Derived neutrophil to lymphocyte ratio predicts prognosis for patients with HBV-associated hepatocellular carcinoma following transarterial chemoembolization

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Abstract. The derived neutrophil to lymphocyte ratio (dNLR) has been proposed as an easily determinable prognostic factor for cancer patients, but the prognostic significance of the dNLR in hepatocellular carcinoma (HCC) has not been investigated. The present study aimed to validate the prognostic power of the NLR and dNLR in HCC patients undergoing transarterial chemoembolization (TACE). The data of 279 consecutive patients who underwent TACE for unresectable HBV-associated HCC between September 2009 and November 2011 at the Department of Hepatobiliary Surgery, Sun Yat-sen University Cancer Center (Guangzhou, China) were retrieved from a prospective database. The cut-off values for the NLR and dNLR were determined by receiver operating characteristic (ROC) analysis. The association between the NLR and dNLR and the clinicopathological characteristics and overall survival (OS) rates and times of patients was analyzed. The area under the curve (AUC) was calculated to evaluate the discriminatory ability of the NLR and dNLR. The median follow-up period was 446 days, the 1, 2 and 3-year OS rates were 38.8, 18.5 and 11.1% respectively, and the median OS time was 264 days. The cut-off values were determined as 2.6 and 1.8 for the NLR and dNLR, respectively. The NLR and dNLR were each associated with patient age, presence of vascular invasion, tumor size, AST level and ALP level. Multivariate analysis showed that the NLR, dNLR, ALT level and AFP level were independent prognostic factors for OS. An elevated NLR or dNLR was associated with a poor prognosis (P=0.001 and P=0.002, respectively). The prognostic power of NLR [AUC=0.539; 95% confidence interval (CI), 0.423-0.656] and dNLR (AUC=0.522; 95% CI, 0.406-0.638) was similar. Elevated dNLR predicted poor prognosis for patients with HBV-associated HCC undergoing TACE, with similar prognostic power to NLR. The dNLR may be used as an alternative to the NLR, as it is easily available and inexpensive.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most frequent cancer and the third most common cause of cancer-associated mortality in the world (1). There are numerous risk factors associated with the causes of liver disease, including hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol abuse (≥80 g/day), iron overload and fatty liver, which results in HCC being heterogeneous and complex (2-4). The prognosis of patients with HCC is poor. Resection, liver transplantation and percutaneous treatment may be curative for an early stage of tumor, which accounts for ≤30% of patients. For intermediate to advanced-stage HCC, transarterial chemoembolization (TACE) is considered to be the standard treatment by certain international guidelines (5). A meta-analysis reveals that TACE improves survival in patients with unresectable HCC, who have been selected as they have intermediate stage, multimodular and Performance Status Test (PST) 0 or advanced stage, portal invasion, lymph node 1, metastasis 1 and PST 1-2, and evidence obtained from randomized controlled trials has confirmed the beneficial effect of TACE in improving survival (6,7). However, studies have also shown that not all patients with unresectable HCC benefit from TACE. Therefore, it is crucial to differentiate between patients that are most likely to benefit from TACE and those that are not in a heterogeneous HCC population.

The pathogenesis of HCC is based on inflammation. Particularly in China, the majority of HCC cases develop due to underlying chronic HBV infection. Tumor inflammation and immunology have previously been identified to enable

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Key words: neutrophil, lymphocyte, hepatocellular carcinoma, transarterial chemoembolization

cancer characteristics, and increasing evidence supports the involvement of inflammation and immunology in cancer progression and metastases (8,9). In addition, the combination of hematological components of the systemic inflammatory response have been shown to have prognostic value in patients with a variety of cancers, including the Glasgow prognostic score (GPS) (10-13), modified GPS (mGPS) (10-13), neutrophil to lymphocyte ratio (NLR) (14-17), prognostic nutritional index (PNI) (18), platelet-lymphocyte ratio (PLR) (19) and prognostic index (PI) (20). Of all these scores, the NLR is the most inexpensive and easily obtained. Studies have previously shown that an elevated NLR indicated a poor prognosis for patients with HCC (14-17). However, in clinical trials, only the white blood cell and neutrophil counts of the patients are commonly entered into clinical trial databases. Therefore, Proctor *et al* (21) recently implemented a derived NLR (dNLR), which is composed of the neutrophil count and the white blood cell count minus neutrophil count. Proctor *et al* evaluated the prognostic value of dNLR on cancer outcome in different cancer types and demonstrated that the dNLR had a similar prognostic value to the well-established NLR, and dNLR was suggested to be a cheaper and more easily determinable parameter than NLR. However, the application of dNLR in HCC patients was not fully validated. The present study was conducted to investigate the prognostic value of the pre-treatment dNLR on overall survival (OS) in patients with unresectable HCC undergoing TACE.

Patients and methods

Patients. Patients treated with TACE for unresectable HCC between September 2009 and November 2011 at the Department of Hepatobiliary Surgery of Sun Yat-sen University Cancer Canter (Guangzhou, China) were identified using the prospective database of the hospital. The present study was approved by the Institutional Review Board of Sun Yat-sen University Cancer Center, and written informed consent was obtained from all patients.

The diagnosis of HCC was based on the diagnostic criteria for HCC used by the American Association for the Study of the Liver guidelines (22). HCC was diagnosed by at least two radiological images showing the characteristic features of HCC, or one radiological image showing characteristic features of HCC associated with elevated serum AFP level (≥400 ng/ml) or histopathological evidence. Patients who met all of the following criteria were included in analysis: i) No previous treatment prior to TACE; ii) HBV-positive; iii) no HCV and HIV expression; iv) liver function Child-Pugh grade A or B; and v) a follow-up period ≥3 months.

All parameters were recorded and evaluated as possible predictors of survival, such as the gender, age, C-reactive protein (CRP) level, white blood cell count (WBC), neutrophil count, lymphocyte count, platelet count (PLT), α-fetoprotein (AFP) level, alkaline phosphatase (ALP) level, total bilirubin level (TBIL), albumin (ALB) level, tumor size and number and vascular invasion status of patients.

TACE procedure. TACE was performed using a previously reported protocol (23). A selective 5-Fr catheter (Terumo Corporation, Tokyo, Japan) was introduced into the hepatic artery and visceral angiography was performed to assess the arterial blood supply to the liver. Depending on the size, location and arterial supply of the tumor, the tip of the catheter was advanced into the right or left hepatic artery; if all the tumors were fed by one enlarged independent hepatic artery branch, the tip of catheter was introduced into this tumor-feeding artery. If the conventional catheter could not enter the hepatic artery due to technical reasons, a 2.9-Fr micro catheter (Terumo Corporation, Tokyo, Japan) was used. Hepatic artery infusion chemotherapy was performed using 300 mg carboplatin (Bristol-Myers Squibb, New York, NY, USA). Subsequently, chemolipiodolization was performed using 50 mg epirubicin (Pfizer, Wuxi, Jiangsu, China), and 6 mg mitomycin C (Zhejiang Hisun Pharmaceutical Co. Ltd., Taizhou, Zhejiang, China) mixed with 5 ml lipiodol (Lipiodol Ultra-Fluide; Guerbet, Villepinte, France). If the chemolipiodolized arterial territory did not show stagnant flow, pure lipiodol was then injected. In certain patients, stasis in a tumor-feeding artery was not achieved, even subsequent to the injection of the maximum amount of iodized oil (25 ml), due to the large size of the tumor. Embolization was then performed in these patients with the injection of absorbable gelatin sponge particles (Gelfoam; Hangzhou ALC Ltd., Hangzhou, China), 1-2 mm in diameter, through the angiographic catheter. This treatment regimen was used consistently in the present study, regardless of tumor type and size.

Follow-up. Patients were followed carefully subsequent to treatment. Patients underwent liver computed tomography (CT) scans 1 month subsequent to TACE, and liver CT scans were performed at 3-month intervals during the first 2 years, then every 6 months thereafter, with physical examination, blood tests for the AFP level and liver function. When metastasis was suspected, CT chest, bone scintigraphy, positron emission tomography (PET) and biopsy, if indicated, were also performed to confirm the presence of metastasis. The end of follow-up was December 2013, which was the time of the last follow-up, or the date of mortality.

Another session of TACE was performed every 4-10 weeks after the original administration of TACE until CT scans and AFP levels indicated stabilization of the tumor, or until TACE was not technically feasible, either due to hepatic artery occlusion or impaired liver function. The OS time was defined as the interval between the date of treatment and the date of mortality or last follow-up of surviving patients. Causes of mortality were determined from death certificates, medical interviews and radiological findings.

Statistical analysis. The receiver operating characteristic (ROC) analysis was used to determine the cut-off values of NLR and dNLR. The OS was calculated using the Kaplan-Meier method and compared by the log-rank test. The prognostic varieties in predicting the OS were assessed by multivariate Cox proportional hazards regression analysis. All covariates that affected survival at the $P<0.10$ level of significance in univariate analysis were included in the multivariate Cox proportional hazards model. A ROC curve was also generated and the area under the curve (AUC) was calculated to evaluate the discriminatory ability. The association between the NLR and dNLR was assessed by Spearman's rank correlation

Table I. Baseline characteristics and univariate analysis for overall survival in 279 patients undergoing transarterial chemoembolization for hepatocellular carcinoma.

Variables	n	Value	Univariate analysis P-value
Median age, years (range)		50 (23-80)	0.049
<50	158		
≥50	121		
Gender, n		NA	0.111
Male	251		
Female	28		
Mean WBC, n x10^9/l (range)		6.6 (2.1-24.6)	0.080
<9	36		
≥9	243		
Mean neutrophil count, n x10^9/l (range)		4.2 (0.7-21.5)	0.019
<7	28		
≥7	251		
Mean lymphocyte count, n x10^9/l (range)		1.5 (0.3-4.8)	0.166
<0.8	268		
≥0.8	11		
Mean PLT count, n x10^9/l (range)		182 (23-548)	0.412
<100	47		
≥100	232		
Mean ALT, μ/l (range)		56.6 (8-304)	0.003
<40	169		
≥40	110		
Mean AST, μ/l (range)		75.8 (19.3-472.6)	<0.001
<45	191		
≥45	88		
Mean albumin, g/l (range)		39 (25-79)	0.052
<35	238		
≥35	41		
Mean total serum bilirubin, μmol/l (range)		17.8 (4.8-222.9)	<0.001
<20	68		
≥20	191		
Mean ALP, IU/l (range)		150 (13-761.5)	<0.001
<110	171		
≥110	126		
Mean AFP, ng/ml (range)		751.2 (1.26-1,210,000)	<0.001
<400	154		
≥400	125		
Mean AFU, U/l (range)		35.2 (13-992)	0.022
<40	105		
≥40	174		
Mean PT, sec (range)		12.5 (9.8-36.8)	0.001
≤13.5	242		
>13.5	37		
Mean diameter of largest lesion, cm (range)		10 (1.4-20.0)	<0.001
<10	109		
≥10	170		
Tumor number, n		NA	0.041
Solitary	83		
Multiple	196		

Table I. Continued.

Variables	n	Value	Univariate analysis P-value
Vascular invasion, n		NA	<0.001
Absent	185		
Present	94		
Child-Pugh grade, n		NA	0.005
A	258		
B	21		
NLR, n		NA	0.001
<2.6	139		
≥2.6	140		
dNLR, n		NA	0.002
<1.8	153		
≥1.8	126		

NA, not applicable; WBC, white blood cell count; PLT, platelet count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; AFP, α-fetoprotein; AFU, α-L-fucosidase; PT, prothrombin time; NLR, neutrophil to lymphocyte ratio; dNLR, derived NLR.

analysis. Data are expressed as the mean ± standard deviation. All statistical tests were two-sided, and P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using the SPSS 13.0 statistical software (SPSS, Inc., Chicago, IL, USA).

Results

Baseline characteristics. A total of 279 consecutive patients that met the inclusion criteria were included in present study. The baseline characteristics of the patients are summarized in Table I. Overall, 251 patients were male (90%) and 28 patients were female (10%), with a median age of 50 years (range, 23-80 years). The majority of the present patients exhibited a good liver functional reserve, classified as Child-Pugh grade A (92.5%).

By applying the aforementioned criteria, a cut-off value of 2.6 for NLR and 1.8 for dNLR was determined by ROC analysis to be best to discriminate between patients' survival in the whole cohort. In total, 140 patients had NLR ≥2.6 (50%) and 126 patients had dNLR ≥1.8 (45.2%). The association between inflammatory scores and clinicopathological features was analyzed (Table II). The NLR and dNLR were each associated with patient age, tumor size, presence of vascular invasion, and aspartate aminotransferase (AST) and ALP levels.

Survival and prognostic factors. The median follow-up period was 446 days. The 1, 2 and 3-year OS rates were 38.8, 18.5 and 11.1% respectively, and the median OS time was 264 days. The univariate and multivariate analyses of prognostic factors for OS were analyzed. In univariate analysis (Table I), age (P=0.049), CRP (P<0.001), alanine aminotransferase (ALT; P=0.003), AST (P<0.001), ALP (P<0.001), LDH (P<0.001), α-L-fucosidase (AFU; P=0.024), TBIL (P<0.001), AFP (P<0.001), prothrombin time (PT; P=0.001), tumor size (P<0.001), tumor number (P=0.041), vascular invasion

(P<0.001), metastasis (P<0.001), Child-Pugh scores (P=0.005), NLR (P=0.001) and dNLR (P=0.002) were prognostic factors for OS.

Multivariate analysis (Table III) showed that NLR [hazard ratio (HR), 1.382; 95% confidence interval (CI), 1.037-1.842; P=0.027], ALT (HR, 1.472; 95% CI, 1.099-1.971; P=0.01), and AFP (HR, 1.677; 95% CI, 1.259-2.233; P<0.001) were independent prognostic factors for OS. When NLR was replaced by dNLR, the multivariate analysis also showed that the dNLR (HR, 1.445; 95% CI, 1.086-1.923; P=0.012) was an independent prognostic factor for OS, along with the ALT and AFP levels. An elevated NLR or dNLR is associated with a poor prognosis (P=0.001 and P=0.002 respectively; Fig. 1).

Association and comparison between the NLR and dNLR. The association between the NLR and dNLR was assessed by Spearman's rank correlation analysis. There was a significant correlation between NLR and dNLR (R=0.875; P<0.001). The prognostic power of NLR and dNLR was compared using AUC analysis. As shown in Fig. 2, the AUC of NLR was 0.539 (95% CI, 0.423-0.656) and the AUC of dNLR was 0.522 (95% CI, 0.406-638), which was similar.

Discussion

In the present study, the prognostic power of the NLR and dNLR was evaluated in patients with HBV-associated HCC undergoing TACE. The present results demonstrated that there was a significant correlation between NLR and dNLR, and NLR and dNLR each predicted the prognosis of patients with a similar prognostic power. Thus, the dNLR may be used as an alternative to NLR.

Previous studies (10-13) have shown that inflammation scores, such as the GPS, mGPS, NLR, PLR, PI and PNI, are associated with the prognosis of patients with HCC undergoing surgical resection, transplantation, TACE and

Table II. Association between NLR or dNLR and clinical variables.

Variables	NLR, n ≤2.6	NLR, n >2.6	P-value	dNLR, n ≤1.8	dNLR, n >1.8	P-value
Age			0.03			0.016
>50 years	87	71		96	62	
≤50 years	52	69		57	64	
Gender			0.437			0.179
Male	127	124		141	110	
Female	12	16		12	16	
Diameter of largest lesion			<0.001			<0.001
>10 cm	29	80		35	74	
≤10 cm	106	56		114	48	
Number of lesions			0.096			0.235
1	35	48		41	42	
>1	104	92		112	84	
Vascular invasion			<0.001			<0.001
Absent	107	78		115	70	
Present	32	62		38	56	
ALT			0.063			0.024
>40 μ/l	76	93		84	85	
≤40 μ/l	63	47		69	41	
AST			<0.001			<0.001
>45 μ/l	81	110		94	97	
≤45 μ/l	58	30		59	29	
Total serum bilirubin			0.049			0.104
>20 μmol/l	25	42		32	35	
≤20 μmol/l	114	98		121	91	
ALP			<0.001			0.001
>110 IU/l	72	98		82	88	
≤110 IU/l	67	42		71	38	
AFP			0.115			0.204
>400 ng/ml	70	83		79	74	
≤400 ng/ml	69	57		74	52	
PT			0.012			0.128
Normal	126	116		137	105	
Abnormal	13	24		16	21	
Child-Pugh grade			0.507			0.489
A	130	128		143	115	
B	9	12		10	11	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; AFP, α-fetoprotein; PT, prothrombin time; NLR, neutrophil to lymphocyte ratio; dNLR, derived NLR.

RFA. Among these inflammation-based scores, NLR is inferior to other measures of the systemic inflammatory response, including mGPS, but it is less expensive and more readily available in day-to-day oncological practice (24). It is therefore notable that the NLR has been shown to have prognostic value in patients with a variety of cancers, and dynamic changes in the NLR may predict the prognosis of patients (25). Proctor *et al* (21) evaluated the prognostic value

of the dNLR in a large cohort of 12,118 patients with different cancer types, including hepatopancreaticobiliary cancer (n=721). This study clearly demonstrated that the dNLR has a similar predictive ability for prognosis as the NLR, with patients with an elevated dNLR demonstrating a poor clinical outcome, which can be equally used to predict survival (21). The advantage of the dNLR compared with the NLR is that the dNLR remains available in the absence of the lymphocyte

Table III. Multivariate analyses of prognostic factors for overall survival in 279 patients undergoing TACE for hepatocellular carcinoma.

Variables	Value	Multivariate analysis	
		Hazard ratio (95% CI)	P-value
NLR[a]			
Mean ALT, μ/l (range)	56.6 (8-304)	1.472 (1.099-1.971)	0.010
Mean AFP, ng/ml (range)	751.2 (1.26-1,210,000)	1.677 (1.259-2.233)	<0.001
NLR, n			
≤2.6	139	1.382 (1.037-1.842)	0.027
>2.6	140		
dNLR[a]			
Mean ALT, μ/l (range)	56.6 (8-304)	1.469 (1.098-1.966)	0.010
Mean AFP, ng/ml (range)	751.2 (1.26-1,210,000)	1.720 (1.294-2.287)	<0.001
dNLR, n			
≤1.8	153	1.445 (1.086-1.923)	0.012
>1.8	126		

[a]NLR and dNLR were used as covariates, adjusted by ALT and AFP. ALT, alanine aminotransferase; ALP, alkaline phosphatase; NLR, neutrophil to lymphocyte ratio; dNLR, derived NLR.
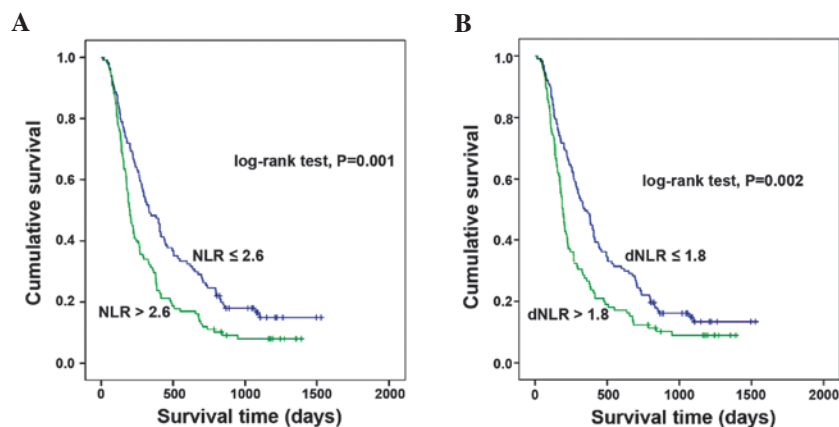

Figure 1. Kaplan-Meier survival curves for the overall survival of 279 patients undergoing transarterial chemoembolization for hepatocellular carcinoma. (A) NLR, (B) dNLR. NLR, neutrophil to lymphocyte ratio; dNLR, derived NLR.
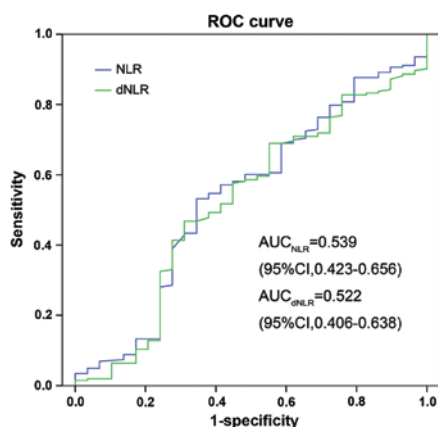

Figure 2. Comparison of AUC for the prediction of the outcome for patients with NLR and dNLR. NLR, neutrophil to lymphocyte ratio; dNLR, derived NLR; ROC, receiver operating characteristic; AUC, area under the curve.

count, and may therefore be widely used on the basis of clinical trial databases.

It is generally accepted that inflammatory processes in the tumor microenvironment play a crucial role in promoting the proliferation, invasion and metastasis of malignant cells (26,27). The infiltrating leucocytes are important factors in this process (26). There are two elements to the dNLR, consisting of the neutrophil count, and the white blood cell count minus the neutrophil count. The latter count is dominated by lymphocytes and monocytes. Neutrophils in the peripheral blood or in the tumor microenvironment have been shown to produce pro-angiogenic factors, including vascular endothelial growth factor, to stimulate tumor development and progression (27). The cytokines involved in cancer-associated inflammation, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα), may induce neutrophilia (28,29).

The para-neoplastic production of myeloid growth factors by cancer cells may act as an additional cause of neutrophilia (30). Therefore, a high peripheral neutrophil level may indicate a cancer-associated inflammation or tumor progression, and predict poor clinical outcome. In addition, the neutrophils and leucocytes are mostly composed of lymphocytes. Immune cells that infiltrate into or around the tumor engage in dynamic and extensive crosstalk with cancer cells (31). Over the past decade, there has been growing evidence that lymphocytes act as crucial components of the adaptive immune system and are the cellular basis of cancer immuno-surveillance and immuno-editing (32,33). Furthermore, infiltrating lymphocytes have been reported to indicate the generation of an effective anti-tumor cellular immune response (34,35). Therefore, a low lymphocyte count may be responsible for an inadequate immunological reaction to the tumor, and consequently, a weakened defense against cancer, resulting in a poor prognosis (35). The peripheral monocyte count is known to be increased in cancer patients (36-38). Schmidt *et al* (36) created a prognostic model in metastatic melanoma based on independent prognostic factors in 321 patients receiving IL-2-based immunotherapy. This study showed that an elevated monocyte count may replace an elevated neutrophil count as an independent prognostic factor for poor survival (36). Leitch *et al* (39) compared the prognostic value of an inflammation-based prognostic score in 149 patients with colorectal cancer and concluded that the monocyte count was independently associated with cancer-specific survival. One possible hypothesis is that macrophages express chemokine (C-X-C motif) (CXC) receptors 1 and 2, corresponding with CXC ligand (CXCL)1, also termed Gro-α, and CXCL8, also termed IL-8, respectively. These chemokines may be involved in tumor invasion and angiogenesis. However, monocytes only account for <8% of leucocytes, with limited effect on the dNLR or NLR.

In the present study the association between the NLR and the dNLR was analyzed by Spearman's rank correlation analysis and it is not notable that a significant correlation was identified between the NLR and the dNLR. The prognostic power of the NLR and the dNLR was also compared, and AUC analysis showed that the prognostic power was similar between the two. In the Cox proportional hazards regression analysis for OS, either NLR or dNLR were considered to be an independent prognostic factor, with a similar hazard ratio. All these results indicated that the dNLR may be used as an alternative to NLR in these patients.

The elevated ALT and AFP levels were also revealed as independent prognostic factors for poor outcome, as has been reported in previous studies (40-42). Notably, in patients with chronic hepatitis and cirrhosis, an increase in the AST/ALT ratio is associated with progressive liver functional impairment (43,44). As a major mammalian embryo-specific and tumor-associated protein, AFP has been used for the diagnosis and screening of HCC worldwide. An increased AFP level is connected with larger tumors and lower hypohepatia, reflecting an aggressive biology (45).

There are potential limitations of the present study, as follows: i) It is a retrospective, small sample, single-institution study; ii) only patients treated with TACE were recruited; and iii) the patient population is biased due to the prevalence of HBV infection, which is unusual in Western countries. Therefore, a large-scale prospective validation study is required to confirm the present results.

The current results revealed that an elevated dNLR predicted poor prognosis with a similar prognostic power to the NLR in patients with HBV-associated HCC undergoing TACE. Due to the dNLR being an easily available and inexpensive marker in clinical studies, the dNLR should be considered as a novel prognostic marker for patients with HBV-associated HCC in routine practice.

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