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Decline in serum albumin concentration is a predictor of serious events in nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is associated with metabolic syndrome, which includes diabetes mellitus and hyperlipidemia. A fraction of NAFLD patients develop nonalcoholic steatohepatitis, leading to cirrhosis associated with various serious complications, including hepatocellular carcinoma, gastroesophageal varices, cardiovascular events, and other organ malignancy. Although the incidence of chronic viral hepatitis with associated complications has gradually decreased as highly effective antiviral therapies have been established, the number of patients with steatohepatitis has been increasing.

This retrospective study examined data of 229 patients from 22 hospitals in our region. We examined 155 cases of chronological data and assessed the development of liver fibrosis and evaluated hepatic reserve-related markers such as platelet count, FIB-4 index, prothrombin time, and serum albumin concentration. We analyzed the relationship of these chronological changes and the incidence of NAFLD related serious complications.

Data related to liver fibrosis progression, albumin, and prothrombin time were significantly associated with the occurrence of serious complications associated with cirrhosis. We compared 22 event and 133 nonevent cases of chronological changes in the data per year and found that serum albumin concentration was significantly lower in the group that developed serious complications (event cases: -0.21 g/dL/year, nonevent cases: –0.04 g/dL/year ($P < .001$)). This albumin decline was only the associated factor with the event incidence by multivariate analysis ($P < .01$).

Annual decline in serum albumin concentration in patients with NAFLD is associated with serious events from the outcome of multicenter retrospective study. This highlights its potential utility as a surrogate marker to assess the efficacy of prediction of NAFLD related serious events.

Abbreviations: ALT = alanine aminotransferase, AST = aspartate aminotransferase, CLD = chronic liver disease, DM = diabetes mellitus, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HL = hyperlipidemia, HT = hypertension, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, PT = prothrombin time.

Keywords: FIB-4 index, gastroesophageal varices, hepatic encephalopathy, hepatocellular carcinoma, liver fibrosis, nonalcoholic steatohepatitis, prothrombin time

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Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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1. Introduction

The modern lifestyle, characterized by an excess of calorie intake and insufficient exercise,[1] has contributed to the increase in patient populations of diabetes mellitus (DM), hypertension (HT) and hyperlipidemia (HL), which are cluster conditions that comprise metabolic syndrome. In a substantial number of patients, metabolic syndrome is also complicated by fatty liver disease, because the liver accumulates and stores excess nutrients.[2] Fatty liver disease includes alcoholic liver disease and nonalcoholic fatty liver disease (NAFLD); the underlying mechanisms are commonly associated in part with lipid accumulation in the hepatocytes.[3] The detailed pathological and clinical features of NAFLD have yet to be fully clarified; in some cases NAFLD progresses to nonalcoholic steatohepatitis (NASH), which is concomitant with inflammation of the liver that may result in liver fibrosis.[4] However, progression to advanced liver disease, particularly liver cirrhosis, occurs in about 10% of NAFLD patients over a 10- to 20-year observation period,[5] and details of the clinical course are yet to be reported. Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection has been the most common cause of chronic liver disease (CLD). Recent therapeutic advances in controlling or eradicating HBV and HCV have succeeded in alleviating the incidence of liver dysfunction, ameliorating advanced hepatic fibrosis, and decreasing the incidence of HCC.[6] In contrast, research and development of antifibrotic treatments aimed at halting NASH progression have been carried out and clinical trials of novel treatments have been conducted[7]; however, no effective treatment for NAFLD/NASH has been established. Therefore, existing therapeutic strategies remain limited to the control of body weight and improvement of lifestyle habits.

The progression of liver fibrosis in NAFLD/NASH is a serious condition because cirrhosis is frequently associated with various other complications, including gastroesophageal varices and HCC, which further worsen prognosis.[8] Some reports have described measures of the extent of fibrosis such as FIB-4 index, serum markers, hyaluronic acid, procollagen type III peptide and the 7S domain of type IV collagen, for assessing the development hepatic fibrosis.[9] Moreover, transient elastography with the FibroScan device (Echosens, Paris, France) for noninvasive liver diagnosis and serum *Wisteria floribunda* agglutinin-positive Mac-2 binding protein, and serum aldo-keto reductase family 1 member B10 have been reported to be useful for assessing advanced disease in NASH.[10,11] Various factors including metabolic disorder-related diseases that are associated with NAFLD/NASH affect the clinical and pathological features.[1,2,12] However, it is not fully clear whether these clinical markers are valuable for assessing the progression of NAFLD/NASH.

Previous studies on the clinical course of NAFLD have shown that 3% of patients developed liver cirrhosis over an observation period of about 8 years, with liver disease-associated death in 1.7% of patients.[13] The average incidence rate of HCC has been found to be 3% per year in patients with NASH-related liver cirrhosis.[14] However, this value may differ by country or region because of differences in lifestyle habits; moreover, the exact diagnosis or characteristics of NAFLD patients is unclear, such as the amount of alcohol consumed and the results of virology and other laboratory tests. Single-center analysis of the risk of serious events after NAFLD diagnosis may have some disadvantage because of clinical bias specific to each institute. For these reasons, multicenter clinical studies are important for assessing what clinical features and data are sensitively associated with the occurrence of serious events related to NAFLD.

Therefore, in this study, we analyzed data of patients with a diagnosis of NAFLD from multiple institutions in our region, by comparing the clinical background of the patients and the incidence of serious events or prognosis. Chronological decline in serum albumin concentration was found to be a valuable indicator of the occurrence of serious complications of NAFLD/NASH.

2. Methods

2.1. Study design and patient population

We collected clinical information on patients with NAFLD including blood examination data, complications, treatments, NAFLD-related events, and treatment-related events observed over a period of at least 1 to 5 years using patients' medical records from April 1, 2006 to December 31, 2019. Most of the participating institutions are core hospitals in their region, and others are outpatient clinics that provide regular health check-ups. NAFLD was diagnosed based on blood examination and imaging tests such as ultrasonography and computerized tomography scanning. In this study, patients diagnosed with NAFLD had an ethanol intake below 70 g/day with accompanying obesity, DM, HL, HT, and hyperuricemia. HBV or HCV infection, alcoholic liver injury, autoimmune hepatitis, primary biliary cholangitis, and drug-induced liver injury were excluded from the diagnosis of NAFLD. This study was approved by the medical ethics committee of Kanazawa University (approval number: 2064), and ethical essential documents were also approved by the relevant authorities of each participating institution.

2.2. Methods of data acquisition

NAFLD-related event case report forms were sent to participating institutions to collect information on sex, age, complications of DM, HL, serial platelet count, prothrombin time (PT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and albumin. We also collected other serological data, imaging test results, liver biopsy results, clinical information on serious events such as incidence of HCC, gastroesophageal varices, cardiovascular events, clinical features of hepatic encephalopathy, other organ malignancies, and death. The data were analyzed to identify any associations with NAFLD-related serious events. Some patients underwent liver biopsy, which was evaluated using the Brunt classification for staging fibrosis. We compared these factors between event and nonevent cases and assessed whether they differ in terms of incidence of HCC, gastroesophageal varices, cardiovascular events, clinical features of hepatic encephalopathy, other organ malignancies, and death.

We also focused on cases that had available blood test data collected for over a course of 1 year to assess chronological changes in any markers in blood.

2.3. Definition of liver fibrosis-related formula

Liver fibrosis was assessed by calculating the FIB-4 index using the following formula: FIB-4 index = Age [years] × AST [IU/L]/(Platelet count [10^9/L] × $\sqrt{}$ALT [IU/L]).

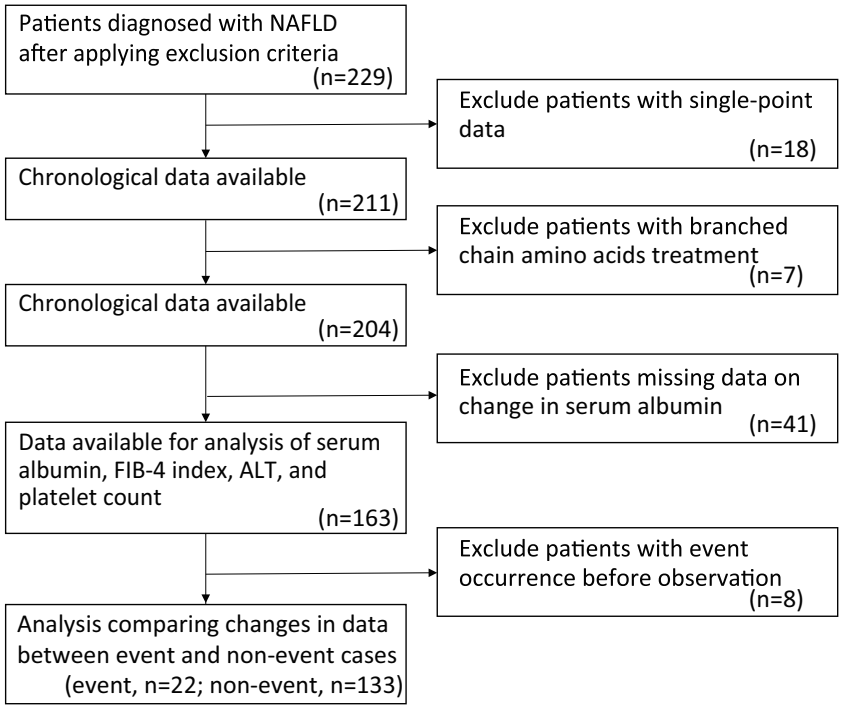

Figure 1. NAFLD patient data collection from multiple institutions. Most of institution participated in providing patients so that we could analyze changes in data before serious NAFLD-related events. ALT = alanine aminotransferase, NAFLD = nonalcoholic fatty liver disease.

2.4. Statistical analyses

For qualitative comparisons, chi-squared or Fischer's exact test were used, and Mann–Whitney *U* test and Student *t* test were used for quantitative comparisons. Cox regression model was used for multivariate analysis. All statistical analysis were performed using EZR software version 1.37 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), GraphPad Prism version 8 (GraphPad Software, San Diego, CA), and IBM SPSS Statistics for Windows software version 23.0 (IBM Corp., Armonk, NY).

3. Results

3.1. Case enrolment and data collection from multiple institutions

We analyzed report sheets for NAFLD patients from each institution and excluded some cases from the analysis of chronological changes in data with regard to incidence of NAFLD-related severe events (Fig. 1). The total number of objective NAFLD cases was 229 patients in 22 institutions; other etiologies of CLD were excluded in all cases. We also excluded 18 cases whose data were available at only a single time point because the major objective of the study is to assess chronological changes in data and occurrence of serious events. All institutions performed blood tests including at least serial platelet count, serum of AST and ALT activity, and serum albumin concentration (these data can be used to calculate FIB-4 index); however, serum fibrosis markers, PT, and liver biopsy findings were not always available. As the treatment of before and within observing periods, branched chain amino acid administered 7 cases were excluded. Next, we excluded single or nonchronological data for serum albumin data, resulting in the exclusion of 46 cases. We also excluded 8 cases for whom data were collected at only the time of or after an event. Finally, the number of event and nonevent cases was 22 and 133, respectively.

3.2. Clinical manifestations in NAFLD cases with serious events

In some patients, NAFLD is associated with hepatic inflammation, leading to liver cirrhosis, which ultimately leads to serious events, such as esophageal and gastric varices posing risk of massive hematemesis, hepatic encephalopathy, cardiovascular events such as acute coronary syndrome, and HCC as well as other organ malignancies. Therefore, we further investigated to identify the characteristic features of NAFLD patients at risk of event occurrence. The subjects were 155 NAFLD cases (Table 1). Mean observation period was 3.2 years. Event cases were 20 cases including 9 varices yet to be treated, 6 HCC cases, 3 hepatic encephalopathy cases, 2 cardiac events, and 2 other organ malignancies, breast cancer. The observation periods were not different between event and nonevent cases, although ages in the event group were relatively high. We could observe histological findings in 56% of cases, even though the timing of liver biopsy was different for each patient. For major complications of metabolic diseases, DM and HL, the frequency of DM was higher in event cases than in nonevent cases, and the frequency of HL was lower in nonevent cases, although not significantly.

3.3. Liver fibrosis index and hepatic reserve-related data predicts NAFLD-related serious events

We compared clinical laboratory data and features between event and nonevent cases at the start of observation. We found that several factors including age, platelet count, ALT, albumin, total

Table 1

Comparison of clinical characteristics of patients between severe NAFLD-related event cases and nonevent cases at the start and end of the observation period.

Clinical data and features	Nonevent cases	Event cases	*P* value
No. of patients	n = 133	n = 22	
Sex (M vs F)	65 vs 68	7 vs 15	.21
Observation period (yr)	3.2	3.1	.70
Cases of liver histological assessment	70/133 (53%)	17/22 (77%)	.05
Liver histology: Brunt Stages 3 and 4	23/77 (30%)	11/17 (65%)	**<.01
Diabetes mellitus cases	45/133 (34%)	11/22 (50%)	.22
Hyperlipidemia cases	80/133 (60%)	9/22 (41%)	.15
Start of observation			
Age at start of observation (yr)	53 (16–83)	66 (49–83)	**<.01
Platelet count ($\times 10^4/\mu L$)	21.7 (5.5–39.2)	11.6 (4.2–22.5)	***<.001
ALT (IU/L)	80 (9–324)	47 (7–152)	*<.05
Albumin (g/dL)	4.5 (3.7–5.1)	4.3 (3.7–4.8)	**<.01
Total bilirubin (mg/dL)	0.9 (0.3–4.1)	1.2 (0.6–3.5)	**<.01
Prothrombin time (%)	95 (63–127)	75 (60–88)	***<.001
Child–Pugh score	5.0 (5–8)	5.4 (5–7)	**<.01
FIB-4 index	1.91 (0.26–8.16)	5.45 (2.21–17.82)	***<.001
End of observation			
Age at end of observation (yr)	57 (18–84)	70 (50–85)	***<.001
Platelet count ($\times 10^4/\mu L$)	20.4 (4.0–38.5)	10.7 (3.3–20.7)	***<.001
ALT (IU/L)	60 (8–498)	32 (9–124)	*<.05
Albumin (g/dL)	4.4 (3.4–5.1)	3.7 (1.6–4.7)	***<.001
Total bilirubin (mg/dL)	0.9 (0.2–4.6)	1.3 (0.6–3.8)	*<.05
Prothrombin time (%)	99 (64–128)	70 (21–95)	***<.001
Child–Pugh score	5.1 (5–7)	6.7 (5–11)	***<.001
FIB-4 index	2.02 (0.27–11.4)	6.74 (2.60–12.97)	***<.001

ALT = alanine aminotransferase, NAFLD = nonalcoholic fatty liver disease.
$^{*}P < .05$.
$^{**}P < .01$.
$^{***}P < .001$.

bilirubin, and FIB-4 index significantly differed between the 2 groups (Table 1). We also histologically assessed liver biopsy specimens. The ratios of Brunt 3 and 4 to Brunt 1 and 2 in the event group was significantly higher than nonevent group. We also compared these data at the end of the observation period and found the same significant and nonsignificant differences for the respective data items. These results indicate that the occurrence of serious events in NAFLD patients was related to clinical data in the context of hepatic fibrosis and hepatic reserve.

3.4. Decline in serum albumin concentration is associated with occurrence of NAFLD-related serious events

Next, we assessed changes in each clinical data set from the start through to the end of the observation period in the nonevent and event groups. We compared the changes in each data set per year on average for at least a 1-year observation period. We found that these chronological changes in serum albumin concentration were significantly different (Fig. 2a). Other clinical and laboratory data such as PT, platelet count, and FIB-4 index, did not significantly differ in event group (Fig. 2b–d). Multivariate analyses using Cox regression model resulted that serum albumin decline was the significant factor of NAFLD related event incidence (Table 2). Moreover, this analysis that was restricted among F3 to 4 cases, exhibited only serum albumin decline and this was the factor of event incidence. We also compared serum albumin changes in cases with each event, such as gastroesophageal varices leading to rupture or required preventive intervention, hepatic failure leading to hepatic encephalopathy, HCC, other organ malignancy, and cardiovascular events. The decline was significantly larger (–0.38 g/dL/year) in cases with hepatic failure, than in nonevent cases (–0.04

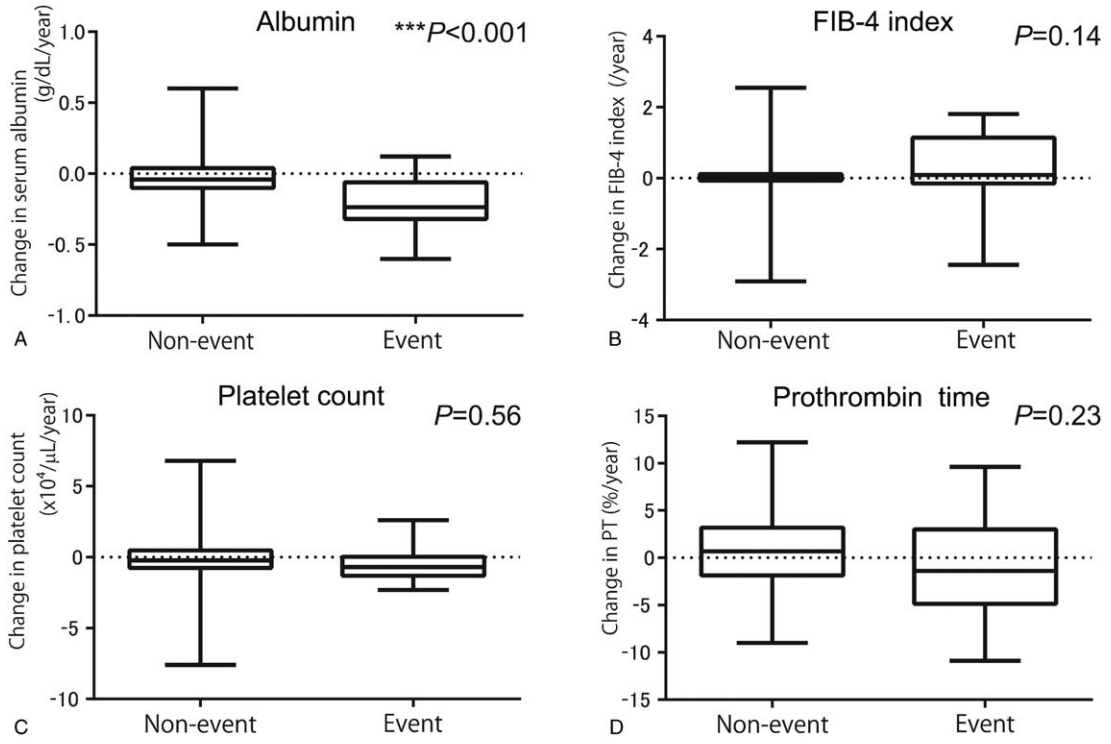

Figure 2. Comparison of changes in laboratory data between NAFLD-related serious event and nonevent cases. (a) Change in serum albumin per year. (b) Changes in FIB-4 index per year. (c) Changes in platelet count per year. (d) Changes in prothrombin time per year. NAFLD = nonalcoholic fatty liver disease.

Table 2

Cox regression analysis of NAFLD related event incidence.

Variable	HR (95% CI)	*P* value
All cases (n = 155)		
Sex (M vs F)	0.360 (0.049–2.650)	.316
Age (≥60 vs <60 yr)	6.38 (0.591–68.82)	.127
Diabetes mellitus (+ vs -)	2.17 (0.466–10.12)	.323
Hyperlipidemia (+ vs -)	0.977 (0.198–4.821)	.978
FIB-4 index (≥0.07/yr vs <0.07/yr)	0.969 (0.268–3.500)	.961
Albumin (≥0.12g/dL/yr vs <0.12g/dL/yr)	0.110 (0.022–0.551)	**.007
Fibrosis (F3–4 vs F1–2)	1.070 (0.191–5.992)	.939
Prothrombin time (≥0.15%/yr vs <0.15%/yr)	0.588 (0.126–2.747)	.499
Selected only F3 to 4 cases (n = 34)		
Sex (M vs F)	23.3 (0.335–1625)	.146
Age (≥60 vs <60 yr)	34.1 (0.910–1277)	.056
Diabetes mellitus (+ vs -)	0.415 (0.032–5.399)	.502
Hyperlipidemia (+ vs -)	29.4 (0.835–1039)	.063
FIB-4 index (≥0.07/yr vs <0.07/yr)	1.027 (0.200–5.284)	.974
Albumin (≥0.12g/dL/yr vs <0.12g/dL/yr)	0.026 (0.001–0.460)	*.013
Prothrombin time (≥0.15%/yr vs <0.15%/yr)	0.103 (0.004–2.370)	.155

NAFLD = nonalcoholic fatty liver disease.
* $P < .05$.
** $P < .01$.

g/dL/year) (Fig. 3). In detail, serum albumin decline was significant in cases with other event complications; gastroesophageal varices (–0.25 g/dL/year), HCC (–0.16 g/dL/year), compared to that in other organ malignancies (–0.08 g/dL/year), and cardiovascular events (–0.11 g/dL/year).

4. Discussion

In this study, we assessed the clinical features and data of NAFLD/NASH patients in 22 regional institutions in our region. We found that serum albumin concentration and changes in the clinical course were significantly associated in the group of patients in whom these events occurred.

NAFLD is one of the emerging forms of CLD that is yet to be fully investigated to clarify the clinical and pathological features in detail. Currently, the established pathological feature of NAFLD is steatosis, which is the accumulation of lipid, primarily triglyceride, in hepatocytes. NAFLD can also be complicated by obesity, DM, HL, HT, and hyperuricemia in many patients. Although these metabolic syndrome-associated conditions in NAFLD patients can be treated, the therapeutic benefit in CLD is uncertain. NASH develops in 10% to 15% of NAFLD patients.[5] Furthermore, 15% of NASH patients develop hepatic fibrosis ultimately leading to cirrhosis. Pathologically, fibrosis staging using Brunt classification[15] but not the NAFLD scoring system has been shown to be an important factor in event occurrence.[16] Clinically, imaging modalities and liver fibrosis scoring systems such as the FIB-4 index are available[9] and are useful to some extent for assessing hepatic fibrosis.[17,18] However, they are not sufficiently accurate for assessing the development of liver fibrosis. Therefore, it is necessary to identify clinical features that are significantly associated with events in the advanced stage of hepatic fibrosis in NAFLD.

A significant decline in serum albumin concentration between the start and end of the observation was found in the event group. In particular, the occurrence of gastroesophageal varices and hepatic failure such as hepatic encephalopathy were strongly associated with decline in serum albumin. Portal vein blood flow is decreased by the development of liver fibrosis in NASH patients.[19] Furthermore, hepatic function is significantly affected by the volume of portal vein blood flow.[20] Decreased portal vein blood flow due to advanced hepatic fibrosis causes extrahepatic blood flow shunt, including gastroesophageal varices and ammonia accumulation. Therefore, it is reasonable that a decline in serum albumin is associated with varices and hepatic encephalopathy as a complication of hepatic fibrosis.

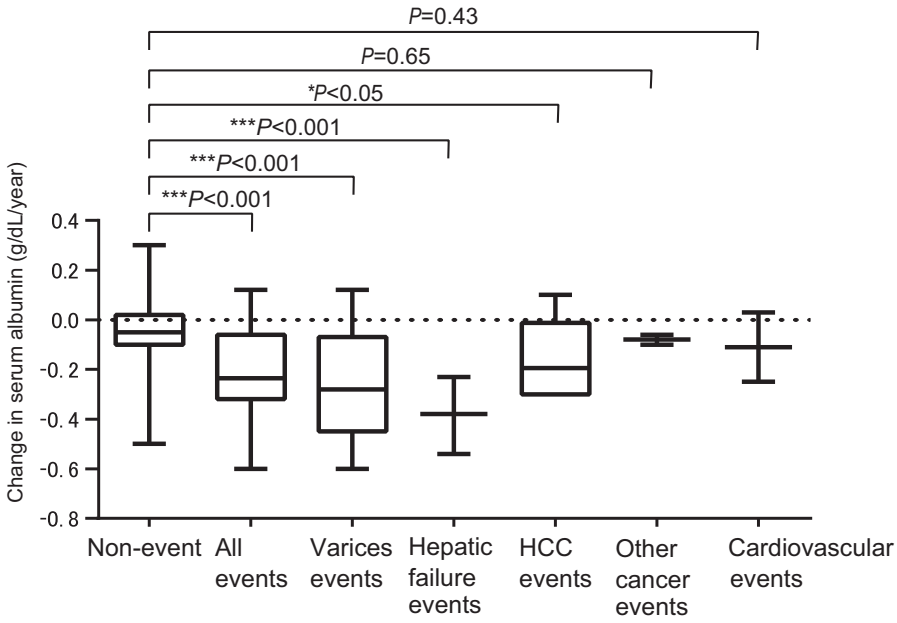

Figure 3. Serum albumin changes per year between event and nonevent groups. Data in the nonevent group was compared to that in the event group; all events, gastroesophageal varices, hepatic failure, HCC, other cancer events, and cardiovascular events. HCC = Hepatocellular carcinoma.

HCC incidence in NAFLD case is often observed, as chronic liver inflammation induces carcinogenesis.[21] NAFLD/NASH is associated with overnutrition, hyperglycemia, insulin resistance, abnormality of lipid metabolism profile, which eventually resulted in chronic liver inflammation, liver fibrosis as well as atherosclerosis.[22] Cardiovascular event occurs as the result of atherosclerosis that was frequently associated with DM, HL, and obesity. This event is observed in NAFLD/NASH cases who exhibited chronic liver injury, and there is possibility of observing chronological albumin decline.[22] Obesity is the risk factor for other cancer malignancies; especially breast cancer, colon cancer, or prostate cancer.[23] In our current study, enrolled patients included the cases of breast cancer incidence, thus, we thought that it also implied the possible relation with NAFLD. Considering these, the long-term albumin decline in NAFLD/NASH is an important factor of events incidence that is also shown by analyzing multivariate analysis.

No report has precisely discussed the factors in which changes reflect disease progression in NAFLD/NASH, such as FIB-4 index, albumin, PT, and platelet count, although the studies have reported changes in data as a consequence of pharmacological treatments for the associated diseases DM and HT.[24–28] Thus, our study showing the importance of decline in serum albumin in advanced CLD due to NAFLD/NASH contributes to clarifying the clinical features.

The fundamental pathogenesis of NAFLD/NASH has not been reported and no treatment is available, in contrast to antiviral treatments for HBV or HCV in chronic viral hepatitis, so further efforts to advance treatments for nonviral CLD are expected. Recently, many clinical trials have been conducted to develop novel treatments for NAFLD/NASH.[29] The possible development of regenerative therapy was reported as a potentially novel treatment for CLD to achieve possible improvement in liver function.[30–32] The development of appropriate clinical markers for assessing progression of NAFLD/NASH is extremely important because NAFLD/NASH is a form of CLD that gradually progresses to cirrhosis with advanced fibrosis as well as dysfunction manifesting as decreased hepatic reserve.

There are several limitations for this multicenter study. Though we assessed serum albumin changes among NAFLD patients by comparing another advanced liver disease factors such as PT, FIB-4 index and platelets count, PT was not measured frequently for some patients. The number of liver histology assessed patient is not sufficient because this is the invasive examination with hospitalization. Transient elastography or liver fibrosis related serum markers had been desired as the candidate of NAFLD related event prediction, but the number of performed institution is limited, this is the main limitation of the multicenter study. However, the results of multivariate analysis that we analyzed might overcome these limitations.

In conclusion, our findings from this retrospective multicenter study show that decline in serum albumin over a clinical course of several years following a diagnosis of NAFLD is an important factor associated with the incidence of severe events. These results highlight the importance of careful monitoring of changes in serum albumin concentration for predicting the occurrence and prognosis of severe events in advanced NAFLD/NASH.

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