Biochemical markers to predict the development of gastrointestinal bleeding and esophageal varices after portoenterostomy in biliary atresia

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Abstract
Purpose Gastrointestinal bleeding (GIB) due to esophageal varices (EV) is one of the factors that negatively impact native liver survival of patients with biliary atresia (BA). Gastrointestinal fibroscopy (GIF) is usually used to determine the presence of EVs; however, it requires general anesthesia. The aim of this study is to search for markers in blood tests obtained during routine check-ups that can predict the development of GIB.
Methods Data of patients with BA who underwent portoenterostomy at our hospital from 2014 to 2020 were retrospectively reviewed. The patients’ data were assigned to three groups according to specific time points: Group B, which included data at GIB; Group NB-T, which included data at GIF and EV treatment; and Group NB-NT, which included data at GIF without treatment. The data in Group B were compared to those of other groups.
Results In our study, GIB occurred in 11 patients, and 12 cases and 8 cases were classified into Groups NB-NT and NB-T, respectively. Compared with the other groups, only ChE and M2BPGi in Group B showed statistically significant differences.
Conclusions ChE and M2BPGi are useful for predicting GIB.

Keywords Biliary atresia · Gastrointestinal bleeding · Esophageal varices · Laboratory test

Background
Biliary atresia (BA) is a disease characterized by progressive fibro-obliteration and obstruction of the biliary tree caused by inflammation of unknown etiology. Kasai portoenterostomy (KP), wherein a Roux-en-Y loop of the jejunum is anastomosed to the porta hepatis, facilitates bile drainage. However, when jaundice is not resolved by KP, liver cirrhosis progresses, and the patient will eventually require liver transplantation (LT) [1]. BA is known to cause portal hypertension due to the progression of hepatic fibrosis even after successful KP [2]. Gastrointestinal bleeding (GIB), which is mostly caused by esophageal varices (EV), is associated with portal hypertension and has a significant impact on the prognosis of BA [3].

Upper gastrointestinal fibroscopy (GIF) is necessary to detect and treat EV and prevent GIB. General anesthesia is essential for performing GIF; however, the procedure carries inherent risks especially in children. Therefore, avoiding unnecessary GIF is preferred. Predicting the onset of GIB or EV from simple indicators, such as blood test results, even before the onset of GIB is essential.

The aim of this study is to identify biochemical markers for predicting GIB and EV by retrospectively reviewing data of patients with BA who underwent KP.
Methods.

Data of patients with BA who underwent KP at our hospital from January 2014 to December 2020 were retrospectively reviewed and approved by our ethics committee (ref no. 2021-0155).

Data of enrolled patients at the first occurrence of GIB and at the first GIF before GIB were collected. Patients who did not develop GIB or undergo GIF were excluded.

The data of patients were assigned to 1) Group B, which included those who developed GIB, and 2) Group NB, which included those who underwent GIF without GIB. GIB was defined as any episode of bloody stools or tarry stools, a positive fecal occult blood test result, and obvious hematemesis.

Group NB was further subdivided based on the presence or absence of EV treatments such as injection sclerotherapy or variceal ligation; Group NB-T was comprised of patients who underwent treatment, whereas Group NB-NT was comprised of patients who did not undergo treatment. For GIB, those who underwent GIF before the occurrence of GIB were assigned to Group NB, whereas those who underwent GIF after the occurrence of GIB were excluded. Patients who underwent LT were terminated from the investigation at the time of LT (Fig. 1).

The patient backgrounds and the latest blood examination results were retrospectively investigated. The blood examination for investigation was collected at the outpatients of routine follow-up. Aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyltransferase, cholinesterase (ChE), total bilirubin, direct bilirubin, total bile acid, and mac-2 binding protein glycosylation isomer (M2BPGi) levels and platelet counts were reviewed. In addition, the APRI score was calculated from the data collected using the following formula: APRI = (measured AST/30; i.e., upper normal limit of AST)/platelet count (10³/μL). To predict the onset of GIB, they were compared among the three groups.

Group NB was further divided into two groups according to the EV findings: Group NR, wherein EVs were either not found by GIF or the EVs did not have the red color (RC) sign; and Group RC, wherein EVs with RC sign were found (Fig. 1). To determine whether the detected EVs required treatment, the data were compared between the two groups.

Statistical analysis was performed by Fisher’s exact test for categorical variables. For continuous variables, the Kruskal–Wallis test was used to compare data among the three groups, and the Mann–Whitney U test with Bonferroni correction was used to compare data between each group. A p value <0.05 was considered statistically significant. Receiver operating characteristic (ROC) analysis was performed for the items in which statistically significant differences were detected, and the thresholds and their sensitivity and specificity were determined.

Results

A total of 55 patients underwent KP for BA during the study period. Among these, 18 patients underwent LT without undergoing GIF and before GIB occurrence, and 10 patients survived with a native liver without undergoing GIF nor GIB; they were excluded from the investigation.

In total, GIB occurred in 11 cases, who were assigned to Group B. They contained 4 males and 7 females. They experienced the first GIB at a median of 469 days old (range 296–1952), and at a median of 405 days (range 207–1897) after KP. The blood specimens for investigation were collected at a median of 32 days before the occurrence of GIB (range 2–96).

Whereas 20 cases underwent GIF before the occurrence of GIB or without an encounter with GIB. GIF without EV treatment was performed in 12 cases and their data were assigned to Group NB-NT. They contained 3 males and 9 females. They underwent GIF at a median of 607 days
old (range 303–1643), and at a median of 569 days (range 231–1591) after KP. The blood specimens for investigation were collected at a median of 43 days before the occurrence of GIB (range 1–57). Among them, 2 developed GIB afterwards. While, EV treatment was performed in 8 cases, and their data were assigned to Group NB-T. They contained 3 males and 5 females. They underwent GIF at a median of 856 days old (range 306–1655), and at a median of 815 days (range 252–1571) after KP. The blood specimens for investigation were collected at a median of 36 days before the occurrence of GIB (range 1–78). Among them, 2 developed GIB afterwards. While, EV treatment was performed in 8 cases, and their data were assigned to Group NB-T. They contained 3 males and 5 females. They underwent GIF at a median of 856 days old (range 306–1655), and at a median of 815 days (range 252–1571) after KP. The blood specimens for investigation were collected at a median of 36 days before the occurrence of GIB (range 1–78).

Among them, 2 developed GIB afterwards. There was no statistical difference in the following three parameters among the three groups: the male-to-female ratio, the age at the event, postoperative day at the event, and the period between specimen collection and the event (Table 1).

Only ChE and M2BPGi in Group B were significantly different from those in the other two groups. No other parameter in Group B showed a statistically significant difference from those in the other two groups (Table 1). Group B had a lower ChE value (median, 152 U/L) and higher M2BPGi value (median, 6.92 C.O.I.) than those of the other two groups (Fig. 2). ROC analysis showed a high risk of GIB when the ChE value was less than 196 U/L (sensitivity; 90.9% and specificity; 90.0%) or the M2BPGi value was more than 5.89 C.O.I. (sensitivity; 81.8% and specificity; 75.0%) (Fig. 3).

Subsequently, the EV findings during GIF were also reviewed. Of the 12 cases in Group NB-NT, EVs were absent in 5 cases, and only slight EVs with negative RC signs were observed in 7 cases. In contrast, of the 8 cases in Group NB-T, severe EVs with RC signs were observed in all cases. In other words, data in Group NB-NT also belonged to Group NR, and all data in Group NB-T also belonged to Group RC.

The comparison between Group RC and Group NR revealed that the M2BPgi values, platelet counts, and APRI scores were significantly different between the groups ($p = 0.0018$, 0.0078, and 0.0186, respectively). Group RC had a higher M2BPgi value (median, 4.24 C.O.I.), lower platelet count (median, $118 \times 10^3/\mu L$), and higher APRI score (median, 2.37) than those of Group NR (Fig. 4). ROC analysis showed that there was a higher risk of RC-positive EVs when 1) the M2BPgi value was higher than 2.91 C.O.I. (sensitivity; 100.0% and specificity; 75.0%), 2) the platelet count was less than $198 \times 10^3/\mu L$ (sensitivity; 87.5% and specificity; 75.0%), and 3) the APRI score was higher than 1.45 (sensitivity; 87.5% and specificity; 75.0%) (Fig. 5).

**Discussion**

BA is a chronic cholestatic disease of unknown etiology that occurs in approximately 1 in 8000–18,000 live births. KP is performed to drain bile, and approximately 60% of

| Table 1 | The patient backgrounds and laboratory data of Groups B, NB-NT, and NB-T |
|---------|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|         | Group B                  | Group NB-NT     | Group NB-T      | $p$ value among 3 groups | $p$ value by Mann Whitney with Bonferroni correction of Group B vs NB-NT | $p$ value by Mann Whitney with Bonferroni correction of Group B vs NB-T |
| N       | 11                        | 12              | 8               |                 | 0.794           | 0.005           |
| male/female | 4/7                      | 3/9             | 3/5             |                 | 0.203           |                  |
| Age (days) | 469 (296–1952)            | 606 (303–1643)  | 856 (306–1655)  |                 | 0.236           |                  |
| POD (days) | 405 (207–1897)            | 569 (231–1591)  | 815 (252–1571)  |                 | 0.839           |                  |
| Specimen (days) | 32 (2–96)                | 43 (1–57)       | 36 (1–78)       |                 | 0.081           |                  |
| AST U/L   | 119 (55–312)              | 88 (38–239)     | 99 (46–125)     |                 | 0.318           |                  |
| ALT U/L   | 69 (41–422)               | 44 (9–215)      | 50 (33–85)      |                 | 0.011           | 0.068           |
| GGT U/L   | 187 (31–486)              | 97 (17–587)     | 160 (27–294)    |                 | 0.122           |                  |
| ChE U/L   | 152 (114–235)             | 267 (152–419)   | 241 (166–398)   | <0.001          |                  | <0.001          |
| T-B mg/dL | 1.0 (0.5–2.9)             | 0.7 (0.4–4.5)   | 1.0 (0.5–1.8)   | 0.083           |                  | 0.036           |
| D-B mg/dL | 0.4 (0.0–1.4)             | 0.1 (0.0–3.1)   | 0.1 (0.0–0.6)   | 0.122           |                  |                  |
| TBA μmol/L| 131.4 (33.7–585.6)        | 107.0 (10.1–288.3) | 126.6 (72.4–409.4) | 0.105 |                  |                  |
| M2BPgi C.O.I | 6.92 (2.26–10.63) | 1.93 (0.62–3.99) | 4.24 (0.94–7.14) | <0.001          |                  | <0.001          |
| Platelet $10^3/\mu L$ | 160 (45–316)         | 241 (141–386)   | 118 (88–259)    | 0.011           | 0.068           | 1                |
| APRI score | 2.38 (0.78–23.1)       | 1.09 (0.42–3.48) | 2.37 (1.00–4.37) | 0.016           | 0.045           | 1                |

The results of the statistical analysis of the three groups are also described. Data are expressed as median (range).
patients achieve successful bile drainage and jaundice resolution. When KP fails, liver cirrhosis progresses, and the patient will ultimately require LT [4]. However, even after reestablishing bile flow, liver fibrosis may progress, which may cause portal hypertension that results in EV. Most EVs develop before 2 years old in more than 50% of these children [5].

EV is a major cause of GIB, and GIB strongly affects the prognosis of children with BA and whether the children can live with their own liver. Duche et al. reported that 75% of

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**Fig. 2** M2BPGi (a) and ChE (b) values of the three groups are represented by a box and whisker plot. The numbers indicate the median of each group.

**Fig. 3** Receiver operating characteristics curve for (a) M2BPGi and (b) ChE in cases with GIB (Group B) and in cases without GIB (other 2 groups). The numbers in each image indicate the threshold (sensitivity and specificity) of each item.
children with BA developed GIB by 2 years old, and when GIB occurred, the probability of LT increased to approximately 20% at 2 years old [5]. Therefore, GIF examination to evaluate EV and prevent GIB should be mandatory for BA patients.

General anesthesia is often required when children undergo GIF. Due to their age, children with BA that will undergo GIF cannot be examined without general anesthesia. In our study, GIB occurred at a median of 1 year after KP. Thus, it is necessary to perform GIF on younger children.
to prevent GIB. However, general anesthesia itself is not completely risk-free [6]. Therefore, it is important to select patients who absolutely require GIF to avoid unnecessary general anesthesia. Based on this, the blood examination results were investigated to determine whether a parameter could predict GIB in patients with BA. After analysis, ChE and M2BPGi were revealed to be predictors of GIB.

For the same purpose, various examinations have been reviewed. In adult portal hypertension due to liver cirrhosis, the APRI and Fib-4 scores are highly regarded as predictors of bleeding from EV [7]. In one study, the sensitivity and specificity of the APRI and FiB-4 scores in predicting EV were 60 and 52.1%, and 65 and 57.4%, respectively. Furthermore, the platelet count was revealed to have the highest sensitivity and specificity at 62.4 and 70%, respectively. In our study, the APRI score and platelet count did not significantly predict GIB. However, M2BPGi or ChE, which both showed statistically significant differences in our study, had higher sensitivity and specificity than the parameters in previous studies. The FiB-4 score, which includes age, was not used in our study because the scores for adults were not applicable to our subjects.

A similar study on children with BA also reported that fibrinogen was found to be a significant predictor of GIB [8], and that a higher incidence of GIB was significantly associated with lower fibrinogen levels. However, its sensitivity, specificity, and threshold were not described in their report. In our study, fibrinogen was not included as it was not examined routinely. Hence, we do not have data regarding fibrinogen as a predictor of GIB.

Some reports have used imaging studies to predict GIB, with some evaluating the combination of spleen size and blood test results [9–11]. Another study reported that transient elastography of the liver with ultrasonography could predict GIB in BA patients [12]. However, because imaging studies are not frequently performed unlike blood tests, results of imaging studies were not as frequently available among our cases. On the other hand, blood examinations, particularly ChE and M2BPGi, can be routinely performed for follow-up observation of BA patients.

It has been reported that preventing GIB could reduce complications associated with portal hypertension and increase the probability of native liver survival in children with BA [5, 13]. Prophylactic treatment for EV in children with BA is effective in preventing spontaneous GIB and should be advocated for high-risk EV [13]. Some studies have reported that a positive RC sign indicates a high-risk EV that is likely to induce GIB [14, 15].

In our study, we assumed that EVs with a positive RC sign required treatment, and the predictors of a positive RC sign were estimated. The results showed that there were statistically significant differences in the M2BPGi values, platelet counts, and APRI scores when EVs with RC signs were found compared to when EVs without RC signs or no EVs were found.

It has been reported that spleen stiffness based on Two-dimensional shear wave elastography is a good predictor of high-risk EVs [3]. It has a sensitivity and specificity of 92.9 and 90.0%, respectively, which are very accurate. On the other hand, our results were not as an accurate predictor of high-risk EV as spleen stiffness.

Two-dimensional shear wave elastography requires a high level of skill to perform especially in children when the child cannot remain still. Of the three parameters that showed statistically significant differences in our study, the platelet count and APRI score were inferior to 2D shear wave elastography in both sensitivity and specificity. However, the M2BPGi seemed to be comparable to the results of 2D shear wave elastography, especially about sensitivity. Sensitivity is important in screening tests. In particular, the purpose of the current study was not to identify the methods for making a definitive diagnosis but to identify biochemical markers for predicting GIB and EV. Therefore, M2BPGi was noteworthy, which is comparable to excellent but arduous imaging examinations. Therefore, the indicators revealed in this study are sufficient for screening for high-risk EV when only common and simple blood examinations are available.

Based on our results, it is necessary to investigate whether detecting and treating high-risk EVs will increase the possibility of native liver survival in children with BA.

Author contributions W.S. wrote the main manuscript text and prepared figures and table. All authors reviewed the manuscript.

Declarations

Competing interests The authors declare no competing interests.

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