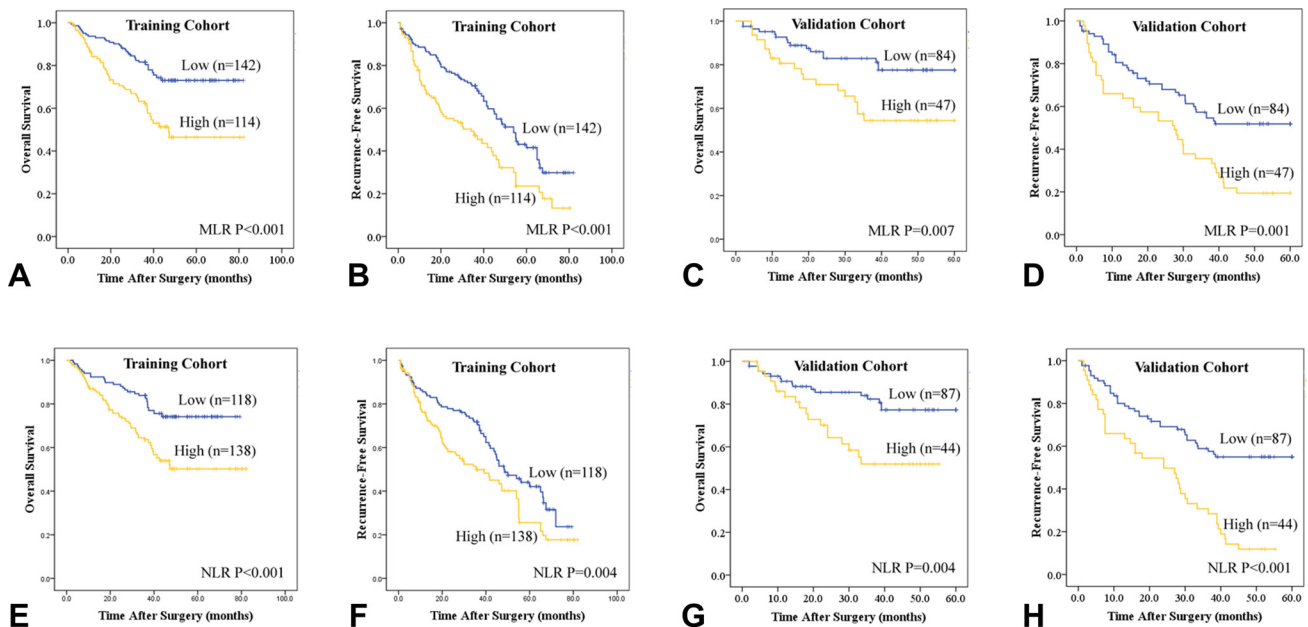
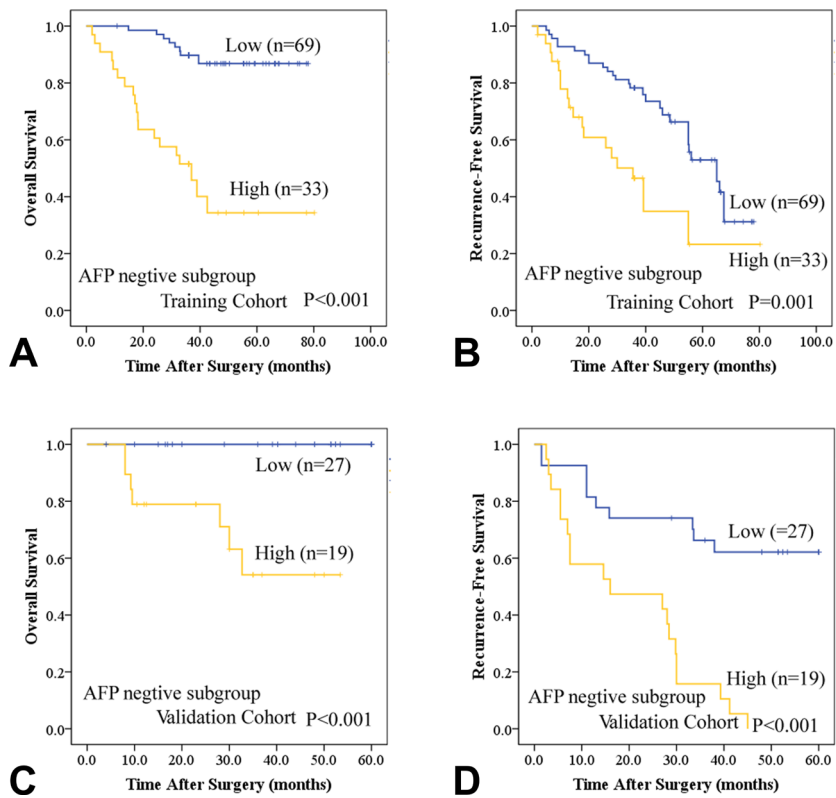


# Systemic and intratumoral balances between monocytes/macrophages and lymphocytes predict prognosis in hepatocellular carcinoma patients after surgery

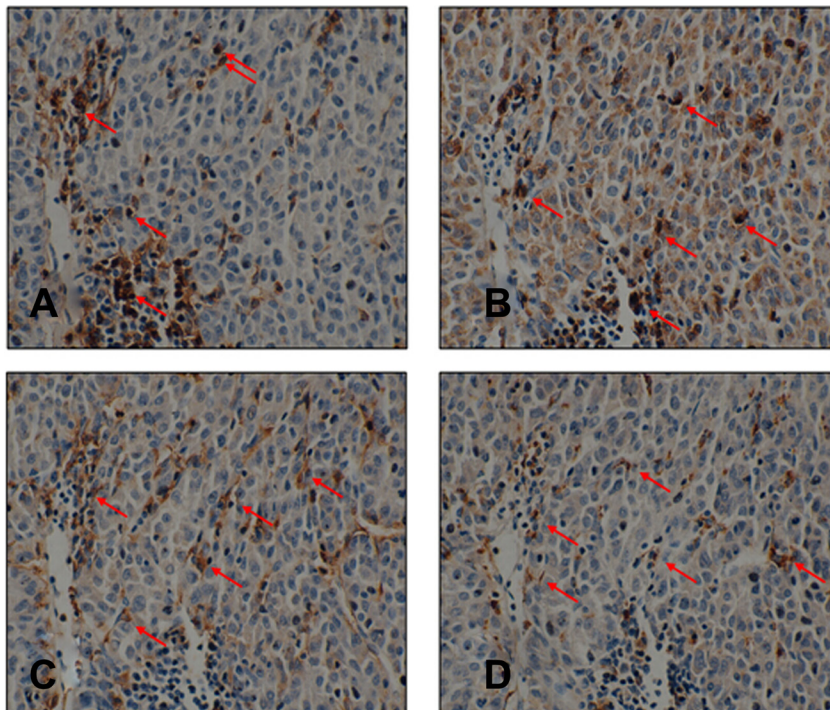
## Supplementary Materials



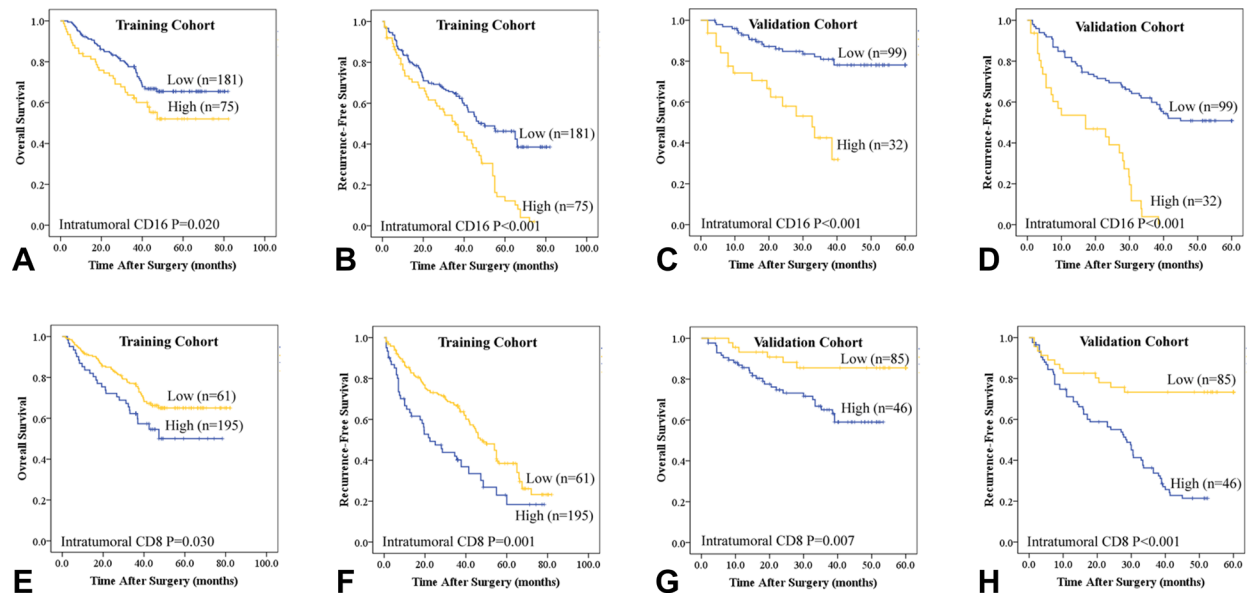
Supplementary Figure S1: Comparison of overall survival (OS, A, C, E, and G) and recurrence-free survival (RFS, B, D, F, and H) based on peripheral monocyte to lymphocyte ratio (MLR, A–D) and neutrophil to lymphocyte ratio (NLR, E–H) in the training (A, B, E, and F) and validation cohorts (C, D, G, and H).



**Supplementary Figure S2: Kaplan-Meier analyses of overall survival (OS, A and C) and recurrence-free survival (RFS, B and D) based on peripheral neutrophil and monocyte to lymphocyte ratio (NMLR) in the negative AFP subgroup in the training (A and B) and validation cohorts (C and D).**



**Supplementary Figure S3: Immunohistochemical staining of intratumoral CD4 (A), CD8 (B), CD16 (C), and CD68 (D) in a representative case. Positive cells are stained brown (red arrows, 200 $\times$ ).**



**Supplementary Figure S4: Prognostic significance of CD16 and CD8 in HCC patients undergoing resection.** Kaplan-Meier analyses showed the overall survival (OS, A, C, E, and G) and recurrence-free survival (RFS, B, D, F, and H) based on CD16 (A–D) and CD8 (E–H) in the training (A, B, E, and F) and validation cohorts (C, D, G, and H).

**Supplementary Table S1: Univariate and multivariate analyses for early recurrence subgroups**

Factors	Training Cohort (n = 81)		Validation Cohort (n = 48)			
	Univariate	Multivariate	Univariate	Multivariate		
	P	HR (95% CI)	P	P	HR (95% CI)	P
Tumor size, cm (≤ 5.0 vs > 5.0)	<b>0.05</b>		0.666	0.135		NA
Tumor number (Single vs Multiple)	0.105		NA	<b>0.016</b>		0.131
Tumor differentiation (I–II vs III–IV)	0.144		NA	<b>&lt; 0.001</b>	0.017 (0.000–0.843)	<b>0.041</b>
BCLC stage (0/A vs B/C)	<b>0.025</b>	1.971 (1.039–3.742)	<b>0.038</b>	<b>0.015</b>		0.052
NLR (≤ 2.5 vs > 2.5)	0.684		NA	<b>0.017</b>		0.189
MLR (≤ 0.3 vs > 0.3)	0.142		NA	<b>0.05</b>		0.183
NMLR (≤ 1.2 vs > 1.2)	<b>0.012</b>	2.072 (1.126–3.811)	<b>0.019</b>	<b>&lt; 0.001</b>	5.478 (1.848–16.238)	<b>0.010</b>

Univariate analysis: Kaplan-Meier method; multivariate analysis: Cox proportional hazards regression model.  
Abbreviations: HR: Hazard Ratio; BCLC: Barcelona clinic liver cancer; NLR: neutrophil to lymphocyte ratio; MLR: monocyte to lymphocyte ratio; NMLR: neutrophil and monocyte to lymphocyte ratio; NA: not adopted.

**Supplementary Table S2: Univariate and multivariate analyses of prognostic factors in the validation cohort (n = 131)**

Factors	RFS			OS		
	Univariate	Multivariate		Univariate	Multivariate	
	<i>P</i>	HR (95% CI)	<i>P</i>	<i>P</i>	HR (95% CI)	<i>P</i>
Age, year (≤ 50 vs > 50)	0.234		NA	0.055		NA
Gender (Female vs Male)	0.645		NA	0.323		NA
ALT, U/L (≤ 40 vs > 40)	0.477		NA	0.340		NA
Liver cirrhosis (Yes vs No)	0.151		NA	0.733		NA
HBsAg (Positive vs Negative)	0.530		NA	0.621		NA
AFP, ng/ml (≤ 20 vs > 20)	0.508		NA	0.055		NA
Platelet count, 109/L (≤ 100 vs > 100)	0.192		NA	0.033		0.161
Tumor encapsulation (Yes vs No)	0.361		NA	0.335		NA
Tumor number (Single vs Multiple)	0.446		NA	0.771		NA
Vascular invasion (Yes vs No)	<b>0.008</b>	2.515 (1.257–5.030)	<b>0.009</b>	0.001	6.408 (2.526–16.254)	<b>&lt; 0.001</b>
Tumor differentiation (I–II vs III–IV)	0.212		NA	0.877		NA
Tumor size, cm (≤ 5.0 vs > 5.0)	<b>0.012</b>	2.192 (1.332–3.610)	<b>0.002</b>	<b>&lt; 0.001</b>	6.154 (2.982–12.699)	<b>&lt; 0.001</b>
TNM stage (I vs II–III)	<b>0.006</b>		0.951	<b>0.001</b>		0.801
BCLC stage (0/A vs B/C)	<b>0.004</b>		0.192	<b>&lt; 0.001</b>		0.988
NLR (≤ 2.5 vs > 2.5)	<b>&lt; 0.001</b>		0.064	<b>0.004</b>		0.699
MLR (≤ 0.3 vs > 0.3)	<b>0.001</b>		0.916	<b>0.007</b>		0.790
NMLR (≤ 1.2 vs > 1.2)	<b>&lt; 0.001</b>	3.044 (1.864–4.972)	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	4.403 (2.104–9.215)	<b>&lt; 0.001</b>
Intratumoral CD16	<b>&lt; 0.001</b>	3.422 (2.018–5.803)	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	2.407 (1.118–5.179)	<b>0.025</b>
Intratumoral CD8	<b>&lt; 0.001</b>	0.412 (0.217–0.784)	<b>0.007</b>	<b>0.007</b>		<b>0.624</b>
Intratumoral CD16/CD8 (≤ 1.1 vs > 1.1)	<b>&lt; 0.001</b>		0.068	<b>0.001</b>	2.337 (1.018–5.364)	<b>0.045</b>

Univariate analysis: Kaplan-Meier method; multivariate analysis: Cox proportional hazards regression model. Abbreviations: RFS: Recurrence-Free Survival; OS: overall survival; ALT: alanine aminotransferase; HBsAg: hepatitis B surface antigen; AFP: alpha fetoprotein; TNM: tumor-nodes-metastasis; NLR: neutrophil to lymphocyte ratio; MLR: monocyte to lymphocyte ratio; NMLR: neutrophil and monocyte to lymphocyte ratio; NA: not adopted.

**Supplementary Table S3: Correlation between NMLR and clinicopathological characteristics of patients in the training and validation cohorts**

Characteristics		NMLR					
		Training Cohort (n = 256)			Validation Cohort (n = 131)		
		≤ 1.2	> 1.2	P	≤ 1.2	> 1.2	P
Age (year)	≤ 50	61	46	0.703	42	20	1.000
	> 50	81	68		47	22	
Gender	Female	21	21	0.498	6	12	<b>0.002</b>
	Male	121	93		83	30	
ALT (U/L)	≤ 40	85	51	<b>0.017</b>	50	19	0.265
	> 40	57	63		39	23	
Liver cirrhosis	Yes	123	100	0.853	83	40	1.000
	No	19	14		6	2	
HBsAg	Positive	110	98	0.107	76	40	0.142
	Negative	32	16		13	2	
AFP (ng/ml)	≤ 20	63	39	0.123	27	19	0.118
	> 20	79	75		62	23	
Platelet count (109/L)	≤ 100	31	25	1.000	28	13	1.000
	> 100	111	89		61	29	
Tumor number	Single	130	92	<b>0.015</b>	80	38	1.000
	Multiple	12	22		9	4	
Vascular invasion	Yes	41	40	0.344	13	5	0.790
	No	101	74		76	37	
Tumor differentiation	I/II	111	81	0.195	61	34	0.149
	I/II	31	33		28	8	
Tumor encapsulation	Yes	83	58	0.256	47	18	0.350
	No	59	56		42	24	
Tumor size (cm)	≤ 5.0	108	64	<b>0.001</b>	62	26	0.427
	> 5.0	34	50		27	16	
TNM stage	I	105	72	0.077	65	22	<b>0.029</b>
	II/III	37	42		24	20	
BCLC stage	0/A	82	37	< <b>0.001</b>	50	20	0.453
	B/C	60	77		39	22	

Abbreviations: NMLR: neutrophil and monocyte to lymphocyte ratio; ALT: alanine aminotransferase; HBsAg: hepatitis B surface antigen; AFP: alpha fetoprotein; TNM: tumor node metastasis; BCLC: Barcelona clinic liver cancer.

**Supplementary Table S4: Correlation between NLR and MLR and clinicopathological characteristics of patients in the training and validation cohorts**

Characteristics		NLR						MLR					
		Training Cohort (n = 256)			Validation Cohort (n = 131)			Training Cohort (n = 256)			Validation Cohort (n = 131)		
		≤ 2.5	> 2.5	P	≤ 2.5	> 2.5	P	≤ 0.3	> 0.3	P	≤ 0.3	> 0.3	P
Age (year)	≤ 50	49	58	1.000	43	19	0.580	44	63	0.374	40	22	1.000
	> 50	69	80		44	25		70	79		44	25	
Gender	Female	15	27	0.176	79	34	0.057	25	17	<b>0.041</b>	76	37	0.070
	Male	103	111		8	10		89	125		8	10	
ALT (U/L)	≤ 40	59	77	0.381	46	23	1.000	54	82	0.103	48	21	0.203
	> 40	59	61		41	21		60	60		36	26	
Liver cirrhosis	Yes	102	121	0.852	81	42	0.717	103	120	0.192	77	46	0.258
	No	16	17		6	2		11	22		7	1	
HBsAg	Positive	91	117	0.148	75	41	0.384	97	111	0.198	13	2	0.083
	Negative	27	21		12	3		17	31		71	45	
AFP (ng/ml)	≤ 20	48	54	0.898	27	19	0.180	41	61	0.304	26	20	0.189
	> 20	70	84		60	25		73	81		58	27	
Platelet count (109/L)	≤ 100	16	40	<b>0.004</b>	27	14	1.000	23	33	0.649	25	16	0.695
	> 100	102	98		60	30		91	109		59	31	
Tumor number	Single	104	118	0.583	80	38	0.359	92	130	<b>0.015</b>	74	44	0.376
	Multiple	14	20		7	6		22	12		10	3	
Vascular invasion	Yes	79	96	0.687	12	6	1.000	42	39	0.137	14	4	0.290
	No	39	42		75	38		72	103		70	43	
Tumor differentiation	I-II	89	103	1.000	60	35	0.221	82	110	0.314	59	36	0.541
	III-IV	29	35		27	9		32	32		25	11	
Tumor encapsulation	Yes	65	76	1.000	43	22	1.000	59	82	0.377	43	22	0.716
	No	53	62		44	22		55	60		41	25	
Tumor size (cm)	≤ 5.0	84	88	0.231	59	29	0.846	65	107	<b>0.002</b>	56	32	1.000
	> 5.0	34	50		28	15		49	35		28	15	
TNM stage	I	82	95	1.000	59	28	0.697	71	106	<b>0.041</b>	62	25	<b>0.021</b>
	II-III	36	43		28	16		43	36		22	22	
BCLC stage	0/A	61	58	0.133	48	22	0.584	38	81	<b>&lt; 0.001</b>	43	27	0.584
	B/C	57	80		39	22		76	61		41	20	

Abbreviations: NLR: neutrophil to lymphocyte ratio; MLR: monocyte to lymphocyte ratio; ALT: alanine aminotransferase; HBsAg: hepatitis B surface antigen; AFP: alpha fetoprotein; TNM: tumor node metastasis; BCLC: Barcelona clinic liver cancer.

**Supplementary Table S5: Correlation between NMLR and other inflammation associated parameters**

Parameters		NMLR ( $\leq 1.2$ vs $> 1.2$ )					
		Training Cohort ( <i>n</i> = 256)			Validation Cohort ( <i>n</i> = 131)		
		<i>n</i>	<i>r</i>	<i>p</i>	<i>n</i>	<i>r</i>	<i>P</i>
CRP	$\leq 10$ mg/L	232	0.089	0.154	114	0.095	0.282
	$> 10$ mg/L	24			17		
NLR	$\leq 2.5$	118	0.513	$< 0.001$	87	0.689	$< 0.001$
	$> 2.5$	138			44		
MLR	$\leq 0.3$	114	0.858	$< 0.001$	84	0.714	$< 0.001$
	$> 0.3$	142			47		
GPS	0	231	0.129	0.039	111	0.168	0.042
	1/2	25			20		
PI	0	232	0.061	0.329	114	0.075	0.392
	1/2	24			17		
PNI	0	220	0.180	0.004	114	0.221	0.011
	1	36			17		

Abbreviations: NMLR: neutrophil and monocyte to lymphocyte ratio; CRP: C-reactive protein; NLR: neutrophil to lymphocyte ratio; MLR: monocyte to lymphocyte ratio; GPS: Glasgow prognostic score; PI: prognostic index; PNI: prognostic nutritional index.

**Supplementary Table S6: Correlation between iMLR and clinicopathological characteristics of patients in the training and validation cohorts**

Characteristics		iMLR					
		Training Cohort ( <i>n</i> = 256)			Validation Cohort ( <i>n</i> = 131)		
		$\leq 1.1$	$> 1.1$	<i>P</i>	$\leq 1.1$	$> 1.1$	<i>P</i>
Age (year)	$\leq 50$	52	55	0.900	24	38	0.114
	$> 50$	71	78		37	32	
Gender	Female	22	20	0.613	52	61	0.803
	Male	101	113		9	9	
ALT (U/L)	$\leq 40$	67	69	0.708	33	36	0.861
	$> 40$	56	64		28	34	
Liver cirrhosis	Yes	112	111	0.092	60	63	0.067
	No	11	22		1	7	
HBsAg	Positive	103	105	0.341	55	61	0.784
	Negative	20	28		6	9	
AFP (ng/ml)	$\leq 20$	50	52	0.898	16	30	0.066
	$> 20$	73	81		45	40	
Platelet count (109/L)	$\leq 100$	32	24	0.133	22	19	0.345
	$> 100$	91	109		39	51	
Tumor number	Single	108	114	0.713	56	62	0.574
	Multiple	15	19		5	8	
Vascular invasion	Yes	34	47	0.226	7	11	0.613
	No	89	86		54	59	
Tumor differentiation	I-II	91	101	0.773	42	53	0.435
	III-IV	32	32		19	17	
Tumor encapsulation	Yes	68	73	1.000	29	36	0.727
	No	55	60		32	34	

Tumor size (cm)	≤ 5.0	85	87	0.595	45	43	0.142
	> 5.0	38	46		16	27	
TNM stage	I	83	94	0.591	47	40	<b>0.026</b>
	II-III	40	39		14	30	
BCLC stage	0/A	66	53	<b>0.033</b>	39	31	<b>0.035</b>
	B/C	57	80		22	39	

Abbreviations: iMLR: intratumoral CD16/CD8 ratio; ALT: alanine aminotransferase; HBsAg: hepatitis B surface antigen; AFP: alpha fetoprotein; TNM: tumor node metastasis; BCLC: Barcelona clinic liver cancer.

**Supplementary Table S7: The area under the receiver operating characteristic curve (AUROC) of patients in the training and validation cohorts**

Characteristics		AUROC (95% CI)			
		Training Cohort (n = 256)		Validation Cohort (n = 131)	
		RFS	OS	RFS	OS
AFP (ng/ml)	≤ 20	0.568	0.557	0.458	0.603
	> 20	(0.497–0.639)	(0.483–0.632)	(0.359–0.558)	(0.498–0.708)
Tumor number	Single	0.533	0.552	0.524	0.510
	Multiple	(0.462–0.603)	(0.475–0.629)	(0.425–0.624)	(0.397–0.623)
Vascular invasion	Yes	0.564	0.592	0.511	0.562
	No	(0.493–0.634)	(0.517–0.668)	(0.411–0.611)	(0.447–0.678)
Tumor differentiation	I/II	0.551	0.544	0.444	0.507
	III/IV	(0.481–0.622)	(0.468–0.621)	(0.343–0.544)	(0.395–0.620)
Tumor encapsulation	Yes	0.533	0.529	0.441	0.546
	No	(0.462–0.604)	(0.453–0.604)	(0.341–0.541)	(0.435–0.658)
Tumor size (cm)	≤ 5.0	0.582	0.654	0.537	0.685
	> 5.0	(0.512–0.652)	(0.581–0.728)	(0.437–0.637)	(0.578–0.793)
TNM stage	I	0.533	0.616	0.591	0.641
	II/III	(0.462–0.604)	(0.541–0.691)	(0.493–0.688)	(0.531–0.752)
BCLC stage	0/A	0.611	0.651	0.564	0.709
	B/C	(0.541–0.680)	(0.581–0.722)	(0.464–0.663)	(0.610–0.808)
MLR	≤ 0.3	0.569	0.620	0.657	0.626
	> 0.3	(0.499–0.640)	(0.547–0.694)	(0.564–0.751)	(0.515–0.736)
NLR	≤ 2.5	0.551	0.613	0.700	0.622
	> 2.5	(0.480–0.622)	(0.540–0.685)	(0.610–0.789)	(0.510–0.733)
NMLR	≤ 1.2	0.593	0.700	0.733	0.691
	> 1.2	(0.523–0.663)	(0.631–0.769)	(0.648–0.819)	(0.583–0.798)
iMLR	≤ 1.1	0.620	0.574	0.686	0.642
	> 1.1	(0.551–0.690)	(0.500–0.648)	(0.593–0.779)	(0.538–0.747)
Combination of NMLR and iMLR	both low	0.646	0.723	0.818	0.755
	both high	(0.579–0.714)	(0.656–0.789)	(0.746–0.889)	(0.665–0.845)

Abbreviations: RFS: recurrence-free survival; OS: overall survival; NMLR: neutrophil and monocyte to lymphocyte ratio; MLR: monocyte to lymphocyte ratio; NLR: neutrophil to lymphocyte ratio; iMLR: intratumoral CD16/CD8 ratio; AFP: alpha fetoprotein; TNM: tumor node metastasis; BCLC: Barcelona clinic liver cancer.