

Original Article

Prognostic value of systemic inflammatory response markers in patients with intrahepatic cholangiocarcinoma

Chenyue Zhang^{1,3}, Haiyong Wang^{1,2}, Zhouyu Ning^{1,3}, Litao Xu^{1,3}, Liping Zhuang^{1,3}, Peng Wang^{1,3}, Zhiqiang Meng^{1,3}

¹Department of Integrative Oncology, Fudan University Shanghai Cancer Center, 270 Dong An Road, Shanghai 200032, China; ²Department of Radiation Oncology, Shandong Cancer Hospital & Institute, 440 Jiyuan Road, Jinan 250117, China; ³Department of Oncology, Shanghai Medical College, Fudan University, 130 Dong An Road, Shanghai 200032, China

Received January 7, 2016; Accepted May 8, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: Inflammation is a key feature of cancer, and systemic inflammatory responses (SIRs) have been associated with poor prognosis in patients with various types of cancers. However, studies concerning the role of SIR markers in intrahepatic cholangiocarcinoma (ICC) are relatively limited. Therefore, in the present study, we analyzed the prognostic value of SIR markers in ICC patients. A total of 187 patients with ICC were retrospectively recruited from January 2011 to August 2015 at the Fudan University Shanghai Cancer Center. The association of SIR markers including white blood cell (WBC) count; absolute neutrophil, lymphocyte, and monocyte counts; platelet count, neutrophil: lymphocyte ratio (NLR), platelet: lymphocyte ratio (PLR), and lymphocyte:monocyte ratio (LMR) with overall survival (OS) were determined by Kaplan-Meier analysis and multivariate Cox proportional hazards regression model. Results revealed that high WBC, neutrophil, monocyte, and platelet counts and high NLR, PLR, and a low LMR were significantly correlated with decreased OS. Multivariate analysis demonstrated that WBC count (hazard ratio [HR]=1.932, 95% confidence interval [CI]: 1.376-2.711; P<0.001), neutrophil count (HR=1.755, 95% CI: 1.258-2.448; P=0.001), platelet count (HR=1.483, 95% CI: 1.069-2.057, P=0.018), monocyte count (HR=1.398, 95% CI: 1.008-1.939, P=0.045), NLR (HR=1.700, 95% CI: 1.222-2.366, P=0.002), PLR (HR=1.390, 95% CI: 1.001-1.930, P=0.049), and LMR (HR=0.670, 95% CI: 0.483-0.931, P=0.017) were independently associated with OS. These findings indicated that SIR markers are independent prognostic factors that might be useful for individual risk assessment in patients with ICC.

Keywords: Intrahepatic cholangiocarcinoma, prognosis, systemic inflammatory response, NLR, PLR, LMR

Introduction

Intrahepatic cholangiocarcinoma (ICC) is a primary malignancy of the biliary tract, and is the second most common liver tumor after hepatocellular carcinoma [1]. ICC is very aggressive and is associated with a dismal prognosis. The incidence and mortality of ICC appear to be increasing [2], and in contrast to other tumors of the liver and gastrointestinal tract, its pathogenesis remains obscure [3]. Median survival is no longer than 1 year even with surgery, systemic chemotherapy, and radiotherapy [4], but clinical studies have shown that the survival of individual ICC patient varies significantly.

Commonly accepted predictors of survival include intrahepatic satellite lesions, lymph node invasion, and distant metastasis [5].

Inflammation is widely acknowledged as a hallmark of cancer [6]. There has been a recent increase in investigations of the promotion of inflammatory processes by tumors, which results in DNA damage and accelerated angiogenesis [7-9]. Inflammatory status can be monitored by the Glasgow Prognostic Score, which is calculated from serum levels of C-reactive protein and albumin [10]. Recently, other markers of inflammation such as the neutrophil:lymphocyte ratio (NLR), platelet: lympho-

Prognostic value of SIR markers in ICC patients

Table 1. Clinical characteristics of intrahepatic cholangiocarcinoma patients

Variables		N=187, %
Age (years)	Mean±SD	58.14±11.72
Gender	Male	117 (62.6)
	Female	70 (37.4)
Stage	Locally advanced	40
	Metastatic	147
Lymph node invasion	No	71 (38.0)
	Yes	116 (62.0)
CA19-9	<500 IU/ml	106 (56.7)
	≥500 IU/ml	81 (43.3)
WBC	Median (Range)	6.3 (2-19.5)
Neutrophil	Median (Range)	4.6 (1-17.7)
Lymphocyte	Median (Range)	1.4 (0.3-3.8)
PLT	Median (Range)	196 (44-614)
Monocyte	Median (Range)	0.5 (0.1-1.3)

cyte ratio (PLR), and lymphocyte:monocyte ratio (LMR) have been associated with poor prognosis in breast, pancreatic, and ovarian cancer patients [11-13]. However, very few studies have reported on the prognostic value of systemic inflammatory response (SIR) markers in ICC until recent studies demonstrated that preoperative NLR and PLR are predictors for prognosis after hepatic resection in ICC patients [14-16]. The aim of this study was to clarify the prognostic value of these parameters in a cohort of patients with ICC.

Materials and methods

Subjects

This study was approved by the Ethics Committee of Fudan University Shanghai Cancer Center, Shanghai, China, and written informed consent was obtained from each participant in accordance with institutional guidelines. A total of 187 patients pathologically diagnosed with ICC were included in this retrospective study. All had been treated between January 2011 and August 2015 at our cancer center. Standard radiological studies included contrast-enhanced abdominal computed tomography scans, magnetic resonance imaging, and magnetic resonance cholangiopancreatography. Given the fact that acute infection could affect the fluctuations in blood cell count, patients with acute infectious diseases were excluded from the analysis. The majority of

excluded patients had been diagnosed with acute pancreatitis or cholangitis.

Laboratory measurements

Routine laboratory measurements included WBC, neutrophil, lymphocyte, monocyte, and platelet counts, which were performed prior to cancer diagnostic interventions or treatments. The NLR, PLR, and LMR were calculated.

Statistical analyses

All data were expressed as mean ± standard deviation. OS was defined as the interval between the date of a definitive diagnosis and death or between the date of a definitive diagnosis and the last observation of surviving patients. The Kaplan-Meier method was used to compare the OS of patients in different groups, and the log-rank test was used to estimate differences in survival. Univariate and multivariate analyses were performed using the Cox proportional hazards regression model using the Statistical Package for Social Sciences version 15.0 (SPSS, Inc.; Chicago, IL, USA). HRs estimated from the Cox analysis were reported as relative risks with corresponding 95% CIs. Statistical significance was taken at the 5% level.

Results

Patient clinical characteristics

The median age of the 187 patients enrolled in this study was 60 years (range, 20-84 years), and 117 (62.6%) were men. Eighty-one patients (43.3%) had CA 19-9 concentrations ≥500 IU/mL, 116 (62.0%) had lymph node invasion. A gemcitabine-based chemotherapy was given to 113 patients (60.4%), while 74 (39.6%) were given 5-fluorouracil-based chemotherapy. The clinicopathological characteristics of the patients are summarized in **Table 1**.

Identification of prognostic systemic inflammatory response (SIR) markers

Descriptive statistics of the SIR markers (white blood cell, neutrophil, lymphocyte, platelet, and monocyte counts) are shown in **Table 1**. The median level of each marker was used as the cutoff value in the analysis of clinical outcome in the ICC patients. The influence of each SIR

Prognostic value of SIR markers in ICC patients

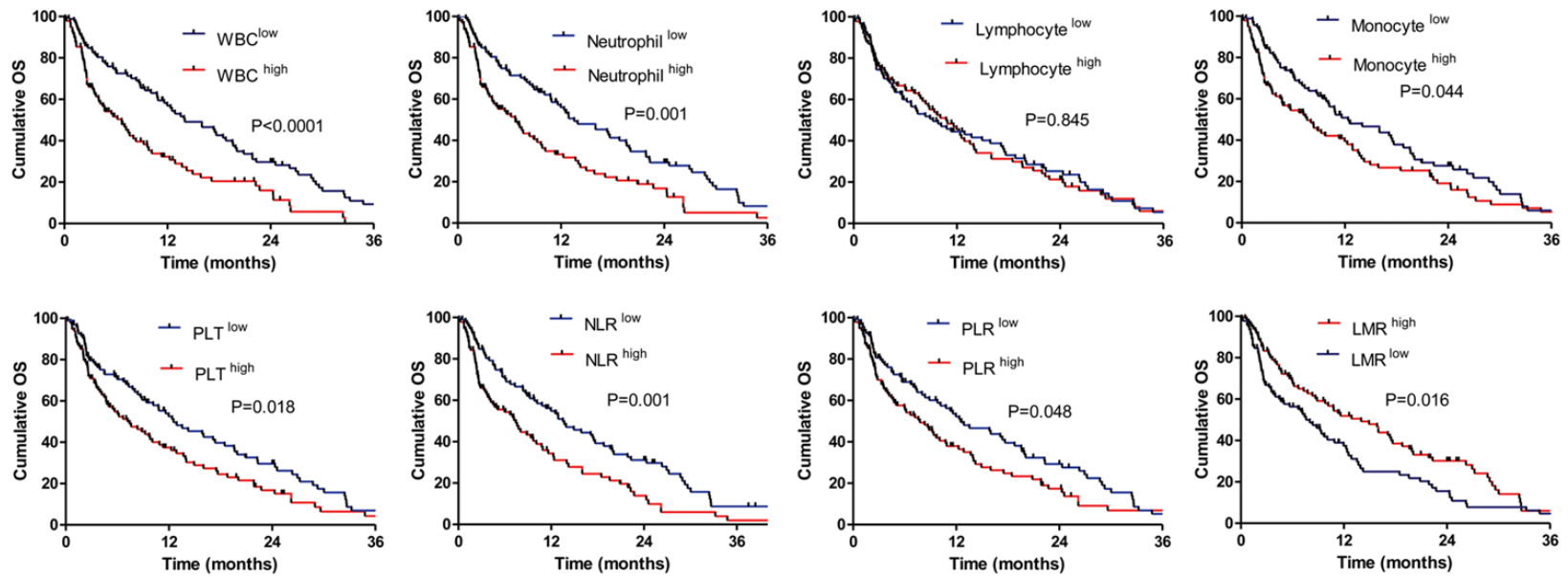


Figure 1. Association between SIR markers and OS in patients with intrahepatic cholangiocarcinoma (N=187). Kaplan-Meier curves for OS of all cases. The median level was selected as the cutoff between low and high levels. The p -value was determined using the log-rank test.

Prognostic value of SIR markers in ICC patients

Table 2. Univariate analysis of prognostic factors in intrahepatic cholangiocarcinoma patients (N=187)

Variables	Subgroup	N	MST (month)	1-year survival rate(%)	p-value*
Age (years)	<60	90	7.4	34.4	0.609
	≥60	97	7.4	33.7	
Gender	Male	117	6.9	31.4	0.415
	Female	70	8.8	38.6	
CA199	<500 IU/ml	106	10.8	44.9	<0.0001
	≥500 IU/ml	81	3.9	19.8	
WBC	<6.3*10 ⁹ /L	91	11.6	47.8	<0.0001
	≥6.3*10 ⁹ /L	96	4.5	21.9	
Neutrophil	<4.6*10 ⁹ /L	92	11.4	46.2	0.001
	≥4.6*10 ⁹ /L	95	4.5	22.1	
Lymphocyte	<1.4*10 ⁹ /L	88	8.4	36.0	0.845
	≥1.4*10 ⁹ /L	99	6.7	32.3	
PLT	<196*10 ⁹ /L	92	9.2	38.7	0.018
	≥196*10 ⁹ /L	95	5.7	29.5	
Monocyte	<0.5*10 ⁹ /L	90	9.8	39.6	0.044
	≥0.5*10 ⁹ /L	97	5.6	28.9	
NLR	<3.21	92	11.4	45.7	0.001
	≥3.21	95	4.9	22.1	
PLR	<138	94	9.2	40.4	0.048
	≥138	93	6.1	27.7	
LMR	<3	91	6.7	26.4	0.016
	≥3	96	9.8	41.2	

marker-WBC, neutrophil, lymphocyte, monocyte, and platelet count; NLR, PLR, and LMR on overall survival (OS) was determined by Kaplan-Meier analysis. All the markers were identified as indicative of OS except for lymphocyte count (Figure 1).

Multivariate regression analysis confirmed SIR markers as independent prognostic factors

Univariate analysis revealed that WBC, neutrophil, platelet, and monocyte counts, NLR, PLR, LMR, and CA19-9 were all significantly associated with OS (Table 2). Multivariate analysis using a Cox regression model that included CA19-9, lymph node invasion, and metastasis, but not the SIR markers identified CA19-9 (HR=2.510, 95% CI: 1.772-3.556; $P<0.0001$) as an independent influence on OS. When adjusted for CA19-9 (<500 vs. ≥500), multivariate analysis confirmed that WBC count (HR:1.610, 95% CI: 1.134-2.285, $P=0.008$), neutrophil count (HR=1.633, 95% CI: 1.165-2.289, $P=0.004$), platelet count (HR=1.573,

95% CI: 1.131-2.188, $p=0.007$), NLR (HR=1.505, 95% CI: 1.075-2.106, $P=0.017$), PLR (HR=1.335, 95% CI: 0.959-1.860, $P=0.087$), and LMR (HR=0.745, 95% CI: 0.534-1.040, $P=0.084$) were independent prognostic markers (Table 3).

SIR markers predicted patient survival within subgroups of ICC patients

WBC, neutrophil, monocyte and platelet counts; NLR, PLR; and LMR were all significantly associated with OS across all subgroups (Figure 2), confirming that these SIR markers could predict ICC patient outcome regardless of age and gender.

Discussion

We confirmed that high WBC, neutrophil, monocyte, and platelet counts, high NLR, and PLR, and low LMR were all significantly correlated with shorter OS.

These markers of SIR were also independent prognostic markers of OS in patients with ICC. They can thus be considered when choosing the optimal treatment strategies for individual patients.

White blood cell, neutrophil, monocyte, and platelet counts were all significantly associated with OS of these ICC patients. However, there was almost no impact of the total lymphocyte count on OS. Further investigation of the role of lymphocytes in ICC would be aided by study of lymphocyte subsets such as CD16+56+, CD4+, CD8+, CD19+, and CD3+, as they have different effects on the dysregulation of immunosurveillance and immunomodified in the development of cancer [17, 18].

NLR, PLR, and LMR have been the focus of extensive research because their levels are affected by combined inflammatory markers, and may thus offer a more comprehensive insight into inflammatory status [11-13]. Of these three, NLR may have the strongest cor-

Prognostic value of SIR markers in ICC patients

Table 3. Correlation of SIR markers with OS in patients with intrahepatic cholangiocarcinoma (N=187)

Variables	Group	Case number	HR (95% CI)	p-value	Ajusted HR (95% CI) ^a	Ajusted p-value ^a
WBC	<6.3/≥6.3	91/96	1.932 (1.376-2.711)	<0.001	1.610 (1.134-2.285)	0.008
Neutrophil	<4.6/≥4.6	92/95	1.755 (1.258-2.448)	0.001	1.633 (1.165-2.289)	0.004
Lymphocyte	<1.4/≥1.4	88/99	0.968 (0.700-1.340)	0.846	1.011 (0.729-1.403)	0.946
PLT	<196/≥196	92/95	1.483 (1.069-2.057)	0.018	1.573 (1.131-2.188)	0.007
Monocyte	<0.5/≥0.5	90/97	1.398 (1.008-1.939)	0.045	1.265 (0.907-1.763)	0.166
NLR	<3.21/≥3.21	92/95	1.700 (1.222-2.366)	0.002	1.505 (1.075-2.106)	0.017
PLR	<138/≥138	94/93	1.390 (1.001-1.930)	0.049	1.335 (0.959-1.860)	0.087
LMR	<3/≥3	91/96	0.670 (0.483-0.931)	0.017	0.745 (0.534-1.040)	0.084

relation with OS in ICC patients. The association between elevated NLR and poor prognosis is complex. One plausible explanation is that an elevated NLR reflects a boosted neutrophil response to tumors. Neutrophils have been shown to promote tumor growth and metastasis by remodeling the extracellular matrix, releasing reactive oxygen species, nitric oxide, and other factors that suppress the cytolytic activity of immune cells such as lymphocytes, natural killer cells, and activated T cells [19]. Tumor-infiltrating neutrophils might also contribute to neovascularization by releasing vascular endothelial growth factor and matrix metalloproteinase-9 (MMP-9), which promote tumor growth [19, 20]. In addition, patients with a high NLR resulting from relative lymphocytopenia often have a weakened lymphocyte-mediated antitumor cellular immune response [21, 22]. Overall, concurrent high neutrophil and low lymphocyte counts might lead to an imbalanced inflammatory cascade and dysregulated immune modulation, thus creating an optimal microenvironment for tumor growth.

We found that the PLR was associated with OS and was of prognostic value in ICC patients. The PLR has been extensively studied in cancers, and was reported to be an independent prognostic factor in patients with ovarian or gastric cancer [23-25]. The adverse effect of a high PLR can be accounted for by the effects of either a high production of platelets or a low production of lymphocytes. In the first instance, platelet aggregation and activation following exposure to inflammatory cytokines and adenosine phosphate from tumor cells could activate invasiveness of tumor cells by enhancing MMP-9 secretion. Therefore, thrombocytosis could not only reflect inflammation status but

also promote tumor invasion and metastasis. Indeed, interactions of cancer cells and platelets are quite complex. Tumor cells can trigger platelet-mediated recognition, during which immune recognition and elimination of cancer cells are suppressed to some degree [26, 27]. Besides, an increased PLR resulting from a low lymphocyte count may reflect a weak immunologic response to the tumor associated with tumor progression and metastasis.

We also evaluated the impact of the LMR on patient outcome. Unlike NLR and PLR, a low LMR indicated an unfavorable prognosis in patients with ICC, and was negatively correlated with OS, which is consistent with reports of the impact of a low LMR in other types of cancer [28, 29]. It should be noted that monocytes may be linked to cancer biology. Tumor-associated macrophages (TAM), derived from circulating monocytes, are actively recruited by chemotactic factors released from tumors. They accumulate at the tumor site and produce cytokines that lead to angiogenesis and promote anti-immune responses. Our results validate those of studies showing that OS was decreased in patients with solid tumors that were highly infiltrated with TAMs, and that a high monocyte count indicated a poor prognosis [30]. Thus, a low LMR, often suggestive of a high TAM count, may provide a favorable niche for tumor growth, contributing to tumor growth and metastasis.

This study has some limitations. First, because a relatively small number of ICC, compared with HCC, patients were available, we could not enroll a larger group, and it would have provided a stronger validation of our findings. Second, factors such as infection, ischemia, and coro-

Prognostic value of SIR markers in ICC patients

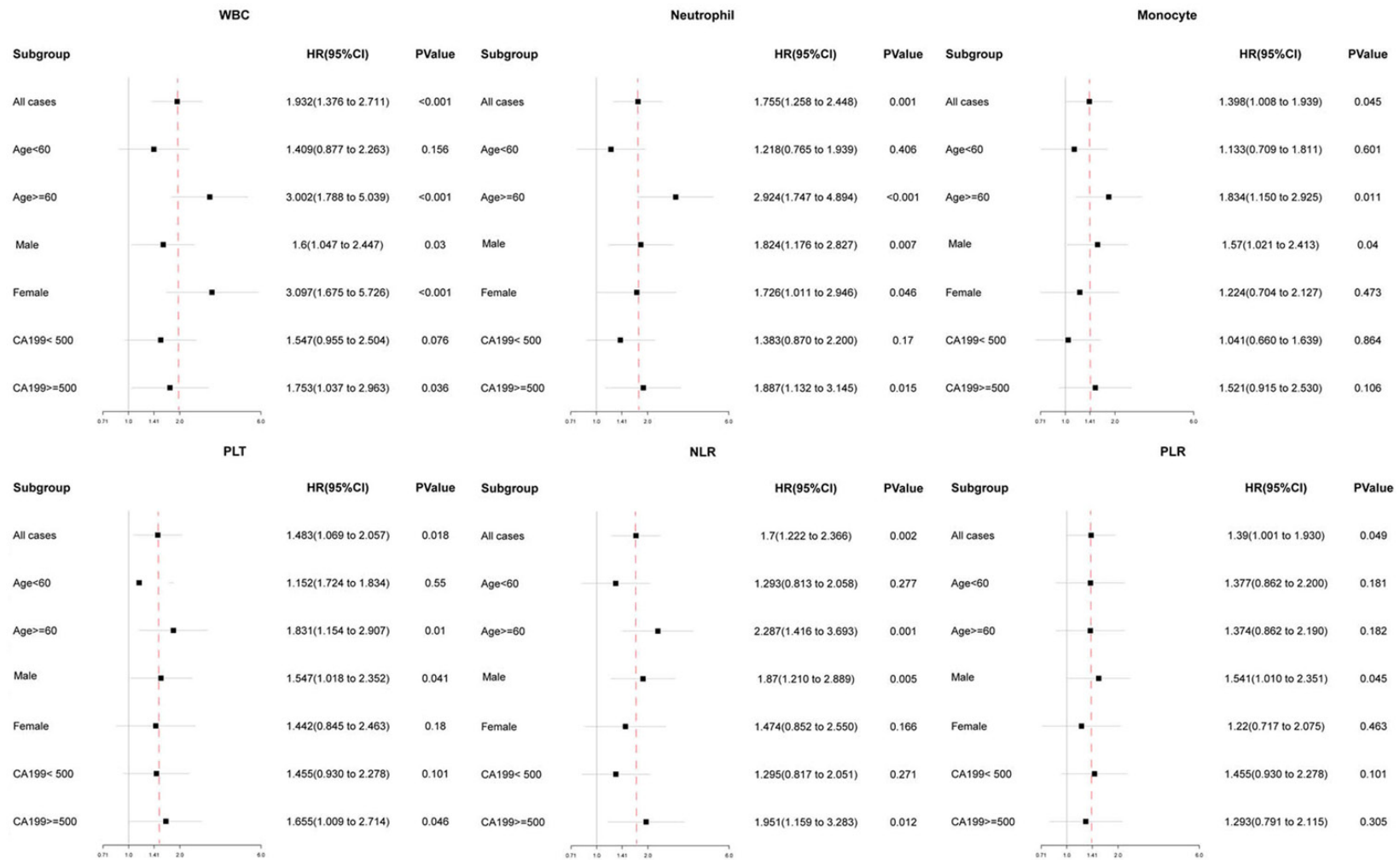


Figure 2. HRs of prognostic SIR markers for OS in different patient subgroups. HRs were calculated by comparing patients with low values to those with high values. HRs >1.0 indicate a worse outcome. The median level for each group was selected as the cutoff.

nary syndrome, which may affect the WBC count, were not taken into consideration. Thus, the results may not be generalizable to ICC patients with those conditions. Third, because C-reactive protein, a widely used inflammation biomarker, was not routinely measured in our clinical practice, we need to further investigate the prognostic value of the SIR markers together with C-reactive protein to enhance our interpretation.

In conclusion, our study showed that high WBC, neutrophil, monocyte, and platelet counts; a high NLR or PLR, and a low LMR were significantly correlated with shorter OS. In addition, these SIR markers were independent prognostic predictors of OS in patients with ICC. The study confirmed that the SIR markers can be used to predict the prognosis of ICC. Their use in tandem with other established prognostic systems may allow more accurate prediction of outcome in patients with ICC.

Acknowledgements

This study was supported by Natural Science Foundation of China (No. 81273954).

Disclosure of conflict of interest

None.

Address correspondence to: Zhiqiang Meng and Peng Wang, Department of Integrative Oncology, Fudan University Shanghai Cancer Center, 270 Dong An Road, Shanghai 200032, China. Tel: 8621-64175590 3628; Fax: 8621-64437657. E-mail: mengzhq@yeah.net (ZQM); wangp413@163.com (PW)

References

- [1] Patel T. Cholangiocarcinoma-controversies and challenges. *Nat Rev Gastroenterol Hepatol* 2011; 8: 189-200.
- [2] Patel T. Worldwide trends in mortality from biliary tract malignancies. *BMC cancer* 2002; 2: 10.
- [3] Braconi C, Patel T. Cholangiocarcinoma: new insights into disease pathogenesis and biology. *Infect Dis Clin North Am* 2010; 24: 871-884.
- [4] Chu KM, Lai EC, Al-Hadeedi S, Arcilla CE Jr, Lo CM, Liu CL, Fan ST, Wong J. Intrahepatic cholangiocarcinoma. *World J Surg* 1997; 21: 301-305; discussion 305-306.
- [5] Kawarada Y, Yamagiwa K, Das BC. Analysis of the relationships between clinicopathologic factors and survival time in intrahepatic cholangiocarcinoma. *Am J Surg* 2002; 183: 679-685.
- [6] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell* 2011; 144: 646-674
- [7] Gunter MJ, Stolzenberg-Solomon R, Cross AJ, Leitzmann MF, Weinstein S, Wood RJ, Virtamo J, Taylor PR, Albanes D, Sinha R. A prospective study of serum C-reactive protein and colorectal cancer risk in men. *Cancer Res* 2006; 66: 2483-2487.
- [8] Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res* 2000; 60: 184-190.
- [9] Jackson JR, Seed MP, Kircher CH, Willoughby DA, Winkler JD. The codependence of angiogenesis and chronic inflammation. *FASEB J* 1997; 11: 457-465.
- [10] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; 340: 448-454.
- [11] Krenn-Pilko S, Langsenlehner U, Thurner EM, Stojakovic T, Pichler M, Gerger A, Kapp KS, Langsenlehner T. The elevated preoperative platelet-to-lymphocyte ratio predicts poor prognosis in breast cancer patients. *Br J Cancer* 2014; 110: 2524-2530.
- [12] Raungkaewmanee S, Tangjitgamol S, Manusirivithaya S, Srijaipracharoen S, Thavaramara T. Platelet to lymphocyte ratio as a prognostic factor for epithelial ovarian cancer. *J Gynecol Oncol* 2012; 23: 265-273.
- [13] Qi Q, Geng Y, Sun M, Wang P, Chen Z. Clinical implications of systemic inflammatory response markers as independent prognostic factors for advanced pancreatic cancer. *Pancreatol* 2015; 15:145-150.
- [14] Chen Q, Yang LX, Li XD, Yin D, Shi SM, Chen EB, Yu L, Zhou ZJ, Zhou SL, Shi YH, Fan J, Zhou J, Dai Z. The elevated preoperative neutrophil-to-lymphocyte ratio predicts poor prognosis in intrahepatic cholangiocarcinoma patients undergoing hepatectomy. *Tumour Biol* 2015; 36: 5283-5289
- [15] Chen Q, Dai Z, Yin D, Yang LX, Wang Z, Xiao YS, Fan J, Zhou J. Negative impact of preoperative platelet-lymphocyte ratio on outcome after hepatic resection for intrahepatic cholangiocarcinoma. *Medicine (Baltimore)* 2015; 94: e574
- [16] Gomez D, Morris-Stiff G, Toogood GJ, Lodge JP, Prasad KR. Impact of systemic inflammation on outcome following resection for intrahepatic cholangiocarcinoma. *J Surg Oncol* 2008; 97: 513-518.

Prognostic value of SIR markers in ICC patients

- [17] Jang H, Chess L. An integrated view of suppressor T cell subsets in immunoregulation. *J Clin Invest* 2004; 114: 1198-1208.
- [18] Ockenga J, Jacobs R, Kemper A, Benschop RJ, Schmidt RE, Manns MP. Lymphocyte subsets and cellular immunity in patients with chronic pancreatitis. *Digestion* 2000; 62: 14-21.
- [19] Ardi VC, Kupriyanova TA, Deryugina EI, Quigley JP. Human neutrophils uniquely release TIMP-free MMP-9 to provide a potent catalytic stimulator of angiogenesis. *Proc Natl Acad Sci* 2007; 104: 20262-20267.
- [20] Pahler JC, Tazzyman S, Erez N, Chen YY, Murdoch C, Nozawa H, Lewis CE, Hanahan D. Plasticity in tumor-promoting inflammation: impairment of macrophage recruitment evokes a compensatory neutrophil response. *Neoplasia* 2008; 10: 329-340.
- [21] Svennevig JL, Lunde OC, Holter J, Bjørgsvik D. Lymphoid infiltration and prognosis in colorectal carcinoma. *Br J Cancer* 1984; 49: 375-377.
- [22] Okano K, Maeba T, Moroguchi A, Ishimura K, Karasawa Y, Izuishi K, Goda F, Usuki H, Wakabayashi H, Maeta H. Lymphocytic infiltration surrounding liver metastases from colorectal cancer. *J Surg Oncol* 2003; 82: 28-33.
- [23] Templeton AJ, Ace O, McNamara MG, Al-Mubarak M, Vera-Badillo FE, Hermanns T, Seruga B, Ocaña A, Tannock IF, Amir E. Prognostic role of platelet to lymphocyte ratio in solid tumors: A systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2014; 23: 1204-1212.
- [24] Zhou X, Du Y, Huang Z, Xu J, Qiu T, Wang J, Wang T, Zhu W, Liu P. Prognostic value of PLR in various cancers: A meta-analysis. *PLoS One* 2014; 9: 101-119.
- [25] Lian L, Xia YY, Zhou C, Shen XM, Li XL, Han SG, Zheng Y, Mao ZQ, Gong FR, Wu MY, Chen K, Tao M, Li W. Application of platelet/lymphocyte and neutrophil/lymphocyte ratios in early diagnosis and prognostic prediction in patients with resectable gastric cancer. *Cancer Biomark* 2015; 15: 899-907.
- [26] Borsig L. The role of platelet activation in tumor metastasis. *Expert Rev Anticancer Ther* 2008; 8: 1247-1255.
- [27] Kyriazi V, Theodoulou E. Assessing the risk and prognosis of thrombotic complications in cancer patients. *Arch Pathol Lab Med* 2013; 137: 1286-1295.
- [28] Nishijima TF, Muss HB, Shachar SS, Tamura K, Takamatsu Y. Prognostic value of lymphocyte-to-monocyte ratio in patients with solid tumors: A systematic review and meta-analysis. *Cancer Treat Rev* 2015; 41: 971-978.
- [29] Shibutani M, Maeda K, Nagahara H, Ohtani H, Sakurai K, Yamazoe S, Kimura K, Toyokawa T, Amano R, Tanaka H, Muguruma K, Hirakawa K. Prognostic significance of the lymphocyte-to-monocyte ratio in patients with metastatic colorectal cancer. *World J Gastroenterol* 2015; 21: 9966-9973.
- [30] Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 2006; 124: 263-266.