Association of serum albumin levels and long-term prognosis in patients with biopsy-confirmed nonalcoholic fatty liver disease

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Abstract

**Background:** The relationship between baseline serum albumin level and long-term prognosis of patients with nonalcoholic fatty liver disease (NAFLD) remains unknown.

**Methods:** This is a sub-analysis of the CLIONE (Clinical Outcome Nonalcoholic Fatty Liver Disease) study. The main outcomes were: death or orthotopic liver transplantation (OLT), liver-related death, and liver-related events (hepatocellular carcinoma [HCC], decompensated cirrhosis, and gastroesophageal varices/bleeding).

**Results:** 1,383 Japanese patients with biopsy-confirmed NAFLD were analyzed. They were divided into 3 groups based on serum albumin: high (>4.0 g/dL), intermediate (3.5–4.0 g/dL), and low (<3.5 g/dL). Unadjusted hazard ratio [HR] of the intermediate albumin group, compared with the high albumin group, were 3.6 for death or OLT, 11.2 for liver-related death, 4.6 for HCC, 8.2 for decompensated cirrhosis, and 6.2 for gastroesophageal varices (all risks were statistically significant). After adjusting confounding factors, albumin remained significantly associated with death or OLT (intermediate vs high albumin group: HR 3.06, 95% confidence interval [CI] 1.59–5.91, \( P < 0.001 \); low vs high albumin group: HR 22.9, 95% CI 8.21–63.9, \( P < 0.001 \)).

**Conclusions:** Among biopsy-confirmed NAFLD patients, those with intermediate or low serum albumin had a significantly higher risk of death or OLT than those with high serum albumin.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common etiology of chronic liver disease, affecting approximately 25% of the adult population worldwide. NAFLD is a clinical consequence of obesity and can progress to nonalcoholic steatohepatitis (NASH). NASH is characterized histologically by the presence of steatosis, inflammatory cell infiltration and hepatocyte ballooning, with or without fibrosis, and can ultimately lead to cirrhosis, hepatocellular carcinoma (HCC), or end-stage liver disease.

Albumin is the most abundant plasma protein and plays a key role in the regulation of plasma colloid osmotic pressure. It also has various other physiologic functions, including solubilization, binding, and transport of endogenous and exogenous molecules; antioxidative, anti-inflammatory, and hemostatic effects; endothelial stabilization; and adjustment of capillary permeability. Importantly, albumin is a major prognostic factor in patients with liver cirrhosis, being reported as a significant predictor of death in over 100 studies. It is also a component of the most important and widely used prognostic score in cirrhosis, the Child-Turcotte-Pugh (CTP) score.

Hypoalbuminemia is frequently observed in patients with advanced cirrhosis and is generally defined as an intravascular albumin level < 3.5 g/dL. The nutritional therapy algorithm in the 2020 guidelines from the Japanese Society of Gastroenterology/Japanese Society of Hepatology recommends...
conducting a nutritional assessment for hypoalbuminemia, Child–Pugh class B or C, and sarcopenia, each of which exerts an adverse impact on clinical outcomes in patients with cirrhosis\textsuperscript{10}. Additionally, Angulo et al. reported that the NAFLD fibrosis score (NFS) could help identify patients with NAFLD at increased risk for liver-related complications or death\textsuperscript{11}. This noninvasive test includes serum albumin levels, which indirectly reflect hepatic synthetic reserve\textsuperscript{11,12}.

Based on these data, we hypothesized that serum albumin levels at the time of liver biopsy could predict the long-term prognosis of patients with NAFLD. Therefore, we used data from a multicenter registry to conduct a cohort study examining the prognostic value of serum albumin in patients with biopsy-proven NAFLD.

**Results**

**Baseline Characteristics**

Baseline characteristics of the cohort (N = 1,383) are presented in Table 1. Mean age was 54.6 years, mean body mass index (BMI) was 27.9 kg/m\textsuperscript{2}, and females accounted for 57.1% (n = 790) of the cohort. The majority (66.9\%) of participants had NASH, and 223 (16\%) had advanced fibrosis (stages 3–4). When patients were divided into 3 categories by albumin levels, we observed a stepwise increase in mean age, aspartate aminotransferase, the Fibrosis-4 (FIB-4), NFS, and prevalence of advanced fibrosis as albumin levels decreased. Conversely, platelet counts decreased significantly with decreasing albumin levels. The relationship between fibrosis stage and albumin levels is shown in Supplementary Fig. 1. The mean values of albumin in stages 0, 1, 2, 3, and 4 were 4.5, 4.4, 4.3, 4.2, and 3.8 g/dL, respectively. When albumin levels were analyzed in relation to the histologic stage of fibrosis, the distribution of albumin levels differed by histologic stage (Kruskal-Wallis test, \( P < 0.001 \)).

Data for clinically important outcomes are presented in Table 2. During a median follow-up of 4.5 years (range, 0.3–21.6 years), there were 46 deaths or orthotopic liver transplantation (OLT) and 20 deaths were liver-related. The occurrence of liver-related events was as follows: HCC, 36 patients; decompensated cirrhosis, 23 patients; and gastroesophageal varices, 17 patients.

**Clinically Important Outcomes According To Albumin Level**

Overall mortality and liver-related events stratified according to albumin are shown in Fig. 1 and Supplementary Fig. 2. Death or OLT, liver-related death, HCC, decompensated cirrhosis, and gastroesophageal varices differed significantly according to the serum albumin category (high, intermediate, or low; log-rank \( P < 0.001 \) for all outcomes).

Next, we calculated the unadjusted hazard risks of clinically important outcomes (Fig. 2). Among patients with biopsy-proven NAFLD, the hazard ratios (HRs) were significantly increased in the intermediate albumin group, compared with the high albumin group, for death or OLT (3.6), liver-related death (11.2),
HCC (4.6), decompensated cirrhosis (8.2), and gastroesophageal varices (6.2). The hazard risks were also higher in the low albumin group, compared with the high albumin group, for death or OLT (20.9), liver-related death (83.6), HCC (4.2), decompensated cirrhosis (45.4), and gastroesophageal varices (38.4). These low albumin group risks were statistically significant for all outcomes except HCC.

Univariate HRs for death or OLT and liver-related death are shown in Supplementary Table 1. Lower albumin levels were significantly associated with death or OLT (intermediate vs high albumin group: HR 3.58, 95% CI 1.91–6.69, \( P < 0.001 \); low vs high albumin group: HR 20.9, 95% CI 7.72–56.8, \( P < 0.001 \)) and liver-related death (intermediate vs high albumin group: HR 11.2, 95% CI 3.88–32.4, \( P < .001 \); low vs high albumin group: HR 83.6, 95% CI 21.1–330, \( P < 0.001 \)). Older age (\( \geq 65 \text{ y} \)) was significantly associated with death or OLT (HR 2.58, 95% CI 1.43–4.68, \( P = 0.002 \)). More advanced fibrosis stage was also significantly associated with liver-related death (stage 3 vs stage 0; HR 5.22, 95% CI 1.08–25.3, \( P = 0.040 \)).

Table 3 shows the multivariate analysis results. Lower baseline serum albumin levels were significantly associated with death or OLT in model 1 (intermediate vs high albumin group: HR 3.51, 95% CI 1.85–6.63, \( P < 0.001 \); low vs high albumin group: HR 23.3, 95% CI 8.42–64.6, \( P < 0.001 \)), model 2 (intermediate vs high albumin group: HR 3.26, 95% CI 1.72–6.20, \( P < 0.001 \); low vs high albumin group: HR 23.6, 95% CI 8.51–65.6, \( P < 0.001 \)), and model 3 (intermediate vs high albumin group: HR 3.06, 95% CI 1.59–5.91, \( P < 0.001 \); low vs high albumin group: HR 22.9, 95% CI 8.21–63.9, \( P < 0.001 \)) (Table 3). Lower baseline serum albumin levels were also significantly associated with liver-related death in model 1 (intermediate vs high albumin group: HR 11.4, 95% CI 1.85–6.63, \( P < 0.001 \); low vs high albumin group: HR 99.8, 95% CI 24.1–412, \( P < 0.001 \)). Older age (\( \geq 65 \text{ y} \)) and male sex were significantly associated with death or OLT in all models (Supplementary Table 2).

Discussion

Our retrospective cohort study yielded at least 2 major findings. First, clinical outcomes of NAFLD could be stratified by the serum albumin level at the time of liver biopsy. Second, the prognostic utility of baseline serum albumin level for predicting the outcome of death or OLT remained significant after adjusting for age, sex, presence of histologic NASH, diabetes mellitus (DM), and fibrosis stage, thus highlighting the importance of aggressive efforts to provide early nutritional intervention.

Hypoalbuminemia is a well-known risk factor for mortality and other clinically important adverse outcomes in a variety of patient populations\(^6\),\(^9\). Hypoalbuminemia has also been associated with poorer prognosis for individuals with liver disease, including NAFLD. For example, Kawanaka et al. followed 489 patients with biopsy-proven NAFLD for 1–22.2 years\(^13\) and found that patients with an albumin level < 3.5 g/dL, platelet counts < 150×10\(^9\)/L, and type IV collagen 7S levels \( \geq 5 \text{ ng/mL} \) indicate a poor prognosis. In particular, the 10-year survival rate is only 43% in patients presenting with all three factors. They concluded that albumin is reported to be one of the most important prognostic factors\(^13\). Vilar-Gomez et al. followed 458 patients with biopsy-proven NAFLD and bridging fibrosis (F3, n = 159) or
compensated cirrhosis (CTP score of A5, n = 222; CTP score of A6, n = 77) for 5.5 years (range, 2.7–8.2 years). The transplantation-free survival rate at 10 years was higher in patients with F3 fibrosis (94%; 95% CI 86–99%) than in those with cirrhosis and a CTP score of A5 (74%; 95% CI 61–89%) or A6 (17%; 95% CI 6–29%). Importantly, lower albumin levels (3.0–3.5 g/dL) would explain the main differences observed between patients with a CTP score of A5 and those with a CTP score of A6. In our analysis, the hazard risk of all clinical events except HCC was 20–80 times higher in the low albumin group than in the high albumin group by Cox proportional hazard regression analysis (Fig. 2); these results are consistent with those of previous reports. As mentioned above, NFS, which includes albumin as a variable, is useful for predicting the prognosis of NAFLD. Furthermore, the Model for End-Stage Liver Disease (MELD), which is a known reliable predictor of short-term survival in patients with end-stage liver disease, was recently updated to MELD 3.0 to improve the accuracy of mortality prediction. This optimized version takes into account new variables, including serum albumin.

Branched-chain amino acids (BCAAs) are a group of essential amino acids consisting of valine, leucine, and isoleucine. BCAA supplementation was originally proposed as a strategy to normalize amino acid profiles and nutritional status. However, large-scale, multicenter, randomized, double-blinded, controlled trials performed in Italy and Japan have demonstrated that BCAA supplementation improves not only nutritional status but also prognosis and quality of life in patients with liver cirrhosis. Specifically, a study from Japan revealed that serum albumin levels increased significantly after 2 years of oral BCAA administration. The 2019 European Society for Clinical Nutrition and Metabolism guideline on clinical nutrition in liver disease recommends long-term oral BCAA supplements (0.25 g × kg$^{-1}$ × d$^{-1}$) for patients with advanced cirrhosis to improve event-free survival or quality of life. In our cohort, the percentage of patients with advanced fibrosis in the intermediate albumin group (albumin 3.5–4.0 g/dL) was only 25% (74/295) (Table 1). Moreover, the onset of clinically important events in this group often occurred approximately 5 years after the start of observation, which tended to be earlier than the time of onset in the high albumin group (Fig. 1). In consideration of previous reports that patients with greater declines in serum albumin during follow-up have a poorer prognosis, BCAA administration to the intermediate albumin group may be beneficial to improve the prognosis of patients with NAFLD. However, the following points should be noted: 1) the inclusion criteria for BCAA use in patients with cirrhosis in the aforementioned Japanese study defined a low albumin concentration as ≤ 3.5 g/dL, so its usefulness in patients with NAFLD, including those without cirrhosis, remains unclear, and 2) in most countries, oral BCAA supplements are not reimbursed, and the combination of cost and poor palatability may affect compliance.

Long-term administration of albumin can modify the course of decompensated cirrhosis by reducing the onset of new complications, improving the quality of life, and probably providing survival benefits. There is, however, a need to rationalize the use of albumin therapy in different types of liver disease and stages of cirrhosis and to determine the optimum dose, duration, and frequency of albumin in each situation. Hypoalbuminemia can develop during acute stress, major operations, trauma, or infection and...
does not always require albumin replacement. Indeed, it has been estimated that 40–90% of albumin prescriptions are unjustifiably given for correcting hypoalbuminemia per se, without considering the underlying disease process.

The main strengths of our study were the large number of included patients; confirmation of all cases of NAFLD by liver biopsy; grading and staging of liver biopsies by a single, experienced liver pathologist, thereby avoiding inter-observer variability; and the use of widely accepted scoring systems, including the FLIP algorithm, to grade and stage liver biopsy features.

This study also had some limitations, most of which are inherent to retrospective studies, including the absence of a specific treatment protocol, lack of follow-up endoscopic evaluations, and lack of imaging results in patients without cirrhosis. Thus, the number of liver-related events was possibly underestimated. Additionally, the follow-up period was shorter than in some other studies. Nevertheless, this study was not meant to be a clinical trial; the goal was to analyze real-world data from patients who underwent liver biopsy.

In conclusion, baseline albumin levels allow appropriate prediction of patients with NAFLD at higher risk of developing death or OLT, liver-related death, HCC, decompensated cirrhosis, and gastroesophageal varices. These results are relevant for patient counseling and early nutritional intervention (especially an albumin level < 4.0 g/dL) in Asian patients with NAFLD.

**Methods**

**Study design and population**

The current study is a sub-analysis of the longitudinal multicenter cohort study called CLIONE (Clinical Outcome Nonalcoholic Fatty Liver Disease) in Asia. After excluding 15 patients with missing serum albumin data, we enrolled 1,383 Japanese patients with biopsy-proven NAFLD. The methodology for establishing the cohort, as well as the main CLIONE study results, are reported elsewhere. The current study was performed according to the 1964 Declaration of Helsinki, and approved by the institutional review board of Saga University Hospital (approval no. 2020-04-R-02, June 30, 2020), which waived the requirement for informed consent because of the use of pre-existing data. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

**Data sources**

We used a database from the Japan Study Group of NAFLD (JSG-NAFLD) to obtain information regarding patients with biopsy-proven NAFLD. All study data were collected and managed using REDCap electronic data capture tools, hosted at the Osaka Metropolitan University.

**Study cohort**
We identified all patients diagnosed with biopsy-proven NAFLD between December 1, 1994 and December 31, 2020. We followed this cohort until March 31, 2021 to identify clinically important outcomes: death or OLT, liver-related death, and liver-related events (HCC, decompensated cirrhosis, or gastroesophageal varices/bleeding). We excluded patients with excessive alcohol intake (> 30 g/day in men; >20 g/day in women) or liver disease of another etiology, including viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cholangitis, or biliary obstruction.

**Clinical assessment**

Data were extracted regarding BMI, blood pressure, daily alcohol intake, smoking habits, past medical history, and current drug history. We also recorded plasma glucose, lipids, and liver biochemistry values, which were measured in venous blood samples obtained after fasting for ≥ 8 h. DM, hypertension, and dyslipidemia were diagnosed according to standard criteria. FIB-4 and NFS were calculated using the available parameters. For this study, we divided the patients into 3 groups according to albumin level: high (> 4.0 g/dL), intermediate (3.5–4.0 g/dL), and low (< 3.5 g/dL).

**Liver histology**

Percutaneous liver biopsy samples were obtained under ultrasound guidance. Formalin-fixed, paraffin-embedded liver sections were stained with hematoxylin and eosin or azan and then transported for central reading by an experienced pathologist (S.A.) at Saga University, who was blinded to the patients’ clinical and laboratory data. NAFLD was defined as the presence of ≥ 5% hepatic steatosis (according to Kleiner et al.). Grading and staging were performed according to Brunt et al. and Kleiner et al. Advanced fibrosis was defined as stage 3 or 4 fibrosis. NASH was diagnosed according to the fatty liver inhibition of progression (FLIP) algorithm.

**Follow-up evaluation**

Details regarding follow-up evaluations were described previously and are summarized briefly here. The follow-up period began on the day of liver biopsy and continued until the last visit, death, or OLT. Patients were followed at 3– to 12–month intervals after NAFLD diagnosis, and anthropometric measurements and metabolic assessments were repeated during each visit. For liver-related events (HCC, gastroesophageal varices, and decompensated cirrhosis), only the first event after liver biopsy was recorded; recurrent events were excluded. Follow-up duration was the period between the date of the biopsy and the date of the most recent follow-up.

**Statistical analysis**

Continuous and ordinal variables are expressed as mean (standard deviation) or median (range) and were compared using the unpaired t-test. Categorical variables were compared using the $\chi^2$ test. Clinically important outcomes are presented as Kaplan–Meier curves, and albumin levels were compared using the log-rank test. Univariate (unadjusted) and multivariate (adjusted) HR estimates (relative risk) of clinically significant outcomes were calculated using Cox proportional hazard regression analysis to control for the
effects of potential risk factors, while considering follow-up duration. In this study, we created 3 models. Model 1 included serum albumin level, age (binarized as ≥ 65 years and < 65 years), and sex (male or female). Model 2 included variables from model 1 plus NASH (based on the FLIP algorithm) and stage. Model 3 included variables from model 1 plus DM and stage. Two-sided $P$ values < .05 were considered significant. All statistical tests were conducted using JMP® 16.0.0 software (SAS Institute Inc., Cary, NC, USA).

**Declarations**

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**Author contributions**

Conceptualization and Methodology: H. Fujii; Statistical analysis: H. Fujii, H. Takahashi, and A. Tokushige; Interpretation of the data: H. Fujii, H. Takahashi, M. Kawanaka, and Y. Kamada; Writing – original draft preparation: H. Fujii; Writing – review and editing: H. Fujii, H. Takahashi, M, Kawanaka, Y. Kamada, A. Tokushige, Y. Kamada, and Y. Sumida. All authors have reviewed and approved the final version of the manuscript.

**Conflict of interest statement:** All authors declare that they have no financial conflicts of interest to disclose.

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**Data availability**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**References**


2. Chalasani, N. *et al.* The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology **67**, 328–357,


Tables 1-3 is available in the Supplementary Files section.

**Figures**

**Figure 1.**

*Clinically important outcomes stratified according to albumin*

- **a.** Death or orthotopic liver transplantation
- **b.** Liver-related death
- **c.** Hepatocellular carcinoma
- **d.** Decompensated cirrhosis

**Figure 1**

Clinically important outcomes stratified according to albumin.
(a) Death or OLT, (b) liver-related death, (c) hepatocellular carcinoma, (d) decompensated cirrhosis. OLT, orthotopic liver transplantation. Albumin groups: high, >4.0 g/dL; intermediate, 3.5–4.0 g/dL; low, <3.5 g/dL.

Figure 2.

Heatmap of univariate unadjusted hazard risks of clinically important outcomes

<table>
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<td>Gastroesophageal varices</td>
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</table>

Significant hazard risks are highlighted in bold. OLT, orthotopic liver transplantation.

Figure 2

Univariate unadjusted hazard risks of clinically important outcomes.

OLT, orthotopic liver transplantation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [20221123SupplementaryInformation.docx](#)
- [Table13.docx](#)