CLINICAL INVESTIGATION

Mac-2-binding protein glycosylation isomer well correlates with the controlling nutritional status score in hepatitis viruses-related liver diseases

Abstract

Purpose: Examining the clinical significance of Mac-2-binding protein glycosylation isomer (M2BPGi), which was recently introduced as a novel liver fibrotic biomarker in chronic liver disease patients with unique fibrosis associated glycol chain protein alteration, other than liver fibrotic marker appears to be of importance. We sought to examine the relevance between M2BPGi and the Controlling Nutrition (CONUT) score in hepatitis B and C viruses-related patients (the HBV-related cohort (Br-cohort, n=249) and the HCV-related cohort (Cr-cohort, n=386)) comparing with other liver fibrotic markers.

Patients and Methods: We checked the correlation between the CONUT score and four liver fibrotic markers (M2BPGi, FIB-4 index, hyaluronic acid, and platelet count) in the two cohorts. Receiver operating characteristics (ROC) analyses associated with elevated CONUT score (CONUT score ≥ 1,2,3,4 or 5) were also conducted.

Results: The median CONUT score (range) were 1 (0-5) in the Br-cohort and 2 (0-8) in the Cr-cohort ($P<0.0001$). In the Br-cohort, advanced fibrosis or more (F3 or F4) was noted in 60 patients (24.1%), while in the Cr-cohort, it was noted in 212 patients (54.9%). In the Br-cohort, the highest correlation coefficient was identified in the FIB-4 index ($r=0.436$, $P<0.0001$), followed by M2BPGi ($r=0.376$, $P<0.0001$). In the Cr-cohort, the highest correlation coefficient was noted in M2BPGi ($r=0.690$, $P<0.0001$), followed by the FIB-4 index ($r=0.598$, $P<0.0001$). For the ROC analyses linked to the elevated CONUT score, in the Cr-cohort, M2BPGi yielded the highest AUC in all ROC analyses, whereas in the Br-cohort, such tendencies were not noted.

Conclusion: M2BPGi can be a useful marker for predicting nutritional condition as determined by the CONUT score especially in chronic hepatitis C patients.

Keywords: controlling nutritional status score • M2BPGi • chronic hepatitis B • chronic hepatitis C • liver fibrotic marker • comparative study • liver fibrosis • liver inflammation

Submitted: 28 January 2019; Accepted: 12 February 2019; Published online: 18 February 2019

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Abbreviations

PEM: Protein-Energy Malnutrition; LC: Liver Cirrhosis; CLD: Chronic Liver Disease; CONUT: Controlling Nutritional Status; M2BPGi: Mac-2-Binding Protein Glycosylation Isomer; HSC: Hepatic Stellate Cell; SLE: Systemic Lupus Erythematosus; CHB: Chronic Hepatitis B; CHC: Chronic Hepatitis C; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; Br-cohort: HBV-related Cohort; Cr-cohort: HCV-related Cohort; ROC: Receiver Operating Characteristics; AUC: Area Under the ROC Curve; COI: Cut-Off Index

Introduction

The liver exerts a significant role in the metabolism of carbohydrate by means of maintaining glucose levels in the normal range because it is the central organ for the metabolism [1-5]. One of the frequently encountered Liver Cirrhosis (LC) related complications is protein-energy malnutrition (PEM) and it may cause high morbidity and mortality [1,6-8]. Appropriate nutritional evaluation and nutritional interventions are therefore essential for the adequate management of Chronic Liver Disease (CLD) patients.

The Controlling Nutritional Status (CONUT) scoring system is a quantitative and objective scoring system which is widely utilized to assess the nutritional condition [9-16]. It is calculated from total cholesterol level, serum albumin level and peripheral lymphocyte count and they are surrogate serum markers of calorie shortage, protein synthesis inability and immune defense disorder [13,14]. As the CONUT score can be calculated easily using laboratory parameters, clinicians can continuously assess the nutritional condition of the subject [13,14]. This scoring system has been shown to well reflect liver functional reserve and clinical outcome in CLD patients [16,17].

Mac-2-Binding Protein Glycosylation Isomer (M2BPGi) was recently introduced as a novel liver fibrotic biomarker in CLD patients with unique fibrosis associated glycochain protein alteration [18-20]. A recent meta-analysis reported that M2BPGi can be a good substitute for liver biopsy and can be helpful for predicting both hepatocellular carcinoma incidence and survival [20]. Furthermore, Bekki, et al. demonstrated that Hepatic Stellate Cells (HSCs) are the source of M2BPGi secretion and M2BPGi from HSCs induces Mac-2 expression in Kupffer cells, which subsequently activates HSCs to be fibrogenic in the liver [21]. On the other hand, we have reported that M2BPGi reflects not only the severity of liver fibrosis but also the severity of liver inflammation in CLD patients [18,22]. The impact of M2BPGi on outcome have been also shown in esophageal cancer, Systemic Lupus Erythematosus (SLE), primary sclerosing cholangitis, heart disease, pancreatitis and pancreatic cancer [23-28]. Thus, clinical evidence of this biomarker have been accumulated in various diseases in these days.

However, currently, there is scarce data regarding the relevance between the CONUT score and M2BPGi in patients with hepatitis virus-related CLD [29]. These data seem to be important for examining the clinical significance of M2BPGi other than liver fibrosis marker. The primary aim of our study is to investigate the relation between M2BPGi and the CONUT score in a patient with Chronic Hepatitis B (CHB) and Chronic Hepatitis C (CHC) comparing with other liver fibrotic markers.

Patients and Methods

Study design

Six hundred and thirty-five patients diagnosed as CHB (n=249, the HBV-related cohort (Br-cohort)) or CHC (n=386, the HCV-related cohort (Cr-cohort)) were admitted at our hospital between September 2005 and July 2015 and they were analyzed in this study. A stored serum sample was collected from all patients after obtaining written informed consent. All analyzed subjects had liver biopsy data. In all Br-cohort patients, seropositivity of HB surface antigen with no proof of co-infection with HCV and no proof of drug-induced liver injury or alcoholic liver injury was confirmed. In all Cr-cohort patients, seropositivity of HCV antibody with no proof of co-infection with HBV and no proof of drug-induced liver injury or alcoholic liver injury was confirmed. We checked the correlation between the CONUT score and four liver fibrotic markers (M2BPGi, FIB-4 index, hyaluronic acid, and platelet count) in the two cohorts. Receiver operating characteristics (ROC) analyses associated with elevated CONUT score (CONUT score ≥ 1, 2, 3, 4 or 5) were also conducted. Our study protocol conformed to every provision of the Declaration of Helsinki and the ethical committee of our hospital acknowledged our study protocol (approval number, 1831).

CONUT score

As noted earlier, the CONUT score indicates the sum of the following three laboratory data; the serum

albumin level (converted to 0,2,4 or 6 points according to each value), the total peripheral lymphocyte count (converted to 0,1,2 or 3 points according to each value) and total cholesterol level (converted to 0,1,2 or 3 points according to each value) [13,14]. Based on the CONUT scores, analyzed subjects were divided into four categories: normal (0 or 1 point), mild malnutritional condition (2, 3 or 4 points), moderate malnutritional condition (5, 6, 7 or 8 points) and severe malnutritional condition (9 or more points).

Measurement of M2BPGi and FIB-4 index

Serum M2BPGi level was tested in preserved blood samples obtained at baseline. M2BPGi quantification was tested as reported elsewhere [30-32]. FIB-4 index was calculated according to previous reports [33,34].

Statistical analysis

In terms of quantitative variables, the statistical analyses in cohorts or subgroups were done by means of Mann-Whitney *U* test, Student's **t**-test, Kruskal-Wallis test, Fisher's exact test or Pearson correlation coefficient **r** as suitable. Data for ROC curve analyses were indicated along with area under the ROC curve (AUC), each optimal cut-off value where the sum of specificity and sensitivity reaches a maximum, sensitivity (%) and specificity (%). Unless otherwise mentioned, data are indicated as median value (range). A significant level of *P* value was set to less than 0.05. We performed statistical analyses with the JMP 13 (SAS Institute Inc., Cary, NC).

Results

Baseline patient data

Table 1, shows the baseline data in our analyses. The median age (range) was 45 years (18-78 years) in the Br-cohort and 62 years (20-87 years) in the Cr-cohort ($P<0.0001$). The median CONUT score (range) was 1 (0-5) in the Br-cohort and 2 (0-8) in the Cr-cohort ($P<0.0001$). In the Br-cohort, normal nutritional condition as defined by the CONUT score was noted in 156 patients (62.9%), mild malnutrition in 88 patients (35.3%), moderate malnutrition in 5 patients (2.0%) and severe malnutrition in none, while in the Cr-cohort, normal nutritional condition was noted in 178 patients (46.1%), mild malnutrition in 168 patients (43.5%), moderate malnutrition in 40 patients (10.4%) and severe malnutrition in none (Figure 1). In the Br-cohort, advanced fibrosis or more (F3 or F4) was noted in 60 patients (24.1%), while in the Cr-cohort, it was noted in 212 patients (54.9%). The median (range) M2BPGi, FIB-4 index and hyaluronic acid were: 1.14 Cut-Off Index (COI)

Table 1. Baseline data in the HBV-related cohort (Br-cohort) and the HCV-related cohort (Cr-cohort)

Parameters	Br-cohort (n=249)	Cr-cohort (n=386)	*P* value
Age (years)	45 (18-78)	62 (20-87)	<0.0001
Gender, male/female	155/94	180/206	0.0001
CONUT score	1 (0-5)	2 (0-8)	<0.0001
AST (IU/l)	29 (11-421)	41 (14-343)	<0.0001
ALT (IU/l)	34 (7-781)	41.5 (7-396)	0.027
Serum albumin (g/dl)	4.2 (3.0-5.1)	4.1 (2.5-4.9)	0.0001
Total bilirubin (mg/dl)	0.8 (0.3-2.2)	0.8 (0.2-2.3)	0.483
Prothrombin time (%)	90.8 (62-125.5)	89.8 (48.1-121.6)	0.253
Platelet count ($\times 10^4/mm^3$)	18.1 (4.5-38.3)	14.0 (3.5-38.7)	<0.0001
Hyaluronic acid (ng/ml)	24 (9-759)	89.5 (9-1420)	<0.0001
Total cholesterol (mg/dl)	185 (94-311)	164.5 (80-314)	<0.0001
Lymphocyte count ($/mm^3$)	1601 (698-3341)	1532 (377-5313)	0.0542
Previous antiviral therapy, yes/no	60/189	287/99	<0.0001
HBV DNA ≥ 5 log copies/ml, yes/no	121/128	NA	NA
HBe antigen positivity, yes/no	93/156	NA	NA
HCV genotype, 1b/2a/2b/others	NA	288/66/23/9	NA
HCV RNA ≥ 5 log copies/ml, yes/no	NA	326/60	NA
M2BPGi (cutoff index, COI)	1.14 (0.25-12.9)	2.12 (0.34-20.0)	<0.0001
FIB-4 index	1.30 (0.28-12.47)	2.92 (0.40-16.52)	<0.0001
Fibrosis stage, F4/3/2/1/0	19/41/51/124/14	122/90/63/103/8	<0.0001
A stage, 0/1/2/3	17/155/62/15	7/154/208/17	<0.0001

Note: Data are expressed as number or median (range).
CONUT score: Controlling Nutritional Score; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; M2BPGi: Mac-2-Binding Protein Glycosylation Isomer; NA: Not Available

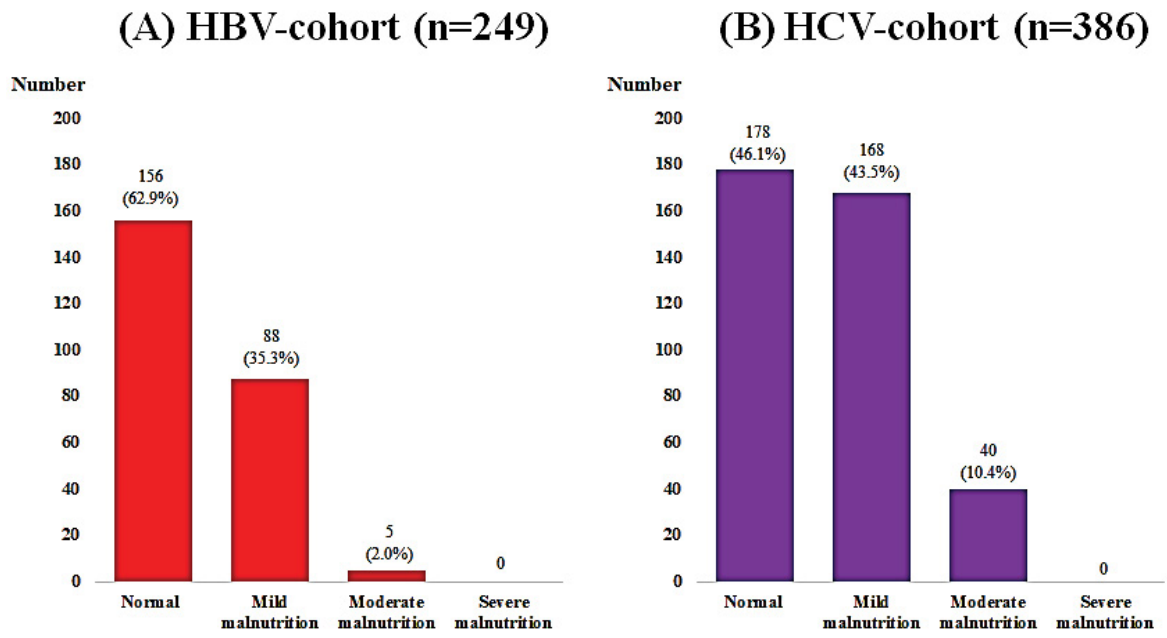

Figure 1: The proportion of normal nutrition (the CONUT score 0 or 1), mild malnutrition (the CONUT score 2, 3 or 4), moderate malnutrition (the CONUT score 5, 6, 7 or 8) and sever malnutrition (the CONUT score 9 or more) in the HBV-related cohort (A) and the HCV-related cohort (C).

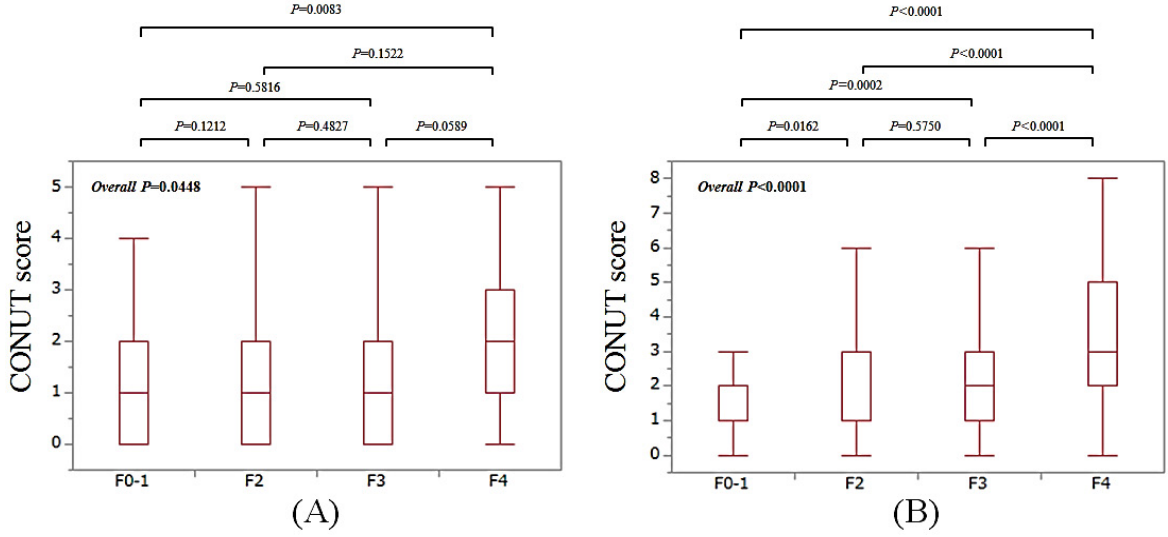

Figure 2: The CONUT score according to liver fibrotic stage in the HBV-related cohort (A) and the HCV-related cohort (B).

(0.25-12.9 COI), 1.30 (0.28-12.47) and 24 ng/ml (9-759 ng/ml) in the Br-cohort and 2.12 COI (0.34-20.0 COI), 2.92 (0.40-16.52) and 89.5 ng/ml (9-1420 ng/ml) in the Cr-cohort (*P* values, all <0.0001).

CONUT score stratified by liver fibrotic stages in the Br-cohort and the Cr-cohort

Figure 2, shows the CONUT score stratified by liver fibrotic stages in the Br-cohort and the Cr-cohort. In the Br-cohort, the median (range) CONUT score in each liver fibrotic stage were: 1 (0-4) in F0-1 (n=138), 1 (0-5) in F2 (n=51), 1 (0-5) in F3 (n=41), and 2 (0-5) in F4 (n=19) (*P* values; 0.1212 in F0-1 and F2, 0.4827 in F2 and F3, 0.0589 in F3 and F4, 0.5816 in F0-1 and F3, 0.1522 in F2 and F4, 0.0083 in F0-1 and F4, overall significance *P*=0.0448) (Figure 2A). In the Cr-cohort, the median (range) CONUT score in each liver fibrotic stage were: 1 (0-7) in F0-1 (n=111), 1 (0-6) in F2 (n=63), 2 (0-8) in F3 (n=90), and 3 (0-

8) in F4 (n=122) (*P* values; 0.0162 in F0-1 and F2, 0.5750 in F2 and F3, <0.0001 in F3 and F4, 0.0002 in F0-1 and F3, <0.0001 in F2 and F4, <0.0001 in F0-1 and F4, overall significance *P*<0.0001) (Figure 2B).

CONUT score stratified by liver inflammation stages in the Br-cohort and the Cr-cohort

Figure 3, shows the CONUT score stratified by liver inflammation stages in the Br-cohort and the Cr-cohort. In the Br-cohort, the median (range) CONUT score in each liver inflammation stage were: 1 (0-5) in A0-1 (n=172), 1 (0-5) in A2 (n=62) and 1 (0-5) in A3 (n=15) (*P* values; 0.3619 in A0-1 and A2, 0.5814 in A2 and A3, 0.7694 in A0-1 and A3, overall significance *P*=0.6252) (Figure 3A). In the Cr-cohort, the median (range) CONUT score in each liver inflammation stage were: 1 (0-8) in A0-1 (n=161), 2 (0-8) in A2 (n=208) and 4 (0-8) in A3 (n=17) (*P* values; <0.0001 in A0-1 and A2, 0.0011 in A2 and A3, <0.0001 in A0-1 and A3, overall significance *P*<0.0001) (Figure 3B).

Relevance between the CONUT score and liver fibrotic markers in the Br-cohort and the Cr-cohort

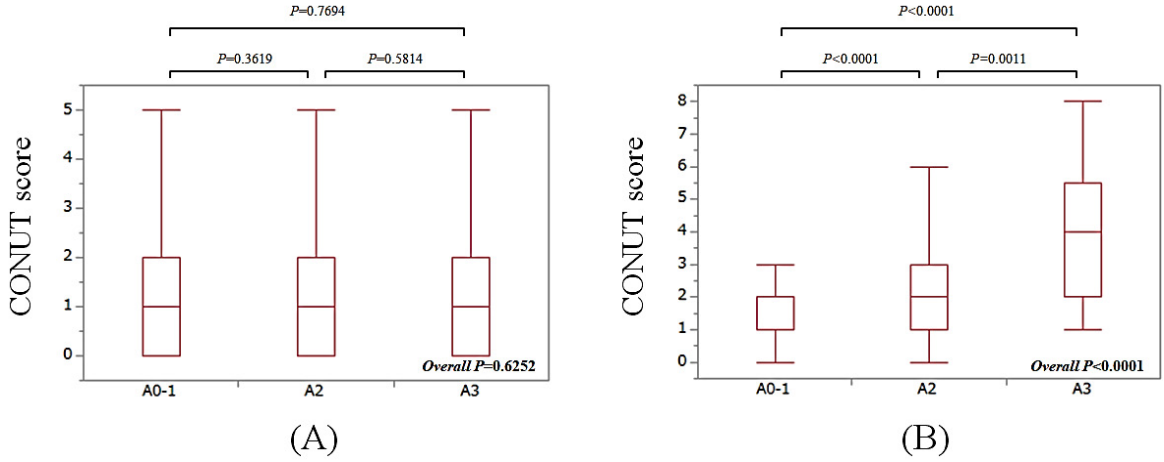

Figure 3: The CONUT score according to liver inflammation stage in the HBV-related cohort (A) and the HCV-related cohort (B).

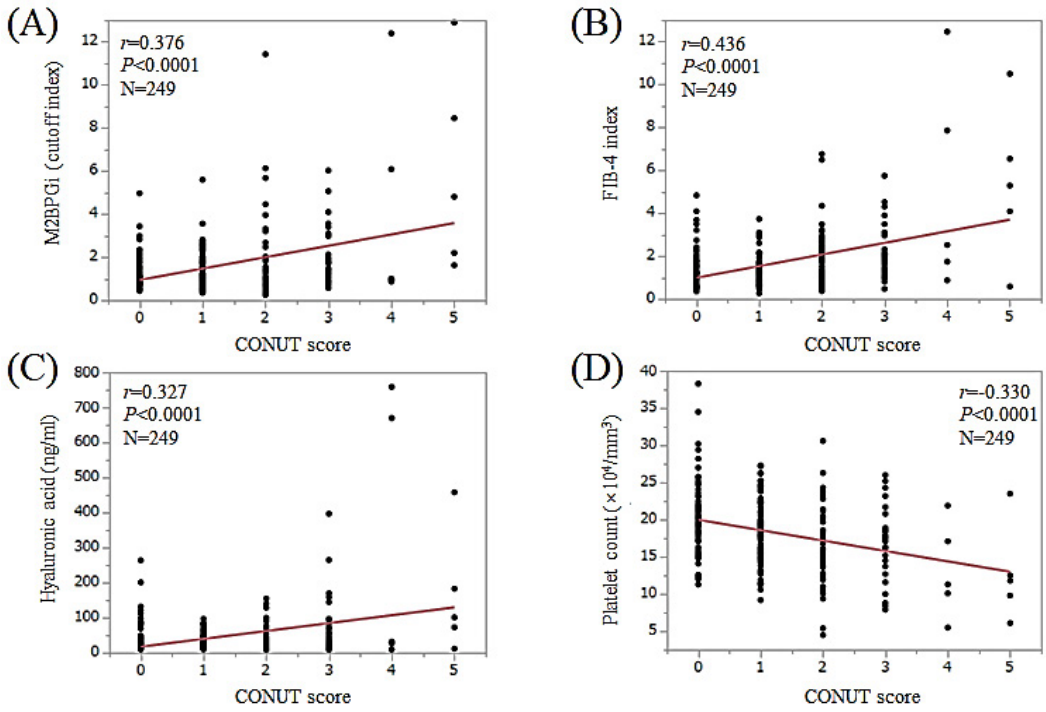

Figure 4: Relevance between the CONUT score and liver fibrotic markers (M2BPGi (A), FIB-4 index (B), hyaluronic acid (C) and platelet count (D)) in the HBV-related cohort.

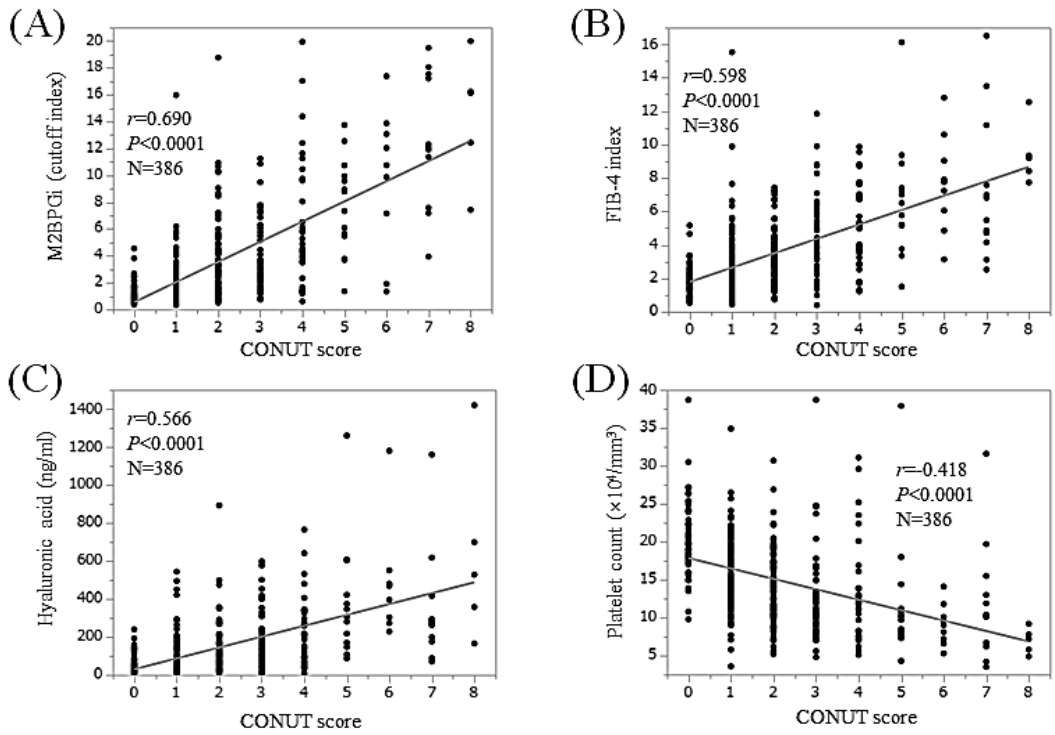

Figure 5: Relevance between the CONUT score and liver fibrotic markers (M2BPGi (A), FIB-4 index (B), hyaluronic acid (C) and platelet count (D)) in the HCV-related cohort.

Figures 4 and 5, shows the relevance between the CONUT score and liver fibrotic markers in the Br-cohort and the Cr-cohort. In the Br-cohort, the highest correlation coefficient was identified in FIB-4 index (r=0.436, P<0.0001), followed by M2BPGi (r=0.376, P<0.0001) (Figure 4). In the Cr-cohort, the highest correlation coefficient was noted in M2BPGi (r=0.690, P<0.0001), followed by the FIB-4 index (r=0.598, P<0.0001) (Figure 5).

Relevance between the CONUT score and liver fibrotic markers in the Br-cohort and the Cr-cohort in each liver fibrotic stage

Table 2, demonstrates the relevance between the CONUT score and liver fibrotic markers in the Br-cohort and the Cr-cohort in each liver fibrotic stage. In the Br-cohort, the highest correlation coefficient was found in M2BPGi for patients with F4 and F0-1, while the highest correlation coefficient was found in FIB-4 index for all other subgroups. In the Cr-cohort, the highest correlation coefficient was found in FIB-4 index for patients with F2 and F0-1, while the highest correlation coefficient was noted in M2BPGi for all other subgroups.

Relevance between the CONUT score and liver fibrotic markers in the Br-cohort and the Cr-cohort in each liver inflammation stage

Table 3, indicates the relevance between the CONUT score and liver fibrotic markers in the Br-cohort and the Cr-cohort in each liver inflammation stage. In the Br-cohort, the highest correlation coefficient was noted in M2BPGi for patients with A3, while the highest correlation coefficient was noted in FIB-4 index for all other subgroups. In the Cr-cohort, the highest correlation coefficient was identified in M2BPGi for all subgroups.

ROC analyses of liver fibrotic markers associated with the CONUT score ≥ 1, 2, 3, 4 or 5 in the Br-cohort and the Cr-cohort

Table 4, shows ROC analyses associated with the CONUT score ≥ 1 in the Br-cohort and the Cr-cohort. In the Br-cohort, platelet count yielded the highest AUC (0.643) for the CONUT score ≥ 1, followed by FIB-4 index (AUC=0.594). In the Cr-cohort, M2BPGi yielded the highest AUC (0.805) for the CONUT score ≥ 1, followed by platelet count (AUC=0.799).

Table 5, shows ROC analyses associated with the CONUT score ≥ 2 in the Br-cohort and the Cr-cohort. In the Br-cohort, FIB-4 yielded the highest AUC (0.688) for the CONUT score ≥ 2, followed by platelet count (AUC=0.668). In the Cr-cohort, M2BPGi yielded the highest AUC (0.813) for the

Table 2. The relationship between liver fibrotic markers and CONUT score in the HBV-related cohort (Br-cohort) and the HCV-related cohort (Cr-cohort) according to the degree of liver fibrosis

Variables		Br-cohort		Cr-cohort	
		r	*P* value	*r*	*P* value
F4	M2BPGi	0.706	0.0007	0.683	<0.0001
	FIB-4 index	0.685	0.0012	0.458	<0.0001
	Hyaluronic acid	0.417	0.0761	0.424	<0.0001
	Platelet count	-0.657	0.0022	-0.186	0.0401
F3	M2BPGi	0.077	0.6311	0.542	<0.0001
	FIB-4 index	0.205	0.1976	0.527	<0.0001
	Hyaluronic acid	-0.050	0.7578	0.536	<0.0001
	Platelet count	-0.198	0.2144	-0.368	0.0004
F2	M2BPGi	0.356	0.0104	0.506	<0.0001
	FIB-4 index	0.546	<0.0001	0.557	<0.0001
	Hyaluronic acid	0.412	0.0027	0.542	<0.0001
	Platelet count	-0.503	0.0002	-0.557	<0.0001
F0-1	M2BPGi	0.163	0.0559	0.378	<0.0001
	FIB-4 index	0.136	0.1117	0.507	<0.0001
	Hyaluronic acid	0.056	0.5144	0.444	<0.0001
	Platelet count	-0.110	0.1995	-0.447	<0.0001
F3 or more	M2BPGi	0.457	0.0002	0.690	<0.0001
	FIB-4 index	0.539	<0.0001	0.532	<0.0001
	Hyaluronic acid	0.348	0.0064	0.502	<0.0001
	Platelet count	-0.413	0.0011	-0.277	<0.0001
F2 or more	M2BPGi	0.419	<0.0001	0.685	<0.0001
	FIB-4 index	0.519	<0.0001	0.558	<0.0001
	Hyaluronic acid	0.372	<0.0001	0.528	<0.0001
	Platelet count	-0.434	<0.0001	-0.345	<0.0001

M2BPGi: Mac-2-binding protein glycosylation isomer

Table 3. Relationship between liver fibrotic markers and CONUT score in the HBV-related cohort (Br-cohort) and the HCV-related cohort (Cr-cohort) according to the degree of liver inflammation

Variables		Br-cohort		Cr-cohort	
		r	*P* value	*r*	*P* value
A3	M2BPGi	0.730	0.0002	0.890	<0.0001
	FIB-4 index	0.723	0.0006	0.862	<0.0001
	Hyaluronic acid	0.596	0.019	0.720	0.0017
	Platelet count	-0.585	0.0221	-0.378	0.1491
A2	M2BPGi	0.212	0.0986	0.600	<0.0001
	FIB-4 index	0.248	0.0518	0.508	<0.0001
	Hyaluronic acid	0.178	0.1675	0.501	<0.0001
	Platelet count	-0.134	0.2997	-0.377	<0.0001
A0-1	M2BPGi	0.394	<0.0001	0.733	<0.0001
	FIB-4 index	0.411	<0.0001	0.579	<0.0001
	Hyaluronic acid	0.295	<0.0001	0.547	<0.0001
	Platelet count	-0.360	<0.0001	-0.349	<0.0001
A2 or more	M2BPGi	0.411	0.0002	0.654	<0.0001
	FIB-4 index	0.467	<0.0001	0.572	<0.0001
	Hyaluronic acid	0.359	0.0013	0.544	<0.0001
	Platelet count	-0.259	0.0231	-0.411	<0.0001

M2BPGi; Mac-2-binding protein glycosylation isomer

CONUT score ≥ 2, followed by hyaluronic acid (AUC=0.777).

Table 6, shows ROC analyses associated with the CONUT score ≥ 3 in the Br-cohort and the Cr-cohort. In the Br-cohort, FIB-4 index yielded the highest AUC (0.737) for the CONUT score ≥ 3, followed by hyaluronic acid (AUC=0.692). In the Cr-

Table 4. Receiver operating characteristics curve analyses linked to CONUT score ≥ 1 in the HBV-related cohort (Br-cohort) and the HCV-related cohort (Cr-cohort)

Variables	Br-cohort			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.567	1.63	34.8	81.7
FIB-4 index	0.594	0.75	87.6	28.2
Hyaluronic acid	0.503	25	51.7	56.3
Platelet count	0.643	18.1	57.9	69.0
Variables	**Cr-cohort**			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.805	1.77	63.3	85.7
FIB-4 index	0.789	2.64	63.6	87.5
Hyaluronic acid	0.768	63	70.3	73.2
Platelet count	0.799	16.9	73.9	82.1

M2BPGi: Mac-2-binding protein glycosylation isomer; AUC: area under the receiver operating characteristics curve

Table 5. Receiver operating characteristics curve analyses linked to CONUT score ≥ 2 in the HBV-related cohort (Br-cohort) and the HCV-related cohort (Cr-cohort)

Variables	Br-cohort			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.600	2.98	21.5	96.2
FIB-4 index	0.688	1.75	54.8	82.1
Hyaluronic acid	0.610	23	62.4	55.1
Platelet count	0.668	18.6	74.2	55.8
Variables	**Cr-cohort**			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.813	2.34	72.1	78.7
FIB-4 index	0.763	3.03	68.8	75.8
Hyaluronic acid	0.777	93	70.2	74.7
Platelet count	0.758	13	63.5	81.5

M2BPGi: Mac-2-binding protein glycosylation isomer; AUC: area under the receiver operating characteristics curve

Table 6. Receiver operating characteristics curve analyses linked to CONUT score ≥ 3 in the HBV-related cohort (Br-cohort) and the HCV-related cohort (Cr-cohort)

Variables	Br-cohort			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.709	1.22	73.7	57.8
FIB-4 index	0.737	1.75	68.4	74.9
Hyaluronic acid	0.692	53	47.4	82.0
Platelet count	0.650	17.7	65.8	58.3
Variables	**Cr-cohort**			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.820	2.97	74.2	74.9
FIB-4 index	0.809	3.12	82.3	71.0
Hyaluronic acid	0.812	145	68.6	82.4
Platelet count	0.757	13.1	75.0	71.8

M2BPGi: Mac-2-binding protein glycosylation isomer; AUC: area under the receiver operating characteristics curve

cohort, M2BPGi yielded the highest AUC (0.820) for the CONUT score ≥ 3, followed by hyaluronic acid (AUC=0.812).

Table 7, shows ROC analyses associated with the CONUT score ≥ 4 in the Br-cohort and the Cr-cohort. In the Br-cohort, FIB-4 index yielded the highest AUC (0.784) for the CONUT score ≥ 4, followed by platelet count (AUC=0.765). In the Cr-cohort, M2BPGi yielded the highest AUC (0.867) for the CONUT score ≥ 4, followed by hyaluronic acid (AUC=0.829).

Table 8, shows ROC analyses associated with the CONUT score ≥ 5 in the Br-cohort and the Cr-cohort. In the Br-cohort, M2BPGi yielded the

Table 7. Receiver operating characteristics curve analyses linked to CONUT score ≥ 4 in the HBV-related cohort (Br-cohort) and the HCV-related cohort (Cr-cohort)

Variables	Br-cohort			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.761	4.81	50.0	97.1
FIB-4 index	0.784	2.53	70.0	87.5
Hyaluronic acid	0.738	73	60.0	85.4
Platelet count	0.765	12.5	70.0	89.1
Variables	**Cr-cohort**			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.867	3.60	85.1	78.5
FIB-4 index	0.809	3.52	79.7	70.2
Hyaluronic acid	0.829	145	77.0	76.3
Platelet count	0.729	13.0	75.7	65.1

M2BPGi: Mac-2-binding protein glycosylation isomer; AUC: area under the receiver operating characteristics curve

Table 8. Receiver operating characteristics curve analyses linked to CONUT score ≥ 5 in the HBV-related cohort (Br-cohort) and the HCV-related cohort (Cr-cohort)

Variables	Br-cohort			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.902	1.63	100	71.3
FIB-4 index	0.801	4.10	80.0	95.9
Hyaluronic acid	0.795	73	80.0	84.8
Platelet count	0.780	12.5	80.0	88.1
Variables	**Cr-cohort**			
	AUC	Cutoff	Sensitivity (%)	Specificity (%)
M2BPGi	0.904	5.47	85.0	86.4
FIB-4 index	0.873	4.72	82.5	80.4
Hyaluronic acid	0.875	145	90.0	72.5
Platelet count	0.788	10.4	70.0	81.5

M2BPGi: Mac-2-binding protein glycosylation isomer; AUC: area under the receiver operating characteristics curve

highest AUC (0.902) for the CONUT score ≥ 5, followed by FIB-4 index (AUC=0.801). In the Cr-cohort, M2BPGi yielded the highest AUC (0.904) for the CONUT score ≥ 5, followed by hyaluronic acid (AUC=0.875).

Best cut-off points, sensitivity (%) and specificity (%) for each liver fibrotic marker are listed in each table.

Discussion

To our knowledge, the current study is the largest study evaluating the relationship between the CONUT score and M2BPGi in hepatitis virus-related CLD patients. As mentioned above, examining the clinical significance of M2BPGi other than liver fibrotic marker appears to be clinically of importance. In that sense, we believe that our data are worthy of reporting.

In our results, M2BPGi well correlated with the CONUT score both in the Br-cohort and the Cr-cohort. For the correlation according to the severity of liver fibrosis, in the Cr-cohort, M2BPGi yielded the strongest correlation with the CONUT score in all subgroups except F2 and F0-1, whereas in the HBV-cohort, FIB-4 index yielded the strongest correlation with the CONUT score in all subgroups except F4 and F0-1. For the correlation according to the severity of liver inflammation, in the Cr-cohort, M2BPGi yielded the strongest correlation with the CONUT score in all subgroups, whereas in the Br-cohort, FIB-4 index yielded the strongest correlation with the CONUT score in all subgroups except A3. For the ROC analyses, in the Cr-cohort, M2BPGi yielded the highest AUC in all ROC analyses, whereas in the Br-cohort, such tendencies were not noted. These results denote that M2BPGi can be helpful for predicting the nutritional condition, especially in CHC patients. While in CHB patients, FIB-4 index may be helpful for predicting the nutritional condition. Malnutrition in CLD patients can be associated with both liver fibrosis progression

and liver inflammation and M2BPGi can reflect not only the severity of liver fibrosis but also the severity of liver inflammation in CLD patients [18,22,35]. In other words, higher M2BPGi level indicates advanced liver fibrosis and liver inflammation and progressive liver disease can cause a decrease in albumin synthesis and cholesterol synthesis and immune dysfunction [6]. Considering these, it is not so surprising that M2BPGi is closely associated with the CONUT score in CLD patients. Although this is beyond the aim of our study, the significant relevance of inflammation and M2BPGi may be associated with the role of M2BPGi as a messenger sent by HSCs to Kupffer cells and its accompanying inflammation [19]. Interestingly, a recent report demonstrated that M2BPGi can contribute to the inflammatory process in patients with SLE [24].

The different results in the Br-cohort and the Cr-cohort need discussion. Our speculation is that the current differences in the two cohorts are attributed to the differences of baseline characteristics in the two cohorts. Age and FIB-4 index in the Cr-cohort was significantly higher than that in the Br-cohort. The proportion of advanced fibrosis (F3 or more) in the Br-cohort (60/249, 24.1%) and the Cr-cohort (212/386, 54.9%) were quite different. When the number of CHB patients with F4 increases, M2BPGi may have the strongest correlation even in the Br-cohort because in F4 patients in the Br-cohort, M2BPGi had the strongest correlation with the CONUT score in our data (r=0.706). On the other hand, as shown in Figure 3, in the Br-cohort, liver inflammation activity did not affect the CONUT score, while in the Cr-cohort, the CONUT score was well stratified according to the severity of liver inflammation stage. The CONUT score may be easily affected by liver inflammation in CHC patients although the reasons for these are unknown.

Appropriate timing of nutritional interventions can be a point of focus. In ROC analyses of M2BPGi for the CONUT score ≥ 2 (i.e., mild, moderate or severe malnutritional condition), the optimal cut-off points of M2BPGi in the Br-cohort and the Cr-cohort were 2.98 COI and 2.34 COI, respectively. In CLD patients with more than those M2BPGi values, nutritional interventions should be considered. On the other hand, it is particularly noteworthy that in ROC analyses linked to the CONUT score ≥ 5 in the Cr-cohort, the AUC was 0.904. In addition, in ROC analyses linked to the CONUT score ≥ 6 or 7 in the Cr-cohort, AUCs were 0.918 and 0.951, respectively (data not shown). Higher predictability of M2BPGi for poor nutrition state may provide useful information for clinicians. Fukushima, et al. demonstrate the usefulness of the CONUT score on survival in patients with end-stage liver disease, which may be associated with our observation [16].

Several limitations with regard to our study warrant mention. Firstly, the study was a single-center observational study with a retrospective nature. Secondly, the study data was derived from a Japanese HBV or HCV-related liver disease population data, and additional investigations on other liver disease etiologies and races are needed to further verify and extend the application to other races. Thirdly, the nutritional condition can vary depending on diet or exercise in daily life. Our data should be therefore cautiously interpreted. Nevertheless, our study results denoted that M2BPGi is closely associated with the CONUT score in hepatitis virus-related CLD patients. In conclusion, M2BPGi can be a useful marker for predicting nutritional condition as determined by the CONUT score, especially in CHC patients.

Conflicts of Interest

We have no conflict of interest to declare. There is no specific funding for the study reported in this paper.

Authorship

Guarantor of the article

S.N.

Author contributions

H.N., H.E. and S.N. participated in the conception and design of the study, participated in acquisition/collection of data, analysis and interpretation of data, and drafted/revised the manuscript for important intellectual content. K.H., Y.I., Y.S., N.I., T.T., NA., R.T., K.Y., N.I., Y.Y., T.N., and H.I. participated in acquisition/collection of data and drafted/revised the manuscript for important intellectual content. All authors approved the final version of the manuscript for submission, including the authorship list.

Acknowledgement

Executive summary

Purpose: Examining the clinical significance of Mac-2-binding protein glycosylation isomer (M2BPGi), which was recently introduced as a novel liver fibrotic biomarker in chronic liver disease patients with a unique fibrosis associated glycochain protein alteration, other than liver fibrotic marker appears to be of importance. We sought to examine the relevance between M2BPGi and the Controlling Nutrition (CONUT) score in hepatitis B and C viruses-related patients (the HBV-related cohort (Br-cohort, n=249) and the HCV-related cohort (Cr-cohort, n=386)) comparing with other liver fibrotic markers.

Patients and Methods: We checked the correlation between the CONUT score and four liver fibrotic markers (M2BPGi, FIB-4 index, hyaluronic acid and platelet count) in the two cohorts. Receiver Operating Characteristics (ROC) analyses associated with elevated CONUT score (CONUT score ≥ 1, 2, 3, 4 or 5) were also conducted.

Results: The median CONUT score (range) were 1 (0-5) in the Br-cohort and 2 (0-8) in the Cr-cohort (P<0.0001). In the Br-cohort, advanced fibrosis or more (F3 or F4) was noted in 60 patients (24.1%), while in the Cr-cohort, it was noted in 212 patients (54.9%). In the Br-cohort, the highest correlation coefficient was identified in FIB-4 index (r=0.436, P<0.0001), followed by M2BPGi (r=0.376, P<0.0001). In the Cr-cohort, the highest correlation coefficient was noted in M2BPGi (r=0.690, P<0.0001), followed by FIB-4 index (r=0.598, P<0.0001). For the ROC analyses linked to the elevated CONUT score, in the Cr-cohort, M2BPGi yielded the highest AUC in all ROC analyses, whereas in the Br-cohort, such tendencies were not noted.

Conclusion: M2BPGi can be a useful marker for predicting nutritional condition as determined by the CONUT score especially in chronic hepatitis C patients.

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We gratefully thank all staff in our department for sample collection and clinical data collection.