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## A novel prognostic index—neutrophil times $\gamma$ -glutamyl transpeptidase to lymphocyte ratio ( $N\gamma LR$ ) predicts outcome for patients with hepatocellular carcinoma

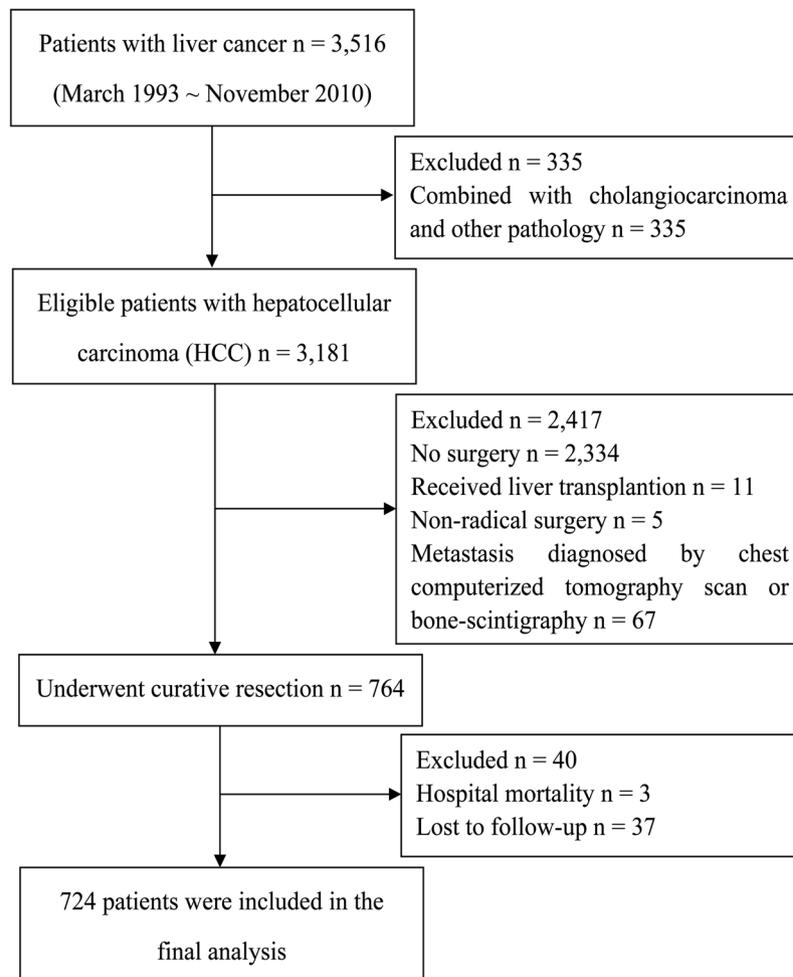
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Clinical outcomes of patients with hepatocellular carcinoma (HCC) are highly variable. This study aims to identify and validate a simple, readily available, and objective prognostic index for the management of HCC. Data from 724 HCC patients undergoing curative resection were evaluated and randomly divided into two cohorts for building and validating the prognostic index. A best model,  $N\gamma LR = (\text{neutrophil count } [10^9/L] \times \gamma\text{-glutamyl transpeptidase } [U/L]) / (\text{lymphocyte count } [10^9/L] \times U/L)$ , was selected. An optimal cut-off value of 103.6 for  $N\gamma LR$  stratified patients into high  $N\gamma LR (>103.6)$  and low  $N\gamma LR (\leq 103.6)$  groups.  $N\gamma LR > 103.6$  was closely associated with HCC malignant characteristics. Elevated  $N\gamma LR$  predicted a worse overall survival (OS) and progression-free survival (PFS) for HCC patients and remained an independent predictor for both types of survival. Moreover, early recurrence rates in patients with  $N\gamma LR > 103.6$  were higher than that in patients with  $N\gamma LR \leq 103.6$  ( $P < 0.0001$ ).  $N\gamma LR$  was an important independent predictor of survival for HCC patients and might be a new promising method to identify patients at different risks of early recurrence and survival after curative resection.

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, with approximately 745,000 deaths each year<sup>1</sup>. Unlike other cancers, the clinical manifestations of HCC are remarkably heterogeneous, and the prognosis of HCC is also complex and multifaceted, leading it to be the third most lethal malignant tumour worldwide<sup>2</sup>. Despite the advances in diagnostic and therapeutic methods, the 5-year risk of recurrence of HCC after surgery is as high as 70%<sup>3</sup>. A high incidence of HCC exists in Southeast Asia and sub-Saharan Africa, where the infection of hepatitis B virus (HBV) is endemic, while the incidence of HCC in western countries has also increased in recent years<sup>2,4</sup>. In the United States, the incidence rate of HCC more than doubled between 1985 and 2002, due to chronic hepatitis C virus infection, alcohol-related cirrhosis, or possibly the prevalence of obesity and diabetes<sup>2</sup>.

It is clear that the high recurrence rate of HCC after surgery is a major obstacle faced by clinicians. In recent decades, a wide range of prognostic staging systems, including the Cancer of the Liver Italian Program<sup>5</sup>, Barcelona Clinic Liver Cancer (BCLC), Tumour-Node-Metastasis (TNM), the Chinese University Prognostic Index<sup>6</sup>, the Tokyo scoring system<sup>7</sup>, and the Hong Kong Liver Cancer staging system<sup>8</sup>, have been proposed, among others. In general, these prognostic models have unique prognostic performance, but none of them is universally accepted<sup>9</sup>. Numerous efforts have been devoted to identify molecular signatures to guide prognosis prediction in HCC

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**Figure 1.** Flow diagram of patients enrolled in this study and reasons for exclusion.

patients, such as microRNA, gene signatures, and epigenetic biomarkers<sup>10–12</sup>. However, gene expression analysis is expensive and highly sophisticated, limiting its applicability to patients in routine clinical practice.

Recently, in the setting of precision medicine, researchers have been trying to find a potential marker or develop a multi-factor model that can predict the risk and prognosis of HCC from the peripheral blood early in the course of the disease. For example, a simple risk score composed of routinely measured parameters was constructed to predict the incidence of HCC in HBV carriers<sup>13</sup>; a simple model based solely on serum bilirubin and albumin levels was developed to assess liver function in patients with HCC<sup>14</sup>; an elevated neutrophil-lymphocyte ratio predicted adverse outcomes for HCC patients after living-donor liver transplantation<sup>15</sup>; a systemic immune-inflammation index [SII] score >330 was related to a higher recurrence rate and a shorter survival in patients with HCC<sup>16</sup>; and the aspartate transaminase (AST) to lymphocyte ratio index (ALRI) was built to predict different prognoses among various subgroups of HCC<sup>17</sup>.

For most clinicians, a simple, objective, and readily-calculated model is preferred. In this study, we identified a novel and simple laboratory index, i.e. neutrophil times  $\gamma$ -glutamyl transpeptidase to lymphocyte ratio ( $N\gamma LR$ ) in large cohorts of patients, and then validated its diagnostic accuracy in another independent cohort. We found the prediction ability of the  $N\gamma LR$  to be generally high throughout the training cohort. The correlation between the  $N\gamma LR$  and clinicopathologic parameters was also explored, and the impact of an elevated  $N\gamma LR$  on postoperative survival and recurrence was systematically evaluated.

## Materials and Methods

**Patients.** Between March 1993 and November 2010, 3,516 patients newly diagnosed with liver cancer at the Affiliated Hospital of Guilin Medical University (Guilin, People's Republic of China) were evaluated retrospectively. In the light of the included and excluded criteria, a total of 724 patients who underwent curative resection for HCC were eligible for this study (Fig. 1). The diagnostic criteria of HCC were based on clinical, serological, ultrasonography (US), computerized tomography (CT), magnetic resonance imaging (MRI), and pathologic examinations according to the Primary Liver Cancer Clinical Diagnosis and Staging Criteria (Ministry of Health, Beijing, China). Curative resection was defined as a complete resection of the tumour, a resection margin of at least 1 cm, no residual tumour based on histological examination, and no residual tumours or new lesions determined by two observations not less than 4 weeks apart<sup>18</sup>. Both intraoperative US and postoperative CT were

used to confirm complete removal of HCC. The baseline information including demographic characteristics, hepatitis B surface antigen (HBsAg), hepatitis C virus antibody, use of nucleoside analogues (NAs), presence of liver cirrhosis, complete blood count, albumin, globulin, total bilirubin, direct bilirubin, alanine aminotransferase (ALT), AST, alpha-fetoprotein (AFP),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT), tumour characteristics, metastasis and recurrence were collected. All laboratory parameters used in the study were measured before curative resection. All methods were carried out in accordance with Affiliated Hospital of Guilin Medical University guidelines and regulations. This study was approved by the research ethics committee of Affiliated Hospital of Guilin Medical University and complied with The Declaration of Helsinki Principles. Informed consent was obtained from all patients.

**Follow-up and postoperative treatment.** All postoperative patients were followed with the regular monitoring of serum AFP concentrations and abdomen US every 2 months and chest radiography every 6 months during the first two postoperative years and at 3–6 month intervals thereafter. Further examinations, including CT and MRI, were performed if recurrence or metastasis was suspected. When recurrence or metastasis was confirmed, further treatment, i.e., a second surgical resection, radiofrequency ablation, transcatheter arterial chemoembolization, percutaneous ethanol injection or sorafenib treatment were suggested. The main clinical endpoint of this study was overall survival (OS), measured from the date of surgery to the date of death or the last follow-up. Progression-free survival (PFS) was calculated from the date of surgery to the date of recurrence, metastasis, death, or last follow-up. The cut-off value between early and late recurrence was set as 2 years.

**Statistical methods.** All 724 patients were randomly assigned to either a training cohort for developing a new prognostic index or a validation cohort for evaluating the obtained prognostic index at an approximately 2:1 ratio. Variables were expressed as the mean  $\pm$  SD unless otherwise stated. Categorical data were compared by the Pearson  $\chi^2$  test or the Fisher exact test, while continuous variables were assessed by Student's *t* test. OS and PFS were calculated using the Kaplan–Meier method and compared with the log-rank test. Univariate analysis was performed to identify significant prognostic factors by using the Cox regression model. The multivariate Cox proportional hazards regression model was performed to identify independent predictors by including all the variables demonstrated to be significant in the univariate analyses. All statistical analyses were performed with SPSS18.0 (SPSS Inc., Chicago, IL). Statistical significance was defined as  $P < 0.05$ .

**Derivation of a prognostic model.** To construct a best prognostic model, an exploratory formula was built by entering different sets of the independent factors into a regression model. The best model based on the preoperative peripheral blood neutrophil count, lymphocyte count, and serum  $\gamma$ -GT level was selected from the training cohort. The calculation formula for the novel prognostic model was as follows:  $N\gamma LR = (\text{neutrophil count } [10^9/L] \times \gamma\text{-GT } [U/L]) / (\text{lymphocyte count } [10^9/L] \times U/L)$ . The diagnostic accuracy of the new index was estimated with the receiver operating characteristic (ROC) curve. Optimal cut-offs for  $N\gamma LR$  were selected in terms of maximizing both the sensitivity and specificity. The ROC curves were compared between different prognostic models for HCC patients using the area under the ROC curves (AUC). Finally, the performance of the  $N\gamma LR$  index was tested in the validation cohort.

**Data availability.** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## Results

**Characteristics of the patients with HCC.** Seven hundred and twenty-four HCC patients, with 463 in the training cohort and 261 in the validation cohort, were enrolled in this study. The clinical and laboratory characteristics of the HCC patients in both training and validation cohorts are shown in Table 1. Overall, the clinicopathologic characteristics between these two cohorts were very similar, except for smoking. The mean age of the patients in the training cohort was  $50.14 \pm 11.58$  years and  $49.56 \pm 10.84$  years in the validation cohort. In both training and validation cohorts, most patients had hepatitis B-related liver disease (85.3% and 82.8%, respectively) and cirrhosis (93.5% and 93.5%, respectively), and the numbers of HBsAg positive patients under NAs were 90 cases and 44 cases, respectively. Microvascular invasion was present in 17.7% of patients in the training cohort and 23.4% in the validation cohort. The median OS times were 47.12 (95% CI, 44.37–50.07) and 51.02 (95% CI, 47.23–54.71) months in the training and validation cohorts, respectively, and the median PFS times were 39.83 (95% CI, 36.78–42.91) and 40.27 (95% CI, 36.29–44.37), respectively. A total of 185 (39.96%) patients in the training cohort and 109 (41.76%) patients in the validation cohort experienced recurrence by the time of the data analysis.

**Comparisons of AUC between  $N\gamma LR$  and other prognostic indices.** Several useful prognostic models resulted from previous research, such as SII and ALRI<sup>16,17</sup>. Therefore, we used the thresholds of these models to assess the prognosis of the HCC patients selected in the present study. The prediction abilities of the  $N\gamma LR$ , SII, ALRI and conventional parameters, such as AFP, were compared. In the training cohort, the AUC for the  $N\gamma LR$  was 0.758 (95% CI, 0.714–0.804), which was higher than those of SII (0.651, 95% CI, 0.602–0.703), ALRI (0.688, 95% CI, 0.641–0.738) and AFP (0.613, 95% CI, 0.562–0.665) (Fig. 2A).

The discrimination ability of  $N\gamma LR$  was further confirmed in the validation cohort. For predicting OS, the AUC of  $N\gamma LR$  index (0.769, 95% CI, 0.712–0.823) was significantly better than those of SII (0.616, 95% CI, 0.549–0.692), ALRI (0.648, 95% CI, 0.583–0.719) and AFP (0.684, 95% CI, 0.620–0.749) (Fig. 2B).

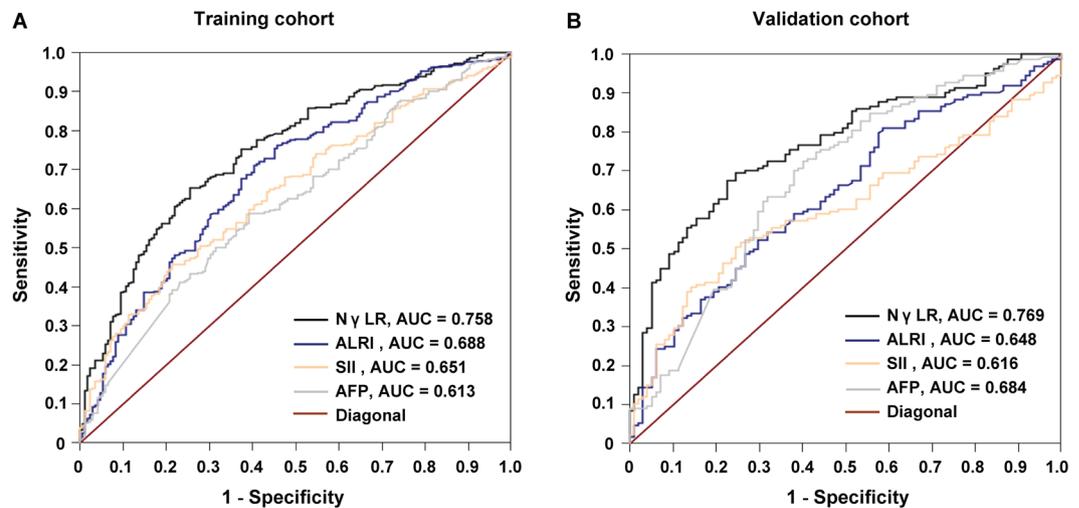
**Association between  $N\gamma LR$  and clinicopathologic parameters.** On the basis of the AUC analysis, an optimal cut-off value of the  $N\gamma LR$  to stratify patients between high and low survival for postoperative patients was

Parameter	Training cohort	Validation cohort	P value
	(n = 463)	(n = 261)	
Age (years)	50.14 ± 11.58	49.56 ± 10.84	0.504
Gender: female/male (n)	62/401	38/223	0.662
Family history: absent/present (n)	400/63	220/41	0.439
Drinking: absent/present (n)	245/218	153/108	0.138
Smoking: absent/present (n)	249/214	167/94	0.008
Cirrhosis: absent/present (n)	30/433	17/244	0.986
HBsAg: negative/positive (n)	68/395	45/216	0.363
HCVAb: negative/positive (n)	454/9	251/10	0.127
MaI: absent/present (n)	381/82	200/61	0.066
N $\gamma$ LR: median, range	137.54, 15.02–1507.96	133.59, 16.73–1236.02	0.746
SII: median, range	327.63, 5.72–2260.07	318.50, 4.35–2168.96	0.833
ALRI: median, range	24.65, 2.47–417.12	23.31, 0.94–320.96	0.540
WBC ( $\times 10^9/L$ )	6.15 ± 1.99	6.23 ± 2.00	0.598
NEUT ( $\times 10^9/L$ )	3.74 ± 1.70	3.80 ± 1.64	0.677
LYMPH ( $\times 10^9/L$ )	1.70 ± 0.63	1.72 ± 0.58	0.685
Platelets ( $\times 10^9/L$ )	175.62 ± 79.28	185.04 ± 82.63	0.131
Albumin (g/L)	39.17 ± 4.46	39.13 ± 4.34	0.915
Globulin (g/L)	30.87 ± 5.29	31.21 ± 4.96	0.388
TBIL ( $\mu\text{mol/L}$ )	17.82 ± 33.40	18.86 ± 41.06	0.719
DBIL ( $\mu\text{mol/L}$ )	7.94 ± 24.72	8.13 ± 25.05	0.850
ALT (U/L)	47.55 ± 43.51	45.39 ± 46.75	0.369
AST (U/L)	54.22 ± 55.06	53.69 ± 57.51	0.801
AFP (ng/ml): median, range	152.33, 0.23–11602.00	161.25, 0.61–10340.00	0.549
$\gamma$ -GT (U/L): median, range	68.60, 10.00–657.30	67.50, 15.10–604.90	0.902
Use of NAs: absent/present (n)	310/93	181/44	0.306
Tumor size (cm)	7.82 ± 4.77	7.43 ± 4.65	0.183
Tumor number: single/multiple (n)	327/136	200/61	0.081
Tumor differentiation: I/II/III/IV (n)	61/180/178/44	43/89/91/38	0.085
Child-Pugh classification: A/B (n)	425/38	237/24	0.648
BCLC stage: 0/A/B/C (n)	20/232/116/95	18/127/78/38	0.076
Type of surgical resection <sup>a</sup> (Anatomical/non-anatomical)	282/181	165/96	0.539

**Table 1.** Clinical and biochemical data of examined patients. <sup>a</sup>Anatomical resection includes hemihepatectomy, sectorectomy, and segmentectomy; non-anatomical resection includes limited resection and tumor enucleation. n, number of patients; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; MaI, microvascular invasion; N $\gamma$ LR, neutrophil cell count times  $\gamma$ -glutamyl transpeptidase to lymphocyte count ratio; SII, systemic immune-inflammation index; ALRI, aspartate transaminase to lymphocyte ratio index; WBC, white blood cell; LYMPH, lymphocyte count; TBIL, total bilirubin; DBIL, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein;  $\gamma$ -GT,  $\gamma$ -glutamyl transpeptidase; NAs, nucleoside analogues; BCLC, barcelona-clinic liver cancer.

103.6 in the training cohort. Subsequently, the N $\gamma$ LR was stratified into  $\leq 103.6$  or  $> 103.6$  for all subsequent analyses. In the training cohort, N $\gamma$ LR  $> 103.6$  was positively correlated with gender ( $P = 0.006$ ), a tumour size  $> 8$  cm ( $P < 0.001$ ), multiple tumour number ( $P = 0.003$ ), poor tumour differentiation ( $P < 0.001$ ), vascular invasion ( $P < 0.001$ ), BCLC stage B and C ( $P < 0.001$ ), early recurrence ( $P = 0.008$ ), AFP ( $> 20$  ng/ml) ( $P = 0.035$ ), ALT ( $> 40$  U/L) ( $P < 0.001$ ), SII ( $> 330$ ) ( $P < 0.001$ ), ALRI ( $> 25.2$ ) ( $P < 0.001$ ), and use of NAs ( $P < 0.001$ ) (Table 2). The relationships between N $\gamma$ LR and the clinicopathologic parameters in the validation cohort were very similar to those in the training cohort (Table 2).

**Prognostic factors and survival in the training cohort.** In the univariate analyses, a tumour size of  $> 8$  cm, multiple tumour number, poor tumour differentiation, vascular invasion, BCLC stage B and C, AFP ( $> 20$  ng/ml), ALT ( $> 40$  U/L), SII ( $> 330$ ), ALRI ( $> 25.2$ ), and N $\gamma$ LR ( $> 103.6$ ) were identified as significant prognostic factors of poor OS and PFS, whereas received NAs as antiviral treatment was associated with better OS and PFS in the training cohort (all  $P < 0.05$ ) (Table 3 and Supplementary Table S1). The Kaplan–Meier analysis showed that a high N $\gamma$ LR was associated with a shorter OS ( $P < 0.0001$ ) and PFS ( $P < 0.0001$ ) (Fig. 3A and Supplementary Fig. S1A). The median OS and PFS times were 62.31 (95% CI, 57.94–66.67) and 56.02 (95% CI, 50.75–61.29) months for patients with N $\gamma$ LR  $\leq 103.6$ , compared with 37.25 (95% CI, 33.86–40.64) and 31.48 (95% CI, 27.95–35.01) months for patients with N $\gamma$ LR  $> 103.6$ . The 1-, 3-, and 5-year OS rates were significantly lower in the subgroup with N $\gamma$ LR  $> 103.6$  (78.68%, 37.12%, and 27.25%, respectively) than in the N $\gamma$ LR  $\leq 103.6$  group (94.12%, 73.02%, and 62.24%, respectively) ( $P < 0.0001$ ).



**Figure 2.** Predictive ability of the  $N\gamma$ LR and the correlation between  $N\gamma$ LR and AFP. The predictive ability of  $N\gamma$ LR was compared with other prognostic parameters by ROC curves in the training (A) and the validation (B) cohorts.

Factors demonstrated to be statistically significant in univariate analysis were entered in the multivariate analysis with the Cox proportional hazards regression model (Table 3 and Supplementary Table S1). Elevated  $N\gamma$ LR was associated with elevated risks for OS (hazard ratio [HR], 1.65; 95% CI, 1.20–2.23;  $P=0.002$ ) and PFS (HR, 1.42; 95% CI, 1.07–1.97;  $P=0.021$ ). Additionally, a tumour size of  $>8$  cm was also identified as an independent predictor for OS (HR, 1.78; 95% CI, 1.36–2.40;  $P<0.001$ ) and PFS (HR, 1.58; 95% CI, 1.17–2.12;  $P=0.003$ ), while presence of vascular invasion (HR, 1.63; 95% CI, 1.18–2.26;  $P=0.004$ ) was an independent predictor for OS. Patients who used NAs as antiviral treatment had a better OS (HR, 0.47; 95% CI, 0.24–0.76;  $P=0.008$ ) in the multivariate model.

**Validating the prognostic value of  $N\gamma$ LR in the validation cohort.** We further evaluated whether  $N\gamma$ LR maintained its prognostic value in another independent cohort. Similar to the results from the training cohort, patients with  $N\gamma$ LR  $>103.6$  had a significantly shorter OS (median, 42.78 months; 95% CI, 38.63–46.94) and PFS (median, 31.41 months; 95% CI, 26.95–35.87) than patients with  $N\gamma$ LR  $\leq 103.6$  (median OS, 64.85 months; 95% CI, 59.92–69.78;  $P<0.0001$ ; median PFS, 55.43 months; 95% CI, 48.61–62.25;  $P<0.0001$ ) (Fig. 3B and Supplementary Fig. S1B). The results of the univariate analyses were very similar between training and validation cohorts (Table 3 and Supplementary Table S1). In the multivariate analysis,  $N\gamma$ LR  $>103.6$  remained an independent predictor for OS (HR, 1.91; 95% CI, 1.31–2.73;  $P=0.001$ ) and PFS (HR, 1.62; 95% CI, 1.10–2.44;  $P=0.015$ ).

**Early recurrence rate and further stratified  $N\gamma$ LR in patients with HCC.** The Kaplan–Meier curves also revealed that the  $N\gamma$ LR  $>103.6$  group was associated with a higher early recurrence rate compared with the  $N\gamma$ LR  $\leq 103.6$  group in the training (Fig. 3C,  $P<0.0001$ ) and validation cohorts (Fig. 3D,  $P=0.007$ ).

To further develop the  $N\gamma$ LR index, this linear predictive index was then categorized into three different groups. In the training cohort, 95 (20.5%), 252 (54.4%), and 116 (25.1%) patients were placed in the low ( $N\gamma$ LR  $\leq 64$ ), intermediate ( $64 < N\gamma$ LR  $\leq 370$ ), and high ( $N\gamma$ LR  $>370$ ) risk groups, respectively, and the median OS times were 65.89 months (95% CI, 60.46–71.32), 47.66 months (95% CI, 43.88–51.44) and 27.29 months (95% CI, 22.41–32.16), respectively (Fig. 3E,  $P<0.0001$ ). The median PFS times were 60.34 months (95% CI, 53.62–67.06), 40.31 months (95% CI, 36.15–44.47), and 23.45 months (95% CI, 18.49–28.41) for patients in the low, intermediate, and high risk groups, respectively (see Supplementary Figure S1C,  $P<0.0001$ ).

Applying the optimized  $N\gamma$ LR index to the validation cohort, 53 (20.3%), 153 (58.6%), and 55 (21.1%) patients were in the  $N\gamma$ LR  $\leq 64$ ,  $64 < N\gamma$ LR  $\leq 370$ , and  $N\gamma$ LR  $>370$  groups, respectively. The mean OS times were 67.69 (95% CI, 61.32–74.05), 52.91 (95% CI, 48.55–57.27), and 30.88 (95% CI, 25.04–36.73) months for patients at low, intermediate, and high risk, respectively (Fig. 3F,  $P<0.0001$ ), while the median PFS times were 58.87 (95% CI, 49.60–68.14), 41.49 (95% CI, 36.29–46.71), and 19.67 (95% CI, 14.97–24.39) months, respectively (see Supplementary Figure S1D,  $P<0.0001$ ).

**Prognostic values of  $N\gamma$ LR in patients with early HCC (BCLC stage 0 and A).** In view of the prognostic values of  $N\gamma$ LR in both training and validation groups, discriminative power of  $N\gamma$ LR was further evaluated in early HCC (BCLC 0 + A) in greater detail. In the early-stage subgroup,  $N\gamma$ LR  $>103.6$  was significantly associated with a shorter OS (median, 48.25 months; 95% CI, 42.94–53.55) and PFS (median, 41.48 months; 95% CI, 35.62–47.35) versus  $N\gamma$ LR  $\leq 103.6$  (median OS, 66.54 months; 95% CI, 61.92–71.16;  $P<0.0001$ ; median PFS, 60.87 months; 95% CI, 55.09–66.64;  $P<0.0001$ ) in the training cohort (Fig. 4A and C). In the validation cohort,  $N\gamma$ LR also significantly correlated with OS and PFS in the BCLC 0 + A subgroup ( $P<0.0001$  and  $P<0.0001$ , respectively, Fig. 4B and D).

Variables		N $\gamma$ LR level					
		Training cohort			Validation cohort		
		$\leq 103.6$	$> 103.6$	<i>P</i> value	$\leq 103.6$	$> 103.6$	<i>P</i> value
Gender	Female	32	30	0.006	21	17	0.020
	Male	135	266		79	144	
Age (years)	$\leq 55$	107	200	0.445	63	114	0.189
	$> 55$	60	96		37	47	
HBsAg	Negative	24	44	0.885	22	23	0.109
	Positive	143	252		78	138	
Tumor size	$\leq 8$ cm	145	149	$< 0.001$	94	93	$< 0.001$
	$> 8$ cm	22	147		6	68	
Tumor number	Single	132	195	0.003	84	116	0.027
	Multiple	35	101		16	45	
Tumor differentiation	I- II	132	109	$< 0.001$	78	54	$< 0.001$
	III- IV	35	187		22	107	
Vascular invasion	Absent	158	223	$< 0.001$	92	108	$< 0.001$
	Present	9	73		8	53	
BCLC stage	0 + A	125	127	$< 0.001$	76	69	$< 0.001$
	B + C	42	169		24	92	
Early recurrence	Absent	135	206	0.008	62	120	0.032
	Present	32	90		38	41	
AFP (ng/ml)	$\leq 20$	59	77	0.035	46	46	0.004
	$> 20$	108	219		54	115	
ALT (U/L)	$\leq 40$	122	103	$< 0.001$	72	67	$< 0.001$
	$> 40$	45	193		28	94	
SII ( $\times 10^9/L$ )	$\leq 330$	122	101	$< 0.001$	62	52	$< 0.001$
	$> 330$	45	195		38	109	
ALRI	$\leq 25.2$	129	103	$< 0.001$	69	75	$< 0.001$
	$> 25.2$	38	193		31	86	
Use of NAs	Absent	89	221	$< 0.001$	58	123	0.012
	Present	45	48		23	21	

**Table 2.** Correlation between the clinicopathologic variables and N $\gamma$ LR level in HCC patients (training cohort,  $n = 463$  and validation cohort,  $n = 261$ ). N $\gamma$ LR, neutrophil cell count times  $\gamma$ -glutamyl transpeptidase to lymphocyte count ratio; HBsAg, hepatitis B surface antigen; BCLC, barcelona-clinic liver cancer; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; SII, systemic immune-inflammation index; ALRI, aspartate transaminase to lymphocyte ratio index; NAs, nucleoside analogues.

**Correlation between N $\gamma$ LR and BCLC stage.** BCLC stage is an important prognostic classification system for patients with HCC. Thus, we further analysed the relationship between the N $\gamma$ LR and the BCLC stage. Box plots of the N $\gamma$ LR in relation to the BCLC stage are presented in Fig. 5. In the training cohort, the severity of the BCLC stage was significantly positively correlated with a gradual increase in N $\gamma$ LR ( $r = 0.452$ ,  $P < 0.001$ ) (Fig. 5A). Furthermore, the results of the validation cohort ( $r = 0.415$ ,  $P < 0.001$ ) were the same as those obtained from the training cohort (Fig. 5B).

## Discussion

In this study, we established a simple and evidence-based prognostic index, namely, the N $\gamma$ LR, which incorporates routinely available laboratory parameters to predict the risk of recurrence and poor survival in HCC patients undergoing curative resection. This prognostic index was both accurate and reproducible. Patients in different subgroups of N $\gamma$ LR levels had distinctly different prognoses after surgery. As a linear risk index, N $\gamma$ LR further categorized patients into low, medium, and high risk groups with significantly different survival rates. The performance of the N $\gamma$ LR in predicting the risk stratification for prognosis was validated in another independent validation cohort with similar accuracy.

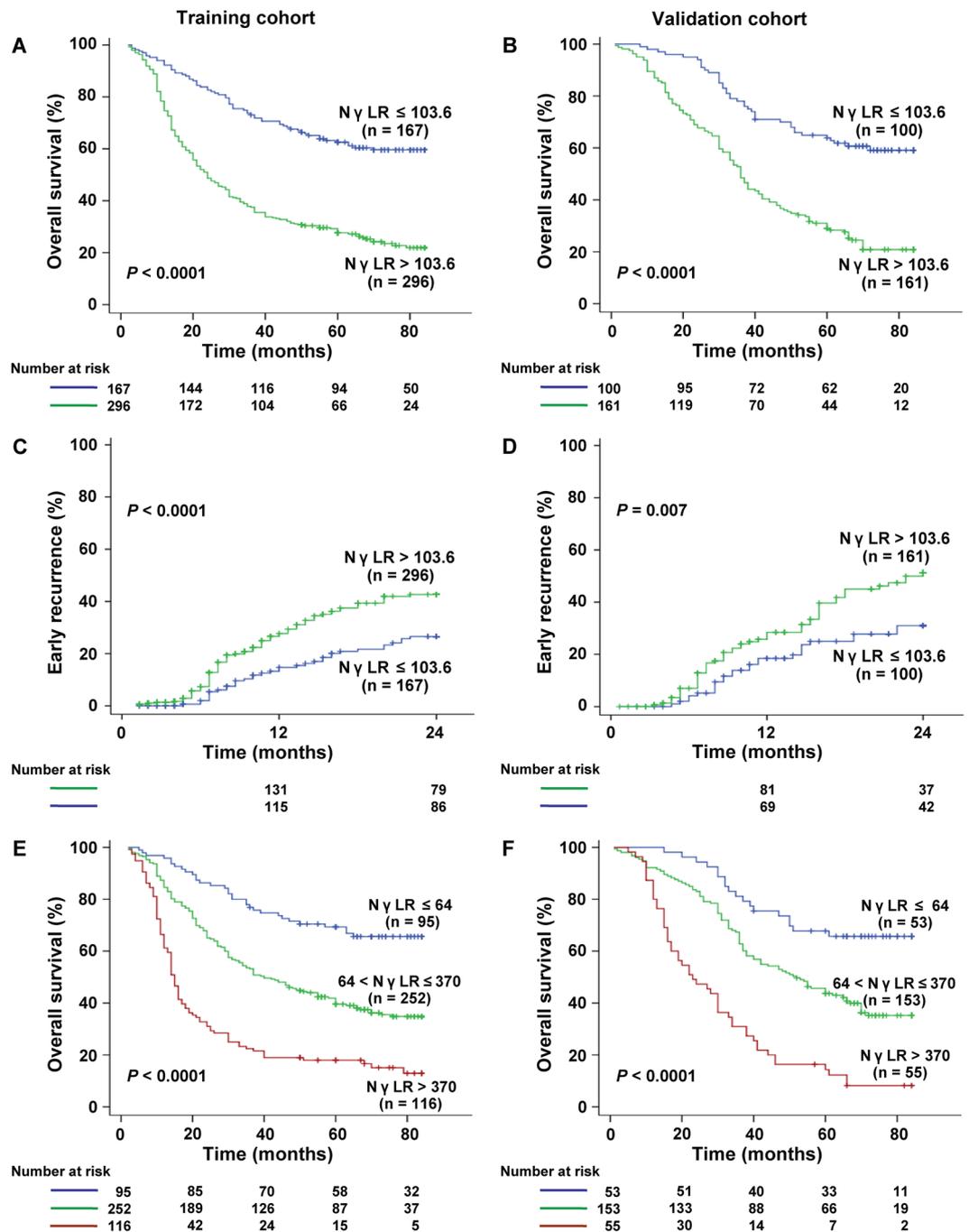
In the current study, the N $\gamma$ LR consisted of three factors: neutrophil count, lymphocyte count, and serum  $\gamma$ -GT level. Neutrophils and lymphocytes are peripheral blood cells that are associated with systemic inflammatory response and immune response. Neutrophils are the key players in inflammatory disorders, while lymphocytes are reflective of the host immune response. Therefore, an increase of N $\gamma$ LR may suggest activation of the inflammatory status and an immune-suppressive response in patients. Elevations of N $\gamma$ LR are usually associated with neutrophilia, elevated  $\gamma$ -GT concentration or lymphopenia. Neutrophils can promote the growth, invasion, and migration of cancer cells by releasing proinflammatory, immunoregulatory, and angiogenic factors<sup>19–21</sup>. Elevated  $\gamma$ -GT was also associated with mortality from many causes, including liver disease, cancer, and diabetes, even when controlling for alcohol consumption and restriction to mild and non-drinkers<sup>22</sup>. Activation of cytotoxic T lymphocytes could induce tumour killing by released cytokines, such as tumour necrosis factor-alpha

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
<i>Training cohort</i>						
Gender (male vs female)	1.26	0.89–1.76	0.092			
Age, y (>55 vs ≤55)	0.81	0.63–1.03	0.087			
HBsAg (positive vs negative)	1.16	0.83–1.61	0.369			
Tumor size, cm (>8 vs ≤8)	3.20	2.58–3.92	<0.001	1.78	1.36–2.40	<0.001
Tumor number (multiple vs single)	1.90	1.51–2.38	<0.001	1.12	0.78–1.49	0.423
Tumor differentiation (III–IV vs I–II)	2.91	2.27–3.65	<0.001	1.21	0.87–1.76	0.362
Vascular invasion (present vs absent)	3.02	2.29–3.96	<0.001	1.63	1.18–2.26	0.004
BCLC (B + C vs 0 + A)	2.70	2.13–3.41	<0.001	1.43	0.92–2.22	0.104
Recurrence (present vs absent)	1.22	0.94–1.61	0.117			
AFP, ng/ml (>20 vs ≤20)	1.38	1.07–1.81	0.014	1.02	0.78–1.31	0.826
ALT, U/L (>40 vs ≤40)	2.20	1.72–2.69	<0.001	1.28	1.07–1.83	0.052
SII, × 10 <sup>9</sup> /L (>330 vs ≤330)	1.86	1.548–2.32	<0.001	1.27	0.97–1.69	0.071
ALRI (>25.2 vs ≤25.2)	2.13	1.71–2.70	<0.001	1.16	0.86–1.59	0.324
N $\gamma$ LR (>103.6 vs ≤103.6)	2.94	2.25–3.86	<0.001	1.65	1.20–2.23	0.002
Use of NAs (present vs absent)	0.52	0.28–0.80	<0.001	0.47	0.24–0.76	0.008
<i>Validation cohort</i>						
Gender (male vs female)	1.11	0.72–1.75	0.521			
Age, y (>55 vs ≤55)	0.82	0.59–1.15	0.246			
HBsAg (positive vs negative)	0.92	0.61–1.36	0.653			
Tumor size, cm (>8 vs ≤8)	3.26	2.41–4.31	<0.001	1.56	1.04–2.35	0.018
Tumor number (multiple vs single)	1.76	1.26–2.47	0.001	1.39	0.91–2.12	0.133
Tumor differentiation (III–IV vs I–II)	3.23	2.38–4.31	<0.001	1.37	0.86–2.18	0.185
Vascular invasion (present vs absent)	3.12	2.30–4.28	<0.001	1.49	0.96–2.34	0.078
BCLC (B + C vs 0 + A)	2.76	2.04–3.77	<0.001	1.41	0.86–2.31	0.171
Recurrence (present vs absent)	1.14	0.86–1.62	0.323			
AFP, ng/ml (>20 vs ≤20)	2.26	1.69–3.27	<0.001	1.46	1.09–2.07	0.086
ALT, U/L (>40 vs ≤40)	1.79	1.35–2.38	0.002	1.11	0.74–1.75	0.522
SII, × 10 <sup>9</sup> /L (>330 vs ≤330)	1.49	1.13–2.01	0.006	0.98	0.69–1.37	0.703
ALRI (>25.2 vs ≤25.2)	1.76	1.29–2.38	0.001	1.19	0.81–1.76	0.335
N $\gamma$ LR (>103.6 vs ≤103.6)	2.89	2.05–4.12	<0.001	1.91	1.31–2.73	0.001
Use of NAs (present vs absent)	0.47	0.22–0.71	<0.001	0.36	0.18–0.59	0.040

**Table 3.** Analysis of overall survival in HCC patients in the training and validation cohort. HBsAg, hepatitis B surface antigen; BCLC, barcelona-clinic liver cancer; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; SII, systemic immune-inflammation index; ALRI, aspartate transaminase to lymphocyte ratio index; N $\gamma$ LR, neutrophil cell count times  $\gamma$ -glutamyl transpeptidase to lymphocyte count ratio; NAs, nucleoside analogues.

and interferon-gamma. Meanwhile, endogenous T cells significantly delay malignant progression by responding to and infiltrating tumours<sup>23</sup>. Thus, it can be speculated that lymphopenia cannot effectively protect against the development of HCC by strengthening the body's immune response. Accordingly, all of these were adverse factors for HCC patients. The N $\gamma$ LR integration of these three factors can better reflect their impact on HCC. In addition, our findings show that high N $\gamma$ LR was positively correlated with larger and multiple tumour number, poor differentiation, vascular invasion, severe BCLC stage, and early recurrence. It is postulated that N $\gamma$ LR represents a systemic inflammatory response; the elevation of N $\gamma$ LR may play an important role in maintaining the malignant phenotype of HCC cells and persistent damage of liver inflammation via internal environment disorders, thereby promoting the recurrence and metastasis of HCC. Our observation is consistent with a gene expression analysis that indicated that HCC metastases and relapse might be promoted by a shift towards anti-inflammatory/immune-suppressive responses<sup>24</sup>.

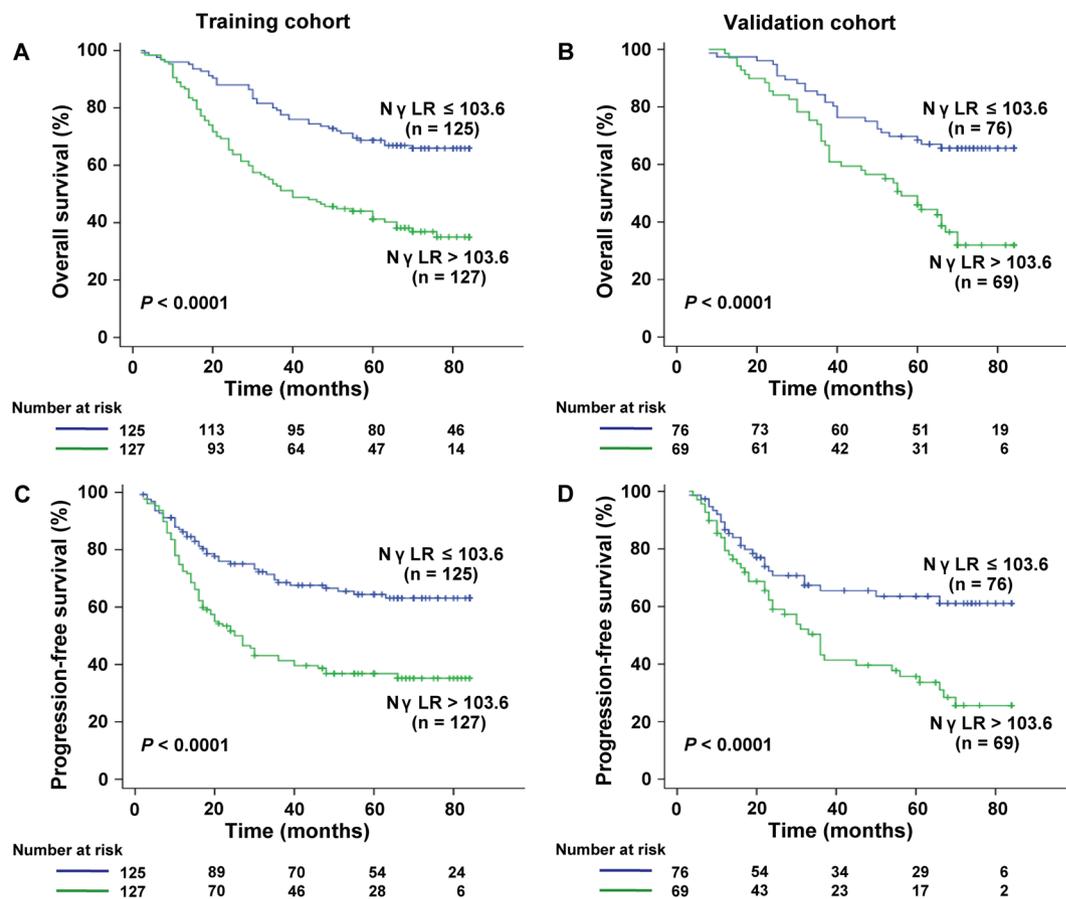
To date, AFP remains the most popular marker for the diagnosis and management of HCC. However, the sensitivity and specificity of AFP are limited; not all HCC patients secrete AFP. The sensitivity and specificity of AFP are only 61% and 81%, respectively, with a cut-off value of 20 ng/mL at the time of diagnosis<sup>25</sup>. Therefore, new markers are greatly needed to improve the ability to predict the prognosis of patients. In this study, we found that AUC for the N $\gamma$ LR was higher than that of AFP, and N $\gamma$ LR may be a novel prognostic marker for HCC patients. Moreover, our data showed that N $\gamma$ LR > 103.6 was also strongly correlated with the early recurrence of HCC. We know that the postoperative recurrence of HCC has two distinct mechanisms: the first is early recurrence via metastasis arising from undetected dissemination of tumour before surgery, and the other is late recurrence via de novo primary HCC in the liver remnant of dysplastic hepatocytes<sup>26</sup>. Postoperative recurrence, especially early recurrence, impacting the outcome of HCC is a very distressing situation for clinicians and patients. To date, no optimal marker has been identified. Although AFP is widely used in the postoperative monitoring of HCC, there



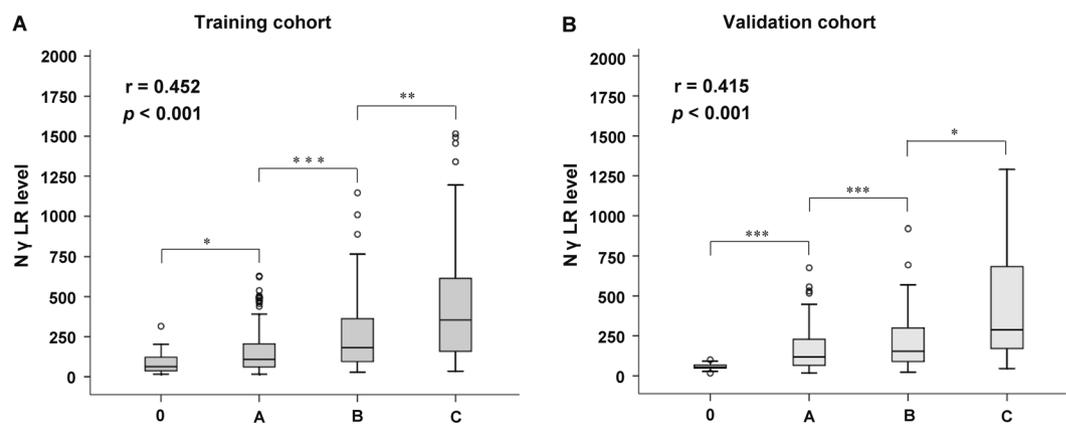
**Figure 3.** Prognostic significance of  $N\gamma LR$  in patients with HCC after surgery. The Kaplan-Meier curves depict overall survival (A,B) and recurrence rates (C,D) in HCC patients with  $N\gamma LR > 103.6$  or  $\leq 103.6$  and estimate the overall survival according to the optimized  $N\gamma LR$  (E-F) in the training and validation cohorts.

are still 30% to 40% of HCC patients with normal serum AFP. Therefore, it is imperative to find other alternative predictor to compensate for this deficiency. Encouragingly, our study suggested that  $N\gamma LR$  could serve as a new marker predicting early recurrence for postsurgical HCC patients. Accordingly,  $N\gamma LR$ , as an inexpensive, objective, and readily available index, might be a promising marker of auxiliary diagnosis and postoperative recurrence monitoring for patients with HCC in clinical practice.

Previous studies have confirmed the prognostic values of SII<sup>16</sup> and ALRI<sup>17</sup> in HCC patients after surgery. The AUC of SII and ALRI to predict survival were lower than that of  $N\gamma LR$  in this cohort; the same studies show inconsistent results, which may be associated with differences in the samples. Meanwhile, the prediction ability of  $N\gamma LR$  was also higher than the conventional AFP. Therefore,  $N\gamma LR$  had fairly good discriminatory power in stratifying patients with HCC into different prognostic groups.



**Figure 4.** Prognostic significance of  $N\gamma LR$  in early HCC patient. Kaplan-Meier curves depict overall survival (A,B) and progression-free survival (C,D) in early HCC patients (BCLC 0 + A) with  $N\gamma LR > 103.6$  or  $\leq 103.6$  in the training and validation cohorts.



**Figure 5.** Box plots of  $N\gamma LR$  values according to the BCLC stage in the training (A) and validation (B) cohorts. (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ )

It is widely accepted that BCLC stage is the most popular staging system with the power of prognostic stratification and therapeutic allocation<sup>27,28</sup>. In this study, when we further explored the relationship between the  $N\gamma LR$  and the BCLC stage, we found that the  $N\gamma LR$  gradually increased with the increasing severity of BCLC stages. Meanwhile, the elevation of  $N\gamma LR$  was associated with increasing risks of death and recurrence of HCC, and the  $N\gamma LR$  had a fairly good prognostic power in both training and validation cohorts. Our study also demonstrated that  $N\gamma LR$  still had a strong prognostic significance in early HCC patients. On the whole, our data indicated that  $N\gamma LR$  could serve as a powerful prognostic marker for patients with HCC. The predictive significance of the

N $\gamma$ LR in these subgroups is of great importance for clinicians to select the appropriate intervention after surgical resection to avoid unproven and futile treatment.

The N $\gamma$ LR was built in a cohort of patients with mainly HBV-related HCC. Effective antiviral therapy may potentially influence the prognosis of these patients. This finding is consistent with previously reported results that antiviral treatment is effective in a better survival<sup>29</sup>. However, antiviral therapy is prescribed in patients with a higher viral load and abnormal serum ALT levels, irrespective of cancer classification. This might explain why the predictive capability of N $\gamma$ LR may not be influenced by antiviral therapy.

The elevation of serum  $\gamma$ -GT concentration is strongly associated with heavy alcohol consumption. However, Ruhl *et al.*<sup>22</sup> reported that a high relative risk of liver disease mortality with elevated  $\gamma$ -GT was not influenced by alcohol consumption. Therefore, alcohol consumption does not diminish the accuracy of the N $\gamma$ LR index in predicting prognosis of HCC. Moreover, HCC patients with alcohol consumption often stop drinking alcohol under doctors' suggestions after being diagnosed with HCC.

We acknowledge that there are some limitations to our study. First, its retrospective nature is a potential limitation. Second, our study included HCC patients only from a single centre with a vast majority of patients having HBV infection. Although internally validated, whether our index can be generalized to different geographical areas remains to be determined. Third, the N $\gamma$ LR has been characterized in HCC patients undergoing curative resection; the prognostic ability of N $\gamma$ LR in palliative settings needs to be further explored in a future study.

In conclusion, we proposed a novel and simple prognostic index, the N $\gamma$ LR, including three readily available laboratory results for HCC patients after curative resection with a relative high degree of accuracy. The concept of the ratios among these three variables in the prediction of prognosis in patients with HCC is novel. N $\gamma$ LR can effectively identify the patients with HCC who are at the greatest risk of poor survival and early recurrence after surgery. It may be a useful tool for clinicians who manage postoperative patients with HCC. A prospective study to validate the N $\gamma$ LR index is being planned.

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### Acknowledgements

This work was supported in part by the National Natural Science Foundation of China (Grant No. 81372163), the Natural Science Foundation of Guangxi (Grant No. 2015GXNSFAA139111), and the Science and Technology Planning Project of Guilin (Grant No. 20150206-1-10), the Program of Guangxi Zhuang Autonomous Region health and Family Planning Commission (Grant No. Z2016562).

### Author Contributions

Weijia Liao designed the research, obtained funding and revised the article; Jun Li, Yan Liao, Pengpeng Zhu, Xinhuang Chen, Wei Dang, Minjun Liao, Liya Suo, Liling Qin and Weijia Liao collected data; Weijia Liao, Jun Li and Yan Liao performed the data analysis, model development and interpretation; Jun Li and Weijia Liao wrote the manuscript. All the authors have read and approved the final manuscript.

### Additional Information

**Supplementary information** accompanies this paper at doi:[10.1038/s41598-017-09696-y](https://doi.org/10.1038/s41598-017-09696-y)

**Competing Interests:** The authors declare that they have no competing interests.

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