RESEARCH ARTICLE

The Blood Neutrophil-to-lymphocyte Ratio Predicts Survival in Patients with Advanced Hepatocellular Carcinoma Receiving Sorafenib

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Abstract

Background and Aim: Increasing evidence correlates the presence of systemic inflammation with poor survival in patients with hepatocellular carcinoma (HCC). The aim of this study was to investigate the prognostic significance of the blood neutrophil-to-lymphocyte ratio (NLR) in patients with advanced HCC who received sorafenib monotherapy. Methods: A total of sixty-five patients with advanced HCC, not eligible for locoregional therapy, treated with sorafenib were enrolled. Potential prognostic factors such as age, gender, tumoral characteristics, performance status and NLR were analyzed. Results: Median OS and TTP for the entire cohort were 10.0 months (95% CI, 7.6-12.3 months) and 4.5 months (95% CI, 4.0-4.9 months). The mean NLR at baseline was 2.89. The median OS of patients with a high NLR (>4) was 6.5 months (95% CI, 5.2-7.7 months) compared with 12.5 months (95% CI, 9.9-15.0) for patients with a normal NLR (≤4) (*P*=0.01). Age ≤65, NLR >4, extrahepatic metastases and vascular invasion were all predictors of poorer overall survival. Multivariate analysis showed that NLR > 4, vascular invasion and extrahepatic metastases were independent predictors of poorer overall survival. The median TTP of patients with a high NLR was 2.5 months (95% CI, 1.4-3.6 months) compared with 4.5 months (95% CI, 3.9-5.1 months) for patients with a normal NLR (*P*=0.012). Conclusions: High baseline NLR was associated with worse OS and TTP for patients with advanced HCC treated with sorafenib.

Keywords: Hepatocellular carcinoma - sorafenib - neutrophil-lymphocyte ratio

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Introduction

Hepatocellular carcinoma (HCC) is one of the leading malignancies worldwide. Despite the increaseing use of surveillance programs, a large proportion of patients with HCC are diagnosed at an advanced stage (Forner et al., 2012). Moreover, a number of patients with early- to intermediate-stage HCC eventually progress to the advanced stage of the disease. Only palliative treatment options are available for these patients (Forner et al., 2012).

Sorafenib is a multikinase inhibitor with effects on tumor proliferation and angiogenesis and was shown to prolong survival of patients with advanced HCC in two placebo-controlled randomized trials (Llovet et al., 2008; Cheng et al., 2009). Many investigators have tried to identify baseline or on-treatment predictive factors for sorafenib because sorafenib does not show the same effect in all HCC patients and the response rate of sorafenib in HCC was modest (Llovet et al., 2008).

In an Italian study, younger age, extrahepatic tumor spread and ECOG performance status were associated with an increased likelihood of non-response after sorafenib (Iavarone et al., 2011). In addition, the side effects caused by sorafenib could also be used as clinical predictive parameters in patients with HCC. Early hand-foot reaction or diarrhea during sorafenib therapy was associated with prolonged TTP or OS than did those without skin toxicity (Vincenzi et al., 2010; Cho et al., 2013).

In the last decade, research on the intersections between inflammation and cancer pathogenesis has blossomed, and the inflammation can be considered as an enabling characteristic for its contributions to the acquisition of core hallmark capabilities of cancer (Hanahan et al., 2011). Recent attention has focused on the systemic inflammatory state as a surrogate for tumor biology in multiple solid tumors. In particular, the ratio of absolute neutrophil count to absolute lymphocyte count (neutrophil-lymphocyte ratio; NLR) has been evaluated as a predictor of recurrence and survival in various malignancies. The NLR is particularly useful, as it can be easily measured by routine complete blood work. Published data also suggest that an elevated NLR may correlate with a worse prognosis in patients with HCC

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who underwent transcatheter arterial chemoembolization (TACE) radiofrequency ablation (RFA) resection or orthotopic liver transplantation (OLT) (Gomez et al., 2008; Chen et al., 2012; Limaye et al., 2012; McNally et al., 2013). To date, there have been no reports regarding NLR in HCC patients treated with sorafenib. The aim of this study was to investigate the prognostic significance of NLR at the time of diagnosis of patients with advanced HCC who received sorafenib monotherapy.

Materials and Methods

Patients

A database of 65 patients with advanced HCC admitted to our Hospital between January 2011 and December 2012 was evaluated retrospectively. Written informed consent was obtained from patients before treatment and the study was approved by the Ethical Committee of our hospital. All patients satisfied the diagnostic criteria for HCC based on radiologic or histologic grounds according to the American Association for the Study of the Liver guidelines (Bruix et al., 2011) and were not suitable candidates for locoregional treatment (resection, OLT, TACE and RFA). All patients received contrast enhanced computed tomography (CT) or magnetic resonance (MR) imaging at baseline as well as blood samples including complete blood routine examination, liver function, and AFP.

NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. Serum complete blood count levels were used to calculate NLR at baseline. NLR measurements were obtained without demonstrable infection. Patients were divided into a high NLR group (>4) and a normal NLR group (≤4) according to the level of NLR before treatment (Motomura et al., 2013).

Treatments and methods

The initial sorafenib dose was 400 mg orally twice daily. Dose reductions of sorafenib and drug interruptions were allowed for toxicities, according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Treatment was continued until unacceptable toxicity, radiological or clinical progression, death or patient refusal. Patients were seen every 4 weeks for toxicity management and clinical assessment. CT or MR imaging was performed at baseline and every 2–4 months. Tumor response was assessed according to response evaluation criteria in solid tumors (RECIST). as complete response (CR) partial response (PR) stable disease (SD) and progressive disease (PD) (Therasse et al., 2000).

Statistical Analyses

Data were evaluated using Statistical Package for Social Sciences, v.13.0. Continuous variables were expressed as mean ± standard deviation, and compared using t test. Categorical variables were expressed as frequency and compared using Chi-square test. The Kaplan-Meier method was used for survival analysis. A multivariate analysis was performed by Cox regression for significant variables on univariate analysis. All p-values were based on two-sided testing and a p-value <0.05 was considered as significant.

Results

Patient Characteristics

A total of sixty-five patients were consecutively treated at Guangdong General Hospital, China, between January 2011 and December 2012. 78.5% were male and mean age was 55±12 years. All of the patients were BCLC stage C. 75.4 percent of the patients were in PS 0-1, and 69.2% had a well preserved liver function (CP-A). A large proportion of patients had highly advanced disease with vascular invasion (61.5%) and extrahepatic metastases (52.3%). Detailed patient characteristics are shown in Table 1.

Tolerability and Safety Aanlysis

The median duration of sorafenib treatment was 123 days (range: 48–424 days). The median maximum administered dose of sorafenib was 800 mg/d (range: 400–800 mg/d). There were no treatment-related deaths. Dose reduction was applied in 28 patients (43.1%) because of side effects related to sorafenib. Dose decreased to 400 mg/d and 400 mg every other day in 21 patients (32.3%) and 7 patients (10.8%) respectively. Sorafenib was discontinued permanently because of tumor progression in 36 patients (55.4%) and side effects in 11 (16.9%). 13 (21%) patients accepted drug withdrawal after dose reduction. Table 2 showed the details of the side effects secondary to sorafenib observed based on CTCAE 4.0.

Table 1. Cliniopathologic and Demographic Features

Characteristic	NLR≤4	NLR>4	*P*
Gender			0.652
Male	40	11	
Female	11	3	
Age			0.217
≤65 years	36	12	
>65 years	15	2	
HBV			0.343
Positive	31	10	
Negative	20	4	
Tumor size			0.552
≤ 5 cm	17	5	
> 5 cm	34	9	
ECOG PS			0.529
0-1	38	11	
2-3	13	3	
Child-Pugh Class			0.559
A	35	10	
B	16	4	
Extrahepatic metastases			0.459
Present	26	8	
Absent	25	6	
Vascular invasion			0.534
Present	31	9	
Absent	20	5	
AFP			0.407
≤ 400 ng/ml	26	6	
> 400 ng/ml	25	8	

NLR, neutrophil–lymphocyte ratio; ECOG PS, Eastern cooperative oncology group performance status; AFP, α-fetoprotein

Table 2. Adverse Effects During Sorafenib Therapy

Adverse effects	NO.	%
Diarrhea	30	46.2
Grade 1	25	38.5
Grade 2	4	6.2
Grade 3	1	1.5
Rash	18	27.7
Grade 1	18	27.7
Hand-foot skin reaction	13	20.0
Grade 1	6	9.2
Grade 2	7	10.8
Fatigue	10	15.4
Grade 1	10	15.4
Anorexia	7	10.8
Grade 1	7	10.8
Nausea	5	7.7
Grade 1	3	4.6
Grade 2	2	3.1
Weight loss	4	6.2
Grade 1	3	4.6
Grade 2	1	1.5
Hair loss	5	7.7
Grade 1	5	7.7
ALT/AST increased	8	12.3
Grade 1	5	7.7
Grade 2	3	4.6

ALT, alanine transaminase; AST, aspartate transaminase; Adverse effects were analysed according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0

Table 3. Univariate and Multivariate Analyses of Risk Factors Affecting Overall Survival

Characteristic	Univariate	Multivariate	HR 95%
	P	*P*	confidence interval
Gender (male/female)	0.53		
Age (≤65 years)	0.014	0.665	0.376-1.868
Tumor size (>5cm)	0.362		
ECOG PS (0-1/2-3)	0.978		
Child-Pugh Class (A/B)	0.291		
Extrahepatic metastases	<0.001	0.006	0.181-0.757
Vascular invasion	0.039	0.046	0.259-0.987
AFP (>400 ng/ml)	0.326		
NLR (>4)	0.01	0.006	1.354-5.970

NLR, neutrophil–lymphocyte ratio; ECOG PS, Eastern cooperative oncology group performance status; AFP, α-fetoprotein

The most common adverse effects related to sorafenib were diarrhea (46.2%) rash (27.7%) and hand-foot skin reaction (20%) most of which were mild to moderate.

Response evaluations

44 patients (67.7%) completed at least 3 months of sorafenib therapy and were evaluable for assessment of tumor response. There were no complete responders. 4 patients (6.2%). had a partial response, 15 (23.1%) had stable disease, and 25 (38.5%). had progressive disease. The objective response rate (complete and partial responses) was 6.2%.

Survival

Median follow-up time was 8.9 months (range: 2 to 25.5 months). At the time of data record, 45 (69.2%) patients were dead. Median OS and TTP for the entire cohort were 10.0 months (95%CI, 7.6-12.3 months) and 4.5 months (95% CI, 4.0-4.9 months). The mean NLR at baseline was 2.89. 51 (78.5%) patients had a NLR >4, and 14 (21.5%) patients had a NLR ≤4. The median OS of patients with NLR>4 was 6.5 months (95%CI, 5.2-7.7 months). compared with 12.5 months (95%CI, 9.9-15.0) for patients with NLR ≤4 (p=0.01) (Figure 1). Age ≤65 (p=0.014) NLR > 4, extrahepatic metastases (p<0.001). and vascular invasion (p=0.039) were all predictors of poorer overall survival. Multivariate analysis showed that NLR > 4 (p=0.006) vascular invasion (p=0.046) and extrahepatic metastases (p=0.006) were independent predictors of poorer overall survival (Table 3). The median TTP of patients with NLR>4 was 2.5 months (95%CI, 1.4-3.6 months) compared with 4.5 months (95%CI, 3.9-5.1 months) for patients with NLR≤4 (p=0.012) (Figure 2). There were no other predictors of shorter TTP.

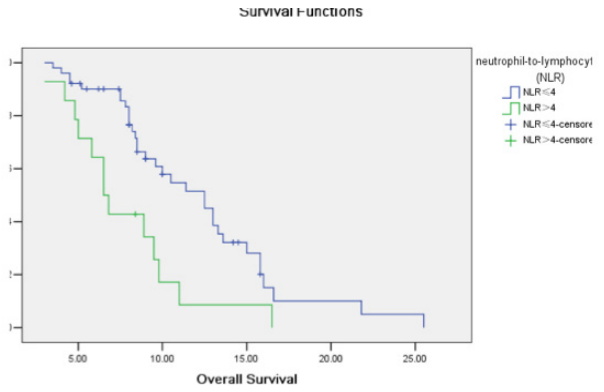

Figure 1. Kaplan-meier Estimates of Overall Survival. NLR≤4 and NLR>4

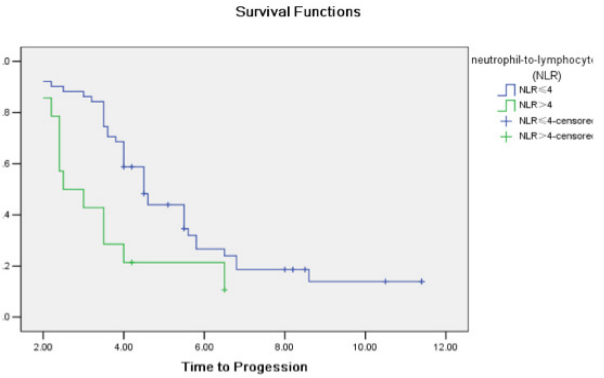

Figure 2. Kaplan-meier Estimates of Overall Survival. NLR≤4 and NLR>4

In patients responded sorafenib treatment (PR, SD, CR) (n=19), 14 patients had NLR≤4 (73.7%) and 5 patients had NLR>4 (26.3%); in patients with progressive disease (n=25), 12 patients had NLR≤4 (48%), and 13 patients had NLR>4 (52%). p=0.03.

Discussion

Currently, sorafenib is the first and only systemic agent to be approved for the treatment of HCC and to have demonstrated an overall survival (OS) benefit in advanced HCC. It can target both cell surface tyrosine kinase receptors and downstream intracellular serine/threonine kinases in the Ras/MAPK cascade (Wilhelm et

al., 2002; Carlomagno et al., 2006). In this retrospective study, we show sorafenib therapy is safe and effective with no unexpected side effects in advanced HCC. In the literature, TTP and OS in sorafenib treated advanced patients were reported as 2.8-7 months and 5.4-12 months, respectively. (Abou-Alfa et al., 2006; Llovet et al., 2008; Cheng et al., 2009; Hsu et al., 2010; Prete et al., 2010). The survival rate in this study is considerably similar to those in literature.

We also found that an elevated NLR, a biomarker comparing tumor inflammation and host immunity, could predict outcome of advanced HCC patients initially treated with sorafenib. The median OS of patients with NLR >4 was much shorter when compared with those with a normal NLR (≤4) 6.5 months and 12.5 months, respectively. This is consistent with the above studies which associated high NLR with poor outcome in HCC. (Gomez et al., 2008; Chen et al., 2012; Limaye et al., 2012; McNally et al., 2013).

Despite significant progress with the advent of sorafenib as a treatment option for advanced HCC, this disease is still a great clinical challenge. Given the heterogeneity of the disease and its causes, there is still much to learn about to whom and when these therapies should be applied. Many prognostic and predictive factors have been developed in HCC patients receiving cytokine and targeted therapy, such as clinical staging systems, the presence of adverse effects, molecular profiling, genetic and epigenetic information (methylation and miRNA profiling) (Minguez et al., 2011). However, the detection of most biomarkers are expensive and not available in clinical practice. While NLR is a cost effective indicator, which can be determined by routine complete blood count (CBC). analyzers.

Recently, a number of studies indicated the prognostic value of inflammatory markers in many cancers. Systemic inflammatory responses have been shown to reflect the promotion of angiogenesis, DNA damage and tumor invasion through upregulation of cytokines (Jaiswal et al., 2000; Coussens et al., 2002). Though measured easily, NLR is more complicated due to its special feature as a combined factor of inflammation and host immune reaction.

An elevated NLR can promote the progression of HCC not only via angiogenesis, but also via some sort of inflammatory microenvironment (Motomura et al., 2013). Motomura et al reported a correlation between elevated NLR and upregulation of IL-17 production in both peritumoral regions of the liver and peripheral blood. IL-17 is a proinflammatory cytokine that promotes the growth of HCC. IL-17 is an initiator of neutrophil recruitment by CXC chemokines, such as CCL2 released from IL-17-producing T cells.

Elevated neutrophils were regarded as a reservoir for VEGF (Kusumanto et al., 2003). Other circulating angiogenesis-regulating chemokines, such as CXCL8 and tissue inhibitors of metalloproteinase can also be produced by neutrophil (Tanigawa et al., 1997; Kusumanto et al., 2003; Halazun et al., 2009; Hung et al., 2011). In addition, high infiltration of peri-tumoral neutrophils can also stimulate angiogenesis by releasing matrix metallopeptidase 9 (MMP-9). and enhance tumor invasion by producing hepatocyte growth factor (Kuang et al., 2011). Besides angiogenesis, neutrophil can contribute to cancer metastasis via promoting motility of cancer cells and adhesion to hepatic sinusoids (McDonald et al., 2009).

On the other hand, high NLR reflects relatively depleted lymphocytes, exhibiting a poor lymphocyte-mediated immune response to malignancy because of decreasing T4/T8 ratio (Chew et al., 2010; Ding et al., 2010). Weaker immune reaction due to relative lymphocytopenia and elevated NLR could explain for depletion of tumor-infiltrating lymphocytes that are independently predictive of cancer specific survival (Ohtani, 2007). Lymphocytes have pivotal roles in cytotoxic cell death and cytokines production that inhibit proliferation and metastatic activity of tumor cells (Ding et al., 2010b). Consequently, patients with weaker lymphocytic infiltration within tumor would have a worse prognosis (Chew et al., 2010b).

There are some limitations of this study. The relative small size and the nature of the retrospective design are main limitations. Besides, there is no universal consensus regarding the threshold that defines high NLR, and this impeded the direct comparison between different studies.

In summary, a high NLR may be used as an available and cheap biomarker for predicting poor survival in advanced HCC patients who received sorafenib. However, concrete molecular mechanisms are still unknown and logical further studies are needed.

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