

Postoperative Low Absolute Lymphocyte Counts may Predict Poor Outcomes of Hepatocellular Carcinoma After Liver Resection

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Abstract

Background: The absolute lymphocyte counts (ALCs) have been reported as one of worse prognostic factors for hepatocellular carcinoma (HCC) patient after liver transplantation. The aim of this study was to assess the influence of ALCs on the outcomes of patients with hepatitis B virus (HBV)-related HCC within the Milan criteria following liver resection.

Methods: Data of patients with HCC within the Milan criteria who received liver resection between January 2007 and June 2013 were reviewed, and perioperative ALCs were carefully monitored. All potential risk factors were statistically analyzed by uni- and multi-variate analyses. The receiver operating characteristic (ROC) curve was used to determine the optimal ALCs cut-off value to predict HCC recurrence after liver resection.

Results: A total of 221 patients were enrolled in the current study. During the follow-up period, 106 patients experienced recurrence, and 38 patients died. Multivariate analysis suggested microvascular invasion (MVI), a tumor grade ≥ 2 , and a low postoperative ALCs in the 1st postoperative month increased the incidence of postoperative recurrence, besides, MVI, intraoperative transfusion, and a low postoperative ALCs in the 1st postoperative month were associated with poor overall survival (OS). An ROC analysis showed that a cut-off value of $1.5 \times 10^9/L$ for ALCs in the 1st postoperative month predicted postoperative recurrence. The 5-year recurrence-free survival (RFS) and OS rates of patients with low postoperative ALCs were 34.5% and 64.8%, respectively, which were significantly lower than those of patients with high postoperative ALC (58.5% for RFS and 86.5% for OS).

Conclusion: Low ALCs in the 1st postoperative month may be associated with high recurrence incidence and poor OS for patients with HBV-related HCC within the Milan criteria after liver resection.

Key words: Absolute Lymphocyte Counts; Hepatocellular Carcinoma; Liver Resection; Outcome

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most frequent malignancy and the third most common cancer-related cause of death worldwide. Chronic hepatitis B virus (HBV) infection is estimated to cause 55–60% of HCC worldwide, and it is the most important etiologic agent of HCC in Asia.^[1] In China, approximately 7% of the population suffer from chronic HBV infection. Owing to this high prevalence, China alone accounts for approximately 55% of the HCC cases in the world.^[2] Liver resection is a curative treatment for patients with HCC, but postoperative recurrence is still the main obstacle to the long-term survival of HCC patients. The incidence of postoperative recurrence for patients with small HCC can be as high as 50–70% after liver resection.^[3]

Lymphopenia is advocated as a surrogate marker of malnutrition and poor anti-tumor immunomodulation

abilities in patients with chronic liver disease.^[4] Previous investigations confirmed that low absolute lymphocyte counts (ALCs) contributed to poor outcomes for many malignancies, such as leukemia, ovarian cancer, Merkel cell carcinoma, and others.^[5,6] Recently, Nagai *et al.* investigation^[7] indicated that perioperative ALCs were a prognostic factor in HCC recurrence after liver transplantation. They suggested that ALCs could reflect host cellular immunity because lymphocytes such as CD4⁺ and natural killer cells play key

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roles in antiviral immunity.^[7] Li *et al.*^[8] also suggested that low preoperative ALCs were powerful prognostic factors for HCC recurrence after thermal ablation. However, whether ALCs could predict HCC recurrence after liver resection for patients with HBV-related HCC was unclear. Accordingly, in this study, we aimed to clarify whether ALCs could predict outcomes for patients with HBV-related HCC within the Milan criteria following liver resection.

METHODS

Study group

Data of patients with HBV-related HCC within the Milan criteria who received liver resection at Department of General Surgery, West China Hospital, Sichuan University (China) between January 2007 and June 2013 were reviewed. Hepatitis B surface antigen was detected in all patients. HCC was confirmed by postoperative pathology. All patients had Child A liver function. Patients who fit any one of the following criteria were excluded from this study: (1) co-infection with hepatitis C virus; (2) simultaneous splenectomy; (3) ruptured HCC; (4) infections during the perioperative period; (5) re-resection; or (6) preoperative anti-tumor treatments. This study was approved by the Ethics Committee of West China Hospital.

Follow-up and definitions

All preoperative blood cell counts and differential counts were taken 2 days before the operation. After liver resection, all patients were regularly followed up in the 1st postoperative month and then every 3 months during the following 3 years and every 6 months in the subsequent years. Liver function, blood cell tests, serum alpha-fetoprotein (AFP), HBV-DNA, visceral ultrasonography, and computed tomography or magnetic resonance imaging and chest radiography were monitored for all patients. Bone scintigraphy was performed whenever HCC recurrence was suspected. Postoperative recurrence was defined as positive imaging findings compared with preoperative examination values and newly rising tumor marker (AFP) values or confirmation by biopsy or resection.^[9] The neutrophil-to-lymphocyte ratio (NLR) was defined as absolute neutrophil counts divided by the lymphocyte counts. The platelet-to-lymphocyte ratio (PLR) was defined as the absolute platelet count divided by the lymphocyte counts. An HBV-DNA higher than 104 copies/ml was considered high.^[10] An AFP >400 ng/ml was considered high.^[9]

Statistical analysis

We used SPSS 21.0 for Windows (SPSS Company, Chicago, IL, USA) to perform statistical analyses. All continuous variables are presented as mean \pm standard deviation (SD) and compared using one-way analysis of variance (ANOVA). Categorical variables were compared using the Chi-square test or Fisher's exact test. The independent risk factors for recurrence-free survival (RFS) and overall survival (OS) were identified by Cox regression. Factors those were significant at a $P < 0.1$ in the univariate analyses were included in the multivariate analysis. RFS and OS

were determined using the Kaplan–Meier method, with comparisons using a log-rank test. A receiver operating characteristic (ROC) analysis was used to establish the cut-off value for postoperative ALCs in predicting recurrence. A $P < 0.05$ was considered statistically significant.

RESULTS

A total of 221 patients were included in the present study. With a mean age of 49.72 ± 11.84 years, there were 191 males and 30 females. The mean tumor size was 3.26 ± 1.04 cm. In total, 32 patients suffered from multiple tumors. Thirty-one patients received intraoperative blood transfusion. Sixty-one patients had a high preoperative HBV-DNA load, and high preoperative AFP levels were observed in 67 patients. Microvascular invasion (MVI) was observed in 42 patients. The mean follow-up time was 36.85 ± 20.65 months. During the follow-up periods, recurrence was observed in 106 patients, and 38 patients died. The 1-, 3-, and 5-year RFS of the whole cohort were 77.4%, 54.9%, and 44.7%, respectively [Figure 1a]. The 1-, 3-, and 5-year OS of the whole cohort were 95.5%, 84.5%, and 76.0%, respectively [Figure 1b].

Risk factors associated with postoperative recurrence

As shown in Table 1, univariate analyses suggested that MVI, intraoperative transfusion, a tumor grade ≥ 2 , a high preoperative HBV-DNA load, postoperative ALCs, and postoperative NLR were all potential influences on RFS. However, only MVI ($P < 0.001$, hazard ratio [HR] = 2.270, 95% confidence interval [CI] = 1.468–3.509), a tumor grade ≥ 2 ($P = 0.002$, HR = 2.056, 95% CI = 1.289–3.280), and postoperative ALCs ($P = 0.004$, HR = 0.604, 95% CI = 0.427–0.854) increased the incidence of postoperative recurrence in the multivariate analysis [Table 2].

Risk factors associated with overall survival

Additional analyses were also performed to identify risk factors associated with postoperative survival by univariate and multivariate Cox regression analyses. In the univariate analyses, age, MVI, intraoperative transfusion, platelet, high preoperative HBV-DNA load, and preoperative and postoperative ALCs and NLRs were associated with mortality after liver resection for patients with HCC within the Milan criteria [Table 3]. However, only MVI ($P = 0.002$, HR = 3.030, 95% CI = 1.519–6.044), intraoperative transfusion ($P = 0.002$, HR = 3.139, 95% CI = 1.536–6.415), and postoperative ALCs ($P < 0.001$, HR = 0.210, 95% CI = 0.116–0.380) were demonstrated as independent prognostic factors for poor OS in the multivariate analysis [Table 4].

Comparative outcomes of patients with high versus low postoperative absolute lymphocyte counts

An ROC analysis showed that the cut-off value of $1.5 \times 10^9/L$ for postoperative ALCs predicted recurrence with a sensitivity of 68.9% and a specificity of 65.2% [Figure 2]. The area under the ROC curve was 0.716 [Figure 2]. The 1-, 3-, and 5-year RFS rates for patients with low ($\leq 1.5 \times 10^9/L$,

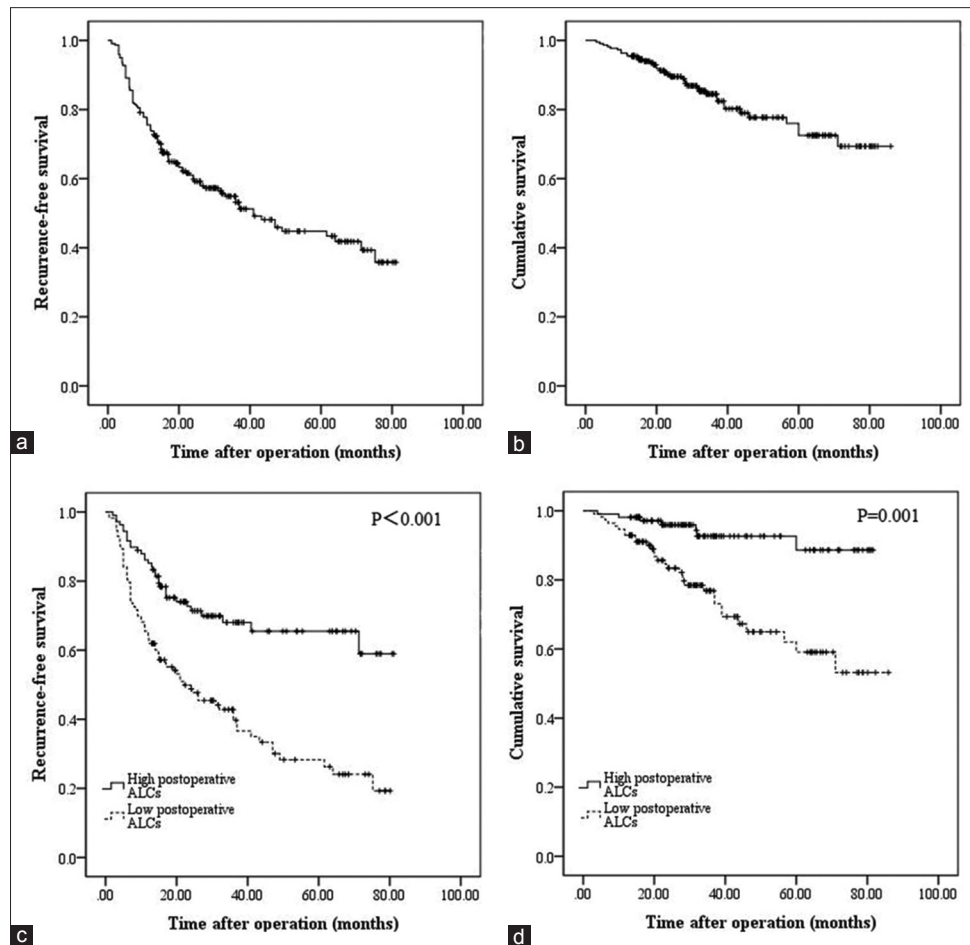


Figure 1: Recurrence-free survival (a) and overall survival (b) curves for all patients. Recurrence-free survival (c) and overall survival (d) comparisons of patients with high and low postoperative absolute lymphocyte counts.

Table 1: Univariate analyses of factors associated with postoperative recurrence

Variables	Recurrence group (<i>n</i> = 106)	Non-recurrence group (<i>n</i> = 115)	Statistics	<i>P</i>
Gender (male/female)	98/8	95/20	4.831*	0.084
Age (years)	49.91 ± 12.40	49.55 ± 11.35	0.224†	0.823
Tumor size (cm)	3.23 ± 1.06	3.29 ± 1.03	0.377†	0.707
Differentiation (well/moderate/poor, <i>n</i>)	8/63/35	15/72/28	3.147*	0.207
Tumor number ≥2 (<i>n</i>)	23	9	8.572*	0.003
MVI (<i>n</i>)	29	13	9.236*	0.002
TB (μmol/ml)	16.03 ± 7.25	15.14 ± 6.57	0.951†	0.343
Platelet (×10 ³ /L)	108.4 ± 43.7	119.7 ± 47.1	0.065†	0.097
Transfusion (<i>n</i>)	20	11	3.958*	0.047
High HBV-DNA load (<i>n</i>)	39	22	8.572*	0.003
High AFP (<i>n</i>)	38	29	2.951*	0.086
Preoperative NLR	1.87 ± 1.07	1.62 ± 1.41	1.440†	0.151
Postoperative NLR	1.99 ± 1.66	1.36 ± 1.04	3.441†	0.001
Preoperative PLR	76.44 ± 38.08	88.14 ± 59.30	1.729†	0.085
Postoperative PLR	76.90 ± 35.27	79.45 ± 46.57	0.456†	0.649
Preoperative ALCs (×10 ⁹ /L)	1.62 ± 0.62	1.69 ± 0.61	0.845†	0.623
Postoperative ALCs (×10 ⁹ /L)	1.38 ± 0.69	1.81 ± 0.57	5.065†	<0.001

* χ^2 values; †*t* values. ALCs: Absolute lymphocyte counts; AFP: Alpha-fetoprotein; HBV: Hepatitis B virus; MVI: Microvascular invasion; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; TB: Total bilirubin.

n = 113) and high ($>1.5 \times 10^9/L$, *n* = 108) postoperative ALCs were 68.9%, 45.0%, and 34.5%; and 87.9%, 63.5%, and

58.6%, respectively. Significant differences were observed [Figure 1c, *P* = 0.001]. The 1-, 3-, and 5-year OS rates of

Table 2: Multivariate analyses of factors associated with postoperative recurrence

Variables	HR	95% CI	P
Tumor number ≥ 2	2.056	1.289–3.280	0.002
MVI	2.270	1.468–3.509	<0.001
Postoperative ALCs	0.604	0.427–0.854	0.004

ALCs: Absolute lymphocyte counts; MVI: Microvascular invasion; HR: Hazard ratio; CI: Confidence interval.

Table 3: Univariate analyses of factors associated with postoperative survival

Variables	Died (n = 38)	Survival (n = 183)	Statistics	P
Age (years)	46.79 \pm 10.60	50.33 \pm 12.01	1.684 [†]	0.094
Gender (male/female)	35/3	158/25	0.946*	0.331
Tumor size (cm)	3.08 \pm 1.05	3.30 \pm 1.04	1.146 [†]	0.253
Differentiation (well/moderate/poor, n)	3/21/14	20/114/49	1.652*	0.438
Tumor number ≥ 2 (n)	5	27	0.065*	0.799
MVI (n)	14/24	28/155	9.486*	0.002
TB ($\mu\text{mol/ml}$)	15.92 \pm 7.54	15.49 \pm 6.78	0.344 [†]	0.731
Platelet ($\times 10^3/\text{L}$)	104.2 \pm 42.4	116.4 \pm 46.2	1.503 [†]	0.095
Transfusion (n)	12	19	11.723*	0.001
High HBV-DNA load (n)	16	45	4.831*	0.028
High AFP (n)	15	52	1.821*	0.177
Preoperative NLR	2.19 \pm 1.07	1.65 \pm 1.28	2.449 [†]	0.015
Postoperative NLR	2.85 \pm 2.24	1.42 \pm 1.00	6.167 [†]	<0.001
Preoperative PLR	73.41 \pm 29.25	84.43 \pm 53.72	1.225 [†]	0.222
Postoperative PLR	79.27 \pm 32.32	78.01 \pm 43.19	0.170 [†]	0.865
Preoperative ALCs ($\times 10^9/\text{L}$)	1.43 \pm 0.52	1.70 \pm 0.62	2.547 [†]	0.006
Postoperative ALCs ($\times 10^9/\text{L}$)	1.06 \pm 0.41	1.71 \pm 0.65	5.952 [†]	<0.001

* χ^2 values; [†]t values. ALCs: Absolute lymphocyte counts; AFP: Alpha-fetoprotein; HBV: Hepatitis B virus; MVI: Microvascular invasion; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; TB: Total bilirubin.

Table 4: Multivariate analyses of factors associated with postoperative survival

Variables	HR	95% CI	P
MVI	3.030	1.519–6.044	0.002
Transfusion	3.139	1.536–6.415	0.002
Postoperative ALCs	0.210	0.116–0.380	<0.001

ALCs: Absolute lymphocyte counts; MVI: Microvascular invasion; HR: Hazard ratio; CI: Confidence interval.

patients with low postoperative ALCs were also significantly lower than were those for patients with high postoperative ALCs [93.4%, 77.5%, and 64.8% vs. 98.0%, 93.2%, and 86.5%, respectively; Figure 1d, $P = 0.001$].

Comparison of predictive ability of inflammation-based prognostic scores

ROC curves were constructed for postoperative recurrence, and the areas under the ROC curve (AUCs) were compared to assess the discrimination ability of each inflammation-based

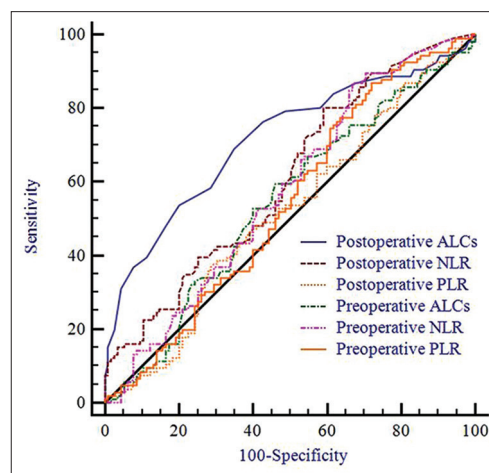


Figure 2: Comparison of the area under the receiver operating characteristics of different inflammation-based scores for recurrence prediction.

prognostic score. As shown in Figure 2, postoperative ALC had the highest AUC (0.716), followed by postoperative NLR (0.609), preoperative NLR (0.575), preoperative ALC (0.545), preoperative PLR (0.541), and postoperative PLR (0.516).

DISCUSSION

In the current study, we found an association between the postoperative prognosis of patients with HCC following liver resection and postoperative ALCs. Our results suggested that low postoperative ALCs in the 1st postoperative month may be a simple and effective marker to use in determining the risks of recurrence and mortality for patients with HCC after liver resection.

T lymphocytes play a key role in anti-tumor immune responses. Previous investigations indicated that tumor-infiltrating lymphocytes represent the host immune response to cancer and further suggested that CD8(+) cytotoxic T-cells have a central role in eliminating tumors and that regulatory T-cells may suppress the immune reaction.^[11] Fu *et al.* study^[12] confirmed that the functional impairment of CD8(+) T-cells may promote HCC progression. Recently, the same group further confirmed that the impairment of CD4(+) cytotoxic T-cells was also associated with poor survival rates and high recurrence of HCC after liver resection.^[13] Yang *et al.*^[14] reported that CD4⁺ CD25⁺ T-cells in the marginal region of HCC may play a critical role in controlling CD8⁺ cytotoxic T-cell activity and, thereby, contribute to the progression of HCC. Unitt *et al.* study^[15] suggested that for patients who underwent liver transplantation as well as who received liver resection and with a reduced lymphocyte infiltration and CD4 to CD8 ratio also played an important role in overcoming HCC recurrence. Our study suggested that patients with low postoperative ALC had poor outcomes after liver resection. One possible explanation for this result was that the anti-tumor immune system was weakened during the perioperative period. Our results also indicated

that we should pay more attention to the changes in the anti-tumor immune system after liver resection because strengthening anti-tumor immune responses may improve outcomes. However, this hypothesis needs additional studies for confirmation.

Many previous studies suggested that a high NLR was an independent risk factor for HCC recurrence after liver resection.^[16,17] However, our study found that both pre- and post-operative NLR had no prognostic power in predicting RFS and OS after liver resection. The potential explanations for NLR in predicting HCC patients' outcomes may be as follows: first, patients with high NLRs had high absolute neutrophil counts and low ALCs; however, some published investigations confirmed that neutrophils could secrete vascular endothelial growth factor (VEGF) which could lead to angiogenesis and increase tumor growth;^[18,19] second, patients' anti-tumor responses mainly depend on lymphocytes. Patients with low ALCs had poor anti-tumor responses.^[20] However, Motomura *et al.*^[21] confirmed that the VEGF, interleukin-8 (IL-8), IL-17, CD68, and CD163 levels were similar between patients with high and low preoperative NLRs who underwent liver transplantation. A high level of IL-17 in the serum and peritumoral tissues was observed in patients with a high NLR. According to Motomura *et al.* results, the NLRs ability to predict tumor recurrence may not be through neutrophil-induced angiogenesis but via the inflammatory tumor microenvironment.^[21] Wu *et al.*^[22] confirmed that both tumors and IL-17 counts from liver infiltrated T-cells were associated with early HCC recurrence and progression after liver resection.

Similar to NLR, some investigations also suggested that high PLR was associated with high risk of postoperative recurrence.^[23] Activated platelets could help tumor cells escape from immune elimination.^[23] Platelet-released factors, such as platelet-derived growth factor, enhance tumor progression and metastasis.^[24] Experimental research even suggested that platelets could stimulate the growth and invasion of a number of HCC cell lines.^[25]

Our study suggested that intraoperative transfusion negatively impacted the OS of patients with HCC after liver resection. Blood loss and secondary transfusion remain major concerns during liver resection. The potential mechanisms that underlie the negative influence of transfusion on postoperative outcomes have been assumed to be associated with suppressive effects of transfusion on the host immune system. Although the exact mechanism is still not well established, a number of investigations have suggested that intraoperative blood transfusion could weaken the function of host natural killer cells, cytotoxic T-cells, macrophages, and monocytes and increase the number of suppressor T-cells.^[26] This may explain why blood transfusion was an independent risk factor of OS after liver resection.

There is no universal consensus regarding the low ALCs cut-off level in predicting the prognoses of patients with HCC after liver resection. In Nagai *et al.* study, they

proposed that $1.0 \times 10^9/L$ was considered a low ALCs for liver transplant recipients.^[7] Li *et al.* study suggested $1.64 \times 10^9/L$ as the optimal ALCs cut-off value to determine OS of patients with recurrent HCC following thermal ablation.^[8] In our study, the optimal ALCs cut-off value in predicting postoperative recurrence were $1.5 \times 10^9/L$. We acknowledge that additional studies are needed to determine the optimal ALCs cut-off point in predicting the outcomes of patients who underwent liver resection for HCC. However, our study confirmed the fact that low postoperative ALCs are associated with a high incidence of postoperative recurrence and poor OS.

There are also some limitations to the current study. This is a single-center retrospective study and, also, it did not exclude patients with portal hypertension. There is no doubt that ALCs may be very low in patients with portal hypertension. We thought the lymphopenia secondary to portal hypertension could be related to poor anti-tumor immunity. Hidaka *et al.*^[27] confirmed that high portal vein pressure was associated with poor long-term outcomes after liver resection for HCC. Chen *et al.*^[28] even suggested that synchronic liver resection and splenectomy could improve the long-term survival of patients with HCC and severe hypersplenism. We also acknowledge that ALC can be affected by numerous factors such as liver function and anti-tumor medicines. In our center, hepatectomy was only performed on patients with Child-Pugh A liver function, and the liver function of most patients who received liver resection recovered during the 1st postoperative month; thus, we think that the influence of liver function on the duration of survival in this study was limited. Moreover, during the 1st postoperative month, the patients in our center did not receive any anti-tumor medicines. Accordingly, we used ALCs measured in the 1st month after liver resection in this study.

In conclusion, our study suggested that low ALCs in the 1st month after liver resection for HBV-related HCC within the Milan criteria were associated with poor outcomes. Low postoperative ALCs might be a surrogate marker for better stratification and management of HCC patients after liver resection. This result also indicated that we should pay more attention to the changes in anti-tumor immune responses in patients with HCC after liver resection.

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Conflicts of interest

There are no conflicts of interest.

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