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ORIGINAL RESEARCH

Neutrophil-to-lymphocyte ratio for predicting palliative chemotherapy outcomes in advanced pancreatic cancer patients

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Abstract

Several previous studies reported that the neutrophil-to-lymphocyte ratio (NLR) could be a promising prognostic factor for patients with cancer. We aimed to determine the prognostic value of NLR in patients with advanced pancreatic cancer (APC) following palliative chemotherapy. We retrospectively reviewed 252 consecutive APC patients receiving palliative chemotherapy between January 2006 and December 2012. We classified the patients according to the pretreatment NLR values (≤5 or >5) into two groups and investigated the difference in treatment outcomes, including time to treatment failure (TTF) and overall survival (OS). A total of 212 patients had pretreatment NLR values of ≤5 (group A), while 40 patients had an NLR of >5 (group B). TTF and OS were significantly shorter in group B than in group A (3.1 vs. 8.7 months and 6.0 vs. 12.8 months, respectively; both $P < 0.01$). After adjustment for putative prognostic factors, including distant metastasis, status of recurrent/unresectable disease, pretreatment carbohydrate antigen 19-9 levels, and carcinoembryonic antigen levels using the Cox regression model, elevated pretreatment NLR remained an independent poor prognostic factor for OS (hazard ratio, 1.92; 95% confidence interval, 1.27–2.90; $P < 0.01$). In addition, patients in group B whose NLR dropped to ≤5 before the second cycle of chemotherapy showed longer TTF and OS compared with those whose NLR remained at >5. Our results support the idea that NLR can be a promising prognostic and predictive marker for APC patients receiving palliative chemotherapy.

Introduction

Pancreatic cancer is one of the most lethal malignancies worldwide [1], and most patients are diagnosed too late for curative resection. Even with curative resection, disease relapse within 2 years occurs in >80% patients [2, 3]. Systemic gemcitabine-based chemotherapy has long been used as a standard therapy for patients with advanced pancreatic cancer (APC). However, the long-term efficiency and prognosis vary greatly among patients [4]. Therefore, it is clinically relevant to identify APC patients who are more likely to benefit from palliative chemotherapy.

Accumulating evidence supports a positive relationship between inflammation and cancer development and progression [5, 6]. The interaction between tumor and host immune system promote tumor cell proliferation, metastasis, and also activate the inflammatory cascade in the

host, which further deteriorates the general condition of cancer patients [6]. Several markers, including neutrophil-to-lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and modified Glasgow prognostic score (mGPS), have been proposed to estimate the magnitude of systemic inflammation in cancer patients [7–9]. Among these markers, a growing body of evidence supports the usefulness of NLR in predicting the prognosis of patients with cancer. Elevated NLR has reportedly been associated with poor survival following resection or chemotherapy in a variety of cancers [10–14]. In pancreatic cancer, an increasing number of studies have reported an association between elevated NLR (>5) and poor prognosis [7, 15–17]. However, most studies included operable pancreatic cancer patients [7, 15, 18], and the prognostic value of NLR in APC patients receiving palliative chemotherapy is still limited. In fact, only one study of a relatively small cohort (n = 89) focused on APC patients receiving chemotherapy and demonstrated that elevated NLR could predict poor survival [16]. Other studies that reported similar results analyzed the pooled data of patients who underwent surgery [17] or did not receive chemotherapy [7]. Therefore, the usefulness of NLR as a prognostic marker for APC patients following chemotherapy should be validated in another large cohort. Furthermore, it is unknown whether the evaluation of NLR kinetics can predict outcomes for APC patients following chemotherapy.

In this study, we aimed to determine whether elevated NLR could be an independent poor prognostic factor in APC patients following chemotherapy and whether the monitoring of decreased NLR before the second cycle of chemotherapy could predict better outcomes.

Patients and Methods

Patients and treatment

Using a prospective cohort database system (CyberOncology®, Cyber Laboratory Inc., Tokyo, Japan) [19] and electronic medical charts, we retrieved the clinical data of 269 consecutive patients with pathologically confirmed pancreatic ductal adenocarcinoma who received at least two cycles of palliative first-line chemotherapy at Kyoto University Hospital (Kyoto, Japan) between January 2006 and December 2012. In principle, NLR was calculated using the neutrophils and lymphocytes counts obtained on the same day of chemotherapy. If blood test was not performed on the same day of chemotherapy, we substituted the data obtained within 2 days of chemotherapy. Sixteen cases were excluded from this study because a set of NLR values before the first and second chemotherapy cycles was not available, and 252 patients were ultimately investigated. Patients who had once undergone radical resection (R0 or R1) for primary tumors and developed recurrent disease were classified into the recurrent group (n = 73), while those who had an initial diagnosis of unresectable disease were placed into the initially unresectable group (n = 179). Palliative chemotherapy regimens included gemcitabine monotherapy (n = 156) [20], gemcitabine and S-1 combination therapy (n = 85) [21], S-1 monotherapy (n = 9) [22], and gemcitabine and erlotinib combination therapy (n = 2) [23]. The standard doses and regimen schedules were adjusted at the discretion of the treating physicians according to incidence of adverse events or the general condition of the individual patient. All patients provided written informed consent for the use of their clinical data in the medical records system for research. This study was approved by the Ethics Committee of Kyoto University Graduate School of Medicine (E1606).

Demographic/clinical and laboratory variables

Baseline patient characteristics, including laboratory data before the first cycle of palliative chemotherapy and the NLR values before the first and second cycles of chemotherapy, were collected for analysis. On the basis of previous studies,[24–26] continuous parameters were categorized for the convenience of prognostic analysis as follows; age (<65 or ≥65 years), Eastern Cooperative Oncology Group Performance Status (ECOG PS) score (0–1 or 2), NLR (≤5 or >5), platelet to lymphocyte ratio (PLR) (<150 or ≥150), levels of carbohydrate antigen 19-9 (CA19-9, <1000 or ≥1000 U/mL), carcinoembryonic antigen (CEA, <5 or ≥5 ng/mL), C-reactive protein (CRP, <0.5 or ≥0.5 mg/dL), lactate dehydrogenase (LDH, <250 or ≥250 IU/L), hemoglobin (<10 or ≥10 g/dL), and albumin (<3.5 or ≥3.5 g/dL).

Statistical analysis

Baseline patient characteristics were compared using the χ^2 test or Fisher's exact test for dichotomous variables or the Mann–Whitney U test for continuous variables. The time to treatment failure (TTF) was calculated from the date of palliative chemotherapy initiation and terminated on the date of palliative chemotherapy discontinuation for various reasons, including treatment toxicity, disease progression, or patient withdrawal. Overall survival (OS) was calculated from the date of palliative chemotherapy initiation and terminated on the date of death for any reason or censored on the last follow-up visit. TTF and OS were estimated using the Kaplan–Meier method, and differences were compared using log-rank tests. Cox

Table 1. Baseline characteristics.

Variables	Total (n = 252)	NLR ≤5 (n = 212)	NLR >5 (n = 40)	P-value
Age				
≥65	148 (58.7%)	122 (57.5%)	26 (65.0%)	0.48
<65	104 (41.3%)	90 (42.5%)	14 (35.0%)	
Gender				
Male	133 (52.8%)	110 (51.9%)	23 (57.5%)	0.61
Female	119 (47.2%)	102 (48.1%)	17 (42.5%)	
PS score				
0–1	242 (96.0%)	204 (96.2%)	38 (95.0%)	0.66
2	10 (4.0%)	8 (3.8%)	2 (5.0%)	
Distant metastasis				
Yes	184 (73.0%)	152 (71.7%)	32 (80.0%)	0.34
No	68 (27.0%)	60 (28.3%)	8 (20.0%)	
Primary tumor location				
Head	146 (57.9%)	127 (59.9%)	19 (47.5%)	0.16
Body and tail	106 (42.1%)	85 (40.1%)	21 (52.5%)	
The status of recurrent or unresectable				
Recurrent	73 (29.0%)	64 (30.2%)	9 (22.5%)	0.45
Unresectable	179 (71.0%)	148 (69.8%)	31 (77.5%)	
Palliative first line				
Gemcitabine monotherapy	156 (61.9%)	130 (61.3%)	26 (65.0%)	0.82
Gemcitabine and S-1	85 (33.7%)	73 (34.4%)	12 (30.0%)	
S-1 monotherapy	9 (3.6%)	7 (3.3%)	2 (5.0%)	
Gemcitabine and Erlotinib	2 (0.8%)	2 (1.0%)	0	
CA19-9 (U/mL)				
<1000	196 (77.8%)	170 (80.2%)	26 (65.0%)	0.04
≥1000	56 (22.2%)	42 (19.8%)	14 (35.0%)	
CEA (ng/mL)				
<5	145 (57.5%)	126 (59.4%)	19 (47.5%)	0.17
≥5	107 (42.5%)	86 (40.6%)	21 (52.5%)	
CRP (mg/dL)				
<0.5	175 (69.4%)	159 (75.0%)	16 (40.0%)	<0.01
≥0.5	77 (30.6%)	53 (25.0%)	24 (60.0%)	
LDH (IU/L)				
<250	219 (86.9%)	190 (89.6%)	29 (72.5%)	0.01
≥250	33 (13.1%)	22 (10.4%)	11 (27.5%)	
Hemoglobin (g/dL)				
<10	26 (10.3%)	20 (9.4%)	6 (15.0%)	0.27
≥10	226 (89.7%)	192 (90.6%)	34 (85.0%)	
Albumin (g/dL)				
≥3.5	183 (72.6%)	157 (74.1%)	26 (65.0%)	0.25
<3.5	69 (27.4%)	55 (25.9%)	14 (35.0%)	
PLR				
≥150	148 (58.7%)	110 (51.9%)	38 (95.0%)	<0.01
<150	104 (41.3%)	102 (48.1%)	2 (5.0%)	
TB (mg/dL)				
Median	0.7	0.7	0.7	0.87
Range	0.2–15.9	0.2–15.9	0.3–6.2	
AST (IU/L)				
Median	24	24	25	0.83
Range	11–466	11.00–466.00	11–122	
ALT (IU/L)				
Median	24	25	24	0.99
Range	7–564	8–564	7–250	
Creatinin (mg/dL)				
Median	0.7	0.7	0.7	0.34
Range	0.2–3.2	0.2–3.2	0.4–1.2	

regression models were used to identify prognostic factors for TTF and OS. Prognostic factors shown to be significant in the univariate analysis were tested via multivariate analysis. The hazard ratio (HR) and 95% confidence interval (CI) were calculated using Cox regression models. A two-tailed P value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS statistical software (version 17.0; SPSS Inc., Chicago, IL).

Results

Patient characteristics

Patient characteristics were stratified by the pretreatment NLR values (≤5 or >5) and are summarized in Table 1. A total of 212 patients had a pretreatment NLR of ≤5, while 40 had an NLR of >5. Most baseline characteristics were comparable between the two groups. However, the

Table 2. Univariate and multivariate analysis of poor prognostic factors for TTF.

			Univariate analysis			Multivariate analysis		
	n	Median TTF (95% CI) (months)	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value
Age (years)								
≥65	148	7.7 (6.0–9.4)	1	0.74–1.27	0.83			
<65	104	8.0 (6.5–9.5)	0.97					
Gender								
Female	119	6.6 (5.0–8.2)	1	0.80–1.36	0.77			
Male	133	8.0 (6.4–9.6)	1.04					
ECOG PS								
0–1	242	7.4 (6.1–8.7)	1	1.03–3.68	0.04	1	0.84–3.15	0.15
2	10	2.2 (0.0–6.4)	1.95			1.62		
Distant metastasis								
No	68	9.0 (6.6–11.4)	1	1.28–2.44	<0.01	1	1.12–2.18	<0.01
Yes	184	6.9 (5.8–8.0)	1.77			1.56		
Primary tumor location								
Head	146	6.7 (5.7–7.7)	1	0.74–1.27	0.84			
Body and tail	106	9.3 (7.1–11.5)	0.97					
The status of initially unresectable/recurrent								
Recurrent	73	11.9 (7.2–16.6)	1	1.34–2.46	<0.01	1	1.17–2.20	<0.01
Initially unresectable	179	6.3 (4.9–7.7)	1.81			1.60		
NLR								
≤5	212	8.7 (7.2–10.2)	1	1.33–2.75	<0.01	1	1.08–2.31	0.02
>5	40	3.1 (2.7–3.5)	1.91			1.58		
PLR								
≤150	104	9.6 (6.8–12.4)	1	0.93–1.59	0.15			
≥150	148	6.3 (4.9–7.7)	1.22					
CA19-9 (U/mL)								
<1000	196	8.8 (7.2–10.4)	1	1.60–3.00	<0.01	1	1.10–2.21	0.01
≥1000	56	4.0 (2.2–5.8)	2.19			1.56		
CEA (ng/mL)								
<5	145	9.4 (7.3–11.5)	1	1.18–2.03	<0.01	1	0.99–1.76	0.06
≥5	107	6.2 (4.9–7.5)	1.55			1.32		
CRP (mg/dL)								
<0.5	175	8.8 (6.9–10.7)	1	1.40–2.47	<0.01	1	1.01–1.87	0.05
≥0.5	77	4.4 (2.8–6.0)	1.86			1.37		
LDH (IU/L)								
≥250	33	3.3 (2.0–4.6)	1	1.03–2.23	0.04	1	0.89–2.00	0.16
<250	219	7.9 (6.4–9.4)	1.51			1.34		
Hemoglobin (g/dL)								
≥10	226	7.5 (6.2–8.8)	1	0.74–1.75	0.57			
<10	26	5.1 (3.4–6.8)	1.13					
Albumin (g/dL)								
≥3.5	183	7.9 (6.3–9.5)	1	0.92–1.68	0.15			
<3.5	69	5.1 (2.4–7.8)	1.24					

Table 3. Univariate and multivariate analysis of poor prognostic factors for OS.

			Univariate analysis			Multivariate analysis		
	n	Median OS (95% CI) (months)	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value
Age (years)								
≥65	148	12.1 (10.6–13.6)	1	0.74–1.29	0.87			
<65	104	11.3 (10.0–12.6)	0.98					
Gender								
Female	119	11.9 (10.5–13.3)	1	0.82–1.43	0.56			
Male	133	11.9 (10.0–13.8)	1.09					
ECOG PS								
0–1	242	12.0 (10.8–13.2)	1	1.09–3.92	0.02	1	0.91–3.46	0.09
2	10	4.4 (3.2–5.6)	2.07			1.78		
Distant metastasis								
No	68	16.7 (11.0–22.4)	1	1.49–2.98	<0.01	1	1.27–2.60	<0.01
Yes	184	11.2 (10.0–12.4)	2.11			1.81		
Primary tumor location								
Body and tail	106	12.2 (10.4–14.0)	1	0.72–1.26	0.72			
Head	146	11.2 (9.9–12.5)	0.95					
The status of initially unresectable/recurrent								
Recurrent	73	15.6 (10.9–20.3)	1	1.22–2.30	<0.01	1	1.08–2.12	0.02
Initially unresectable	179	11.1 (9.8–12.4)	1.67			1.51		
NLR								
≤5	212	12.8 (10.7–14.9)	1	1.50–3.15	<0.01	1	1.27–2.90	<0.01
>5	40	6.0 (2.8–9.2)	2.17			1.92		
PLR								
≤150	104	15.0 (13.3–16.7)	1	1.05–1.85	0.02	1	0.79–1.49	0.63
≥150	148	10.6 (9.6–11.6)	1.39			1.08		
CA19-9 (U/mL)								
<1000	196	13.4 (11.4–15.4)	1	1.78–3.45	<0.01	1	1.24–2.56	<0.01
≥1000	56	6.5 (4.6–8.4)	2.48			1.78		
CEA (ng/mL)								
<5	145	14.8 (12.5–17.1)	1	1.31–2.32	<0.01	1	1.11–2.04	0.01
≥5	107	10.1 (8.9–11.3)	1.74			1.50		
CRP (mg/dL)								
<0.5	175	13.4 (11.3–15.5)	1	1.37–2.48	<0.01	1	0.99–1.88	0.06
≥0.5	77	7.6 (4.6–10.6)	1.84			1.36		
LDH (IU/L)								
<250	219	12.3 (10.8–13.8)	1	1.00–2.22	0.05	1	0.84–1.98	0.24
≥250	33	6.6 (2.7–10.5)	1.49			1.29		
Hemoglobin (g/dL)								
≥10	226	12.0 (10.6–13.4)	1	0.74–1.88	0.48			
<10	26	9.6 (5.7–13.5)	1.18					
Albumin (g/dL)								
≥3.5	183	12.2 (10.6–13.8)	1	0.99–1.83	0.06			
<3.5	69	10.0 (6.7–13.3)	1.34					

following factors, including CA19-9 (≥1000 U/mL) levels, CRP (≥0.5 mg/dL) levels, LDH (≥250 IU/L) levels, and PLR (≥150) were more common in the NLR >5 group.

Prognostic factors for poorer TTF and OS

Univariate analysis identified eight prognostic factors associated with poorer TTF, including an ECOG PS of 2, distant metastasis, the status of unresectable disease, a pretreatment NLR of >5, CA19-9 levels of ≥1000 U/mL, CEA levels of ≥5 ng/mL, CRP levels of ≥0.5 mg/dL, and LDH levels of ≥250 IU/L. All these factors were subsequently analyzed in multivariate analysis. A total of five factors, including distant metastasis, status of unresectable disease, a pretreatment NLR of >5, CA19-9 levels of ≥1000 U/mL, and CRP levels of ≥0.5 mg/dL, remained

Table 4. The NLR thresholds and relationship with survival.

			Median OS (95% CI) (months)	Univariate analysis			Multivariate analysis[1]		
		n (%)		Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value
NLR	≤1	14 (5.6)	12.8 (9.4–16.2)	1	0.86–3.29	0.13	1	0.69–2.71	0.37
	>1	238 (94.4)	11.7 (10.4–13.0)	1.68			1.37		
	≤2	83 (32.9)	14.8 (11.5–18.2)	1	1.13–2.05	0.01	1	0.88–1.66	0.24
	>2	169 (67.1)	10.7 (9.3–12.1)	1.52			1.21		
	≤3	158 (62.7)	13.4 (11.1–15.7)	1	1.26–2.23	<0.01	1	1.18–2.11	<0.01
	>3	94 (37.3)	8.6 (6.2–11.0)	1.68			1.57		
	≤4	194 (77.0)	13.3 (11.4–15.2)	1	1.44–2.78	<0.01	1	1.36–2.67	<0.01
	>4	58 (23.0)	7.3 (5.6–9.0)	2.00			1.91		
	≤5	212 (84.1)	12.8 (10.7–14.9)	1	1.50–3.15	<0.01	1	1.49–3.15	<0.01
	>5	40 (15.9)	6.0 (2.8–9.2)	2.17			2.16		

[1]Multivariable analysis was adjusted for distant metastasis, status of recurrent, CA19-9, and CEA.

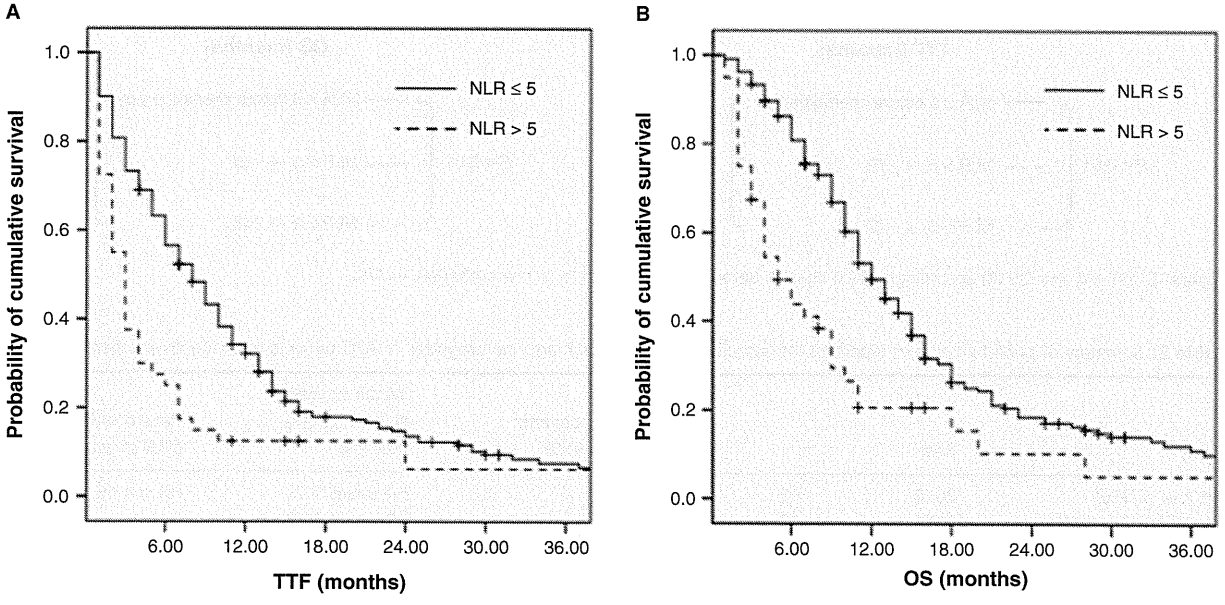

Figure 1. TTF (A) and OS (B) according to basal NLR in APC patients following palliative chemotherapy.

independent prognostic factors for poorer TTF in APC patients following chemotherapy (Table 2).

The same analysis was performed for OS, and a total of five factors, including distant metastasis, status of unresectable disease, a pretreatment NLR of >5, CA19-9 levels of ≥1000 U/mL and CEA levels of ≥5 ng/mL, remained independent prognostic factors after multivariate analysis (Table 3).

Relationship between NLR thresholds and OS

Table 4 shows the relationship between different thresholds of NLR and OS. An NLR cutoff value of 5 could discriminate patients with poorer survival and the highest HR in our cohort.

Comparison of TTF and OS stratified by pretreatment NLR

The median TTF and OS in patients with a pretreatment NLR of >5 was 3.1 (95% CI, 2.7−3.5) months and 6.0 (95% CI, 2.8–9.2) months, respectively, which were significantly shorter compared with those of patients with an NLR of ≤5 (TTF and OS, 8.7 [95% CI, 7.2−10.2] months and 12.8 [95% CI, 10.7−14.9] months, respectively; both $P < 0.01$; Fig. 1A and B).

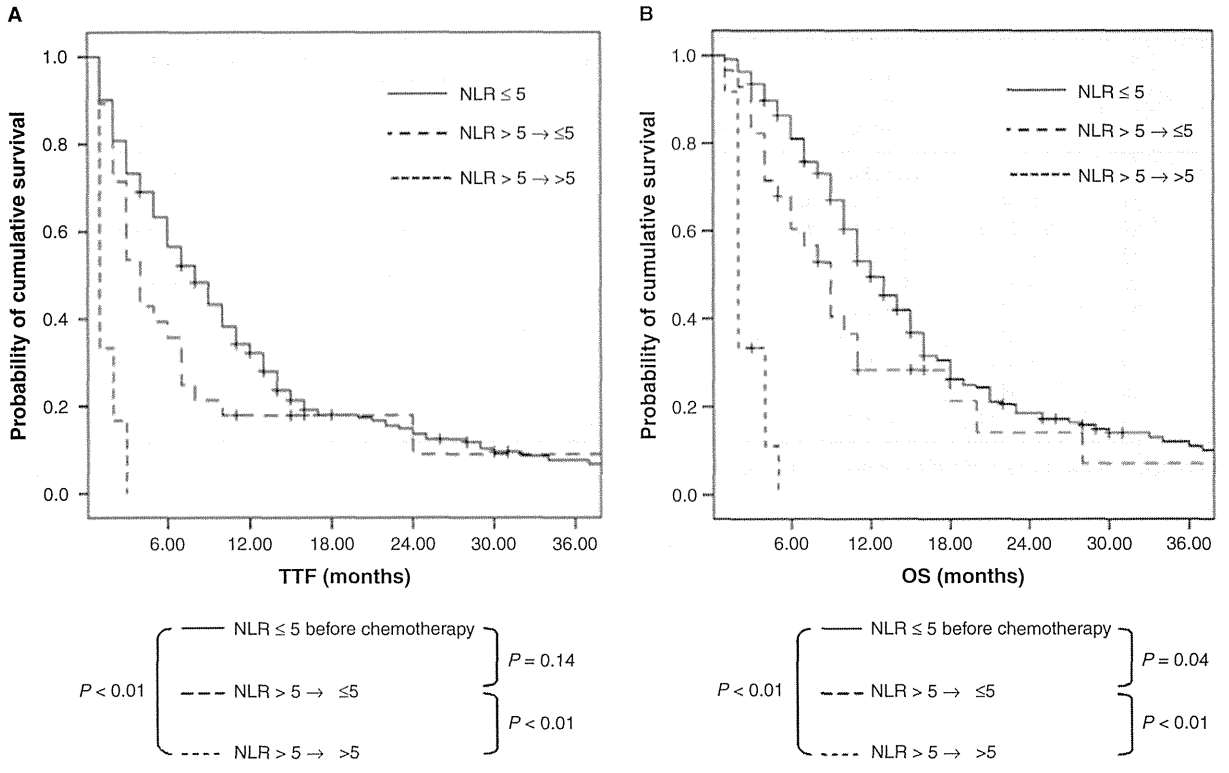

Figure 2. TTF (A) and OS (B) according to NLR change before the second cycle of chemotherapy in APC.

Table 5. Summary of published studies reporting the association between NLR and the prognosis of APC patients receiving chemotherapy.

Study	Year	*n*	Number of patients with NLR >5 (%)	Overall survival (NLR >5 vs. ≤5) (months)	Hazard ratio (NLR ≤5 was set at 1)
An X et al. [16]	2010	89	16 (17.9)	2.4 versus 7.7	HR = 4.49, $P = 0.013$
Wang DS et al. [17]	2012	86	12 (13.9)	5.8 versus 10.2	NA
Stotz M et al[1] [7]	2013	261	79 (30.3)	NA	HR = 2.53, $P < 0.01$
Our study	2013	253	40 (15.8)	6.0 versus 12.8	HR = 1.95, $P < 0.01$

NA, not available.
[1]This study ($n = 261$) pooled the data from patients who received chemotherapy ($n = 179$) and no chemotherapy ($n = 82$).

NLR drop (≤5) before the second cycle of chemotherapy predicted favorable TTF and OS

To test whether the monitoring of the drop in NLR before the second cycle of chemotherapy could predict better outcomes, patients with a pretreatment NLR of >5 were categorized into two groups according to their NLR levels before the first and second cycles of chemotherapy as follows: group 1, NLR >5 at baseline and drop to ≤5 before the second cycle of chemotherapy ($n = 28$); and group 2, NLR >5 before both the first and second cycles of chemotherapy ($n = 12$). Patients in group 1 demonstrated significantly improved TTF and OS compared with those in group 2 (4.3 vs. 1.4 months and 9.3 vs. 2.7 months, respectively; both $P < 0.01$; Fig. 2A and B).

Discussion

Growing evidence supports a positive relationship between inflammation and cancer development and progression [5, 6]. NLR is attracting more and more researchers' attention because it is readily measurable in peripheral blood and is likely to reflect the magnitude of the systemic inflammatory response. An increasing number of studies have reported that elevated NLR can be a marker of poorer

prognosis in a variety of cancers [10–14]. Elevated NLR is often accompanied by elevated neutrophil levels and relative lymphocytopenia. Elevated neutrophil levels can promote tumor cell progression by upregulating a variety of inflammatory cytokines and providing a suitable microenvironment for tumor growth [27, 28]. Furthermore, lymphocytopenia arising from numerous inhibitory immunologic mediators released by tumor cells represents an immunosuppressive condition in cancer patients and contributes to poorer outcome [29].

In this study, we aimed to determine whether elevated pretreatment NLR was associated with poorer prognosis for APC patients receiving palliative chemotherapy. Cox regression analysis identified a total of five factors, including distant metastasis, status of unresectable disease, a pretreatment NLR of >5, CA19-9 levels of ≥1000 U/mL, and CEA levels of ≥5 ng/mL, that were associated with poorer OS in our cohort. We observed significantly shorter TTF and OS among patients with a pretreatment NLR of >5 compared with those among patients with an NLR of ≤5. The median OS was 6.0 months in patients with an NLR of >5 and 12.8 months in patients with an NLR of ≤5. In addition, the NLR cutoff value of 5 was determined to be optimal in our cohort. Dexamethasone is commonly used for antiemetic purpose in systemic chemotherapy; however, the mean dose of dexamethasone used for antiemetic purpose was almost equal (2.2 mg) between group A and group B and it was unlikely that this affected our current results. The present results are in line with those of previous studies [16, 17] reporting that elevated NLR was an independent prognostic factor for OS in APC patients receiving palliative chemotherapy; these data from published studies are summarized in Table 5. The proportion of patients with a pretreatment NLR of >5 in existing research are comparable across studies. To the best of our knowledge, our current study comprised the largest number of APC patients who received palliative chemotherapy, and our results strongly support the hypothesis that elevated NLR (>5) can be a reliable and reproducible marker for identifying a subgroup of APC patients with poorer prognosis following palliative chemotherapy.

We also demonstrated that NLR kinetics could predict treatment outcome in APC patients following palliative chemotherapy. Patients whose pretreatment NLR values of >5 dropped to ≤5 before the second cycle of chemotherapy demonstrated significantly longer TTF and OS compared with those whose NLR values remained at >5 before the second cycle of chemotherapy. A total of five patients developed grade 3 or higher neutropenia during the first cycle of chemotherapy in group B. A persistent NLR of >5 before the second cycle of chemotherapy remained an independent poor predictive marker of TTF and OS (both $P < 0.01$) after adjusting the incidence of grade 3 or higher neutropenia during the first cycle of chemotherapy. Persistent elevation of NLR may reflect the severe systemic inflammatory response in the body and aggressive tumor features. Our results are in line with those of the previous study by Chua et al. [11] They investigated a total of 162 patients with metastatic colorectal cancer who received palliative chemotherapy and reported that patients whose pretreatment NLR values of >5 dropped to ≤5 before the second chemotherapy cycle demonstrated significantly longer progression-free survival and a trend toward longer OS compared with patients with a persistent NLR of >5. Therefore, evaluation of NLR before the second cycle of chemotherapy can help physicians to predict chemotherapy resistance and reconsider the treatment strategy at an earlier time point in daily clinical practice.

In contrast to NLR, we were unable to validate the prognostic value of PLR or mGPS in our cohort, although some researchers reported that these play prognostic roles in patients with cancer [8, 9]. This study was limited by its retrospective design. In addition, chemotherapy regimens differed among patients; however, it is unlikely that chemotherapy regimen heterogeneity affected the current results because almost 99% patients received gemcitabine, S-1, or gemcitabine/S-1 combination therapy, and the efficacies of these three regimens were not statistically different in a large randomized phase III study [30].

In summary, our results strongly support the idea that NLR can be a promising prognostic marker for APC patients receiving palliative chemotherapy. Furthermore, evaluation of NLR before the second cycle of chemotherapy can help physicians to predict response to palliative chemotherapy at an earlier time point. Future prospective studies are warranted to verify the usefulness of monitoring NLR in treating patients with APC.

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Conflict of Interest

None declared.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (10): Alcoholic liver disease

Fibrogenesis in alcoholic liver disease

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Abstract

Alcoholic liver disease (ALD) is a major cause of morbidity and mortality worldwide. In developed countries, ALD is a major cause of end-stage liver disease that requires transplantation. The spectrum of ALD includes simple steatosis, alcoholic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Alcohol abstinence is the most effective therapy for ALD. However, targeted therapies are urgently needed for patients with severe ALD (*i.e.*, alcoholic hepatitis) or those who do not abstain from alcohol. The lack of studies and the availability of animal models that do not reflect all the features of this disease in humans inhibit the development of new drugs for ALD. In ALD-associated fibrosis, hepatic stellate cells are the principal cell type responsible for extracellular matrix production. Although the mechanisms underlying fibrosis in ALD are largely similar to those observed in other chronic liver diseases, oxidative stress, methionine metabolism abnormalities, hepatocyte apoptosis, and endotoxin lipopolysaccharides that activate Kupffer cells may play unique roles in disease-related fibrogenesis. Lipogenesis during the early stages of ALD has recently been implicated as a risk factor for the progression of cirrhosis. Other topics include osteopontin, interleukin-1 signaling, and genetic polymorphism. In this review, we discuss the basic pathogenesis of ALD and focus on liver fibrogenesis.

Key words: Stellate cell; Kupffer cell; Steatohepatitis; Fibrosis; Cytokine; Oxidative stress

Core tip: Alcoholic liver disease (ALD) is a major cause of preventable morbidity and mortality worldwide. In ALD-associated fibrosis, hepatic stellate cells are the principal cell type responsible for extracellular matrix production. Although the mechanisms underlying ALD-associated fibrosis are largely similar to those observed in other chronic liver diseases, oxidative stress, abnormal methionine metabolism, hepatocyte apoptosis, and endotoxin lipopolysaccharides that activate Kupffer cells play unique roles in fibrogenesis in ALD. Recently, lipogenesis during the early stages of ALD has been implicated as a risk factor for progression of cirrhosis. Other critical factors include osteopontin, interleukin-1 signaling, and genetic polymorphisms.

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INTRODUCTION

Although the incidence of alcoholic liver disease (ALD) varies widely worldwide, the burden of ALD and ALD-induced death remains dominant in most countries[1]. ALD is the third highest risk factor for disease and disability worldwide. Almost 4% of all deaths in the world result from ALD, which is greater than deaths caused by

the human immunodeficiency virus/acquired immune deficiency syndrome, violence, or tuberculosis[1]. Furthermore, alcohol is associated with many serious social problems, including violence, child neglect, and abuse, and absenteeism in the workplace. A recent nationwide survey revealed that ALD was the third highest cause of liver cirrhosis in Japan (13.6%)[2], and the associated cost of medical care was estimated to be 6.9% of the total national medical expenditure[3]. Overall, ALD is recognized as a major but preventable public health problem.

The spectrum of ALD is broad: asymptomatic fatty liver, steatohepatitis, progressive fibrosis, end-stage cirrhosis, and hepatocellular carcinoma[4,5]. ALD may often resolve in those who become abstinent. However, for patients with severe ALD and those who do not completely abstain from alcohol, targeted therapies are urgently needed[4].

Patients with ALD can develop progressive liver fibrosis because of the accumulation of extracellular matrix (ECM) materials, including type I collagen, as generated by activated hepatic stellate cells (HSCs) and hepatic myofibroblasts. When liver injury occurs, HSCs are activated and differentiate into myofibroblast-like cells[6,7]. Activated Kupffer cells, infiltrating monocytes, activated and aggregated platelets, and damaged hepatocytes are the sources of platelet-derived growth factor and transforming growth factor-β1 (TGF-β1); these cells initiate intracellular signaling cascades leading to HSC activation. Although the key pathways of HSC activation are common to all forms of liver injury and fibrosis, disease-specific pathways also exist. Some specific signaling pathways regulating HSC activation in ALD are discussed below (Figure 1).

CLASSICAL MECHANISMS UNDERLYING FIBROGENESIS IN ALD

Alcohol metabolism

Approximately 90% of ingested alcohol is metabolized in the cytosol of hepatocytes. Cytosolic alcohol dehydrogenase[8] oxidizes alcohol to acetaldehyde that is then converted to acetate by acetaldehyde dehydrogenase. Acetaldehyde is considered the key toxin in alcohol-mediated liver injury that includes cellular damage, inflammation, ECM remodeling, and fibrogenesis[9]. Moreover, acetaldehyde triggers TGF-β1-dependent late-phase response in HSCs that maintains a pro-fibrogenic and pro-inflammatory cellular state[10]. Recently, Liu *et al*[10] indicated that, in vitro, leptin potentiates acetaldehyde-induced HSC activation and alpha-smooth muscle actin (SMA) expression by interleukin-6 (IL-6)-dependent signals such as p38 and phosphorylated-extracellular signal-regulated kinase 1/2. This report discusses the importance of a synergistic effect of leptin and acetaldehyde in the activation of HSCs in ALD.

Oxidative stress

Alcohol consumed in chronic and heavy drinkers is also oxidized *via* the hepatocytic cytochrome P450 (CYP); previously termed inducible microsomal ethanol-oxidizing system[11]. CYP2E1 metabolizes various substances, including multiple drugs, polyunsaturated fatty acids, acetaminophen, and most organic solvents, and plays a critical role in the generation of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide anions[11,12]. ROS are also generated from nitric oxide and reduced form of nicotinamide adenine dinucleotide phosphate oxidase by Kupffer cells[13]. ROS trigger inflammatory cascades and recruit neutrophils and other immune cells to the site of alcohol-induced hepatocyte damage, increasing levels of circulating pro-inflammatory cytokines, notably tumor necrosis factor (TNF)-α[14].

Accumulation of lipid peroxidation products, such as 4-hydroxynonenal (4-HNE), has been reported both in patients as well as animal models of ALD[15,16]. Several studies have shown that the lipid peroxidation reaction in the liver precedes the initial stages of fibrosis and is associated with the increased production of pro-fibrogenic TGF-β1 by Kupffer cells[14]. Nieto reported that ethanol-induced lipid peroxidation triggers the nuclear factor kappa B (NF-κB) transactivation of the collagen 2(I) gene promoter in HSCs by stimulating kinase cascades, including protein kinase C, phosphoinositide 3 kinase (PI3K), and protein kinase B/Akt[17]. These observations are agreement with the findings of previous reports, indicating that 4-HNE is pro-fibrogenic for collagen production in human HSCs[14] and that oxidative stress directly promotes collagen synthesis in HSCs over-expressing the CYP2E1 gene[14].

Methionine metabolism

Decreased intracellular levels of antioxidants such as vitamin C, vitamin E, and glutathione (GSH) in the blood and liver modify the process of alcohol-induced liver injury[18]. Excessive acute alcohol intake reduces GSH synthesis, and the acetaldehyde produced from alcohol metabolism inhibits GSH activity. Alcohol also disturbs the intracellular transport of GSH and preferentially depletes mitochondrial GSH, leading to apoptosis[18]. Levels of S-adenosylmethionine (SAMe), a universal methyl donor, are also markedly reduced in ALD due to the reduced activity of SAMe synthetase[18]. This fact is clinically important because therapy using SAMe increases survival of patients with alcohol-induced cirrhosis[18].

Hepatocyte apoptosis

Hepatocyte apoptosis is pathophysiologically important in the progression of ALD[19]. There are two important apoptotic pathways: extrinsic (death receptor-mediated) and intrinsic (organelle-initiated)[20]. Most recently, Petrasek *et al*[21] revealed that interferon regulatory factor 3 (IRF-3) mediates ALD by linking endoplasmic reticulum (ER) stress with the mitochondrial pathway of hepatocyte apoptosis. Interestingly, ethanol induces ER stress and triggers the association of IRF-3 with the ER adaptor, stimulator of interferon genes, as well as the subsequent phosphoryla-

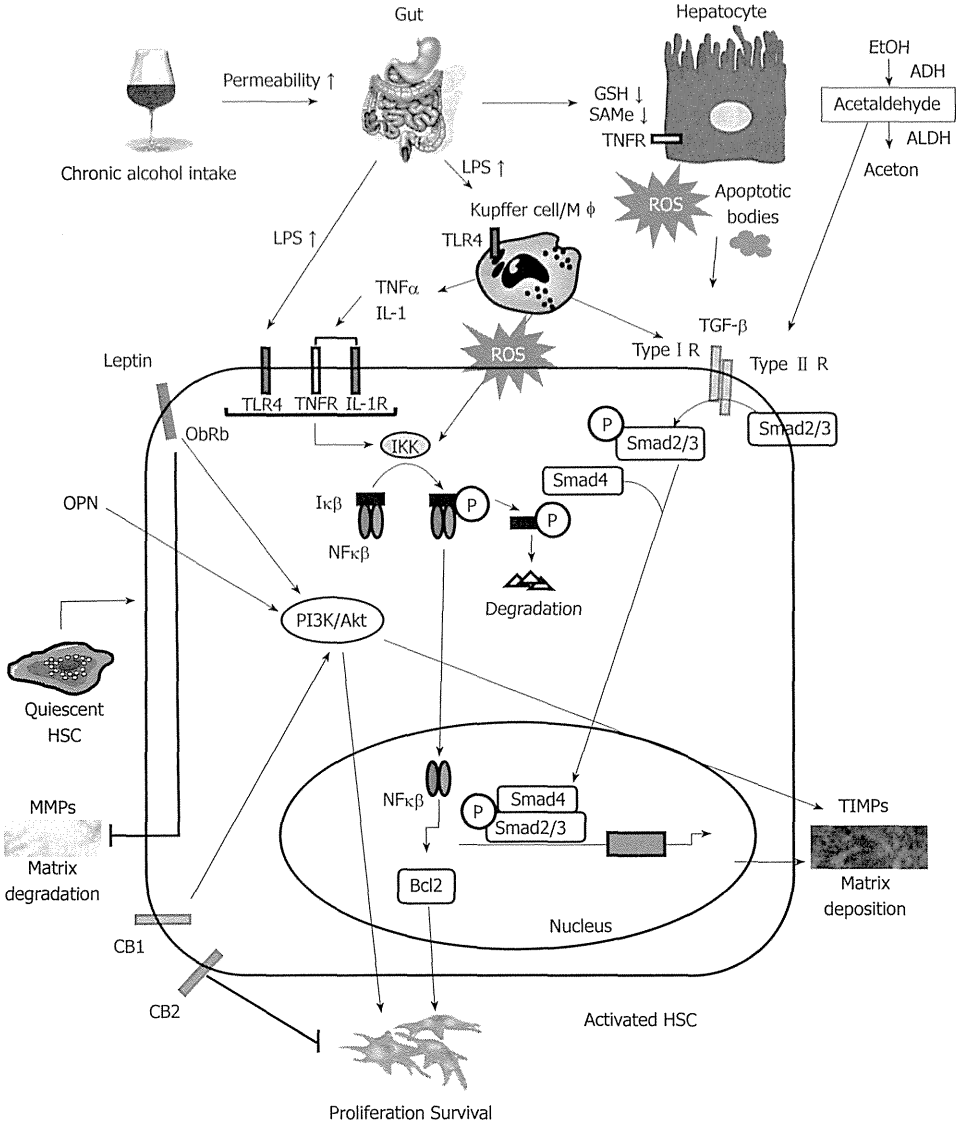

***Figure 1* Signaling pathways regulating hepatic stellate cell activation in alcoholic liver disease.** Alcohol consumption causes hepatocyte damage, which subsequently induces apoptosis. Alcohol dehydrogenase (ADH) oxidizes alcohol to acetaldehyde that is converted to acetate by acetaldehyde dehydrogenase (ALDH). Acetaldehyde directly targets hepatic stellate cells (HSCs). Alcohol reduces glutathione (GSH) synthesis and acetaldehyde inhibits GSH activity in hepatocytes. Levels of the S-adenosylmethionine (SAMe) are also markedly reduced. Alcohol consumption increases permeability of the intestine to bacterial endotoxin that in turn, elevates serum lipopolysaccharide (LPS) levels. LPS directly enhances HSCs activation by upregulating transforming growth factor (TGF)- signaling. TGF-β1 derived from activated Kupffer cells and damaged hepatocytes binds to TGF receptors. Phospho-Smad2/3 and Smad4 complexes translocate into the nucleus, display DNA-binding activity, and activate expression of genes related to fibrosis. Extracellular molecules, such as LPS, tumor necrosis factor (TNF)-α, interleukin (IL)-1, and reactive oxygen species (ROS), activate IκB kinase (IKK) that, in turn, phosphorylates IκB, resulting in ubiquitination, dissociation of IκBα from nuclear factor kappa B (NF-κB), and eventually, degradation of IκB by the proteasome. The activated NF-κB is then translocated into the nucleus and binds to specific DNA response elements. NF-B-dependent pathways are involved in the expression of the anti-apoptotic protein B-cell lymphoma 2 (Bcl2). Leptin binds ObRb, activating the phosphoinositide 3-kinase (PI3K)/Akt pathway and inducing matrix deposition by increasing expression of tissue inhibitor of metalloproteinases (TIMPs). Leptin also inhibits matrix degradation by decreasing expression of Matrix metalloproteinases (MMPs). Osteopontin (OPN) positively stimulates the PI3K/Akt pathway. The cannabinoid receptor CB2 mediates antifibrotic actions; in contrast, activation of CB1 receptors positively stimulates the PI3K/Akt pathway to promote the proliferation and apoptosis of HSCs. TLR: Toll-like receptor.

tion of IRF-3. Activated IRF-3 is associated with the proapoptotic molecule Bax (B-cell lymphoma 2-associated X protein) and contributes to hepatocyte apoptosis[21]. Apoptotic bodies induced by alcohol are phagocytosed by Kupffer cells and HSCs, which then produce TGF-β1 and subsequently activate HSCs[19,22]. Finally, increased serum levels of caspase-digested cytokeratin-18 fragments, a useful marker of hepatocyte apoptosis, are independent factors in predicting severe fibrosis in patients with ALD[23].

Lipopolysaccharide

Increased serum lipopolysaccharide (LPS) levels are commonly found in patients with ALD[19]. Toll-like receptor (TLR) 4 is one of the multiple pattern recognition receptors that recognize both pathogen- and host-

derived factors that modulate inflammatory signals[24]. LPS interacts with TLR4 to activate the MyD88-independent toll-interleukin-1 receptor domain-containing adaptor-inducing interferon-β/IRF-3 signaling pathway that produces oxidative stress and proinflammatory cytokines (including TNF-α) causes hepatocellular damage and contributes to alcoholic steatohepatitis[15,22,25]. Recent studies have revealed that activation of TLR4 and complement factors also stimulates Kupffer cells to produce hepatoprotective cytokines, such as IL-6, and anti-inflammatory cytokines, such as IL-10[15,22,25,26]. These cytokines activate signal transduction and activator of transcription 3 in hepatocytes and macrophages/Kupffer cells, respectively, to prevent alcohol-induced liver injury and inflammation[15,22,26]. On the other hand, previous studies have reported that activation of TLR4 signaling in HSCs and liver sinusoidal endothelial cells (LSECs) promoted liver fibrogenesis[22,25], and that activation of TLR4 signaling in LSECs regulates angiogenesis through the MyD88-effector protein that regulates extracellular protease production, in turn, results in the development of liver fibrosis[27].

Experimental models of ALD have revealed that translocation of bacterial products across the intestinal barrier to the portal circulation triggers inflammatory responses in the liver and contributes to steatohepatitis[28,29]. Most recently, Hartmann *et al*[30] investigated the role of the intestinal mucus layer and found that mucin (Muc) 2 was involved in the development of alcohol-associated liver disease. The authors reported that *Muc2*$^{-/-}$ mice have significantly lower plasma levels of LPS than wild-type mice after alcohol administration. In addition, it was shown that *Muc2*$^{-/-}$ mice are effectively protected from intestinal bacterial overgrowth and the microbiome in response to alcohol administration[30]. This study clearly showed that the alcohol-associated alteration in the microbiome, and in particular, the overgrowth of intestinal bacteria contributes to the progression of ALD.

EMERGING MECHANISMS UNDERLYING FIBROGENESIS IN ALD

Lipogenesis in the early stages of ALD

The development of steatosis due to chronic alcohol consumption is an important contributor to the progression of hepatic fibrogenesis[15]. Recent studies have found that direct or indirect alcohol exposure regulates transcription factors associated with lipid metabolism. Alcohol also stimulates lipogenesis and inhibits fatty acid oxidation[31]. There are two well-known pathways of lipogenesis: sterol regulatory element binding protein (SREBP)-1 activation and adenosine monophosphate kinase (AMPK) inhibition[15,31].

Alcohol consumption directly upregulates SREBP-1c gene expression through its metabolite acetaldehyde[19] or indirectly upregulates activating processes and factors such as ER stress[32], adenosine[33,34], endocannabinoids[35], LPS signaling *via* TLR4, and its downstream proteins, such as IRF-3, early growth response-1, and TNF-α.

AMPK is a key player in cellular and organism survival in metabolic stress through its ability to maintain metabolic homeostasis[36]. Chronic ethanol exposure inhibits AMPK activity, which increases activity of acetyl-CoA carboxylase and suppresses the rate of palmitic acid oxidation through the inhibition of liver kinase B1 phosphorylation[31,36].

The endocannabinoids, which are similar to the major active ingredient in marijuana, are endogenous lipid mediators that participate in the complex neural circuitry that controls energy intake[37]. There are at least two different cannabinoid receptors: CB1 and CB2. Recent studies indicate that while CB2 receptors mediate antifibrotic actions, the activation of CB1 receptors contribute to the development of fibrosis[37,38]. Both cannabinoid receptors are expressed in HSCs, and the inactivation of CB1 receptors decrease fibrogenesis by lowering TGF-β1 levels and reduce the accumulation of fibrogenic cells *via* downregulation of the PI3K/Akt signaling pathway[37]. Intriguingly, alcoholic liver steatosis is mediated mainly through HSC-derived endocannabinoids and their hepatocytic receptor[22,37]. Chronic alcohol consumption stimulates HSCs to produce 2-arachidonoylglycerol and its interaction with the CB1 receptor upregulates the expression of SREPB1c and fatty acid synthase, but downregulates the activities of AMPK and carnitine palmitoyltransferase 1[37,39].

Osteopontin

Osteopontin (OPN) is a secreted, 44-66 kDa adhesive glycophosphoprotein that has involvement in both normal processes, such as bone development and immune system regulation, and pathologic processes, such as inflammation, cell transformation, tumor invasiveness, and metastasis[40]. OPN plays additional roles in ALD. In animal models, hepatic mRNA levels of OPN increased in ALD[15] and stimulated HSC activation in an autocrine and paracrine fashion[41]. Recently, Urtasun *et al*[42] investigated the mechanism of OPN in HSC activation. Recombinant OPN upregulated type I collagen production in primary HSCs in a TGF-β independent fashion, whereas it down-regulated matrix metalloprotease (MMP)-13. OPN induction of type I collagen occurred *via* integrin $\alpha_v\beta3$ engagement and activation of the PI3K/pAkt/NF-κB-signaling pathway[42]. On the other hand, recent studies indicate that OPN participates in the pathogenesis of hepatic steatosis, inflammation, and the fibrosis that results from non-alcoholic steatohepatitis[43]. OPN regulates steatohepatitis by stimulating the Hedgehog-signaling pathway[43]. In human ALD, hepatic mRNA levels of OPN correlate with hepatic neutrophil infiltration and the severity of fibrosis[44]. Finally, immunohistochemical detection of OPN is used as a prognostic biomarker to discriminate outcomes in some transplant patients with hepatocellular carcinoma derived from ALD[45].

IL-1 signaling

Emerging data have provided evidence for the role of

IL-1 signaling in acute and chronic liver injury resulting from various causes, including acetaminophen-induced liver damage[46], nonalcoholic steatohepatitis[47], liver fibrosis[48], and immune-mediated liver injury[49]. However, the significance of IL-1 signaling in ALD has yet to be evaluated. A recent study from Petrasek *et al*[50] showed that activation of inflammasome-IL-1 signaling also plays a critical role in ethanol-induced liver injury in mice. Using IL-1 receptor antagonist-treated mice as well as 3 different mouse models deficient in regulators of IL-1β activation [caspase-1 (Casp-1) and ASC] or signaling (IL-1 receptor), they showed that IL-1β signaling is required for the development of alcohol-induced liver steatosis, inflammation, and injury. Interestingly, several fibrotic markers such as procollagen III N-terminal propeptide (PIIINP), tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), and hyaluronic acid were downregulated in ethanol-fed Casp-1 knockout mice or in response to IL-1Ra treatment. Although the roles of inflammasome in HSC activation are not fully elucidated[51], it is suggested that targeting the inflammasome and/or IL-1 signaling pathways have therapeutic potential in ALD management. However, further studies are required to discover direct evidence of the relationship between IL-1 signaling and fibrogenesis in ALD.

Genetic variants associated with the fibrosis of ALD

With the genotyping technique becoming more widely available, a great number of genetic case-control studies have evaluated candidate gene-variants that code proteins involved in the hepatic fibrosis[52]. Although two fibrosis-associated genes, including TGF-β and MMP 3, were evaluated in ALD[52], these genotypes are not associated with alcoholic liver cirrhosis[53,54]. Recent whole genome analyses of large numbers of genetic variants have identified novel yet unconsidered candidate genes[55]. Romeo *et al*[56] reported that the single-nucleotide polymorphism [rs738409(G), encoding I148M] in the patatin-like phospholipase domain-containing (PNPLA) 3 gene is a significant risk factor for increased hepatic fat accumulation and inflammation in nonalcoholic fatty liver disease. Subsequently, the strong association between the PNPLA3 I148 M allele and an increased risk of clinically evident alcoholic cirrhosis and liver cancer were confirmed in individual studies[57-60]. Most recently, Burza *et al*[61] reported that an increased age at onset of at-risk alcohol consumption and the PNPLA3 I148 M allele were independent risk factors for alcoholic liver cirrhosis (HR = 2.76; $P < 0.01$ *vs* 1.53; $P = 0.021$).

CONCLUSION

In this review, several aspects potentially contributing to the mechanisms underlying fibrogenesis in ALD are discussed. Since there are no FDA-approved treatments for ALD at present, development of novel therapies for inhibiting inflammation and/or fibrogenesis associated with early stages of ALD will be beneficial for slowing disease progression and improving patient outcomes[51]. To achieve these objectives, animal models that accurately reflect the metabolic and histological characteristics of human ALD are needed.

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Branched-Chain Amino Acids Prevent Hepatocarcinogenesis and Prolong Survival of Patients With Cirrhosis

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BACKGROUND & AIMS: **Although a low plasma level of branched-chain amino acids (BCAAs) is a marker of cirrhosis, it is not clear whether BCAA supplements affect disease progression. We performed a multicenter study to evaluate the effects of BCAA supplementation on hepatocarcinogenesis and survival in patients with cirrhosis.**

METHODS: **We enrolled 299 patients from 14 medical institutions in Japan in a prospective, multicenter study in 2009; 267 patients were followed through 2011. Patients were given BCAA supplements (5.5–12.0 g/day) for more than 2 years (n = 85) or no BCAAs (controls, n = 182). The primary end points were onset of hepatocellular carcinoma (HCC) and death. Factors associated with these events were analyzed by competing risk analysis.**

RESULTS: **During the study period, 41 of 182 controls and 11 of 85 patients given BCAAs developed HCC. On the basis of the Cox and the Fine and Gray models of regression analyses, level of α-fetoprotein, ratio of BCAA:tyrosine, and BCAA supplementation were associated with development of HCC (relative risk for BCAAs, 0.45; 95% confidence interval, 0.24–0.88; $P = .019$). Sixteen controls and 2 patients given BCAAs died. Factors significantly associated with death were Child–Pugh score, blood level of urea nitrogen, platelet count, male sex, and BCAA supplementation (relative risk of death for BCAAs, 0.009; 95% confidence interval, 0.0002–0.365; $P = .015$) in both regression models.**

Abbreviations used in this paper: AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCAA, branched-chain amino acid; BMI, body mass index; BUN, blood urea nitrogen; CT, computed tomography; HbA1c, glycosylated hemoglobin; HCC, hepatocellular carcinoma; HOMA-IR, homeostasis model assessment of insulin resistance; MRI, magnetic resonance imaging; TIBC, total iron binding capacity.

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CONCLUSIONS: **On the basis of a prospective study, amino acid imbalance is a significant risk factor for the onset of HCC in patients with cirrhosis. BCAA supplementation reduces the risk for HCC and prolongs survival of patients with cirrhosis.**

Keywords: Liver Cancer; Hepatoma; Nutrition; Treatment Outcome.

The liver is a central organ in the metabolism of many nutrients. Thus, cirrhosis of the liver frequently results in metabolic disarray.[1,2] Decreased serum levels of branched-chain amino acids (BCAAs) are a hallmark of cirrhosis[1,2] that are contributed to by several factors, including reduced nutritional intake, hypermetabolism, and ammonia detoxification in skeletal muscle.[3] Low serum levels of BCAAs are also important in the pathogenesis of hepatic encephalopathy and hypoalbuminemia and are associated with overall mortality.[1-3]

BCAAs are a source of glutamate, which detoxifies ammonia via glutamine synthesis in skeletal muscle.[4] BCAAs have recently been considered as pharmacologic nutrients in cirrhotic patients.[1] In vitro studies have demonstrated that BCAAs prevent the proliferation of hepatocellular carcinoma (HCC) cells by inducing apoptosis.[5] In addition, BCAA supplementation was shown to stimulate antioxidant DNA repair in a rat model of liver injury[6] and to prevent hepatocarcinogenesis in an animal model.[7] In HCC patients, BCAA supplementation reduces early recurrence of HCC after hepatic resection or radiofrequency thermal ablation.[8,9] To investigate the effects of BCAA supplementation on the development of cirrhosis-related complications, multicenter randomized controlled trials were conducted in Italy and Japan at the end of the 1990s.[10,11] Although these studies showed that BCAA supplementation prevented hospital admissions related to cirrhosis complications and improved the quality of life of cirrhotic patients,[10,11] the effects of BCAA supplementation on hepatocarcinogenesis remain unclear.

BCAAs are also known to enhance hepatic regeneration and immunity.[1,2] BCAAs stimulate the production of hepatocyte growth factor in hepatic stellate cells[12] and increase hepatic parenchymal cell mass.[13] In addition, BCAA supplementation increases lymphocyte counts and improves the phagocytic function of neutrophils in cirrhotic patients.[14] BCAAs also reverse functional impairment and stimulate the maturation of myeloid dendritic cells, leading to the production of interleukin-12, a potent activator of natural killer cells.[15] Recently, bacterial infection has become one of the major causes of death in cirrhotic patients.[16] Taken together, these findings suggest that BCAA supplementation may prevent hepatic failure and bacterial infection, leading to prolonged survival in cirrhotic patients. Hitherto, a survival benefit of BCAA supplementation has not been demonstrated.[17]

In Japan, BCAA supplementation is an approved medication for decompensated liver cirrhosis, and thus, a randomized control trial that uses BCAA supplementation cannot ethically be performed. Moreover, the onset of HCC and death are considered as competing risks. Therefore, the aim of this study was to evaluate the effects of BCAA supplementation on the onset of HCC and survival in cirrhotic patients by competing risk analysis.

Methods

Study Design and Ethics

This study was designed in 2009 by the steering committee as a multicenter investigation for evaluating the effects of BCAA supplementation on hepatocarcinogenesis and prognosis in cirrhotic patients. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the prior approval given by the institutional review board of each institution. None of the subjects were institutionalized.

Subjects and Observation Period

In 2009, 299 cirrhotic patients without HCC were enrolled from 14 medical institutions in Japan. Diagnosis of liver cirrhosis was based on an aspartate aminotransferase (AST)-to-platelet ratio index >1.5,[18] morphologic changes of the liver such as hypertrophy of the left lateral and caudate lobes and atrophy of the right posterior hepatic lobe as evidenced by ultrasonography, computed tomography (CT), and/or magnetic resonance imaging (MRI), or a pseudo-lobule formation finding on histopathologic examination. The etiologies of liver cirrhosis were hepatitis C virus infection (n = 171), hepatitis B virus infection (n = 31), alcohol intake (n = 24), autoimmune hepatitis (n = 22), nonalcoholic fatty liver disease (n = 12), and others (n = 7).

Enrolled patients were followed up until 2011. The median observation period was 728 days (range, 22–1069 days), and the mean observation period was 677.9 ± 220.0 days. During the course of the study, 32 patients could not be followed up because of a change of residence (n = 12), failure to attend appointments (n = 17), or data unavailability (n = 3). The remaining 267 cirrhotic patients were analyzed (follow-up rate, 89.3%) (Supplementary Figure 1). A total of 16 patients had hepatic encephalopathy at study entry and were treated with BCAA supplementation, and 4 patients developed hepatic encephalopathy during the study period.

Classification

BCAA supplementation (BCAA granules and BCAA-enriched nutrients) is an approved medication for decompensated liver cirrhosis in Japan. Thus, according to the indication criteria, BCAAs were administered to cirrhotic patients with hepatic encephalopathy or hypoalbuminemia. Patients treated with BCAA supplementation (5.5–12.0 g/day) for >2 years were classified as the BCAA group (n = 85), whereas those without hepatic encephalopathy or hypoalbuminemia (n = 152) or in whom administration was difficult (n = 30) were classified as the non-BCAA group (n = 182). The reasons for BCAA administration difficulty were noncompliance because of the bitterness of the supplement or supplement-related adverse effects such as gastrointestinal discomfort and diarrhea.

Definition of an Event

In this study, an event was defined as the onset of HCC or death from any cause. Subjects were regularly followed up by doctors specializing in liver disease. The follow-up examinations included routine physical examinations, biochemical tests, and HCC screening with 4 monthly tests of serum α-fetoprotein (AFP) levels and diagnostic imaging studies including ultrasonography, CT, or MRI.

HCC was diagnosed by using a combination of the levels of serum tumor markers, such as AFP and des-gamma-carboxy prothrombin, and findings of imaging studies such as ultrasonography, CT, MRI, and angiography. For deceased subjects, the disease directly causing death was defined as the cause of death. Chronic hepatic failure was defined as having jaundice, refractory ascites, and/or hepatic encephalopathy. No patient died of acute liver failure or acute chronic liver failure.

A censoring case was defined as a subject who was followed up until the end of the study period without onset of HCC or death.

Data Collection

Data on the following parameters were collected at study entry: age, sex, daily alcohol intake (none, <60 g, or >60 g), body mass index (BMI), platelet count, serum levels of AST, alanine aminotransferase (ALT), albumin, total bilirubin, total cholesterol, triglycerides, fasting blood glucose, insulin, blood urea nitrogen (BUN), creatinine, sodium, potassium, zinc, iron, total iron binding capacity (TIBC), ferritin, AFP, prothrombin time, proportion of glycosylated hemoglobin (HbA1c), homeostasis model assessment of insulin resistance (HOMA-IR), blood ammonia level, BCAA-to-tyrosine ratio, and Child–Pugh score. Intake of BCAA supplementation was evaluated at every visit.

Statistics

Differences between the BCAA and non-BCAA groups were analyzed by using the Wilcoxon rank sum test. Factors associated with the onset of HCC and death were analyzed by competing risk analysis as previously described.[19]

Bivariate analyses were performed by using the multiple Cox regression model with stepwise selection (enter and exit probabilities are $P = .05$) for the cause-specific hazard of HCC onset and death and the Fine and Gray regression model for the subdistribution hazard of HCC onset and death. Covariates were selected by using a stepwise procedure adapted to multiple imputation methodology.[19] The Fine and Gray model provides complementary competing risk data to the Cox proportional hazards model by considering the subdistribution hazard.[19] P values <.05 were considered significant. All analyses were performed by using the R statistical programming language and computing environment with survival, cmprsk, and MICE packages.[20]

Results

Patient Characteristics

At baseline, no significant differences were noted in age, sex, daily alcohol intake, BMI, platelet count, fasting blood glucose levels, HOMA-IR values, and serum levels of AST, ALT, creatinine, sodium, and AFP between the BCAA and non-BCAA groups (Table 1). In contrast, serum levels of albumin, total cholesterol, ferritin, and the BCAA-to-tyrosine ratio were significantly lower in the BCAA group than in the non-BCAA group (Table 1). In addition, blood ammonia levels and Child–Pugh scores were significantly higher in the BCAA group than in the non-BCAA group (Table 1).

Incidence Rate of Events

During the study, 52 patients developed HCC (41 patients in the non-BCAA group and 11 patients in the BCAA group), and 18 patients died (16 patients in the non-BCAA group and 2 patients in the BCAA group) (Table 2). The incidence rates of HCC onset and death in the overall study sample were 19.5% (52 of 267) and 6.7% (18 of 267), respectively.

The causes of death and the incidence rates for each group are summarized in Supplementary Table 1. Chronic hepatic failure and bacterial infection accounted for 27.8% (5 of 18) and 22.2% (4 of 18) of all deaths, respectively. Of the 16 deaths in the non-BCAA group, 4 (25%) were due to hepatic failure, and 4 (25%) were due to bacterial infection. Of the 2 deaths in the BCAA group, 1 (50%) was due to hepatic failure, and none were due to bacterial infection (Supplementary Table 1).