Risk factors of recurrence and poor survival in curatively resected hepatocellular carcinoma with microvascular invasion

Li Zhou¹–⁴, Shao-Bin Wang⁵, Shu-Guang Chen⁶, Qiang Qu⁷, Jing-An Rui⁸–¹⁰

Department of General Surgery, Chinese Academy of Medical Sciences/Peking Union Medical College, Beijing, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Abstract

Background. Microvascular invasion (MVI) is a significant sign of the invasive property and a strong predictor of poor prognosis in hepatocellular carcinoma (HCC), a life-threatening malignancy. However, recurrence-associated and post-surgical long-term prognosis-associated factors in HCC with MVI remain unknown.

Objectives. To address the abovementioned issues, based on a Chinese patient cohort with HCC after curative hepatic resection.

Material and methods. The patient cohort consisted of 62 consecutive patients with HCC and MVI who underwent curative hepatic resection. The associations between clinicopathologic variables and recurrence, as well as patient overall/disease-free survival, were univariate and multivariately evaluated.

Results. Univariate χ² test identified hepatitis B surface antigen (HBsAg) positivity, high Edmondson–Steiner grade and male gender as risk factors of recurrence, whereas Edmondson–Steiner grade and HBsAg positivity were significant or marginally significant in the multivariate stepwise logistic regression analysis. Subsequently, univariate log-rank test showed that Edmondson–Steiner grade, HBsAg positivity and Child–Pugh grade were associated with overall and/or disease-free survival. Among them, the independent prognostic impact of Edmondson–Steiner grade and HBsAg positivity for both overall and disease-free survival were proven in the multivariate Cox regression analysis.

Conclusions. Our data suggested that Edmondson–Steiner grade and HBsAg positivity might serve as useful indicators of recurrence and pessimistic prognosis in HCC with MVI.

Key words: prognostic factor, recurrence, survival, hepatocellular carcinoma, hepatic resection
Introduction

Hepatocellular carcinoma (HCC) is well-recognized as a malignant tumor with high incidence and mortality worldwide. Therefore, identification of prognostic markers of HCC became a research hotspot. Except for some clinical and pathological variables, such as portal vein tumor thrombosis (PVTT), tumor size, alpha-fetoprotein (AFP), and Child–Pugh grade, that were previously summarized as the most robust predictors of death, microvascular invasion (MVI), one of signs of unfavorable histological features of HCC (especially the invasive property), was also revealed to be of strong predictive potential for poor prognosis in HCC after several treatment methods, such as resection, transplantation and ablation. However, it was also reported that some patients who carried HCC with MVI acquired actual ten-year survival. Therefore, there is an urgent need and particular interest for the identification of prognostic determinants in this highly invasive subtype of HCC.

The current investigation aimed to address the issues through identification of factors that were associated with recurrence and long-term survival in this subtype of HCC.

Material and methods

Patients

In total, 62 patients undergoing curative hepatic resection for HCC with MVI were included in the study. Their age ranged from 24 to 76 years (mean ± standard deviation (M ±SD) 56.8 ±12.5 years). Tumor sizes, regarding the largest dimensions, ranged from 2 cm to 26 cm (M ±SD 8.5 ±4.9 cm). Microvascular invasion was diagnosed through the postoperative routine pathological examinations. Portal vein tumor thrombosis was defined as tumor involvement in the main branches of portal vein. Satellite nodules were macroscopic. Histological grading was given based on Edmondson–Steiner criteria. The baseline characteristics of patients are shown in Table 1. The acquisition of clinico-pathologic and follow-up data was approved by the institutional Ethics Committee of Peking Union Medical College Hospital, Beijing, China.

Evaluated variables and endpoints

Twelve variables related to general situation of the patient (age and gender), hepatic background (hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), liver cirrhosis, and Child–Pugh grade) and tumor phenotypes (serum AFP value, tumor size, tumor number, PVTT, satellite nodule, and Edmondson–Steiner grade) were chosen for analyses. Post-surgical recurrence, as well as overall and disease-free survival, served as the endpoints. Disease-free survival was defined as the patient survival interval from surgery to recurrence or distant metastasis occurrence.

Follow-up

The follow-up for all patients, ranging from 3 to 108 (Me: 14) months, was performed through imaging examinations and serum AFP level detection, as previously reported. Follow-up intervals ranged from 1 to 3 months.

Statistical analyses

The uni- and multivariate risk factors of recurrence were identified using \( \chi^2 \) and stepwise logistic regression tests. Survival analyses were adopted using Kaplan–Meier method and log-rank test. Cox regression (proportional hazard model) was used for multivariate analysis of prognostic factors. Statistical software package SPSS v. 11.5 (SPSS Inc., Chicago, USA) was applied for all the analyses. Statistical significance was indicated when a p-value was less than 0.05.
Results

Risk factors of recurrence in patients with hepatocellular carcinoma and microvascular invasion after curative hepatic resection

Using univariate χ² test, gender, HBsAg and Edmondson–Steiner grade were associated with postoperative recurrence (p < 0.05; Table 2), but other parameters were not of significance (p > 0.05; Table 2). Multivariate stepwise logistic regression showed that Edmondson–Steiner grade was the single independent risk factor of recurrence (hazard ratio (HR) = 6.374, 95% confidence interval (95% CI) = 1.196–33.963, p = 0.030; Table 2), while HBsAg positivity was of marginal significance (HR = 4.933, 95% CI = 0.789–30.833, p = 0.088; Table 2).

Prognostic factors in patients with hepatocellular carcinoma and microvascular invasion after curative hepatic resection

Univariate log-rank test revealed that HBsAg and Edmondson–Steiner grade were significantly associated with overall survival (p < 0.05; Fig. 1 and Table 3), whereas Edmondson–Steiner grade, HBsAg and Child–Pugh grade were significant for disease-free survival (p < 0.05; Fig. 2 and Table 3). In multivariate Cox regression analyses, Edmondson–Steiner grade and HBsAg were identified as independent prognostic indicators for both overall and disease-free survival (HR = 2.905 and 2.942, 95% CI = 1.263–6.683 and 1.021–8.474, p = 0.012 and 0.046 for overall survival, respectively; HR = 3.407 and 2.944, 95% CI = 1.577–7.360 and 1.124–7.710, p = 0.002 and 0.028 for disease-free survival, respectively; Table 4).

Table 2. Univariate and multivariate factors associated with recurrence in HCC with MVI

| Variables                              | n   | Univariate analysis | Multivariate analysis |
|----------------------------------------|-----|---------------------|-----------------------|
|                                        |     | with recurrence     | without recurrence    | p-value  | HR      | 95% CI       | p-value |
| Age                                    |     |                     |                       |          |         |              |         |
| ≥65 years                              | 19  | 14                  | 5                     | 0.147    | N/A     | N/A          | N/A     |
| <65 years                              | 43  | 38                  | 5                     |          |         |              |         |
| Gender                                 |     |                     |                       |          |         |              |         |
| male                                   | 53  | 47                  | 6                     | 0.045*   | 4.780   | 1.0.671–34.066 | 0.118   |
| female                                 | 9   | 5                   | 4                     |          |         |              |         |
| HBsAg                                  |     |                     |                       |          |         |              |         |
| positive                               | 52  | 47                  | 5                     | 0.001    | 4.933   | 1.0.789–30.833 | 0.088   |
| negative                               | 10  | 5                   | 5                     |          |         |              |         |
| HCV                                    |     |                     |                       |          |         |              |         |
| positive                               | 5   | 4                   | 1                     | N/A      | N/A     | N/A          | N/A     |
| negative                               | 57  | 48                  | 9                     |          |         |              |         |
| Cirrhosis                              |     |                     |                       |          |         |              |         |
| present                                | 51  | 45                  | 6                     | 0.119*   | N/A     | N/A          | N/A     |
| absent                                 | 11  | 7                   | 4                     |          |         |              |         |
| Child–Pugh grade                       |     |                     |                       |          |         |              |         |
| grade A                                | 58  | 48                  | 10                    | 0.838*   | N/A     | N/A          | N/A     |
| grade B                                | 4   | 4                   | 0                     |          |         |              |         |
| Tumor size                             |     |                     |                       |          |         |              |         |
| ≥5 cm                                  | 53  | 45                  | 8                     | 0.962*   | N/A     | N/A          | N/A     |
| <5 cm                                  | 9   | 7                   | 2                     |          |         |              |         |
| PVTT                                    |     |                     |                       |          |         |              |         |
| present                                | 19  | 17                  | 2                     | 0.672*   | N/A     | N/A          | N/A     |
| absent                                 | 43  | 35                  | 8                     |          |         |              |         |
| Satellite nodule                       |     |                     |                       |          |         |              |         |
| present                                | 9   | 8                   | 1                     | 1.000*   | N/A     | N/A          | N/A     |
| absent                                 | 53  | 44                  | 9                     |          |         |              |         |
| Tumor number                           |     |                     |                       |          |         |              |         |
| solitary                               | 55  | 45                  | 10                    | 0.493*   | N/A     | N/A          | N/A     |
| multiple                               | 7   | 7                   | 0                     |          |         |              |         |
| AFP level                              |     |                     |                       |          |         |              |         |
| >20 ng/mL                              | 49  | 42                  | 7                     | 0.732*   | N/A     | N/A          | N/A     |
| ≤20 ng/mL                              | 13  | 10                  | 3                     |          |         |              |         |
| Edmondson–Steiner grade                |     |                     |                       |          |         |              |         |
| I–II                                   | 17  | 10                  | 7                     | 0.004*   | 1       | 6.374        | 0.030   |
| III–IV                                 | 45  | 42                  | 3                     |          |         | 1.196–33.963 |         |

HR – hazard ratio; 95% CI – 95% confidence interval; N/A – not applicable; * χ² test with continuity correction; values in bold indicate statistically significant differences.
Table 3. Univariate analysis for overall and disease-free survival of HCC with MVI patients

| Variables                | n   | Overall survival | Disease-free survival |
|--------------------------|-----|------------------|-----------------------|
|                          |     | M ±SE            | 95% CI                | p-value  | M ±SE      | 95% CI      | p-value  |
| Age                      |     |                  |                       |          |            |             |          |
| ≥65 years                | 19  | 40 ±10           | 20–60                 | 0.408    | 34 ±10     | 14–53       | 0.250    |
| <65 years                | 43  | 24 ±3            | 18–30                 |          | 16 ±2      | 12–19       |          |
| Gender                   |     |                  |                       |          |            |             |          |
| male                     | 53  | 28 ±5            | 19–37                 | 0.772    | 19 ±4      | 12–27       | 0.213    |
| female                   | 9   | 22 ±4            | 14–30                 |          | 22 ±5      | 13–31       |          |
| HBsAg                    |     |                  |                       |          |            |             |          |
| positive                 | 52  | 22 ±3            | 16–27                 | 0.006    | 14 ±2      | 11–17       | 0.002    |
| negative                 | 10  | 69 ±15           | 40–97                 |          | 59 ±15     | 29–89       |          |
| HCV                      |     |                  |                       |          |            |             |          |
| positive                 | 5   | 12 ±3            | 6–18                  | 0.063    | 9 ±2       | 4–14        | 0.167    |
| negative                 | 57  | 30 ±5            | 20–39                 |          | 22 ±4      | 14–30       |          |
| Cirrhosis                |     |                  |                       |          |            |             |          |
| present                  | 51  | 27 ±5            | 18–36                 | 0.286    | 18 ±3      | 11–24       | 0.080    |
| absent                   | 11  | 27 ±4            | 19–36                 |          | 22 ±4      | 14–30       |          |
| Child–Pugh grade         |     |                  |                       |          |            |             |          |
| grade A                  | 58  | 30 ±5            | 20–39                 | 0.058    | 22 ±4      | 14–31       | 0.017    |
| grade B                  | 4   | 11 ±5            | 1–21                  |          | 7 ±3       | 1–12        |          |
| Tumor size               |     |                  |                       |          |            |             |          |
| ≥5 cm                    | 53  | 27 ±5            | 18–37                 | 0.101    | 21 ±4      | 12–29       | 0.147    |
| <5 cm                    | 37  | 74 ±8            | 58–90                 |          | 21 ±3      | 15–27       |          |
| PVTT                     |     |                  |                       |          |            |             |          |
| present                  | 19  | 20 ±5            | 11–30                 | 0.095    | 13 ±3      | 7–19        | 0.095    |
| absent                   | 43  | 34 ±6            | 21–46                 |          | 26 ±5      | 16–37       |          |
| Satellite nodule         |     |                  |                       |          |            |             |          |
| present                  | 9   | 16 ±5            | 7–25                  | 0.051    | 11 ±3      | 5–18        | 0.012    |
| absent                   | 53  | 31 ±5            | 21–41                 |          | 23 ±4      | 12–32       |          |
| Tumor number             |     |                  |                       |          |            |             |          |
| solitary                 | 55  | 31 ±5            | 20–41                 | 0.203    | 23 ±4      | 14–32       | 0.337    |
| multiple                 | 7   | 17 ±5            | 7–28                  |          | 12 ±4      | 5–20        |          |
| AFP level                |     |                  |                       |          |            |             |          |
| >20 ng/mL                | 49  | 26 ±4            | 17–34                 | 0.517    | 19 ±4      | 11–26       | 0.260    |
| ≤20 ng/mL                | 13  | 31 ±8            | 16–46                 |          | 22 ±5      | 12–32       |          |
| Edmondson–Steiner grade  |     |                  |                       |          |            |             |          |
| I–II                     | 17  | 56 ±13           | 31–80                 | 0.001    | 46 ±12     | 24–69       | <0.001   |
| III–IV                   | 45  | 19 ±2            | 15–23                 |          | 12 ±1      | 10–14       |          |

M – mean; SE – standard error; values in bold indicate statistically significant differences.

Fig. 1. The significant influence of the variables on overall survival of patients with HCC and MVI after curative hepatic resection. A. Edmondson–Steiner grade (solid line – grade I–II, n = 17; dashed line – grade III–IV, n = 45; p = 0.001); B. HBsAg positivity (solid line – negative, n = 10; dashed line – positive, n = 52; p = 0.006).
Microvascular invasion, as a marker of the invasive growth, remarkably influences prognosis of HCC in patients who underwent curative treatments, liver resection and transplantation, when recurrence and/or overall as well as disease-free survival served as endpoints. However, prognostic factors of HCC with MVI remain to be explored. In the present study, factors associated with tumor recurrence were first identified. It was found that gender, Edmondson–Steiner grade and HBsAg were of statistical significance in univariate \( \chi^2 \) test, while these 3 variables were significant or marginally significant in multivariate logistic regression, respectively. Previously, gender and Edmondson–Steiner grade, along with MVI, were suggested to be correlated with post-surgical recurrence of HCC. This study first showed their roles in HCC with MVI. Moreover, the more important finding of this work is to define HBsAg as a potential risk factor of recurrence in HCC with MVI, based on univariate and multivariate analyses. It was shown that high hepatitis B viral load predicted the recurrence of small HCC after curative resection. We provided 2 novel predictors, HBsAg, which is routinely detected, and gender that was not predictive for recurrence in MVI-absent HCC, in addition to Edmondson–Steiner grade.

Table 4. Multivariate analysis for overall and disease-free survival of HCC with MVI

| Variables          | Overall survival | Disease-free survival |
|--------------------|------------------|-----------------------|
|                    | HR               | 95% CI                | p-value | HR               | 95% CI                | p-value |
| Edmondson–Steiner grade | 2.905            | 1.263–6.683           | 0.012 | 3.407            | 1.577–7.360           | 0.002 |
| HBsAg              | 2.942            | 1.021–8.474           | 0.046 | 2.944            | 1.124–7.710           | 0.028 |
| Child–Pugh grade   | N/A              | N/A                   | N/A | 2.043            | 0.718–5.813           | 0.181 |

N/A – not applicable; values in bold indicate statistically significant differences.

Discussion

in multivariate logistic regression, respectively. Previously, gender and Edmondson–Steiner grade, along with MVI, were suggested to be correlated with post-surgical recurrence of HCC. This study first showed their roles in HCC with MVI. Moreover, the more important finding of this work is to define HBsAg as a potential risk factor of recurrence in HCC with MVI, based on univariate and multivariate analyses. It was shown that high hepatitis B viral load predicted the recurrence of small HCC after curative resection. We provided 2 novel predictors, HBsAg, which is routinely detected, and gender that was not predictive for recurrence in MVI-absent HCC, in addition to Edmondson–Steiner grade. Some authors found
the association between HBsAg positivity and high AFP level, whereas AFP is reported to be involved in many malignant phenotypes of hepatoma cells, even in the activity of dendritic cells, through different mechanisms.23–25 Furthermore, the finding that knockdown of HBsAg expression inhibits HCC growth provides the direct evidence for the role of HBsAg.26 Therefore, our results about HBsAg and tumor recurrence might have a molecular basis.

On the other hand, it is well-known that HCC carries unsatisfactory prognosis. Thus far, its prognostic indicators, including MVI, have been identified.4–15 However, prognostic indicators in HCC with MVI remain unclear and need in-depth exploration, because different survival status was also found in patients who carried HCC with MVI.15 Our results showed that HBsAg and Edmondson–Steiner grade univariately and multivariately predicted both overall and disease-free survival, in consistence with reports on other types of HCC.27,28 Considering the fact that these variables were independent or marginally independent risk factors for recurrence, their impact on patient survival of HCC with MVI might be understandable. Previously, the impact of HBsAg positivity on prognosis of HCC after radical or palliative therapies was also suggested.27 This study first links HBsAg and HCC with MVI. Here, the authors preliminarily speculate on the possible route of its action in this subtype of HCC, as this protein stimulates tumor growth, then facilitates recurrence, and finally causes worse prognosis. Of course, this hypothesis needs to be extensively validated.

Conclusions

Our data suggests that Edmondson–Steiner grade and HBsAg positivity might function as significant predictors for recurrence and poor prognosis in HCC with MVI after curative resection.

References

1. Pisani P, Parkin DM, Bray F, Ferlay J. Estimating the world cancer burden: Globocan 2000. Int J Cancer. 2001;94(2):153–156.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics (2002). CA Cancer J Clin. 2005;55(2):74–108.
3. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China (2015). CA Cancer J Clin. 2016;66(2):115–132.
4. Tandon P, Garcia-Tsao G. Prognostic indicators in hepatocellular carcinoma: A systematic review of 72 studies. Liver Int. 2009;29(4):302–510.
5. Ng KM, Yan TD, Black D, Chu FC, Morris DL. Prognostic determinants for survival after resection/ablation of a large hepatocellular carcinoma. HPB (Oxford). 2009;11(4):31–32.
6. Belli G, Fantini C, Belli A, Limongelli P. Laparoscopic liver resection for hepatocellular carcinoma in cirrhosis: Long-term outcomes. Dig Surg. 2011;28(2):134–140.
7. Bertuzzo VR, Cescos M, Ravaiolli M, et al. Analysis of factors affecting recurrence of hepatocellular carcinoma after liver transplantation with a special focus on inflammation markers. Transplantation. 2011;91(11):1279–1285.
8. Lim KC, Chow PK, Allen JC, et al. Microvascular invasion is a better predictor of tumor recurrence and overall survival following surgical resection for hepatocellular carcinoma compared to the Milan criteria. Ann Surg. 2011;254(1):108–113.
9. Fan ST, Poon RT, Yeung C, et al. Outcome after partial hepatectomy for hepatocellular cancer within the Milan criteria. Br J Surg. 2011;98(9):1292–1300.
10. Adam R, Bhangui P, Vibert E, et al. Resection or transplantation for early hepatocellular carcinoma in a cirrhotic liver: Does size define the best oncological strategy? Ann Surg. 2012;256(6):883–891.
11. Shindoh J, Hasagawa K, Inoue Y, et al. Risk factors of post-operative recurrence and adequate surgical approach to improve long-term outcomes of hepatocellular carcinoma. HPB (Oxford). 2013;15(13):31–39.
12. Abdel-Wahab M, Sultan AM, Fathy OM, et al. Factors affecting recurrence and survival after living donor liver transplantation for hepatocellular carcinoma. Hepatogastroenterology. 2013;60(128):1847–1853.
13. Li SH, Wei W, Guo RP, et al. Long-term outcomes after curative resection for patients with macroscopically solitary hepatocellular carcinoma without macrovascular invasion and an analysis of prognostic factors. Med Oncol. 2013;30(4):696.
14. Zhao H, Chen C, Fu X, et al. Prognostic value of a novel risk classification of microvascular invasion in patients with hepatocellular carcinoma after resection. Oncotarget. 2017;8(3):5474–5486.
15. Zheng J, Kuk D, Gønæ M, et al. Actual 10-year survivors after resection of hepatocellular carcinoma. Ann Surg Oncol. 2017;24(5):1358–1366.
16. Edmondson HA, Steiner PE. Primary carcinoma of the liver: A study of 100 cases among 48,900 necropsies. Cancer. 1954;7(3):462–503.
17. Zhou L, Rui JA, Wang SB, Chen SG, Qu Q. The significance of serum AFP cut-off values, 20 and 400 ng/mL in curatively resected patients with hepatocellular carcinoma and cirrhosis might be of difference. Hepatogastroenterology. 2012;59(115):840–843.
18. Zhou L, Rui JA, Wang SB, Chen SG, Qu Q. Risk factors of poor prognosis and portal vein tumor thrombosis after curative resection of solitary hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int. 2013;12(1):68–73.
19. Jeng KS, Sheen IS, Tsai YC. Circulating messenger RNA of alpha-feto-protein: A possible risk factor of recurrence after resection of hepatocellular carcinoma. Arch Surg. 2004;139(10):1055–1060.
20. Qu LS, Jin F, Huang XW, Shen XZ. High hepatitis B viral load predicts recurrence of small hepatocellular carcinoma after curative resection. J Gastrointest Surg. 2010;14(7):1111–1120.
21. Xia F, Lai EC, Lai WY, et al. High serum hyaluronic acid and HBV viral load are main prognostic factors of local recurrence after complete radiofrequency ablation of hepatitis B-related small hepatocellular carcinoma. Ann Surg Oncol. 2012;19(4):1284–1291.
22. Zhou L, Rui JA, Zhou WX, Wang SB, Chen SG, Qu Q. Edmondson–Steiner grade: A crucial predictor of recurrence and survival in hepatocellular carcinoma without microvascular invasion. Pathol Res Pract. 2017;213(7):824–830.
23. Peng SY, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC. High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: Significance of hepatitis virus infection, age, p53 and beta-catenin mutations. Int J Cancer. 2004;112(1):44–50.
24. Pardee AD, Shi JJ, Butterfield LH. Tumor-derived alpha-fetoprotein impairs dendritic cells, through different mechanisms. J Immunol. 2010;36:257–263.
25. Lim KC, Chow PK, Allen JC, et al. Microvascular invasion is a better predictor of tumor recurrence and overall survival following surgical resection for hepatocellular carcinoma compared to the Milan criteria. Ann Surg. 2011;254(1):108–113.
26. Fan ST, Poon RT, Yeung C, et al. Outcome after partial hepatectomy for hepatocellular cancer within the Milan criteria. Br J Surg. 2011;98(9):1292–1300.
27. Adam R, Bhangui P, Vibert E, et al. Resection or transplantation for early hepatocellular carcinoma in a cirrhotic liver: Does size define the best oncological strategy? Ann Surg. 2012;256(6):883–891.
28. Liu XY, Xu JF. Liver resection for young patients with large hepatocellular carcinoma: A single center experience from China. World J Surg Oncol. 2014;12:175.