The preoperative lymphocyte ratio and postoperative C-reactive protein are related to the surgical outcome in biliary atresia: an analysis of serial ubiquitous markers of inflammation

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Abstract

Purpose Various prognostic predictors for biliary atresia (BA) have been identified. This study aimed to evaluate the serial changes in the preoperative and postoperative ubiquitous inflammatory biomarkers and their relationship with the outcomes in patients with BA.

Patients and methods Forty-three BA patients were retrospectively reviewed to investigate serial levels of ubiquitous inflammatory biomarkers, including C-reactive protein (CRP) and lymphocyte ratio, and outcomes. The patients with BA were divided based on their outcomes into two prognostic groups: the native liver survivor group (n = 30) and the survivors with living-donor liver transplant group (n = 13).

Results The area under the receiver operating characteristic (ROC) curve analysis showed that a preoperative lymphocyte ratio of < 61% and CRP value > 0.1 mg/dl predicted a poor outcome. In the ROC curve analysis, the timing of reaching the cut-off value of CRP after Kasai portoenterostomy was postoperative day (POD) 57. The third postoperative week, which was the timing of the discontinuation of steroid therapy, was the branchpoint of inflammatory markers between the two prognostic groups.

Conclusion The POD 57 CRP level predicts the surgical outcome of Kasai portoenterostomy. The postoperative anti-inflammatory management of BA can be monitored by the ubiquitous inflammatory biomarkers CRP and the preoperative lymphocyte ratio.

Keywords Biliary atresia · C-reactive protein · Lymphocyte ratio · Native-liver survival rate · Kasai portoenterostomy

Introduction

Various laboratory data, such as total bilirubin (TB), γ-glutamyltransferase (GT), albumin, prothrombin time international normalized ratios (PT-INR) and platelet counts, have been known to reflect the prognosis of biliary atresia (BA) [1–3]. Davenport and other researchers have reported that various immunological biomarkers, such as T helper 17 (Th17) and regulatory T (Treg) cells, serum levels of adhesion molecules (ICAM and VCAM), predict the outcome of BA [4–6]. These immune biomarkers are mainly driven by key cytokine, interleukin-6 (IL-6). As C-reactive protein (CRP) is another biomarker, which is a clinically available and ubiquitous laboratory test, we retrospectively reviewed CRP as well as lymphocyte ratio as ubiquitous inflammatory biomarkers and assessed their correlation with the prognosis of BA.
Methods

Study population and surgical management

The medical records of 43 patients with BA (male: female = 20: 23) who underwent Kasai portoenterostomy (KPE) at our institution from 2000 to 2020 were reviewed to investigate the preoperative and postoperative inflammatory biomarkers and their relationship to the outcomes. The biomarkers included CRP, the lymphocyte count and ratio (lymphocyte count of total WBC count), and the TB level. Each parameter was also compared between patients with native liver survival (NLS) and living-donor liver transplant (LDLT) recipients, using serial values obtained before surgery, and in the 1st, 2nd, 3rd, 6th, 12th, and 24th weeks after KPE.

KPE undertaken in this series included reconstruction with a long Roux-en-Y jejunal limb (60 cm) following portal dissection. Postoperative management included the administration of ursodeoxycholic acid and the administration of corticosteroids (initial dose: 4 mg/kg/day) starting intravenously on postoperative day (POD) 3, with gradual tapering over the following three weeks.

Statistical analyses

The predictive ability of postoperative CRP and the preoperative lymphocyte ratio for the postoperative outcome over 10 years was assessed by area under the receiver operating characteristic (AUROC) curve analyses. All cut-off values were determined based on the sensitivity and specificity. The Kaplan–Meier method was used to calculate native liver survival. The age at KPE and postoperative TB levels were also evaluated and compared according to these inflammatory markers to determine their predictive value for outcome. Other statistical analyses were performed using Fisher’s exact probability test, and the Mann–Whitney U-test for paired comparisons of serial inflammatory parameters and the TB levels between the NLS and the LDLT groups. Serial changes of the mean preoperative and postoperative inflammatory biomarkers and their relationship to the outcomes in patients with BA were analyzed using a repeated measures ANOVA. P values of <0.05 were considered to be statistically significant. Data are expressed as the mean ± standard deviation. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics [7].

Ethical approval

A retrospective chart review and data collection were performed after receiving institutional review board approval in accordance with the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Ministry of Health, Labor, and Welfare of Japan in 2014. The study complied with the 1964 Declaration of Helsinki (revised in 2013) and was approved by the local ethics committee of our institution (registration number: 27–133). All participants or their parents provided their informed consent for registration in this study.

Results

Background characteristics of the patients

Table 1 showed the background characteristics of the patients. All patients underwent KPE, and none died during this study period. A total of 43 patients with BA (males, n = 20; females, n = 23) were included in this study. The median age at KPE was 60 days (range 44–143 days). There were 7 (16.3%) patients with Type I and 36 (83.7%) patients with Type III. The median follow-up period was 11.2 years (range 1.1–21.2 years).

The 43 patients were divided based on their outcomes into two prognostic groups: the NLS group (n = 30) and the LDLT group (n = 13). None of the patients underwent primary LDLT.

A comparison of the time-course changes in the serial inflammatory parameters and TB levels between the two prognostic groups

Serial pre-and postoperative inflammatory parameters during the postoperative 6 month between the two groups were shown in Fig. 1. There were no significant differences in preoperative inflammatory parameters between the NLS and

| Number of patients (male/female) | 43 (20/23) |
| Days of age at KPE surgery (days (range)) | 60 (44–143) |
| Type of BA (I/III) | 7 (16.3%)/36 (83.7%) |
| Follow up period (years) | 11.2 (1.1–21.2) |
| Prognosis | |
| Native-liver survivor | 30 (69.8%) |
| Living donor liver transplantation | 13 (30.2%) |
| Death | 0 (0%) |

KPE Kasai portoenterostomy, BA Biliary atresia
LDLT groups, with the exception of the lymphocyte ratio. The serial TB levels remained significantly higher in the LDLT group than in the NLS group from the 3rd week after KPE (Fig. 1a). Significant differences were observed in the lymphocyte ratio and CRP value (Fig. 1c, d).

**Lymphocyte ratio and relationship to outcome**

ROC curve of preoperative lymphocyte ratio and the native liver survival curve of the two cohort were shown in Fig. 2. We analyzed the native liver survival rate with the AUROC curve of the preoperative lymphocyte ratio (Fig. 2a). In the AUROC curve analysis, the cut-off value of the lymphocyte ratio was 61%, with an area under the AUROC curve of 0.699 (95% confidence interval [CI] 0.529–0.869). We then divided patients into two groups according to this cut-off value to analyze their long-term prognosis using the Kaplan–Meier method (Fig. 2b). We found that a preoperative lymphocyte ratio of > 61% was significantly associated with long-term native liver survival ($p < 0.05$).

Postoperatively, the lymphocyte ratio continued to be higher throughout the 6 month period in the NLS group in comparison to the LDLT group, with significant differences observed in the 1st, 3rd, 6th, 12th and 24th weeks after KPE (Fig. 1c).

**Postoperative CRP and relationship to outcome**

CRP was increased immediately after KPE, then subsequently showed a gradual decrease to the lowest level to 0.11 mg/dl at week 24 post-KPE in NLS group, whereas in LDLT group, the lowest level was 0.4 mg/dl at week 3 (Fig. 1d). Thereafter, CRP remained significantly higher in comparison to NLS group. The 3rd postoperative week was around the time of discontinuation of corticosteroids administration.

We further analyzed the native liver survival rate with the AUROC curve on the day after KPE when the CRP value decreased to 0.1 mg/dl (Fig. 3a). In the ROC curve analysis, the cut-off value for the day after KPE was POD 57, with an area under the AUROC curve of 0.722 (95% CI 0.545–0.898). In addition, we also analyzed the native liver survival rate with the AUROC curve on the day after KPE when the CRP value became < 1.0 mg/dl (area under the AUROC curve: 0.641, 95% CI 0.435–0.847) and < 0.3 mg/dl (area under the AUROC curve: 0.551, 95% CI 0.348–0.755). We then divided patients with CRP levels ≥ 0.1 and < 0.1 on POD 57, and their long-term prognosis using the Kaplan–Meier method (Fig. 3b). We found that patients with a CRP value of ≥ 0.1 higher on POD 57 were significantly more likely to undergo liver transplantation ($p < 0.05$).
Comparison with previously known prognostic factors for BA

Age at KPE and its predictability of outcome

We analyzed the native liver survival rate with the AUROC curve for age (in days) at KPE (Fig. 4a). In the AUROC curve analysis, the cut-off value of the age at KPE was 60 days, with an area under the AUROC curve of 0.683 (CI 0.518–0.848). We then divided patients into two groups according to this cut-off value to analyze their long-term prognosis using the Kaplan–Meier method (Fig. 4b). We found that < 60 days of age at KPE was significantly associated with long-term native liver survival ($p < 0.05$).

TB level and its predictive ability for the outcome

We also analyzed the native liver survival rate with the AUROC curve of the TB level at the 6th week after KPE (Fig. 5a). In the AUROC curve analysis, the cut-off value...
of the TB value was 2.7 mg/dl, with an area under the AUROC curve of 0.841 (CI 0.681–1.0). We then divided patients into two groups according to this cut-off value to analyze their long-term prognosis using the Kaplan–Meier method (Fig. 5b). We found that a TB value of < 2.7 mg/dl higher at the 6th week after KPE was significantly associated with long-term native liver survival ($p < 0.05$).

**Discussion**

We evaluated the preoperative and serial postoperative ubiquitous laboratory biomarkers, CRP, lymphocyte ratio of the peripheral blood in BA patients. We revealed two intriguing findings: first, the CRP value on POD 57 after
KPE as a significant prognostic biomarker for BA and the second, the preoperative lymphocyte ratio as a predictor of the outcome. The predictive value of these ubiquitous inflammatory biomarkers for predicting the outcome was compared to that of well-known prognostic factors, which demonstrated that these markers were inferior to the TB level but equivalent to the age of KPE. This observation implies that the fibroinflammatory process of the bile duct in BA essentially starts before surgical correction and continues to various degrees after surgery.

Plasma CRP, an acute-phase protein, is a sensitive marker of inflammation and tissue damage. It is produced only by hepatocytes and is induced under transcriptional control of IL-6 [8]. IL-6 is produced by dendritic cells and macrophages upon antigen stimulation and is a key cytokine that promotes B cell differentiation, enhances soluble cellular adhesion molecules (e.g., soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular cell adhesion molecule-1 [sVCAM-1]), and stimulates CD4+ T cells to inhibit the Treg cell function and promote Th17 differentiation [9]. In BA, sICAM was reported as the earliest marker to show a significant difference between outcome groups at one month post-KPE [6]. Th17 cell infiltration is greater in the BA liver [6]. Th17 cells are elevated whereas Treg cells are decreased in the peripheral blood mononuclear cells with the increased expression of IL-6 [5]. Based on these reports, the IL-6/CRP axis appears upregulated in patients with BA.

An increased preoperative lymphocyte ratio is an unexpected finding, but seemed to be a potential prognostic factor for BA based on the findings of our analysis. Dong et al. [10] reported that the overexpression of interleukin-8 (IL-8) in BA caused inflammation and led to disease progression because IL-8 is a chemotactic factor that attracts neutrophils during the inflammatory process. Our results might indirectly reflect the suppression of preoperative inflammatory reaction, however, whether the increase in the preoperative lymphocyte count is a primary or a relative phenomenon should be defined.

In our series, we used a fixed protocol of corticosteroid therapy that is prednisone was started on the POD 3 with 4 mg/kg/day and was tapered gradually over the subsequent three weeks. Postoperative CRP value showed the lowest at around that time of discontinuation in each patient. Indeed, the third week is the branchpoint of the lymphocyte ratio and serum TB level between the two prognostic groups. In other words, the patient outcome seems to depend on whether or not the inflammation continuously diminished after discontinuation of steroids. Therefore, more individualized and aggressive use of steroids might be indicated, targeting CRP levels of <0.1 mg/dl and increased lymphocyte ratio during the postoperative two months. This might result in lowering the subsequent risk of flare-up of cholangitis and avoiding liver transplantation.

To our knowledge, we showed for the first time that the ubiquitous inflammatory biomarkers postoperative CRP and the preoperative lymphocyte ratio are related to the immunodynamics in BA, reflecting the surgical outcome.

A major limitation of our study is the lack of phenotypes of peripheral blood lymphocytes. Further investigations are warranted to clarify any impact of glucocorticoids on the Treg cell physiology in BA.

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Author contributions T.H., T.M. and S.I. wrote the main manuscript text. T.H., S.K., M.M., K.T. and S.I. did the data analysis and data interpretation. M.M., K.Y. and S.O. prepared figures 1-5. K.Y., W.Y. and M.M. prepared Table1. S.I. made critical revision. All authors reviewed the manuscript.

Declarations

Conflict of interest The authors declare no conflicts of interest in association with the present study.

References

1. Tomita H, Okhuma K, Masugi Y, Hosoe N, Hoshino K, Fuchimoto Y, Fujino A, Shimizu T, Kato M, Fujimura T, Ishihama H, Takahashi N, Tanami Y, Ebinuma H, Saito H, Sakamoto M, Nakano M, Kuroda T (2016) Diagnosing native liver fibrosis and esophageal varices using liver and spleen stiffness measurements in biliary atresia: a pilot study. Pediatr Radiol 46(10):1409–1417
2. Freeman RB Jr, Wiensner RH, Harper A, McDermott SV, Lake J, Edwards E, Merion R, Wolfe R, Turcotte J, Teperman L, Unos OL, Disease Severity Score UOL, Intestine CUOPT (2002) The new liver allocation system: moving toward evidence-based transplantation policy. Liver Transpl 8(9):851–858
3. Colecchia A, Festi D, di Biase AR (2012) Noninvasive parameters for predicting esophageal varices in children: their sequential use provides the best accuracy. Gastroenterology 142(2):e32 (author reply e32-33)
4. Narayanaswamy B, Gondev C, Tredger JM, Hussain M, Vergani D, Davenport M (2007) Serial circulating markers of inflammation in biliary atresia–evolution of the post-operative inflammatory process. Hepatology 46(1):180–187
5. Yang Y, Liu YJ, Tang ST, Yang J, Cao GQ, Zhang JH, Wang XX, Mao YZ (2013) Elevated Th17 cells accompanied by decreased regulatory T cells and cytokine environment in infants with biliary atresia. Pediatr Surg Int 29(12):1249–1260
6. Hill R, Quaglia A, Hussain M, Hadzic N, Mieli-Vergani G, Vergani D, Davenport M (2015) Th-17 cells infiltrate the liver in human biliary atresia and are related to surgical outcome. J Pediatr Surg 50(8):1297–1303
7. Kanda Y (2013) Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant 48(3):452–458

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8. Pepys MB, Hirschfield GM (2003) C-reactive protein: a critical update. J Clin Invest 111(12):1805–1812
9. Akira S, Taga T, Kishimoto T (1993) Interleukin-6 in biology and medicine. Adv Immunol 54:1–78
10. Dong R, Zheng S (2015) Interleukin-8: a critical chemokine in biliary atresia. J Gastroenterol Hepatol 30(6):970–976

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