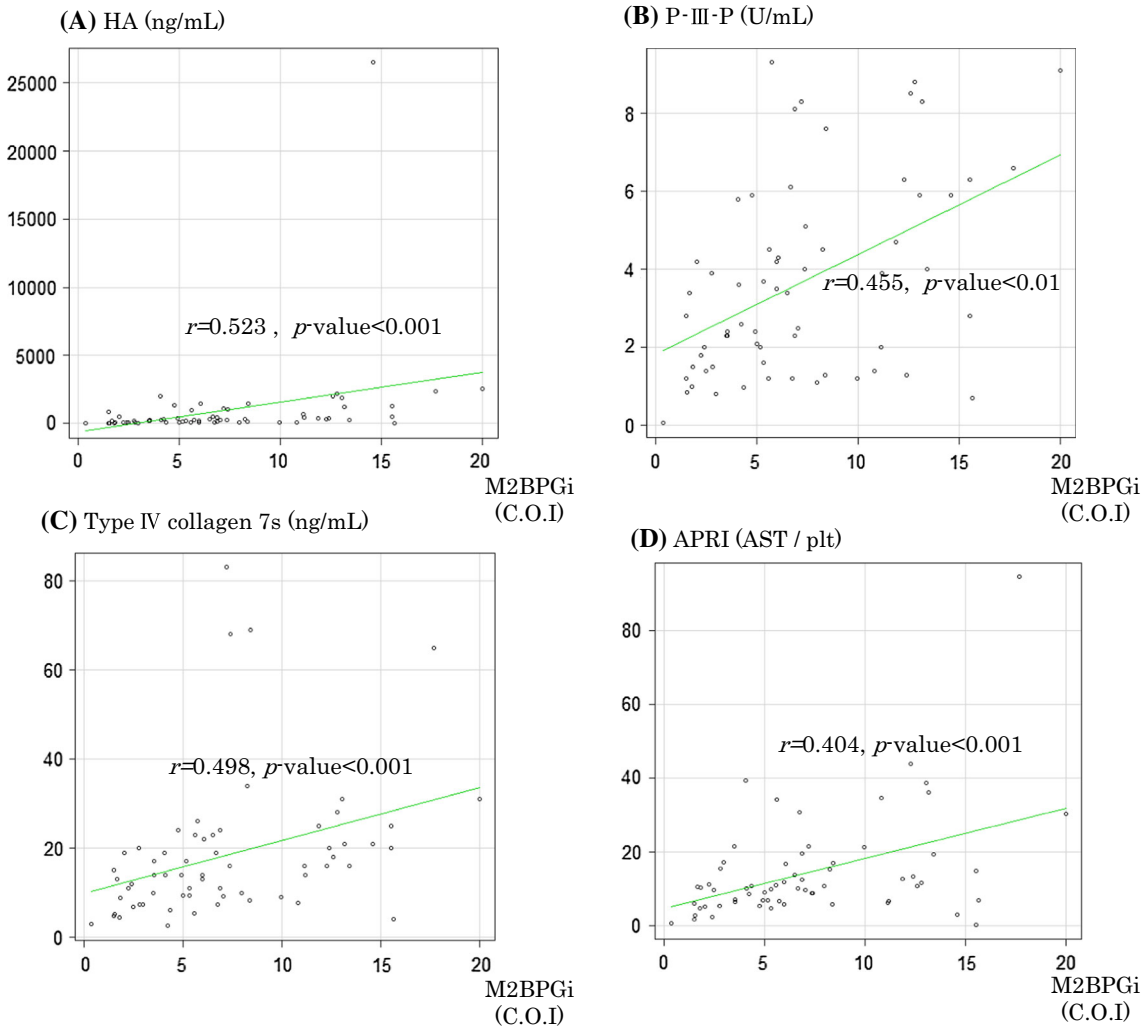

Fig. 3 **a–d** Spearman's rank correlation between M2BPGi and conventional fibrosis markers. All conventional fibrosis markers were positively related with M2BPGi

Ethics

Written informed consent was obtained from each patient and/or their parents included in this study as concerned the use of plasma and liver specimens. This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institution's ethical committee.

Results

Patients' characteristics are shown in Table 1. For patients with BA, the median age and number of male patients were 1.1 years (range, 0.4–16.0) and 16 (25.0 %), respectively. The median pediatric end-stage liver disease score was 9 (range, −14 to 33) and the indications for LT were cholestatic liver cirrhosis in 48 patients (75.0 %), recurrent cholangitis in eight patients, (15.0 %), and intractable portal hypertension (including gastrointestinal varices and hypersplenism) in eight patients (15.0 %).

In patients with BA, their METAVIR fibrosis scores were F0 (none), F1(one patient; 1.6 %), F2 (one patient; 1.6 %), F3 (11 patients; 17.2 %), and F4 (51 patients; 79.7 %); the median M2BPGi level was 6.02 (range, 0.36–20.0). In the control group, we noted patients with scores of F0 (ten patients; 83.3 %), and F1 (two patients; 16.7 %), and the median M2BPGi value was 0.65 (range, 0.17–2.31). In patients with BA, values for another fibrosis markers were HA at a median of 238 (9–26,500); P-III-P at a median of 3.1 (0.1–9.3); type IV collagen 7 s at a median of 14.5 (2.6–83.0); and APRI at a median of 10.3 (0.2–94.4).

We divided the patients based on the METAVIR fibrosis score and compared the values of each marker between

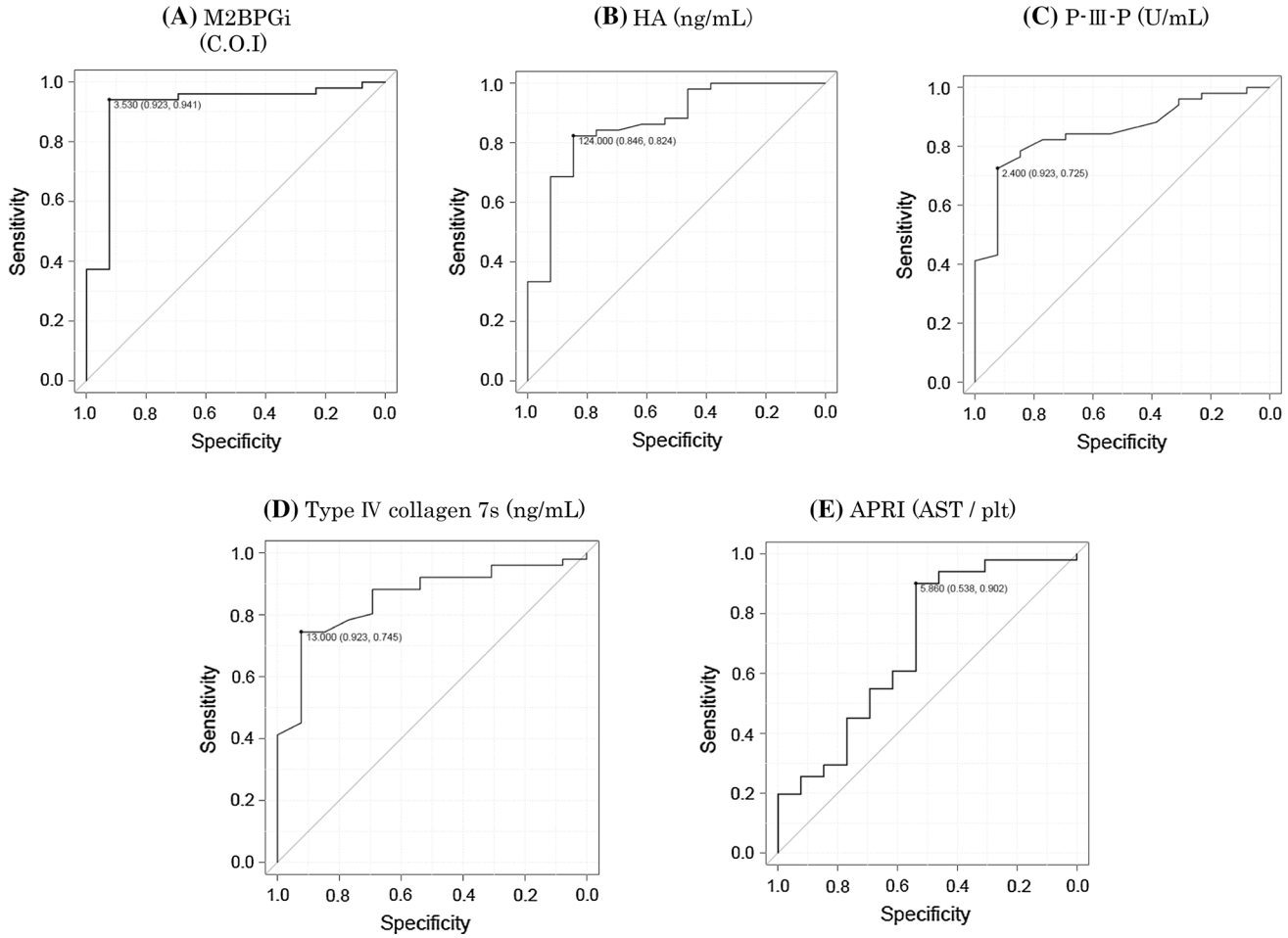

Fig. 4 **a–e** ROC analysis of the ability to detect fibrosis of grade F4 or less. The data shown in the figures are expressed as cutoff values (specificity, sensitivity). The AUC value of M2BPGi was the widest (0.917); there was high sensitivity (94.1 %) and specificity (92.3 %) when the cutoff value was defined 3.53

patients with F3 and F4 disease (Fig. 1a–e). The median M2BPGi value in patients with F4 grade fibrosis was 6.88 (quartile; 5.235, 12.10), which was significantly higher than in patients with F3 fibrosis, who had a median M2BPGi level of 2.42 (quartile; 1.93, 2.895, $p < 0.001$) (Table 2). Similarly, as shown in Table 2, HA, P-III-P, and type IV collagen 7 s values were significantly higher in patients with F4 fibrosis than in patients with F3 fibrosis. However, APRI was not significantly different for patients with F4 and F3 fibrosis. Both graft survival rate and patient survival rate after LDLT were not significantly different between patients with F4 and F3 fibrosis.

Spearman's correlations between M2BPGi and liver function parameters are shown in Fig. 2. Alanine aminotransferase (ALT) and total bilirubin (T-Bil) showed significant positive correlation with M2BPGi. (ALT, $r = 0.298$, p value $= 0.018$, T-Bil, $r = 0.481$, p value <0.001). Conversely, Alb showed a negative correlation to M2BPGi. ($r = -0.260$, p value $= 0.042$). Spearman's correlations between M2BPGi and each conventional marker are shown in Fig. 3. All conventional serum biomarkers, including HA, P-III-P, type IV collagen 7 s, and APRI, showed a positive correlation with M2BPGi.

We also performed ROC analysis to evaluate the ability of each marker to detect METAVIR F4, as shown in Fig. 4. The area under the curve (AUC) was the widest for M2BPGi (0.917); its cutoff value was 3.53, with a high specificity (0.923) and sensitivity (0.941). The AUC for HA (0.867), P-III-P (0.849), type IV collagen 7 s (0.856), and APRI (0.697) were also evaluated (Table 3).

Discussion

In 1986, Iacobelli et al. first reported M2BP as a tumor-associated antigen [17]. Later, M2BP was proven to be associated with the membrane protein that enhances cell

Table 3 The ability of each fibrosis markers to predict F4 or less

	Cutoff value	Specificity	Sensitivity	AUC	95 % CI
M2BPGi	3.53	0.923	0.941	0.917	0.817–1.000
HA (ng/mL)	124.0	0.846	0.824	0.867	0.754–0.980
P-III-P (U/mL)	2.40	0.923	0.725	0.849	0.744–0.955
Type4 collagen 7 s (ng/mL)	13.00	0.923	0.745	0.856	0.752–0.960
APRI (AST/plt)	5.860	0.538	0.902	0.697	0.516–0.878

AUC area under the curve, *CI* confidence interval

adhesion and the extracellular matrix to promote fibrosis of the liver [18, 19]. Recently, M2BPGi has been in the limelight because it is a strong predictor of progression of liver fibrosis that can be assessed in a non-invasive and rapid manner. Various reports have stated that novel serum biomarkers predict fibrosis of the liver for chronic liver diseases, including chronic hepatitis C viruses, chronic hepatitis B viruses, NAFLD, and primary biliary cirrhosis. In addition, biomarkers have the efficacy to predict the development of hepatocellular carcinoma in patients with chronic hepatitis-C [14, 20].

This is the first report to focus on value of examining M2BPGi levels in patients with BA in comparison with histological findings and conventional markers of liver fibrosis. In addition, this is the first report of M2BPGi evaluated in pediatric patients, and our findings confirm the results previously reported in adult patients. It is worth noting that our histological evaluation was performed using multiple specimens from the whole liver, not with the needle LB; therefore, it includes less sampling error than previous reports. Our study demonstrated that M2BPGi value in BA patients is significantly higher in those without liver cirrhotic disease. In patients with BA, ROC analysis demonstrates that serum M2BPGi has better diagnostic ability to detect fibrosis of the native liver with grade F4 fibrosis than other conventional serum biomarkers such as HA, P-III-P, type IV collagen 7 s, or APRI. M2BPGi had a wide AUC and extremely high sensitivity (94.1 %) and specificity (92.3 %) when the cutoff value was defined as 3.53. In general, grade F4 fibrosis is estimated as decompensated cirrhosis, and young patients with BA, including children, are candidates for LT. Our results clearly showed that M2BPGi is a highly useful biomarker for evaluating the status of a native liver, and may also be an important factor in determining indication for LT.

This study has some limitations. Out institute, as a department of transplantation, treated patients who were candidates for LT, and the data were collected during LT. The patients were in an advanced stage of fibrosis such as F3 or F4, and there were few patients at grade F1 or F2. Therefore, not all of the circumstances encountered by BA patients were included in this study. To clarify the value of examining M2BPGi in patients with BA, we should collect M2BPGi data for histological examination from patients who live with a native liver and do not need LT, or we should collect data from each patient over time as degree of fibrosis lessens or strengthens. Ultrasonic transient elastography (Fibroscan) is another less invasive method to evaluate the liver fibrosis that has recently been reported as highly useful [21, 22]. We did not perform elastography and could not compare the M2BPGi data. Further analysis will be needed.

In conclusion, M2BPGi is a novel fibrosis marker to evaluate the status of the liver in patients with BA. It is especially useful for predicting a fibrosis score of F4 or less.

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Compliance with ethical standards

Conflict of interest The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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