

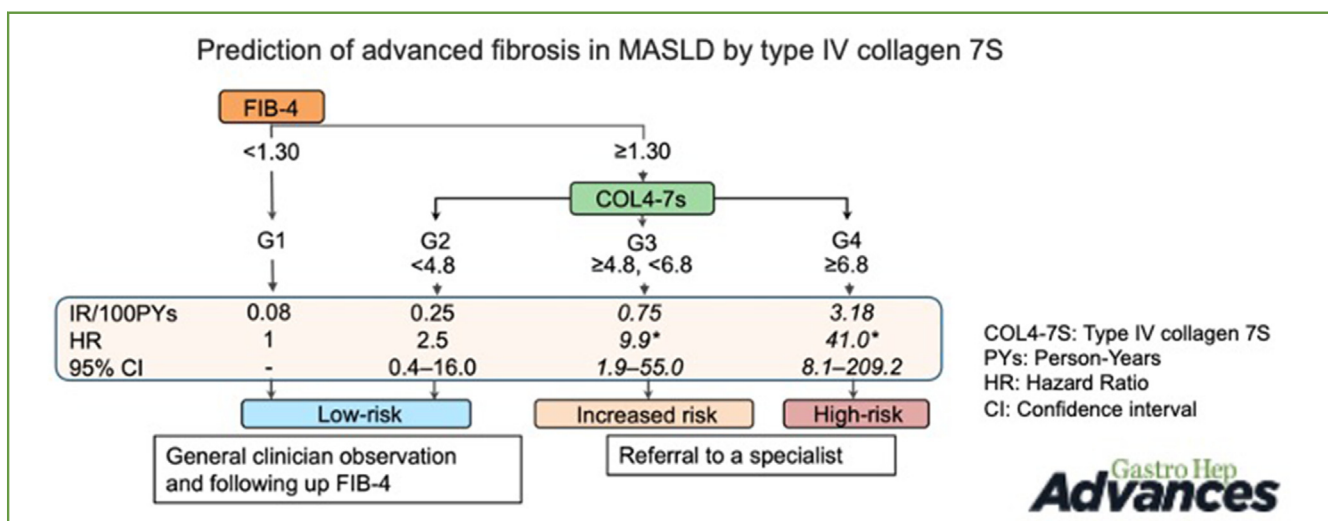
ORIGINAL RESEARCH—CLINICAL

Prediction of Advanced Fibrosis in Metabolic Dysfunction-Associated Steatotic Liver Disease by Type IV Collagen 7S



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BACKGROUND AND AIMS: Type IV collagen 7S (COL4-7S) is a simple, noninvasive biomarker for liver fibrosis. However, whether COL4-7S can detect advanced fibrosis (AF) and predict the prognosis of metabolic dysfunction-associated steatotic liver disease (MASLD) is unclear. We examined the clinical efficacy of COL4-7S in diagnosing AF and determining MASLD prognosis. **METHODS:** Overall, 881 Japanese patients with biopsy-proven nonalcoholic fatty liver disease between 1994 and 2020 were enrolled. Serum COL4-7S levels were measured by radioimmunoassay, and 2 cutoff points were set as 5.1 ng/mL and 7.2 ng/mL. The patients were assigned to 3 groups based on the COL4-7S level. Cox regression analysis was used to estimate the predictive performance of COL4-7S for liver-related events (LREs). **RESULTS:** Overall, 866 MASLD patients were enrolled. The median follow-up period was 4.3 years. Thirty-one patients developed LREs. The area under the curve for COL4-7S in patients with AF was 0.847. The adjusted hazard ratios for LREs in $4.8 \leq \text{COL4-7S} < 6.8$ and $\text{COL4-7S} \geq 6.8$ patients were 6.0 ($P = .009$) and 27.9 ($P < .001$) compared with $\text{COL4-7S} < 4.8$, and the adjusted hazard ratio of AF on liver biopsy was 1.6 ($P = .286$). The incidence rate of LREs was low when the Fibrosis-4 Index (FIB-4) < 1.30 . When the $\text{FIB-4} > 1.30$, effective stratification of the LRE risk group was possible by stratification of COL4-7S. A combination of FIB-4 and COL4-7S stratified risk groups for future LRE development more effectively than when used singly. **CONCLUSION:** COL4-7S accurately diagnosed AF and predicted LREs. COL4-7S and a combination of FIB-4 and COL4-7S might help physicians estimate the prognosis of future LRE risk.

Keywords: Type IV Collagen 7S; Fibrosis-4 Index; Metabolic Dysfunction-Associated Steatotic Liver Disease; Liver-Related Event

Introduction

The prevalence of nonalcoholic fatty liver disease (NAFLD) (25%–30%)^{1,2} is increasing.³ Approximately 5% of NAFLD patients progress to severe liver disease.⁴ NAFLD prognosis worsens with liver fibrosis progression,^{5,6} and all-cause and liver-related mortality increases with increasing fibrosis stage.⁷ Liver fibrosis progression is an important predictor of NAFLD-related outcomes; thus, early identification of NAFLD and advanced fibrosis (AF) (stage 3–4) is recommended.^{8–10} Liver fibrosis is generally detected by liver biopsy,¹¹ which has some limitations (eg, procedural invasiveness, sampling error, high cost).¹²

A systematic review comparing metabolic dysfunction-associated steatotic liver disease (MASLD—a new disease entity) with NAFLD reported high concordance and similar outcomes, suggesting some similarities, although approximately 5% of NAFLD cases did not meet MASLD criteria.^{13–15} Therefore, previous NAFLD research might be applicable to MASLD.

Noninvasive tests (NITs), including the Fibrosis-4 Index (FIB-4), evaluated age and other clinical parameters and accurately diagnosed AF^{16,17} and predicted NAFLD prognosis.^{18–21} However, diagnostic accuracy decreased

with age and diabetes mellitus (DM).^{22–24} Although the low cutoff threshold for FIB-4 (1.30) was a good exclusion criterion for AF and a good predictor of NAFLD prognosis, many patients still required referral to a hepatologist, which is expensive.²⁵ Two-step algorithms including FIB-4 followed by enhanced liver fibrosis (ELF) tests or vibration-controlled transient elastography (VCTE) have identified patients at risk for AF.^{26,27}

Surrogate noninvasive fibrosis markers related to extracellular matrix turnover products (type IV collagen 7S (COL4-7S)) were used to accurately diagnose liver fibrosis^{23,28,29} and study fibrogenic activity in animal models³⁰ and patients with chronic liver disease.³¹ COL4-7S had similar diagnostic accuracy to ELF when detecting fibrosis stage (stage) ≥ 3 and might be superior to ELF when detecting stage ≥ 2 .³²

However, its use to diagnose liver fibrosis and predict MASLD prognosis remains unclear. We evaluated the accuracy of COL4-7S to diagnose liver fibrosis and predict MASLD prognosis.

Methods

Study Design

This registry-based, multicenter, historical cohort study (CLIONE-Asia study) was approved by the Institutional Review Board of Saga University Hospital (approval no. 2020-04-R-02, June 30, 2020) and followed the Strengthening the Reporting of Observational Studies in Epidemiology reporting guidelines. We used a database of patients with biopsy-proven NAFLD from the Japan Study Group of NAFLD, which includes data from 15 hepatology centers in Japan.³³ All data were collected and managed using REDCap electronic data capture tools hosted at Osaka Metropolitan University.³³ The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the *a priori* approval by our institution's human research committee. The requirement for informed consent was waived owing to the use of pre-existing data.

Patients

Patients underwent liver biopsy to diagnose NAFLD between December 1, 1994, and December 31, 2020. They were

Abbreviations used in this paper: AF, advanced fibrosis; aHR, adjusted hazard ratio; CI, confidence interval; CLEIA, chemiluminescent enzyme immunoassay; COL4-7S, type IV collagen 7S; DM, diabetes mellitus; ELF, enhanced liver fibrosis; FIB-4, Fibrosis-4 Index; FPR, false-positive rate; HCC, hepatocellular carcinoma; HR, hazard ratio; LREs, liver-related events; MASLD, metabolic dysfunction-associated steatotic liver disease; NAFLD, non-alcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NITs, noninvasive tests; PYs, person-years; RIA, radioimmunoassay; ROC, receiver operating characteristic; VCTE, vibration-controlled transient elastography.

 Most current article

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followed up until March 31, 2021, to examine the following clinical outcomes: death; liver-related events (LREs) defined by cirrhosis-related complications, incident of hepatocellular carcinoma (HCC), and bleeding varices; HCC incidence; cardiovascular events; and stroke.³³ Of these NAFLD patients, those with MASLD were selected based on MASLD diagnostic criteria¹³ (evidence of hepatic steatosis by imaging, blood biomarkers or scores, or liver biopsy) and at least one of the following cardiometabolic criteria: body mass index ≥ 23 kg/m² in Asians, waist circumference >94 cm in men and >80 cm in women (with ethnic-specific adjustments), fasting glucose ≥ 100 mg/dL, 2-hour postload glucose ≥ 140 mg/dL, HbA1c $\geq 5.7\%$, diabetes treatment, blood pressure $\geq 130/85$ mmHg, hypertension treatment, serum triglycerides ≥ 150 mg/dL, dyslipidemia treatment, or high-density lipoprotein-cholesterol <40 mg/dL for men and <50 mg/dL for women.

Histology

All patients underwent percutaneous liver biopsy under ultrasonic guidance. Liver specimens were diagnosed by an experienced pathologist (S.A.) at Saga University, who was blinded to clinical and laboratory data for central pathology reading and scoring. The Kleiner scoring system was used for histological assessment to grade steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis (stage 0–4).^{34,35} MASH was diagnosed according to the fatty liver inhibition of progression algorithm.¹¹

Clinical, Laboratory, and Outcome Data

Physical characteristics, medical history, lifestyle habits, and clinical laboratory data were collected. Blood samples were obtained using standard techniques. Type 2 DM, hypertension, and dyslipidemia were diagnosed according to standard criteria.^{36,37} Patients were diagnosed with impaired fasting glucose when they were not treated with hypoglycemia agents and fasting plasma glucose concentrations were 110–126 mg/dL.^{38,39} Follow-up was from biopsy to the most recent follow-up date, last visit, death, or liver transplantation. All clinical events were collected using data from patients' electronic medical records.³³ LREs were recorded during the entire follow-up period and defined as the development of ascites, bleeding varices, encephalopathy, jaundice, or incident HCC. When estimating prognosis using LREs, patients with a history of HCC were excluded. In cases of death, the date and cause of death were recorded. COL4-7S levels were measured by double-antibody radioimmunoassay (RIA). Two cut-off thresholds were set so that the sensitivity and specificity for advanced fibrosis were at 90% in this overall patients. All enrolled patients were divided into 3 groups using the new cut-off thresholds: the low-risk group at the lower COL4-7S cut-off (sensitivity: 90%), the high-risk group at the higher COL4-7S cut-off (specificity: 90%), and the intermediate-risk group. The cutoff points for predicting stage 3–4 were 4.8 ng/mL (low) and 6.8 ng/mL (high).²³ These cutoff points were validated with 10-fold cross validation (Table A1).

Statistical Analysis

Descriptive statistics of clinical outcomes are expressed as the incidence rate (number/100 person-years [PYs]). The relationship between the fibrosis stage and COL4-7S level was assessed using the Jonckheere–Terpstra test. Cumulative

incidence curves were constructed and compared using Gray's test with Bonferroni correction. Outcomes were estimated over the entire observation period after liver biopsy. To evaluate the prognostic significance of each COL4-7S group and stage 3–4, we performed multivariate Cox proportional hazards regression, including COL4-7S, with adjustment for stage 3–4. We evaluated 2-step algorithm (FIB-4 and COL4-7S) accuracy to predict LREs. Heatmaps show incidence rates of stratification by fibrosis stage and COL4-7S group. Statistical analyses were performed using R, version 4.1.2. Nominal 2-sided *P* values < 0.05 were considered statistically significant.

Results

Patients' Baseline Characteristics

Overall, 1760 patients were enrolled in the CLIONE-Asia study, and 879 who met the exclusion criteria or lacked COL4-7S values were excluded. Data from 866 MASLD patients were evaluated (Figure A1). Baseline patient characteristics are shown in Table 1. Twenty-two patients died during the study. The median observation period was 4.3 years (0.25–21.1 years) and the median age was 56 years (range, 17–86 years). Stage 3–4 was observed in 139 (16.1%) patients. During the observation period, 9 patients (1.0%) died from LREs, which were observed in 21 (2.4%) and 28 (3.2%) patients with HCC and vascular disease, respectively. Additionally, 31 patients experienced LREs: 4 (12.9%) had decompensated liver cirrhosis (ascites, hepatic encephalopathy), 17 (54.8%) had HCC, and 10 (32.3%) had bleeding varices. No FIB-4 <1.30 patients developed LREs 10 years after liver biopsy and the LRE incident rate was only 0.08/100 PYs over the entire observation period (Table 2). LRE incident rates of COL4 <4.1 or stage ≤ 2 patients were 0.13/100 PYs and 0.35/100 PYs, respectively.

Receiver Operating Characteristic (ROC) Curve Analysis and Liver Fibrosis Diagnostic Ability of COL4-7S and FIB-4

The area under the receiver operating characteristic (AUROC) curve for COL4-7S to diagnose stage 3–4 was 0.845 (95% confidence interval [CI]: 0.811–0.880) and 0.796 (0.757–0.836) for FIB-4 (Figure 1A). The COL4-7S concentrations increased significantly with increased liver fibrosis stages as shown by the Jonckheere–Terpstra test (Figure 1B). In addition, ROC analysis was performed for the differentiation of Stage 2 or higher, and the AUROC values for FIB-4 and COL4-7S were 0.779 and 0.788, respectively ($P = .600$) (Figure A2). The cutoff values for sensitivity 90% and specificity 90% were 0.90 and 2.55 for FIB4 and 3.8 and 5.9 for COL4-7S.

Occurrence of LREs and Prognostic Accuracy of COL4-7S and Liver Fibrosis Stage

During the observation period, 31 patients experienced LREs, with incidence rates of 0.12, 0.58, and 2.94/100 PYs in the low-, intermediate-, and high-risk COL4-7S groups, respectively, and incidence rates of 0.35 and 2.32/100 PYs

Table 1. Patients' Baseline Characteristics

Characteristics	n = 866
Age, y	56 [17, 86]
Sex, male	384 (44.3)
BMI (kg/m ²)	28.03 [10.29, 53.25]
DM	307 (35.5)
Hypertension	345 (39.9)
Dyslipidemia	535 (61.8)
COL4-7S (ng/mL)	4.50 [1.00, 329.00]
FIB-4 index	1.49 [0.09, 12.56]
Steatosis 0: 1: 2: 3 (%)	4 (0.5): 588 (67.9): 174 (20.1): 100 (11.5)
Inflammation 0: 1: 2: 3 (%)	45 (5.2): 553 (63.9): 226 (26.1): 42 (4.8)
Ballooning 0: 1: 2 (%)	301 (34.8): 349 (40.3): 216 (24.9)
Stage 0: 1: 2: 3: 4 (%)	48 (17.1): 339 (39.1): 240 (27.7): 127 (14.7): 12 (1.4)
MASH (%)	557/866 (64.3)
All-cause mortality	22 (2.5)
Liver-related mortality	9 (1.0)
Liver-related events	31 (3.6)
Incidence of HCC	21 (2.4)
Incidence of vascular disease	28 (3.2)
Entire observation period, y	4.30 [0.25, 21.08]
Outcome	
Liver-related mortality (all-cause mortality, %)	9 (40.9)
Cirrhosis complication	4 (18.2)
HCC	4 (18.2)
CCC	1 (4.5)
Liver-related events	
Cirrhosis complications	144 (45.2/12.9)
Incident of HCC	17 (54.8)
Bleeding varices	10 (32.3)

The data are presented as n (%) or median [range].
 BMI, body mass index; CCC, cholangiocellular carcinoma, Cirrhosis complications including ascites, bleeding varices, encephalopathy, jaundice; MASH, metabolic dysfunction-associated steato-hepatitis.

in the stage 0–2 and stage 3–4 groups (Table 3). Cumulative incidence curves for LREs according to baseline COL4-7S in the entire study cohort are shown in Figure 2A. The cumulative incidence in the high-risk group was significantly

higher than that in the low- and intermediate-risk groups ($P < .01$). For stage 3–4 or 4, the cumulative incidence of LREs increased significantly as the COL4-7S risk group increased (Figure A3). Univariate analysis indicated hazard

Table 2. Incident Rate (100 PYs) of LRE Stratified by Each Stage and NIT

Stage and NIT	Entire observation period	5 y after LB	10 y after LB
Stage ≤ 2	0.35 (16/727)	0.34 (9/727)	0.33 (13/727)
Stage ≥ 3	2.32 (15/139)	2.08 (10/139)	2.47 (15/139)
FIB4 < 1.3	0.08 (2/387)	0 (0/387)	0 (0/387)
$1.30 \leq \text{FIB4} \leq 2.67$	0.23 (4/281)	0.19 (2/281)	0.20 (3/281)
FIB4 ≥ 2.67	2.45 (25/198)	2.46 (17/198)	2.70 (25/198)
COL4-7S < 4.8	0.13 (4/475)	0.11 (2/475)	0.11 (3/475)
$4.8 \leq \text{COL4-7S} < 6.8$	0.58 (8/241)	0.46 (4/241)	0.49 (6/241)
COL4-7S ≥ 6.8	2.94 (19/150)	2.71 (13/150)	3.11 (19/150)
FIB-4 < 1.30	0.08 (2/384)	0 (0/384)	0 (0/384)
FIB-4 ≥ 1.30 and COL4-7S < 4.8	0.25 (3/178)	0.29 (2/178)	0.29 (3/178)
FIB-4 ≥ 1.30 and $4.8 \leq \text{COL4-7S} < 6.8$	0.75 (7/168)	0.65 (4/168)	0.70 (6/168)
FIB-4 ≥ 1.30 and $6.8 \leq \text{COL4-7S}$	3.18 (19/136)	2.91 (13/136)	3.36 (19/136)

LB, liver biopsy.

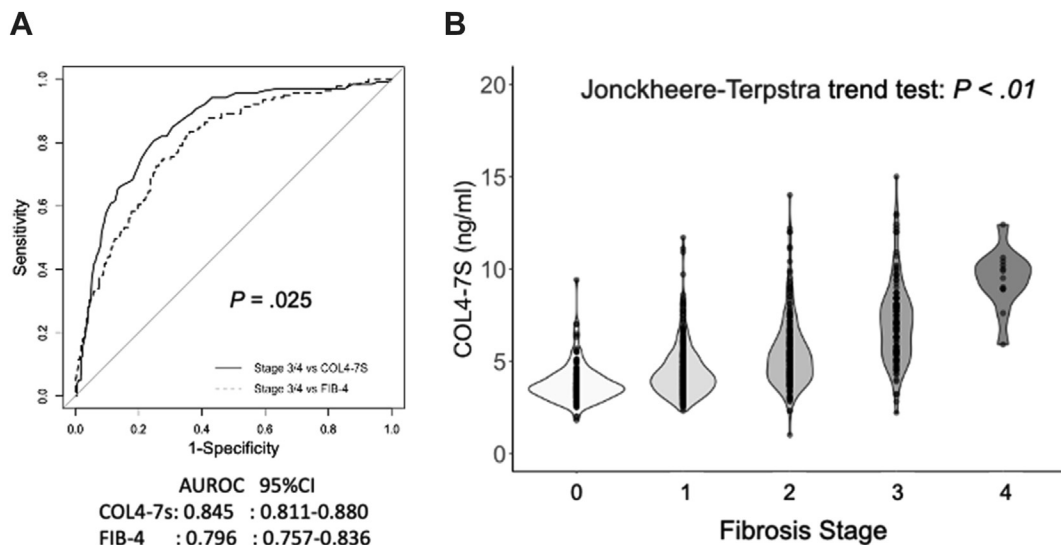


Figure 1. Diagnostic performance of COL4-7S for liver fibrosis in patients with MASLD. (A) ROC curve analysis of COL4-7S and FIB-4 Index to detect stage 3–4 fibrosis. (B) Violin plot of COL4-7S concentrations for each fibrosis stage. The Jonckheere–Terpstra trend test analyzed trends in COL4-7S concentrations for fibrosis stage.

ratios (HRs) for LREs were 6.33 for stage 3–4, and 6.30 and 35.67 for intermediate- and high-risk COL4-7S groups, which were significantly higher than the stage 0–2 and low-risk COL4-7S groups (Table 3). Multivariate analysis indicated the low cutoff threshold of COL4-7S was a significant independent predictor (adjusted HR (aHR) = 5.98 and 27.85 in intermediate- and high-risk COL4-7S groups, respectively, compared with the low-risk COL4-7S group), and the aHR of stage 3–4 was 1.56 ($P = .286$ compared with stage 0–2).

Comparison Between FIB-4 and COL4-7S for Prognostic Prediction

Patients were divided into 2 groups based on FIB-4 (1.30, 2.67) and COL4-7S (4.8, 6.8 ng/mL) cutoff

thresholds, and the prognostic ability of FIB-4 and COL4-7S to predict LREs in high- and low-risk groups was analyzed (Table A2). Multivariate Cox hazard analysis indicated each cutoff threshold was a significant independent predictor of LRE incidence (low cutoff: COL4-7S aHR 8.57, $P < .001$; FIB-4 aHR 6.63, $P = .012$; high cutoff: COL4-7S aHR 4.73, $P = .001$; FIB-4 aHR 10.96, $P < .001$). The incidence of LREs became higher as the COL4-7S risk group increased within the same FIB-4 risk group. Additionally, the incidence rate in the FIB-4 low risk group was almost zero (Figure 2B).

Patients were divided into 4 groups: FIB-4 <1.30 [G1], FIB-4 ≥ 1.30 and COL4-7S <4.8 ng/mL [G2], FIB-4 ≥ 1.30 and 4.8 ng/mL $\leq$ COL4-7S <6.8 ng/mL [G3], and FIB-4 ≥ 1.30 and COL4-7S ≥ 6.8 ng/mL [G4] (Table 3). The stratification algorithm (FIB-4-IV algorithm) and cumulative

Table 3. Unadjusted HRs and aHRs for Predicting LREs According to baseline COL4-7S Concentrations

Stage and NIT	Events	Incidence rate (100 PY)	Univariate analysis			Multivariate analysis ^a		
			HR	95% CI	P	HR	95% CI	P
Stage 0–2	16/727	0.35	1	–	–	1	–	–
Stage 3–4	15/139	2.32	6.33	3.08–12.99	<.001	1.56	0.69–3.50	.286
COL4-7S low (<4.8 ng/mL)	4/475	0.13	1	–	–	1	–	–
COL4-7S intermediate	8/241	0.58	6.30	1.67–23.80	.007	5.98	1.57–22.71	.009
COL4-7S high (≥ 6.8 ng/mL)	19/150	2.94	35.67	10.27–123.92	<.001	27.85	7.29–106.43	<.001
			Univariate analysis			Multivariate analysis ^b		
FIB-4 <1.30 (G1)	2/384	0.08	1	–	–	1	–	–
FIB-4 ≥ 1.30 and COL4-7S <4.8 ng/mL (G2)	3/178	0.25	2.86	0.48–17.20	.251	2.51	0.39–16.02	.329
FIB-4 ≥ 1.30 and 4.8 $\leq$ COL4-7S <6.8 ng/mL (G3)	7/168	0.75	10.98	2.13–56.53	.004	9.88	1.78–54.96	.009
FIB-4 ≥ 1.30 and COL4-7S ≥ 6.8 ng/mL (G4)	19/136	3.18	53.08	11.41–246.90	<.001	41.01	8.05–209.16	<.001

^aThe covariates in the multivariate Cox regression model were the groups stratified by fibrosis stage and COL4-7S risk groups.

^bThe covariates in the multivariate Cox model were age, DM, and the groups stratified by FIB-4 Index and COL4-7S.

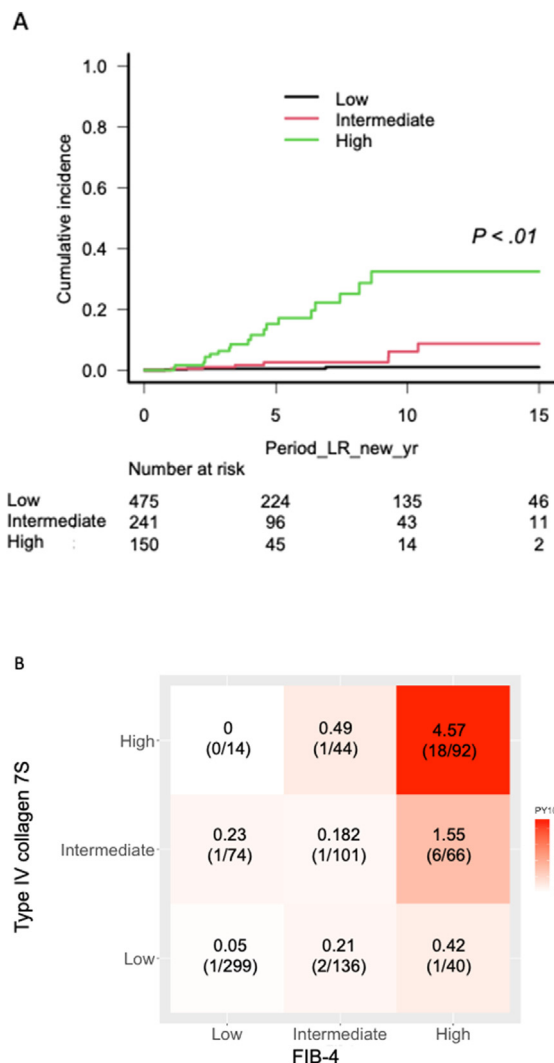


Figure 2. Cumulative incidence of LREs and incident rates by COL4-7S and FIB-4 groups. (A) Cumulative incidence curve of LREs according to groups defined by COL4-7S thresholds. Black line: low-risk group; red line: intermediate-risk group; green line: high-risk group. yr: year. (B) Heatmaps of liver-related incident rates categorized by COL4-7S and FIB-4 groups. Values are incidence rate per 100 PYs (number of incidents per total number in each categorized group).

incidence curves for LREs in the entire study cohort were calculated (Figure 3A and B). Cumulative incidence rates of G3 and G4 were significantly higher compared with G2 and G1 ($P < .01$). HR adjusted by age and DM for LREs was 2.51 for G2 (95% CI: 0.39–16.02, $P = .324$), 9.88 for G3 (95% CI: 1.78–54.96, $P = .009$), and 41.01 for G4 (95% CI: 8.05–209.16, $P < .001$) compared with G1 (Table 3). And next, patients were divided into 3 groups: FIB-4 < 2.67 [G'1], FIB-4 ≥ 2.67 and COL4-7S < 4.8 ng/mL [G'2], FIB-4 ≥ 2.67 and $4.8 \text{ ng/mL} \leq \text{COL4-7S ng/mL}$ [G'3] (Table A3). In the stratification algorithm (FIB-4-IV-high algorithm), the cumulative incidence rates of G'3 were significantly higher compared with G'2 and G'1 ($P < .01$). HR adjusted by age and DM for LREs was 5.48 for G'2 (95% CI: 0.57–52.44,

$P = .140$), 30.43 for G'3 (95% CI: 10.10–91.68, $P < .01$), compared with G'1.

Comparisons of LRE prediction using the cutoff points showed FIB-4 had the highest sensitivity (0.935) and negative predictive value (0.995), but highest false-positive rate (FPR) (0.539). FIB-4-IV and FIB4-IV-high, which includes COL4-7S, had improved specificity (FIB-4 0.461 vs FIB-4-IV 0.667, and FIB4-IV-high 0.840) and FPR (FIB-4 0.539 vs FIB-4-IV 0.333, FIB4-IV-high 0.160). The sum of FPR and FNR, which indicate inappropriate diagnoses, was improved for FIB-4-IV (FIB-4 0.603 vs FIB-4-IV 0.494, and FIB4-IV-high 0.386) (Table A4). Compared FIB-4-IV and FIB-4-IV-high, FNR of FIB-4-IV was lower than that of FIB-4-IV-high.

Discussion

We showed that COL4-7S reflected significant changes in the fibrosis stage and was more useful than FIB-4 for excluding a diagnosis of fibrosis stage ≥ 3 . Additionally, its cutoff values of 4.8 and 6.8 ng/mL stratified the risk of LREs better than histological diagnosis. A 2-step algorithm with FIB-4 and COL4-7S to stratify the risk group for LREs reduced the number of patients requiring referral to a hepatology specialist compared with diagnostic algorithms based on FIB-4 or COL4-7S alone.

COL4-7S values increased with increasing fibrosis stage and COL4-7S was superior to histological assessment for LRE prediction, possibly because liver fibrosis progression is “continuous,” and COL4-7S is a continuous variable. Correlations between COL4-7S and NAFLD activity score were weak (correlation coefficient: 0.356; $P < .01$) indicating their relationship was not as favorable as with fibrosis, possibly because COL4-7S reflects the outcome of liver fibrosis.²⁹

The diagnostic accuracy of FIB-4 for AF is affected by age and DM-related complications.^{22–24} The diagnostic accuracy of COL4-7S for AF was significantly better compared with FIB-4 for NAFLD patients with DM, and was similar to FIB-4 for those without DM.²³ We showed no significant difference between patients with/without DM (AUROC without DM 0.863 vs with DM: 0.801 $P = .144$). FIB-4 was expected to replace histological evaluation for MASLD^{40–42}; however, it has a high FPR (Table A3D). The high FPR was reduced when using COL4-7S, and its predictive performance for LREs was not inferior to FIB-4.

Identifying groups at high risk for LREs using FIB-4 followed by COL4-7S might improve the diagnostic ability of FIB-4 because COL4-7S compensates for the high FPR of FIB-4. The 2-step algorithm stratified the risk for LREs more accurately than single algorithms.

A FIB-4-VCTE stepwise algorithm accurately stratified LRE risk.¹⁸ Compared with patients with FIB-4 < 1.30 , those with FIB-4 ≥ 1.30 and VCTE < 8.0 kPa had similar LRE risks, whereas LRE risk was significantly higher in patients with FIB-4 ≥ 1.30 and VCTE 8.0–12.0 kPa, and even higher for those with FIB-4 ≥ 1.30 and VCTE > 12.0 kPa. Similarly, we

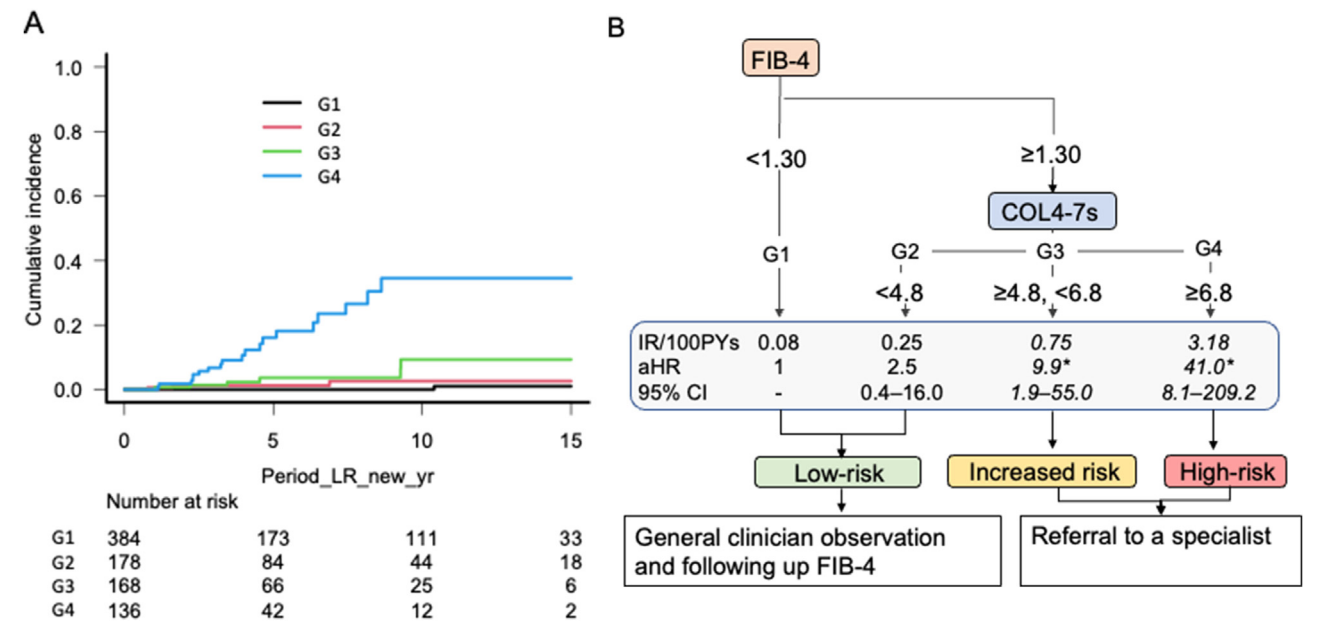


Figure 3. Performance of the FIB-4 Index and COL4-7S for the selection of risk groups for LREs. (A) Cumulative incidence curve analysis of LREs according to group divided by FIB-4 Index and COL4-7S concentration. (B) Flow chart of risk group selection with FIB-4 Index and COL4-7S concentration. IR, incidence rate; yr, year.

showed that compared with patients with FIB-4 <1.30, those with FIB-4 ≥1.30 and COL4-7S <4.8 ng/mL had a similar LRE risk, whereas LRE risk was significantly higher for FIB-4 ≥1.30 and COL4-7S 4.8–6.8 ng/mL, and even higher for FIB-4 ≥1.30 and COL4-7S ≥6.8 ng/mL. The diagnostic performances of VCTE and COL4-7S were not directly compared; therefore, we did not evaluate superiority between the 2 algorithms, although they are probably equivalent at predicting LREs.

The American Gastroenterology Association suggests VCTE for patients with FIB-4 1.30–2.67²⁷; however, the FIB-4 1.30–2.67 group is so large that it is not cost-effective to perform VCTE for all patients. Screening patients with FIB-4 1.30–2.67 and DM might reduce the requirement for testing with VCTE by 73.8% (from 14.5 to 3.8 million).²⁵ However, the high FPR of FIB-4 associated with age remains. Using the FIB-4-IV algorithm with COL4-7S might compensate for the high FPR at high cutoff values, while maintaining the high negative predictive value of FIB-4 at low cutoff values. As a first step, it is a difficult question whether the cutoff value for FIB-4 should be 1.30 or 2.67. In this study, we examined the risk of developing LREs at 2 cutoff values, but when looking at the number of person-years of onset, there appears to be no difference between the 2. However, the FIB-4-IV model has a lower false negative rate than the FIB-4-IV-high model, so from the aspect of the purpose of this model, which is to connect to a liver specialist, the FIB-4-IV model with FIB of 1.30 as the first step may be considered appropriate. Second screening using COL4-7S might reduce the size of this risk group.

The ELF test has an area under the receiver operating characteristic curve of 0.90 for distinguishing fibrosis stage

≥3²⁸ and is the recommended first step for identifying NAFLD risk groups.⁹ As we reported in 2025, serum COL4 levels had comparable diagnostic power for fibrosis stage ≥3 and at-risk MASLD compared with ELF scores but were superior to ELF when diagnosing fibrosis stage ≥2. Thus, diagnosing fibrosis stage ≥2 using noninvasive markers may be influenced by differences in histologic background, resulting in ELF scores being less useful for diagnosing early-stage fibrosis than COL4-7S.³² The diagnostic performance of COL4-7S for AF may be equivalent to that of the ELF test.

The COL4-7S-chemiluminescent enzyme immunoassay (CLEIA; Fujirebio Inc, Tokyo, Japan) is approved for use in Japan.⁴³ Significant correlations were observed between values generated using COL4-7S-CLEIA and COL4-7S-RIA for patients with NAFLD ($r = 0.888$, $P < .01$; $\text{COL4-7S-CLEIA} = 1.28 \times \text{COL4-7S-RIA} - 1.15$). Differences between CLEIA and RIA increased with increased variability (<3.6 ng/mL with 10% coefficient variant).⁴⁴ Therefore, CLEIA and RIA may be used to detect fibrosis development.

Study strengths included the large-sample MASLD cohort, which was diagnosed histologically by a single pathologist. We estimated relationships between serum COL4-7S and histological diagnosis and between COL4-7S and NAFLD prognosis. Unlike FIB-4 and ELF tests, COL4-7S is a single marker of liver fibrosis, and unlike FIB-4, COL4-7S levels were not affected by age or DM. Additionally, COL4-7S concentrations were easily measured by CLEIA.

In addition, the latest guidelines recommend the low cutoff point of 1.30 for FIB-4 for people under 65 years of age and 2.0 for those over 65 years of age. When we examined

the predictive diagnostic performance of LREs using these criteria, we found that the FIB-4 alone only showed a slight improvement in the FPR (specificity) compared to the conventional 1.30. When we looked at the 2-step algorithm using these criteria, there was almost no difference compared to the examination in the main text. At least from the perspective of LREs prediction, if COL4-7S is used in the 2-step algorithm, FIB-4 may be acceptable even at 1.30 (Tables A3 and A4B and G).

This study had some limitations. It was a registry-based retrospective study, and 879 patients were excluded because they lacked COL4-7S values or external validation of COL4-7S efficacy in other independent cohorts. It was a hospital-based study and might have selection bias related to the clinical characteristics of MASLD patients. Additionally, we could not estimate the influence of diet, exercise, and medication use. How NITs such as COL4-7S change with therapeutic intervention is important and should be addressed in future studies. Comparisons of diagnostic accuracy for stage 3–4 and prognostic performance of COL4-7S with VCTE, and magnetic resonance elastography were not performed. Also, to identify individuals at risk of MASLD at an early stage, it is necessary to use NITs of F2 or higher, but the diagnostic performance of conventional serum markers and scoring systems is lower than that of differentiating between F3 or higher. In the future, it will be necessary to consider more effective markers that can be used to evaluate F2 or higher.

Conclusion

Fibrosis stage 3–4 and the predicted prognosis of MASLD were distinguished by COL4-7S with excellent performance compared with histological diagnosis. COL4-7S may be an alternative diagnostic method to liver biopsy and other NITs. The 2-step FIB-4-COL4-7S algorithm might help physicians stratify LRE risk in clinical practice.

Supplementary Materials

Material associated with this article can be found in the online version at <https://doi.org/10.1016/j.gastha.2025.100668>.

References

- Armstrong MJ, Houlihan DD, Benthall L, et al. Presence and severity of non-alcoholic fatty liver disease in a large prospective primary care cohort. *J Hepatol* 2012; 56:234–240.
- Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011;34:274–285.
- Estes C, Anstee QM, Arias-Loste MT, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016–2030. *J Hepatol* 2018;69:896–904.
- Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease: meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73–84.
- Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2015;149:389–397.e10.
- Sanyal AJ, Van Natta ML, Clark J, et al. Prospective study of outcomes in adults with nonalcoholic fatty liver disease. *N Engl J Med* 2021;385:1559–1569.
- Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. *Hepatology* 2017; 65:1557–1565.
- Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;67:328–357.
- European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). *J Hepatol* 2024;81:492–542.
- Tokushige K, Ikejima K, Ono M, et al. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. *J Gastroenterol* 2021;56:951–963.
- Bedossa P, FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. *Hepatology* 2014;60:565–575.
- Myers RP, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. *Liver Int* 2008;28:705–712.
- Rinella ME, Lazarus JV, Ratziu V, et al. NAFLD Nomenclature consensus group. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Ann Hepatol* 2024;29:101133.
- Younossi ZM, Paik JM, Stepanova M, et al. Clinical profiles and mortality rates are similar for metabolic dysfunction-associated steatotic liver disease and non-alcoholic fatty liver disease. *J Hepatol* 2024;80:694–701.
- Kamada Y, Fujii H, Suzuki Y, et al. Clinical characteristics of steatotic liver disease categories in a large cohort of Japanese health checkup participants. *Gastro Hep Adv* 2024;3:1148–1156.
- Sumida Y, Yoneda M, Hyogo H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012;12:2.
- Xiao G, Zhu S, Xiao X, et al. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: a meta-analysis. *Hepatology* 2017; 66:1486–1501.
- Boursier J, Hagström H, Ekstedt M, et al. Non-invasive tests accurately stratify patients with NAFLD based on

- their risk of liver-related events. *J Hepatol* 2022; 76:1013–1020.
19. Johnson AL, Hayward KL, Patel P, et al. Predicting liver-related outcomes in people with nonalcoholic fatty liver disease: the prognostic value of noninvasive fibrosis tests. *Hepatol Commun* 2022;6:728–739.
 20. Loosen SH, Kostev K, Keitel V, et al. An elevated FIB-4 score predicts liver cancer development: a longitudinal analysis from 29,999 patients with NAFLD. *J Hepatol* 2022;76:247–248.
 21. Vieira Barbosa J, Milligan S, Frick A, et al. Fibrosis-4 Index as an independent predictor of mortality and liver-related outcomes in NAFLD. *Hepatol Commun* 2022; 6:765–779.
 22. Ishiba H, Sumida Y, Tanaka S, et al. The novel cutoff points for the FIB4 index categorized by age increase the diagnostic accuracy in NAFLD: a multi-center study. *J Gastroenterol* 2018;53:1216–1224.
 23. Ishiba H, Sumida Y, Seko Y, et al. Type IV Collagen 7S is the most accurate test for identifying advanced fibrosis in NAFLD with type 2 diabetes. *Hepatol Commun* 2020; 5:559–572.
 24. McPherson S, Hardy T, Dufour JF, et al. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. *Am J Gastroenterol* 2017; 112:740–751.
 25. Udompap P, Therneau TM, Canning RE, et al. Performance of American Gastroenterological Association Clinical Care Pathway for the risk stratification of patients with nonalcoholic fatty liver disease in the US population. *Hepatology* 2023;77:931–941.
 26. Archer AJ, Belfield KJ, Orr JG, et al. EASL clinical practice guidelines: non-invasive liver tests for evaluation of liver disease severity and prognosis. *Frontline Gastroenterol* 2022;13:436–439.
 27. Kanwal F, Shubrook JH, Adams LA, et al. Clinical care pathway for the risk stratification and management of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2021;161:1657–1669.
 28. Guha IN, Parkes J, Roderick P, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008; 47:455–460.
 29. Fan W, Torok NJ. Editorial: noninvasive fibrosis biomarkers in patients with NASH with diabetes. *Hepatol Commun* 2021;5:553–555.
 30. Leeming DJ, Nielsen MJ, Dai Y, et al. Enzyme-linked immunosorbent serum assay specific for the 7S domain of Collagen Type IV (P4NP 7S): a marker related to the extracellular matrix remodeling during liver fibrogenesis. *Hepatol Res* 2012;42:482–493.
 31. Stefano JT, Guedes LV, de Souza AAA, et al. Usefulness of collagen type IV in the detection of significant liver fibrosis in nonalcoholic fatty liver disease. *Ann Hepatol* 2021;20:100253.
 32. Ishiba H, Fujii H, Kamada Y, Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD). Accuracy of type IV collagen 7S versus Enhanced Liver Fibrosis score for diagnosing fibrosis in patients with metabolic dysfunction-associated steatotic liver disease. *Hepatol Commun* 2024;9(1):e0563.
 33. Fujii H, Iwaki M, Hayashi H, et al. Clinical outcomes in biopsy-proven nonalcoholic fatty liver disease patients: a multicenter registry-based cohort study. *Clin Gastroenterol Hepatol* 2023;21:370–379.
 34. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313–1321.
 35. Brunt EM, Janney CG, Di Bisceglie AM, et al. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; 94:2467–2474.
 36. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; 33:S62–S69.
 37. Umemura S, Arima H, Arima S, et al. The Japanese Society of Hypertension guidelines for the management of hypertension (JSH 2019). *Hypertens Res* 2019; 42:1235–1481.
 38. Teramoto T, Sasaki J, Ueshima H, et al. Diagnostic criteria for dyslipidemia. Executive summary of Japan Atherosclerosis Society (JAS) guideline for diagnosis and prevention of atherosclerotic cardiovascular diseases for Japanese. *J Atheroscler Thromb* 2007;14:155–158.
 39. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 1997; 20:1183–1197.
 40. Ishiba H, Sumida Y, Kamada Y, et al. Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD). Noninvasive tests predict liver-related events and mortality in patients with nonalcoholic fatty liver disease: sub-analysis of the CLIONE-Asia study. *J Gastroenterol Hepatol* 2023;38:896–904.
 41. Hagström H, Nasr P, Ekstedt M, et al. Accuracy of noninvasive scoring systems in assessing risk of death and liver-related endpoints in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2019;17:1148–1156.e4.
 42. Angulo P, Bugianesi E, Björnsson ES, et al. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013;145:782–789.e4.
 43. Otakaki Y, Yuhara A, Moriyama K, et al. Performance of a highly sensitive sandwich immunoassay for type IV collagen 7S in serum and plasma. *Igaku to Yakugaku* 2020;77:785–792.
 44. Shima T, Ohtakaki Y, Kikuchi H, et al. A novel rapid immunoassay of serum type IV collagen 7S for the diagnosis of fibrosis stage of nonalcoholic fatty liver diseases. *Hepatol Res* 2021;51:263–276.

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acquisition. Hideki Hayashi: Data acquisition, statistical analysis. Hidenori Toyoda: Study concept and design, data acquisition. Satoshi Oeda: Data acquisition. Hideyuki Hyogo: Data acquisition. Miwa Kawanaka: Data acquisition. Asahi Morishita: Data acquisition. Kensuke Munekage: Data acquisition. Kazuhito Kawata: Data acquisition. Tsubasa Tsutsumi: Data acquisition. Koji Sawada: Data acquisition. Tatsuji Maeshiro: Data acquisition. Hiroshi Tobita: Data acquisition. Yuichi Yoshida: Data acquisition. Masafumi Naito: Data acquisition. Asuka Araki: Data acquisition. Shingo Arakaki: Data acquisition. Takumi Kawaguchi: Data acquisition. Hidenao Noritake: Data acquisition. Masafumi Ono: Data acquisition. Tsutomu Masaki: Data acquisition. Satoshi Yasuda: Data acquisition. Eiichi Tomita: Data acquisition. Masato Yoneda: Data acquisition. Akihiro Tokushige: Statistical analysis. Hirokazu Takahashi: Data acquisition. Shinichiro Ueda: Statistical analysis. Shinichi Aishima: Material support. Atsushi Nakajima: Study concept and design, study supervision. Takeshi Okanoue: Study supervision.

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The authors disclose no conflicts.

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Study materials will not be made available to other researchers.

Reporting Guidelines:

Helsinki Declaration, STROBE.