Blood indices and circulating tumor cells for predicting metastasis of hepatocellular carcinoma after liver transplantation

Li Zhuang

Zhejiang University School of Medicine

Xiang-yan Liu

Shulan(Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College

Heng-kai Zhu

NHC Key Laboratory of Combined Multi-Organ Transplantation

Zhuo-yi Wang

Shulan(Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College

Wu Zhang

Shulan(Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College

Guo-ping Jiang

Shulan(Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College

Shu-sen Zheng (✉ Lily_zhuang@yeah.net)

Zhejiang University School of Medicine First Affiliated Hospital

Research

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Abstract

Background: Liver transplantation (LT) can benefit the long-term survival of hepatocellular carcinoma (HCC) patients. We hypothesized that circulating tumor cell (CTC) levels and subtypes are intimately associated with metastasis status of HCC patients, and this study was designed to test that composite hematological indices including CTC can provide sensitive and accurate prediction of post-LT metastasis.

Methods: Between 2017 and 2018, HCC patients receiving LT were included for analysis. The 24-month follow-up was mainly conducted by outpatient and telephone. Blood samples were collected, and hematological indices were examined. The outcomes such as PFS, recurrence, metastasis, location of recurrence/metastasis, and number of metastases were recorded.

Results: The follow-up analysis showed that microvascular invasion (MVI) classification at the baseline is associated with metastasis. Next, AFP level was another useful indicator of postoperative metastasis, especially at the third or fourth month; the PIVKA-II level 3 months after LT was significantly higher for those who had later metastasis. The mesenchymal CTC level at the 45th day was increased for in the metastasis group. Using two-ends Logistic regression, the calculated value MP (metastasis predictor, by above factors). Had an AUC of 0.858 in the ROC curve, with a cutoff value of 0.328. In conclusion, microvascular invasion, AFP level at the third or fourth month, PIVKA-II level at the third month, and mesenchymal CTC level at day 45 were associated with post-LT metastasis.

Conclusion: Using Logistic regression based on above variables, the 2-year metastasis can be predicted with satisfactory sensitivity and accuracy.

Background

Hepatocellular carcinoma (HCC) is a devastating malignant tumor, as one of the top common cancer and a primary cause of cancer-related mortality. Among all viable therapeutic options for HCC, liver transplantation (LT) can benefit the long-term survival of HCC patients, with satisfactory results of 1-, 5-, and 10-year survivals [1], for it addresses both HCC and underlying primary liver diseases (the major cause of HCC) at the same time. However, increasing evidences have shown that tumor may recur after LT, especially for patients with multinodular and large tumors [2]. Different criteria (e.g. Milan criteria) were established to distinguish who can benefit from LT, but there is large insufficiency in metastasis prediction. Metastasis, an important hallmark of malignant tumors, causes more than 90% of cancer mortality. Therefore, post-LT monitoring of metastasis merits more attention in clinical practice. Many novel techniques have been proposed to estimate the recurrence and metastasis of HCC [3], and the forefront methods commonly take advantages of the circulating tumor cell (CTC). Generally, presence of CTC implies metastasis to extra-hepatic organs. Several studies have claimed that the quantification of CTCs is an emerging tool for diagnosing, stratifying, and assessing extra-hepatic metastasis in HCC patients [4]. But for post-LT monitoring in HCC patients, the clinical value of CTC, as well as CTC subtypes, remains unclear. We hypothesized that CTC levels and subtypes are intimately associated with

metastasis status of HCC patients, and this study was designed to test that available hematological indices including CTC data can provide sensitive and accurate prediction of post-LT metastasis.

Methods

Patients

This study was approved by the Institutional Review Board of Shulan (Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College. For each patient, the informed consent had been acquired. Between 2017 and 2018, HCC patients who received LT were included for analysis. Besides the base-line demographic and disease information, the data of surgical procedures, immunosuppressive treatments, and pre-LT anti-tumor treatments were prospectively recorded. The exclusion criteria were as follow: (1) combination with other tumors, (2) with possible metastasis before LT, (3) receiving re-transplantation, and (4) receiving combined liver and kidney transplantation. The validation cohort was composed of 37 patients, and their data were analyzed retrospectively.

Monitoring And Follow-up

The 24-month follow-up was mainly conducted by outpatient and telephone for discharged individuals, and those receiving continuous hospital treatment were monitored. Regular ultrasonography and CT examination were performed when necessary within 24 postoperative months. MRI examination and PET-CT were further conducted towards a specific site if necessary. Using above examination, tumor recurrence or metastasis were confirmed. Follow-up lasted until the patient died or at the 24th month after LT.

Blood samples were collected at appointed time and processed within 24 h. Before surgery and during the first 6 months, following hematological indices were examined. The AFP level, neutrophil to lymphocyte ratio (NLR), platelet level, and PIVKA-II level were tested preoperatively, after 1, 3, 4 and 6 months. Additionally, the circulating tumor cell (CTC) counts (per ml), as well as different types of CTCs, were probed before surgery and at postoperative day 45 and 120. For CTC analysis, The Nano-Velcro CTC system was used which could capture total CTCs, epithelial type CTCs, mesenchymal CTCs (mCTC) and mixed type CTCs through the multi-marker capture cocktail. CTCs were immune-stained using multi-color cytometric assessment.

The outcomes such as PFS (months), metastasis (yes or no within 24 months), location of recurrence/metastasis, and number of metastases were recorded.

Statistical analysis

The SPSS software (25.0) was used for statistical analysis. Categorical variables expressed as frequencies (also as percent) were analyzed using Chi-squared test. Continuous data were expressed in mean $\pm$ standard deviation (SD). The t-test or One-way ANOVA was used for comparison of continuous

data. Logistic regression was used to predict postoperative metastasis within 24 months. A p value < 0.05 was set as the significance level.

Results

Baseline features of enrolled patients

A total of 37 HCC patients (34 males and 3 females) were included in this study, with an average age of 51.89 ± 9.439 . They had an average period of 22.36 months (min 0.3 and max 132.0) from diagnosed to LT. There were 7 cases receiving surgery before LT, 13 cases treated by RFA before LT, 14 cases with a history of TACE, and 4 cases receiving targeted therapy before LT. Besides, there were 1 case in stage I, 15 cases in stage II, 14 cases in stage III, and 2 cases in stage IV.

Factors Associated With Postoperative Metastasis

According to our follow-up observation, a total of 8 patients were found to have postoperative metastasis in 24 months; and different factors were associated with postoperative metastasis. No differences in sex distribution and ages were found between two groups. As Table 1 shown, microvascular invasion (MVI) classification at the baseline is associated with metastasis. There are three MVI classifications: MVI-0 (no microvascular invasion), MVI-1 (≤ 5 MVI counts, and it occurred near the cancer ≤ 1 cm), MVI-2 (> 5 MVI counts, and it occurred far from the cancer > 1 cm). As expected, higher grade means more frequent metastasis in the following 24 months, and the whole MVI-2 group were found metastasis ($p < 0.05$). Next, AFP level was another useful indicator of postoperative metastasis (Table 2), especially at the third or fourth month ($p < 0.01$), while the postoperative NLR or platelet level was not associated with the metastasis outcome (Table 3 and 4, $p > 0.05$ at each time point). Interestingly, the PIVKA-II level 3 months after LT was significantly higher for those who had later metastasis (Table 5, $p < 0.05$). However, only this time point showed a significant correlation, and the 4-month / 6-month levels did not exhibit any predictive value. Further, the total CTC, as well as different subtypes, could not effectively indicate the potential metastasis, except the mesenchymal CTC level at the 45th day (mCTC45d) (Table 6).

Table 1
Association between microvascular invasion classification and postoperative metastasis

			No metastasis	metastasis
MVI classification	0	Number	8	0
		%	27.6%	0.0%
	1	Number	6	0
		%	20.7%	0.0%
	2	Number	15	8
		%	51.7%	100.0%
Total		Number	29	8
Chi-square		6.213	P value	0.0447

Table 2
Association between AFP levels (ng/ml) and postoperative metastasis

Time points	No metastasis (n = 27–29)	Metastasis (n = 7–8)	t	p
Preoperative	348.17 ± 1462.96	552.50 ± 888.19	-0.351	0.728
Postoperative 1 M	159.53 ± 796.77	29.462 ± 51.76	0.457	0.651
Postoperative 3 M	5.33 ± 7.83	74.62 ± 137.59	-2.802	0.008*
Postoperative 4 M	5.27 ± 10.51	99.94 ± 180.06	-2.924	0.006*
Postoperative 6 M	5.42 ± 11.20	141.43 ± 341.11	-2.207	0.034*

Table 3
Association between NLR levels (%) and postoperative metastasis

Time points	No metastasis (n = 27–29)	Metastasis (n = 7–8)	t	p
Preoperative	3.65 ± 2.94	5.39 ± 2.44	-1.526	0.136
Postoperative 1 M	4.86 ± 3.28	4.70 ± 2.89	0.112	0.912
Postoperative 3 M	2.30 ± 0.94	2.38 ± 1.74	-0.161	0.911
Postoperative 4 M	2.03 ± 1.20	1.98 ± 0.87	0.120	0.905
Postoperative 6 M	2.40 ± 1.14	2.34 ± 0.97	0.135	0.893

Table 4
Association between platelet levels and postoperative metastasis

Time points	No metastasis (n = 27–29)	Metastasis (n = 7–8)	t	p
Preoperative	122.79 ± 111.31	152.75 ± 99.41	0.688	0.496
Postoperative 1 M	185.55 ± 96.18	152.75 ± 53.05	0.920	0.364
Postoperative 3 M	133.59 ± 66.98	119.25 ± 38.01	0.576	0.568
Postoperative 4 M	125.38 ± 64.52	117.38 ± 46.31	0.327	0.746
Postoperative 6 M	129.68 ± 63.64	99.14 ± 23.46	1.237	0.225

Table 5
Association between PIVKA-Ⅱ levels and postoperative metastasis

Time points	No metastasis (n = 27–29)	Metastasis (n = 7–8)	t	p
Preoperative	6550.28 ± 16614.56	7468.88 ± 10681.92	0.147	0.884
Postoperative 1 M	60.07 ± 142.97	24.13 ± 21.05	0.702	0.487
Postoperative 3 M	24.86 ± 7.75	47.13 ± 55.89	2.149	0.039*
Postoperative 4 M	43.45 ± 72.61	81.50 ± 133.00	1.082	0.286
Postoperative 6 M	42.31 ± 59.54	116.00 ± 205.46	1.737	0.091

Table 6
Association between interstitial type CTC levels and postoperative metastasis

Time points	No metastasis (n = 27–29)	Metastasis (n = 7–8)	t	p
Preoperative	1.69 ± 2.35	1.38 ± 1.41	0.3595	0.7213
Postoperative 1.5 M	0.83 ± 0.97	2.88 ± 2.95	3.2516	0.0025*
Postoperative 4 M	1.14 ± 1.36	1.33 ± 1.53	0.2356	0.8154

Prediction Of 2-year Metastasis

Based on above analysis, three variables had the closest relationship with 2-year metastasis: mCTC45d, the AFP level at the 4th month (AFP4M), and the PIVKA-Ⅱ level at the third month (PIVKA-Ⅱ3M). Using the two-ends Logistic regression, we named the calculated value MP (metastasis predictor, by above three factors). As the ROC curve shown (Fig. 1), the AUC of MP was 0.858, with a cutoff value of 0.328 (sensitivity = 0.75, specificity = 0.97). Together, the hematological indexes from day 45 to 4 months are useful references in prediction of LT metastasis.

Discussion

There have been a few widely known predictive factors for survival and tumor progression in HCC patients with LT, such as cumulative tumor size [5], histological grade of differentiation [6], vascular invasion [7], lymph node involvement [8], higher TNM stages [9], and high dosage of cyclosporine [10]. In particular, microvascular invasion, macrovascular invasion, tumor number, and total tumor diameter were among the strongest prognostic factors in Chinese patients [11]. In the aspect of postoperative monitoring, different hematological indices have also been investigated (e.g. platelet counts [12], CD133 and CD90 [3]). Moreover, some preventing treatments (e.g. Licartin administration, Sorafenib treatment) may be helpful for blocking HCC recurrence or metastasis [13]. Theoretically, CTCs may serve as a liquid biopsy for metastatic tumors, with adequate and unique advantages: collection of peripheral blood is easy, rapid, lowly invasive, cost-effective, and feasible for serial real-time monitoring. Extensive studies have demonstrated that CTCs are useful prognostic biomarkers. Currently, the index CTC (especially mCTC) has seldom used in monitoring metastasis or recurrence after LT. We here for the first time found that combination of CTC/AFP/PIVKA-II can effectively indicate the later metastasis.

However, we failed to find clear links between post-LT metastasis and traditional factors, such as Milan criteria, Child-Pugh-Turcotte classification, lymph node involvement, cirrhosis background or age [14]. Also, postoperative preventing treatment did not show significant benefit in our work. This may be due to a limited sample size. Overall, the positive results we discovered are consistent with most published research. Similar to the study of Zavaglia [6], we also noticed microvascular invasion classification influenced the postoperative metastasis, which is as theoretically expected. Nevertheless, this variable has a relatively lower association ($p = 0.045$) in comparison with postoperative hematological indices. Adler et al reported that AFP above 100 ng/mL and vascular involvement of the tumor on the explant were predictors of recurrence [15]. Zhang et al pointed out that AFP can be an independent predictor of OS and AFP above 200 ng/ml indicates poorer outcomes. This was repeatedly reported by other independent studies [16]. We also noticed that AFP monitoring helps reflect the postoperative progression of HCC. Especially, the 4th month time point is more valuable. PIVKA-II is an important biomarker for HCC surveillance in conjunction with AFP [17]. AFP and PIVKA-II are associate with giant lymph node metastasis in hepatoid gastric cancer [18]. Another study showed AFP level, PIVKA-II level, portal vein invasion were the most important prognostic factors for HCC patients [19]. Ultra-high PIVKA-II level is correlated with hepatic portal lymph node metastasis [20]. Our work firstly demonstrated that the PIVKA-II level at postoperative 3 month is elevated in the metastasis group, which emphasizes its value of metastasis monitoring.

Still, there are some limitations in this study. For the scarcity of HCC patients with LT treatment, the sample size was small, and no significant results were found regarding the progression free survival and overall survival. Besides, also for the limited sample size, we did not observe a regular trend of changes in hematological indices. Some indices were significantly changed at one time point (e.g. 3rd month) but turned negative at another (e.g. the 4th month). Although it may be due to a key stage of post-LT

metastasis (e.g. at round day 45), the clear trend, changing curves, and burst-out time point of metastasis are still to be explored through larger sample size.

Conclusions

In conclusion, microvascular invasion, AFP level at the third or fourth month, PIVKA-Ⅱ level at the third month, and mesenchymal CTC level at day 45 were associated with post-LT metastasis. Using Logistic regression based on above variables, the 2-year metastasis can be predicted with satisfactory sensitivity and accuracy.

Abbreviations

LT: Liver transplantation; HCC: hepatocellular carcinoma; CTC: circulating tumor cell; MVI: microvascular invasion; NLR: neutrophil to lymphocyte ratio; SD: standard deviation; MMF: mycophenolate mofetil; AFP4M: AFP level at the 4th month; PIVKA-Ⅱ3M: PIVKA-Ⅱ level at the third month.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Writing - original draft: Li Zhuang;

Conceptualization, Supervision, Writing - review & editing: Shu-Sen Zheng;

Data curation, Methodology, Investigation: Xiang-Yan Liu;

Data curation, Formal analysis, Investigation: Heng-Kai Zhu;

Data curation, Investigation: Zhuo-Yi Wang, Wu Zhang;

Writing - review & editing: Guo-Ping Jiang.

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Availability of data and materials

Data is available from the authors by request.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Shulan (Hangzhou) Hospital Affiliated to Zhejiang Shuren University Shulan International Medical College.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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Figures

Figure 1

The receiver operating characteristic (ROC) curve for efficacy analysis of risk factors in post-LT metastasis in 2 years. The variable MP is calculated by the two-ends Logistic regression model, based on variables including mesenchymal CTC level at the 45th day (mCTC45d), the AFP level at the 4th month (AFP4M), and the PIVKA-II level at the third month (PIVKA-II3M).