Tumor necrosis as a poor prognostic predictor on postoperative survival of patients with solitary small hepatocellular carcinoma

Yi-hong Ling
Sun Yat-sen University Cancer Center
Jie-wei Chen
Sun Yat-sen University Cancer Center
Shi-hong Wen
First affliated Hospital of Sun Yat-sen university
Chao-yun Huang
Sun Yat-sen University Cancer Center
Peng Li
Sun Yat-sen University Cancer Center
Liang-he Lu
Sun Yat-sen University Cancer Center
Jie Mei
Sun Yat-sen University Cancer Center
Shao-hua Li
Sun Yat-sen University Cancer Center
Wei Wei
Sun Yat-sen University Cancer Center
Muyan Cai (caimy@sysucc.org.cn)
Sun Yat-sen University Cancer Center
Rong-ping Guo
Sun Yat-sen University Cancer Center

Research article

Keywords: Tumor necrosis; prognosis; small hepatocellular carcinoma

Posted Date: June 11th, 2020

DOI: https://doi.org/10.21203/rs.2.17667/v2

Version of Record: A version of this preprint was published on June 29th, 2020. See the published version at https://doi.org/10.1186/s12885-020-07097-5.

Abstract

Background: Small hepatocellular carcinoma (sHCC) is a special subtype of HCC with the maximum tumor diameter ≤ 3 cm and favorable long-term outcomes. Surgical resection or radiofrequency ablation offer the greatest chance for cure; however, many patients still undergo tumor recurrence after primary treatment. So far, there is no clinical applicable method to assess biological aggressiveness in solitary sHCC.

Methods: In the present study, we retrospectively evaluated tumor necrosis of 335 patients with solitary sHCC treated with hepatectomy between December 1998 and 2010 from Sun Yat-sen University Cancer Center.

Results: In the current study, the presence of tumor necrosis was observed in 157 of 335 (46.9%). Further correlation analysis showed that the presence of tumor necrosis in sHCC was significantly correlated with tumor size and vascular invasion (P = 0.026, 0.003, respectively). The presence of tumor necrosis was associated closely with poorer cancer-specific overall survival (OS) and recurrence-free survival (RFS) as evidenced by univariate (P < 0.001;hazard ratio, 2.821; 95% CI, 1.643-4.842) and multivariate analysis (P = 0.005; hazard ratio, 2.208; 95% CI, 1.272-3.833). More importantly, the combined model by tumor necrosis, vascular invasion and tumor size can significantly stratify the risk for RFS and OS and improve the ability to discriminate sHCC patients' outcomes (P < 0.0001 for both).

Conclusions: Our findings provide evidence that tumor necrosis has the potential to be a parameter for cancer aggressiveness in solitary sHCC. The combined prognostic model may be a useful tool for identifying solitary sHCC patients with worse outcomes.

Background

As the second leading cause of cancer mortality worldwide, hepatocellular carcinoma (HCC) has a high prevalence in Southeast Asia and Africa, and its incidence has been increasing in Europe and America [1-3]. Because of the high prevalence of hepatitis B virus (HBV) infection, HBV-related liver cirrhosis and/or HCC has become a main disease burden in China [4]. Currently, surveillance and the advances in imaging techniques have led to the discovery of more small HCCs (sHCC, ≤ 3 cm in diameter) [5, 6].

Solitary sHCC is a special type of HCC with excellent long-term outcomes, for which surgical resection offers the optimal curative chance [7, 8]. Nevertheless, many patients still suffer from recurrence after primary resection and less is known about the factors correlated with aggressive biological phenotype of sHCC [5-8]. It may be ideal to identify patients at high risk of tumor recurrence and/or poorer outcome, and to target close follow-up or postoperative adjuvant therapies in these sub-populations [9, 10]. Currently, the known clinicopathological factors for sHCC enable the identification and screening the patients at high risk, however, the reliable factors remain ill-defined [11, 12].

Traditionally, pTNM stage and histological grading systems are recognized as the most useful prognostic factors of sHCC. Besides, other features such as tumor size and vascular invasion also utilized in clinical setting and found to be prognostic assessment of sHCC patients. Tumor necrosis is a common pathological feature of solid tumors, which is found to be correlated with chronic ischemic injury due to the rapid growth of tumor [13-15]. The extent of tumor necrosis reflects the level of intra-tumor hypoxia. Moreover, increased cellular hypoxia is linked to the increased metastatic potential and worse outcome in solid tumors, as well as resistance to radio-chemotherapy [13, 16]. To date, the clinical and prognostic implication of tumor necrosis in solitary sHCC remain elusive [17, 18]. In the present study, we proposed to assess the prognostic value of tumor necrosis in solitary sHCC following hepatectomy and to demonstrate whether tumor necrosis can be regarded as a parameter for sHCC aggressiveness. In addition, we further aimed to construct a clinicopathologic model with risk factors to predict the prognosis of sHCC patients.

Methods

Case selection

Data were obtained from the 335 cases of the pathologically proven and non-distant-metastasis solitary sHCC between December 1998 and 2009 in Sun Yat-sen University Cancer Center (Guangzhou, China). The patients underwent surgical resection (not ablation or transplantation) as the first therapy course were included in this study. Cases were acquired following the eligibility criteria: (1) solitary sHCC (diameter ≤ 3 cm) only; (2) positive for HBV surface antigen; (3) with primary and curative hepatectomy; (4) absence of metastasis and residual disease; (5) without preoperative adjuvant therapy; having complete follow-up information.

We collected clinicopathologic data including patient age, gender, alfa-fetoprotein (AFP) level, alanine aminotransferase (ALT) level, tumor size, tumor capsule, histological differentiation, liver cirrhosis, vascular invasion and necrosis. These data are described in Table 1. Tumor differentiation was determined based on the criteria proposed by WHO classification of Tumors of the Digestive System (2010 version). The Institute Research Medical Ethics Committee of Sun Yat-sen University Cancer Center granted approval for this study.

Table 1

Correlation of tumor necrosis with patients' clinicopathological features in primary small hepatocellular carcinomas

Characteristics	Cases	Necrosis (-)	Necrosis (+)	*P* value*
Gender				0.555
Male	295	155 (52.5%)	140 (47.5%)	
Female	40	23 (57.5%)	17 (42.5%)	
Age (years)				0.694
≤ 48.0†	166	90 (54.2%)	76 (45.8%)	
> 48.0	169	88 (52.1%)	81 (47.9%)	
AFP (ng/ml)				0.485
≤ 20	139	77 (55.4%)	62 (44.6%)	
> 20	196	101 (51.5%)	95 (48.5%)	
ALT (μ/l)				0.504
≤ 40	192	99 (51.6%)	93 (48.4%)	
> 40	143	79 (55.2%)	64 (44.8%)	
Tumor size (cm)				0.026
≤ 2.5‡	188	110 (58.5%)	78 (41.5%)	
> 2.5	147	68 (46.3%)	79 (53.7%)	
Differentiation				0.675
Well	56	32 (57.1%)	24 (42.9%)	
Moderate	208	111 (53.4%)	97 (46.6%)	
Poor-undifferentiated	71	35 (49.3%)	36 (50.7%)	
Vascular invasion				0.003
Absent	255	147 (57.6%)	108 (42.4%)	
Present	80	31 (38.8%)	49 (61.3%)	
Envelope				0.872
Absent	214	113 (52.8%)	101 (47.2%)	
Present	121	65 (53.7%)	56 (46.3%)	
Liver cirrhosis				0. 166
Absent	201	113 (56.2%)	88 (43.8%)	
Present	134	65 (48.5%)	69 (51.5%)	

*Chi-square test; †Median age; ‡Median size; AFP indicates alpha-fetoprotein; ALT indicates alanine aminotranferease.

Pathological Evaluation

Patient records and original histopathologic slides were independently reviewed by two experienced pathologists (Y.-H. Ling and M.-Y. Cai) who were blinded to the pathological diagnoses and outcome data. Discrepancies were solved by simultaneous re-examination of the slides by both pathologists with a double-headed microscope. A mean of 4.2 (median 4, range 2-8) paraffin-embedded tissue blocks per tumor were available for evaluation, and all of the 335 patients had at least 3 tissue blocks available.

The presence of necrosis was carefully assessed on hematoxylin and eosin (H&E)-stained slides. Necrosis consisted of homogenous clusters of sheets of dead cells, or coalescing groups of cells forming a coagulum, containing nuclear and cytoplasmic debris as previously described [19]. Coagulative tumor necrosis was found to be present without regard to the area of tumor involved, and the extent of involvement was not assessed. Vascular invasion in each HCC specimen was identified in several serial

cross sections, defined as infiltration of vessel walls or the existence of tumor emboli [20]. The criteria include the following: macroscopic and/or microscopic tumor emboli within the large capsular vessels, the central hepatic vein, or the portal vein [21].

Follow-up

After partial hepatectomy, patients were followed up by the Sun Yat-sen University Cancer Center, every 3 months by AFP and ultrasound or computed tomography or magnetic resonance imaging at least every 6 months for more than two years. The last date of follow-up is January 18th, 2014. Patients who had tumor recurrence were treated with re-resection when possible or by transcatheter arterial chemoembolization, percutaneous ethanol injection or radiofrequency ablation. Cancer-specific overall survival (OS) was defined as the number of months from the date of surgery to the date of the last follow-up visit or time of death attributed to sHCC. Recurrence-free survival (RFS) was defined as the number of months from the date of surgery to the first documentation of cancer recurrence.

Statistical analysis

The correlation between necrosis and the clinicopathologic features of the sHCC patients was evaluated by a $\chi 2$-test. For univariate analysis, survival curves were obtained with the Kaplan-Meier method, and the differences between groups in survival were tested by the log-rank test. Multivariate survival analyses were performed with the Cox proportional hazard regression model. A difference was considered significant if the *P* value from a two-tailed test was less than 0.05. Statistical analysis was performed with SPSS statistical software package (SPSS Standard version 13.0; SPSS, Chicago, IL, USA).

Results

Patient characteristics

Our selection criteria identified 335 adult patients with resected solitary sHCC. All the patients were long-term carriers of HBV and treated with curative surgical resection, which was, in some cases, followed by second-line treatments at the time of recurrence. Demographic and clinical characteristics for the patients are presented in Table 1.

Of the 335 patients, there were 295 (88.1%) males and 40 (11.9%) females, with a median age of 48 years. Among the 335 patients with pre-operation serum AFP level record, 196 (58.5%) patients had serum AFP level >20 ng/ml. 143 (42.7%) patients had serum ALT level >40 μ/l. The median size of the tumors was 2.5 cm. A total of 264 (78.8%) patients had well-differentiated or moderate-differentiated tumors. 214 (63.9%) tumors were encapsulated. Vascular invasion was presented in 80 (23.9%) cases. Liver cirrhosis was observed in 134 (40%) cases.

The patterns of tumor necrosis in solitary sHCC

Presence of tumor necrosis was observed in 157 of 335 (46.9%) of sHCC (Figure 1). Further correlation analysis demonstrated that the presence of tumor necrosis was significantly correlated with tumor size and vascular invasion in sHCC (P = 0.026, 0.003, respectively; Table 1).

The relationship between tumor necrosis and patients' survival: univariate analysis

Assessment of survival of sHCC patients revealed that some clinical pathological parameters indicated a significant impact of prognosis, such as tumor size (*P* = 0.001) and vascular invasion (*P* < 0.001, Table 2), which was reported in our previous study [22]. The result demonstrated that patients with tumor necrosis displayed a poor overall survival (Table 2; Figure 2A) and recurrence-free survival (Figure 2B) than patients without tumor necrosis (*P* < 0.0001).

Table 2

Univariate and multivariate analyses of tumor necrosis and clinicopathologic variables in patients with primary small hepatocellular carcinoma*

Characteristics	*P* value	Hazard Ratio (95% CI)
Univariate analysis		
Gender (Male vs. Female)	0.632	0.825 (0.374–1.816)
Age (≤ 48.0† vs. > 48.0)	0.957	1.014 (0.615–1.672)
AFP (≤ 20 ng/ml vs. > 20 ng/ml)	0.432	1.230 (0.734–2.059)
ALT (≤ 40 μ/l vs. > 40 μ/l)	0.253	1.337 (0.812–2.201)
Tumor size (≤ 2.5‡ cm vs. > 2.5 cm)	0.001	2.431 (1.443–4.093)
Differentiation (well-moderate vs. poor-undifferentiated)	0.512	1.215 (0.679–2.175)
Vascular invasion (absent vs. present)	< 0.001	3.033 (1.827–5.035)
Envelope (absent vs. present)	0.758	0.920 (0.544–1.559)
Liver cirrhosis (absent vs. present)	0.102	1.516 (0.921–2.495)
Tumor necrosis (absent vs. present)	< 0.001	2.821 (1.643–4.842)
Multivariate analysis		
Tumor size (≤ 2.5 cm vs. > 2.5 cm)	0.006	2.083 (1.229–3.529)
Vascular invasion (absent vs. present)	< 0.001	2.663 (1.598–4.437)
Tumor necrosis (absent vs. present)	0.005	2.208 (1.272–3.833)

*The analyses were performed with the use of Cox proportional-hazards regression; †Median age; ‡Median size; AFP indicates alpha-fetoprotein; ALT indicates alanine aminotranferease.

Multivariate Cox regression analysis

Since variables examined to have prognostic influence by univariate analysis may covariate, the presence of tumor necrosis as well as other clinicopathologic features (tumor size and vascular invasion) was tested in multivariate analysis (Table 2). The presence of tumor necrosis was associated closely with poorer cancer-specific OS and RFS as evidenced by univariate (*P* < 0.001; hazard ratio, 2.821; 95% CI, 1.643-4.842) and multivariate analysis (*P* = 0.005; hazard ratio, 2.208; 95% CI, 1.272-3.833). As reported in our previous study [22], of the other parameters, vascular invasion was evaluated as an independent prognostic factor for patient survival (P < 0.001; hazard ratio, 2.663; 95% CI, 1.598-4.437) and tumor size was evaluated as an independent prognostic factor for patient survival (P = 0.006; hazard ratio, 2.083; 95% CI, 1.229-3.529).

The relationship between tumor necrosis and postoperative survival of patients with sHCC stratified according to different risk factors

Kaplan-Meier survival curve comparing tumor necrosis affecting postoperative survival of patients with sHCC was stratified according to different tumor size, differentiation, serum AFP level and vascular invasion. As shown in Figure 3A and 3E, tumor necrosis was associated with a decrease in OS of patients with tumor size ≤ 2.5 cm, and a decrease in RFS of patients with tumor size ≤ 2.5 cm as well as > 2.5 cm (P = 0.0200, 0.0020 and 0.0240, respectively). Meanwhile, tumor necrosis was associated with a decrease in OS and RFS of patients with AFP level ≤ 20 ng/ml and > 20 ng/ml (P = 0.0090, 0.0030, 0.0060 and 0.0020, respectively. Figure 3B and 3F). Tumor necrosis was associated with a decrease in OS and RFS of patients with different tumor differentiation (P = 0.0210, < 0.0001, = 0.0110 and < 0.0001, respectively. Figure 3C and 3G). Tumor necrosis was associated with a decrease in OS and RFS of patients with or without vascular invasion (P = 0.0380, 0.0040, 0.0210 and 0.0030, respectively. Figure 3D and 3H).

New prognostic model with tumor necrosis, tumor size and vascular invasion in sHCC

According to the results of our univariate and multivariate analyses, we proposed a new clinicopathologic prognostic model with three poor prognostic factors: tumor necrosis, tumor size and vascular invasion. Thus, we designated four subtypes based on the presence of the three factors (including tumor necrosis, tumor size > 2.5 cm and vascular invasion): subtype 1, absence of any risk factor; subtype 2, absence of any two risk factors; subtype 3, absence of any one risk factor; subtype 4, presence of three risk factors. The model could significantly stratify risk (low, intermediate and high) for OS (Fig. 4, P < 0.0001) and RFS (Fig. 4, P < 0.0001) in our study based upon a combination of tumor necrosis, tumor size and vascular invasion.

Discussion

In the current study, we assessed a retrospective collection of data and determine the prognostic value of tumor necrosis for sHCC patients after hepatectomy. Our results demonstrated that tumor necrosis was frequently observed in sHCC. Further correlation analysis revealed that the presence of tumor necrosis was significantly associated with vascular invasion. In univariate analysis, tumor necrosis, vascular invasion and tumor size were poor prognostic factors. Furthermore, multivariate analysis evaluated that the presence of tumor necrosis was a prognostic factor independent of certain well-established clinical factors, including tumor size, serum AFP level, vascular invasion and clinical stage.

Tumor necrosis has been shown prognostic impact in lung, breast, thyroid, colorectal, pancreatic, and kidney malignancies, but also in mesenchymal tumors, such as malignant mesothelioma, gastrointestinal stromal tumors and Ewing sarcoma [13, 14, 16, 23-26]. Besides, Soini et al. reported that the survival of sHCC patients showing a high proliferation and simultaneously a low degree of apoptosis and necrosis was significantly shorter than with other patients [17]. Martino et al. found that in the non-cirrhotic patients with HCC tended to be well to moderately differentiated, may presented with certain areas of necrosis but did not demonstrate the relative prognosis [18]. Thus, data regarding the incidence

and prognostic impact of necrosis in HCC are scarce and limited. Our study has characterized, for the first time, tumor necrosis in sHCC and demonstrates that the presence of tumor necrosis is associated with poor survival. This implies a relationship where increased tumor cell death indicates a more aggressive cancer. Tumor microvessels are fragile and susceptible to hypoxia, which suggests that the degree of tumor necrosis reflects the level of intratumoral hypoxia [13, 14, 27, 28]. Measured experimentally with a polarographic needle, intratumoral hypoxia correlates with poor prognosis and sensitivity to radiotherapy and chemotherapy in solid tumors [13]. In breast cancer, tumor necrosis has been shown to correlate with increased tumor size, high-grade disease, negative estrogen receptor status, high microvessel density, and infiltrates of macrophages that express vascular endothelial growth factor [23, 29, 30]. These findings suggest that, in rapidly growing tumors, a hypoxic environment that results in tumor necrosis stimulates angiogenesis due to the release of angiogenic growth factors from infiltrating macrophages.

HCC is characterized by a tendency for vascular invasion that is believed to be a strong predictor of outcome following hepatic resection and liver transplantation of HCC [31, 32]. We previously showed that vascular invasion had an adverse impact on long-term survival in sHCC patients which led to a significant decrease in OS and RFS at 5 years. The association of tumor necrosis and vascular invasion is consistent with studies in breast cancer and malignant mesothelioma. It was observed microvessel hot spots were situated away from areas of tumor necrosis in these two neoplasms [23, 33]. It is possible to explain this apparently paradoxical relationship by rapid tumor growth outstripping the vascular supply, causing ischemic damage to the microvasculature and thereby increased tumor necrosis. Tumor size is a well-known risk factor for poor survival following hepatectomy of HCC, and tumor size > 2.5 cm was correlated with a worse OS or RFS even in the patients with tumors ≤ 3 cm in our previous study [22]. In breast cancer, tumor necrosis correlated with increasing tumor size [23], while the association between T stage and necrosis remains unclear in other solid tumors. It was also confirmed that increasing mass was associated with hypoxia in the experimental murine allograft model [34]. Similarly, we also demonstrated that the tumor necrosis was associated with increasing tumor size in sHCC.

The reported OS rates for patients with HCC following resection varies, with five-year OS rates ranging from 35% to 70%. Patients with sHCC are generally thought to have a good outcome and are often considered as a relatively homogeneous group, while tumor recurrence has become the main factors influencing the sHCC patients' survival. The pTNM stage and histological grading systems are important risk factors affecting the prognosis of HCC. However, these two variables, based on specific clinicopathologic features and extent of disease, may have reached their limits for patients with early HCC in providing critical information influencing choice of follow-up strategies and salvage therapy as well as guiding future studies. Data from our study revealed that even patients with early HCC could be stratified into subgroups with distinct long-term prognoses. Thus, there is a need for new objective strategies that can effectively distinguish between patients with favorable and unfavorable outcome. After analyzed data on one large population-based cohort of patients with the pathologically proven sHCC, our data support the concept that tumor necrosis can identify sHCC patients with or without aggressive clinical course and/or poor outcome. Thus, evaluation of tumor necrosis may become a factor for predicting prognosis and rendering a more tailored treatment strategy in sHCC patients. What's more,

the proposed prognostic model with tumor necrosis, tumor size and vascular invasion could reflect the aggressive phenotype of sHCC. Thus, this combined model may be a useful prognostic index for sHCC. There are also strong efforts to integrate biomarkers into established clinicopathologic models to further improve their predictive ability. Generally, our findings support the idea that the pN classification supplemented by tumor necrosis, vascular invasion and tumor size might improve the ability to discriminate sHCC patients' outcome.

This retrospective study may be considered its major limitation; however, the study was strengthened by the fact that all of the histopathological slides were re-evaluated by two gastrointestinal pathologists. Although, we believe that our results contribute to the literature because it includes only patients with sHCC.

Conclusions

In summary, we observed that presence of tumor necrosis was a strong and independent predictor of adverse survival for sHCC patients. The proposed new prognostic model (combined tumor size, vascular invasion and tumor necrosis) might improve the ability to discriminate sHCC patients' outcome. Thus, the examination of tumor necrosis could be used as an additional effective instrument in identifying those patients at increased risk of tumor progression, which might also help the clinician to choose a suitable therapy for the individual patient, for example, favoring a more aggressive treatment in patients with tumor necrosis.

Abbreviations

sHCC: small Hepatocellular Carcinoma; HBV: Hepatitis B Virus; AFP: Alfa-fetoprotein; ALT: Alanine aminotransferase; OS: Overall survival; RFS: Recurrence-free survival

Declarations

Ethics approval and consent to participate: The Institute Research Medical Ethics Committee of Sun Yat-sen University Cancer Center granted approval for this study. All the participants provided written informed consent.

Consent for publication: Not applicable.

Availability of data and materials: The data that support the findings of this study are available from Sun Yat-sen University Cancer Center, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available.

Competing interests: The authors declare that they have no competing interests.

Funding: This work was supported by Guangdong Natural Science Funds for Distinguished Young Scholar (No. 2015A030306001) and the Nature Science Foundation of China (No. 81672407, 81872001 and 81801895). The funding sources had no role in writing, data collection, analysis, or interpretation, or any aspect pertinent to the study.

Authors' contributions: Concept: R-PG, M-YC. Study design: M-YC, Y-HL, J-WC, S-HW. Statistical analysis: C-YH, WW. Data analysis and interpretation: PL, L-HL, JM, S-HL. Writing of manuscript: Y-HL, J-WC, S-HW. Review and feedback of manuscript: WW, R-PG, M-YC. All authors read and approved the final manuscript.

Acknowledgements: Not applicable.

References

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet. 2012; 380:2095-128.
2. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med. 1999; 340:745-50.
3. Ince N, Wands JR. The increasing incidence of hepatocellular carcinoma. N Engl J Med. 1999;340:798-9.
4. Cai MY, Tong ZT, Zheng F, Liao YJ, Wang Y, Rao HL, Chen YC, Wu QL, Liu YH, Guan XY, et al: EZH2 protein: a promising immunomarker for the detection of hepatocellular carcinomas in liver needle biopsies. Gut. 2011, 60:967-76.
5. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. The Lancet. 2003; 362:1907-17.
6. An F-Q, Matsuda M, Fujii H, Tang R-F, Amemiya H, Dai Y-M, Matsumoto Y. Tumor heterogeneity in small hepatocellular carcinoma: Analysis of tumor cell proliferation, expression and mutation of p53 AND β-catenin. Int J Cancer. 2001;93:468-74.
7. Bruix J, Sherman M, Practice Guidelines Committee AAftSoLD. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208-36.
8. Yamamoto M, Takasaki K, Otsubo T, Katsuragawa H, Katagiri S, Yoshitoshi K, et al. Favorable surgical outcomes in patients with early hepatocellular carcinoma. Ann Surg. 2004;239:395-9.
9. Villanueva A, Hoshida Y, Toffanin S, Lachenmayer A, Alsinet C, Savic R, et al. New strategies in hepatocellular carcinoma: genomic prognostic markers. Clin Cancer Res. 2010, 16:4688-94.
10. Nathan H, Schulick RD, Choti MA, Pawlik TM. Predictors of survival after resection of early hepatocellular carcinoma. Ann Surg. 2009; 249:799-805.
11. Ko CJ, Chien SY, Chou CT, Chen LS, Chen ML, Chen YL. Factors affecting prognosis of small hepatocellular carcinoma in Taiwanese patients following hepatic resection. Can J Gastroenterol.

2011; 25:485-91.

12. Edmondson HA, Steiner PE. Primary carcinoma of the liver.A study of 100 cases among 48,900 necropsies. Cancer. 1954; 7:462-503.
13. Swinson DEB, Jones JL, Richardson D, Cox G, Edwards JG, O'Byrne KJ. Tumour necrosis is an independent prognostic marker in non-small cell lung cancer: correlation with biological variables. Lung Cancer. 2002; 37:235-40.
14. Langner C, Hutterer G, Chromecki T, Leibl S, Rehak P, Zigeuner R. Tumor necrosis as prognostic indicator in transitional cell carcinoma of the upper urinary tract. J Urol. 2006;176:910-3.
15. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. Nat Rev Immunol. 2004; 4:641-8.
16. Richards CH, Mohammed Z, Qayyum T, Horgan PG, McMillan DC. The prognostic value of histological tumor necrosis in solid organ malignant disease: a systematic review. Future Oncology. 2011; 7:1223-35.
17. Soini Y, Virkajarvi N, Lehto VP, Paakko P. Hepatocellular carcinomas with a high proliferation index and a low degree of apoptosis and necrosis are associated with a shortened survival. Br J Cancer. 1996;73:1025-30.
18. Di Martino M, Saba L, Bosco S, Rossi M, Miles KA, Di Miscio R, et al. Hepatocellular carcinoma (HCC) in non-cirrhotic liver: clinical, radiological and pathological findings. Eur Radiol. 2014;24:1446-54.
19. Sengupta S, Lohse CM, Leibovich BC, Frank I, Thompson RH, Webster WS, et al. Histologic coagulative tumor necrosis as a prognostic indicator of renal cell carcinoma aggressiveness. Cancer. 2005;104:511-20.
20. Li H, Zhang Q, Xu L, Chen Y, Wei Y, Zhou G. Factors predictive of prognosis after esophagectomy for squamous cell cancer. J Thorac Cardiovasc Surg. 2009;137:55-9.
21. Mete O, Asa SL. Pathological definition and clinical significance of vascular invasion in thyroid carcinomas of follicular epithelial derivation. Mod Pathol. 2011;24:1545-52.
22. Cai M-Y, Wang F-W, Li C-P, Yan L-X, Chen J-W, Luo R-Z, et al. Prognostic factors affecting postoperative survival of patients with solitary small hepatocellular carcinoma. Chin J Cancer. 2016;35:80.
23. Leek RD, Landers RJ, Harris AL, Lewis CE. Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. Br J Cancer. 1999;79:991-5.
24. Hiltzik D, Carlson DL, Tuttle RM, Chuai S, Ishill N, Shaha A, et al. Poorly differentiated thyroid carcinomas defined on the basis of mitosis and necrosis: a clinicopathologic study of 58 patients. Cancer.2006;106:1286-95.
25. Greenson JK, Huang SC, Herron C, Moreno V, Bonner JD, Tomsho LP, et al: Pathologic predictors of microsatellite instability in colorectal cancer. Am J Surg Pathol. 2009;33:126-33.
26. Lam JS, Shvarts O, Said JW, Pantuck AJ, Seligson DB, Aldridge ME, et al: Clinicopathologic and molecular correlations of necrosis in the primary tumor of patients with renal cell carcinoma. Cancer.

2005;103:2517-25.
27. Rhee H, Chung T, Yoo JE, Nahm JH, Woo HY, Choi GH, et al. Gross type of hepatocellular carcinoma reflects the tumor hypoxia, fibrosis, and stemness-related marker expression. Hepatol Int. 2020;14:239-48.
28. Rhee H, Nahm JH, Kim H, Choi GH, Yoo JE, Lee HS, et al. Poor outcome of hepatocellular carcinoma with stemness marker under hypoxia: resistance to transarterial chemoembolization. Mod Pathol. 2016;29:1038-49.
29. Lee AH, Happerfield LC, Bobrow LG, Millis RR. Angiogenesis and inflammation in invasive carcinoma of the breast. J Clin Pathol. 1997; 50:669-73.
30. Lewis JS, Landers RJ, Underwood JCE, Harris AL, Lewis CE. Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. J Pathol. 2000;192:150-8.
31. McHugh PP, Gilbert J, Vera S, Koch A, Ranjan D, Gedaly R. Alpha-fetoprotein and tumour size are associated with microvascular invasion in explanted livers of patients undergoing transplantation with hepatocellular carcinoma. Hpb. 2010;12:56-61.
32. Min Du LC, Jing Zhao. Microvascular invasion (MVI) is a poorer prognostic predictor for small hepatocellular carcinoma. BMC cancer. 2014;14:38.
33. Shindoh J, Andreou A, Aloia TA, Zimmitti G, Lauwers GY, Laurent A, et al: Microvascular invasion does not predict long-term survival in hepatocellular carcinoma up to 2 cm: reappraisal of the staging system for solitary tumors. Ann Surg Oncol. 2013;20:1223-9.
34. De Jaeger K, Merlo FM, Kavanagh MC, Fyles AW, Hedley D, Hill RP. Heterogeneity of tumor oxygenation: relationship to tumor necrosis, tumor size, and metastasis. Int J Radiat Oncol Biol Phys. 1998;42:717-21.

Figures

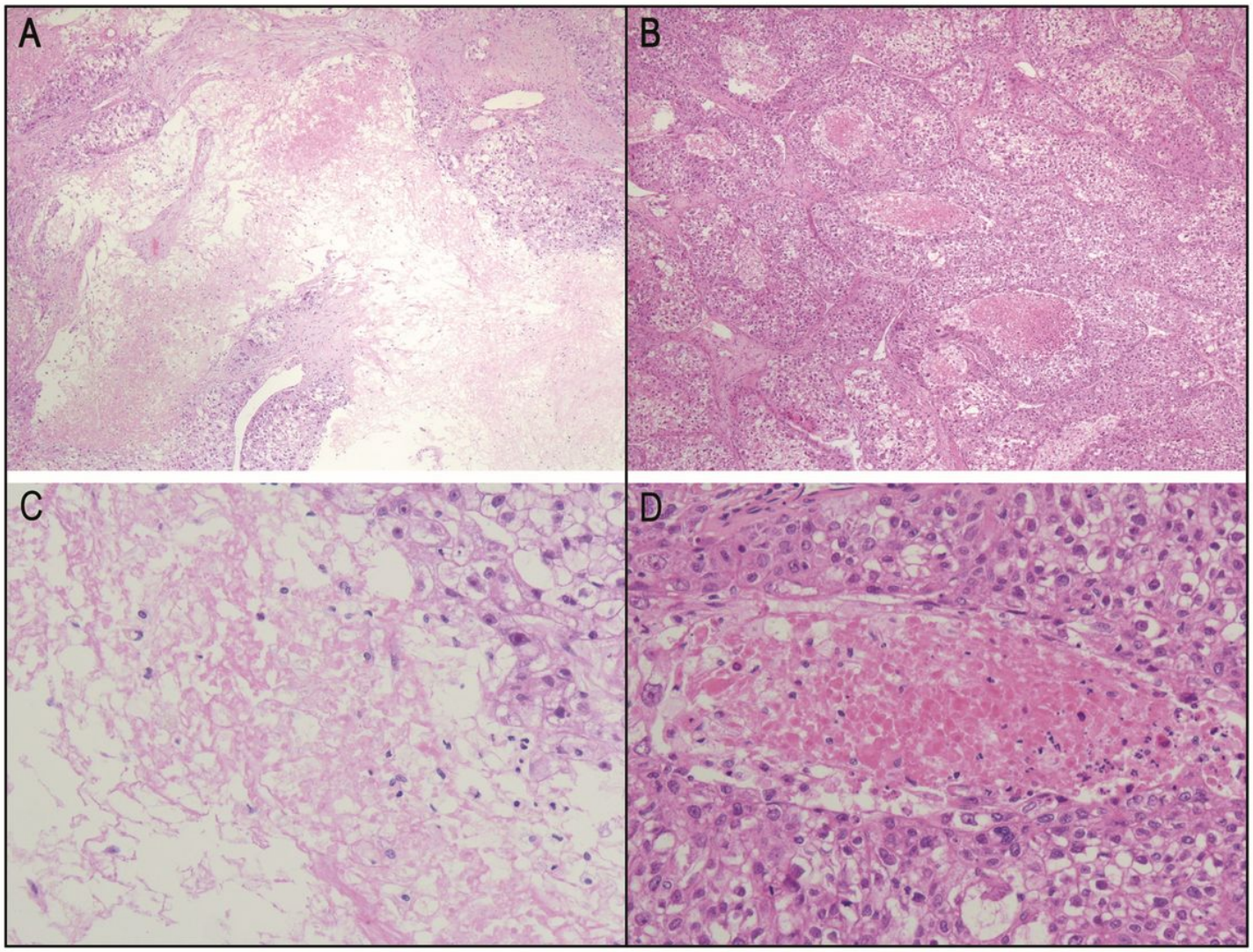

Fig. 1

Figure 1

Histopathological features of tumor necrosis in primary solitary small hepatocellular carcinoma. Tumor necrosis in sHCC consisted of homogenous clusters of sheets of degenerating and dead cells, or coalescing grups of cells forming a coagulum, containing nuclear and cytoplasmic debris, with membrane integrity, intracellular organelle swelling (A-B, hematoxylin and eosin [H&E], original magnification ×4; C-D, H&E, ×20).

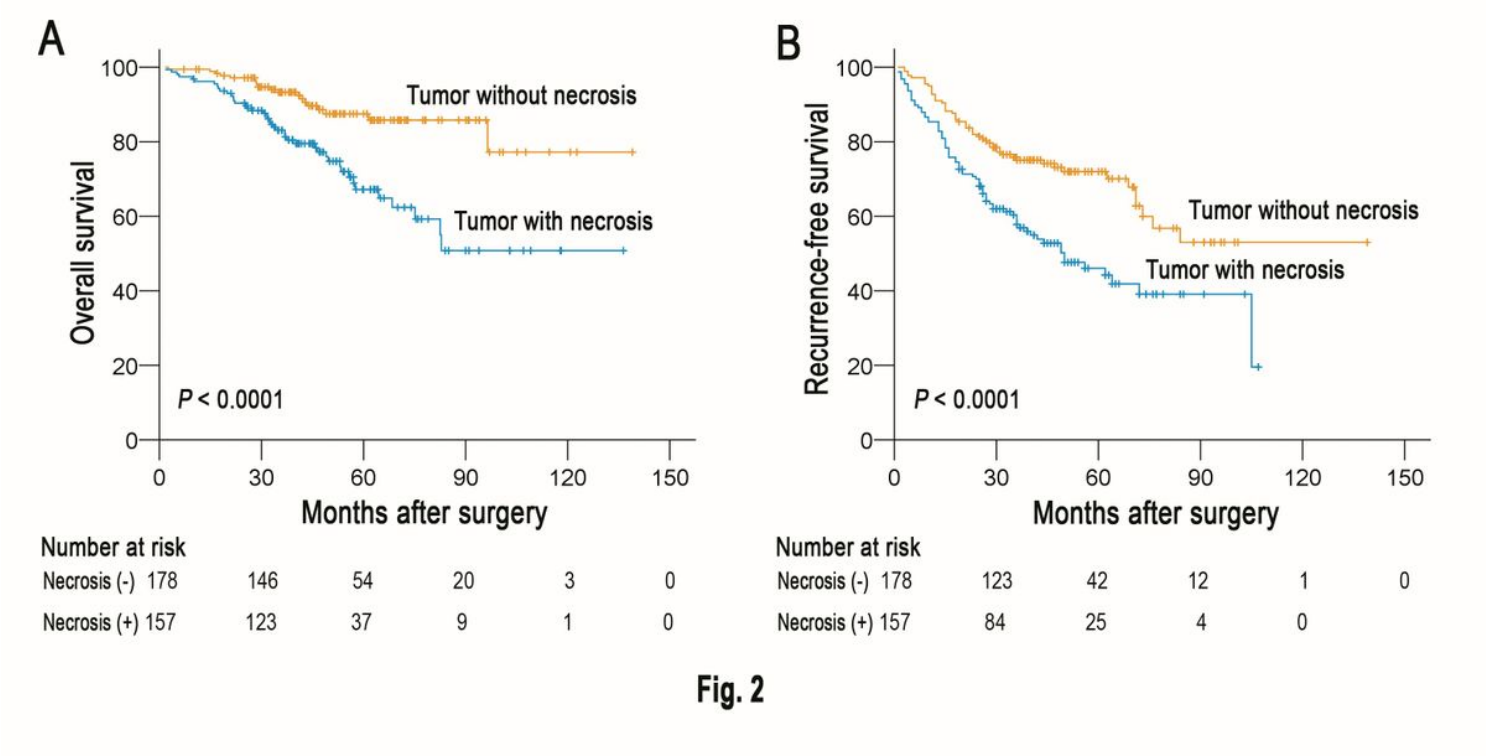

Figure 2

Tumor necrosis affecting postoperative survival of patients with small hepatocellular carcinoma (sHCC) (log-rank test). A, Tumor necrosis was associated with a decrease in overall survival (OS) of patients (P < 0.0001). B, Tumor necrosis was associated with a decrease in recurrence-free survival (RFS) of patients (P < 0.0001).

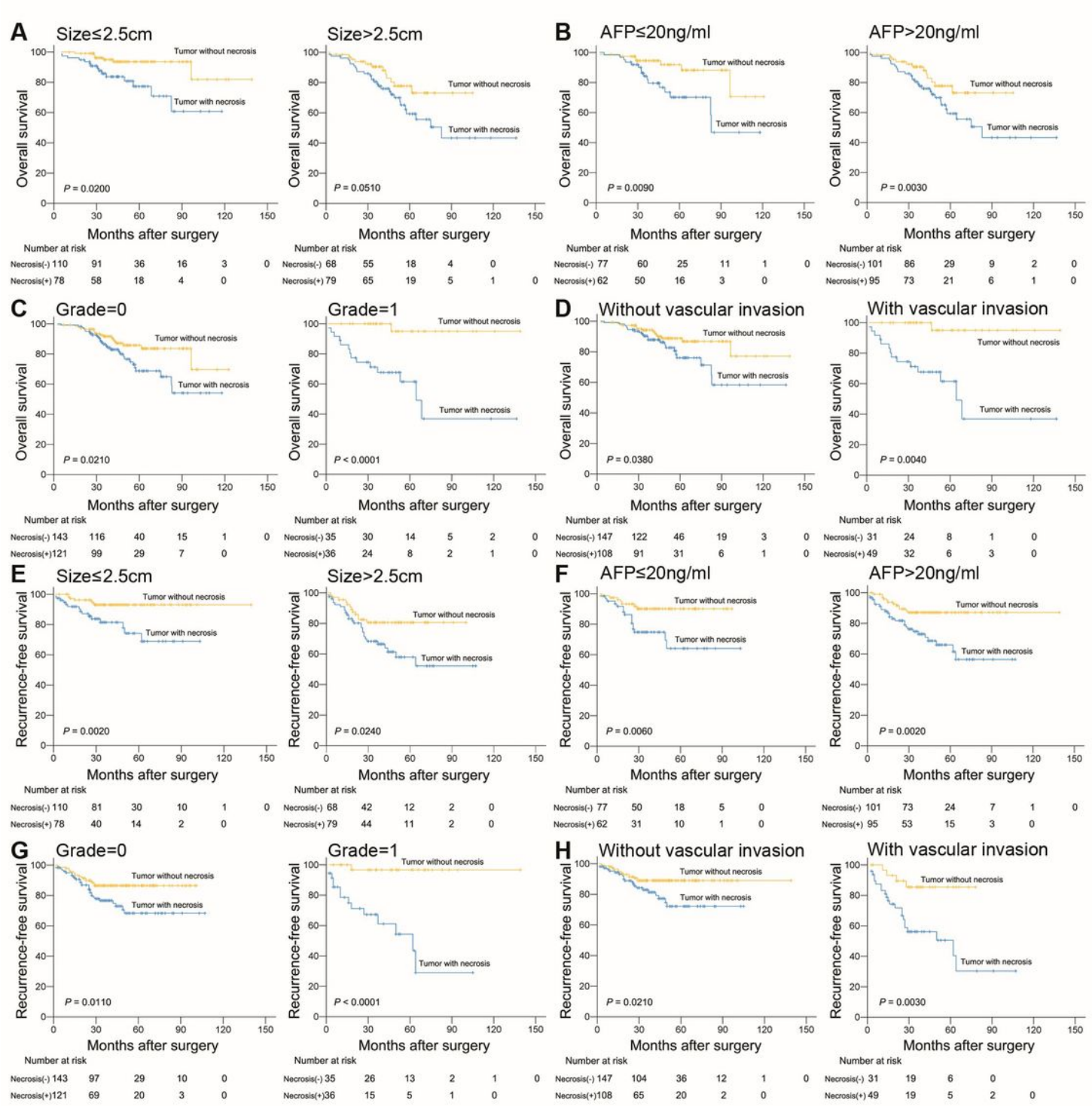

Fig. 3

Figure 3

Kaplan-Meier survival curve comparing tumor necrosis affecting postoperative survival of patients with small hepatocellular carcinoma (sHCC) stratified according to different tumor size, differentiation, serum AFP level and vascular invasion. A, E: Tumor necrosis was associated with a decrease in overall survival (OS) of patients with tumor size ≤ 2.5 cm, and a decrease in recurrence-free survival (RFS) of patients with tumor size ≤ 2.5 cm as well as > 2.5 cm (P = 0.0200, 0.0020, 0.0240, respectively). B, F: Tumor

necrosis was associated with a decrease in OS and RFS of patients with AFP level ≤ 20 ng/ml and > 20 ng/ml (P = 0.0090, 0.0030, 0.0060, 0.0020, respectively). C, G: Tumor necrosis was associated with a decrease in OS and RFS of patients with different tumor differentiation (P = 0.0210, < 0.0001, = 0.0110, < 0.0001, respectively). D, H: Tumor necrosis was associated with a decrease in OS and RFS of patients with or without vascular invasion (P = 0.0380, 0.0040, 0.0210, 0.0030, respectively).

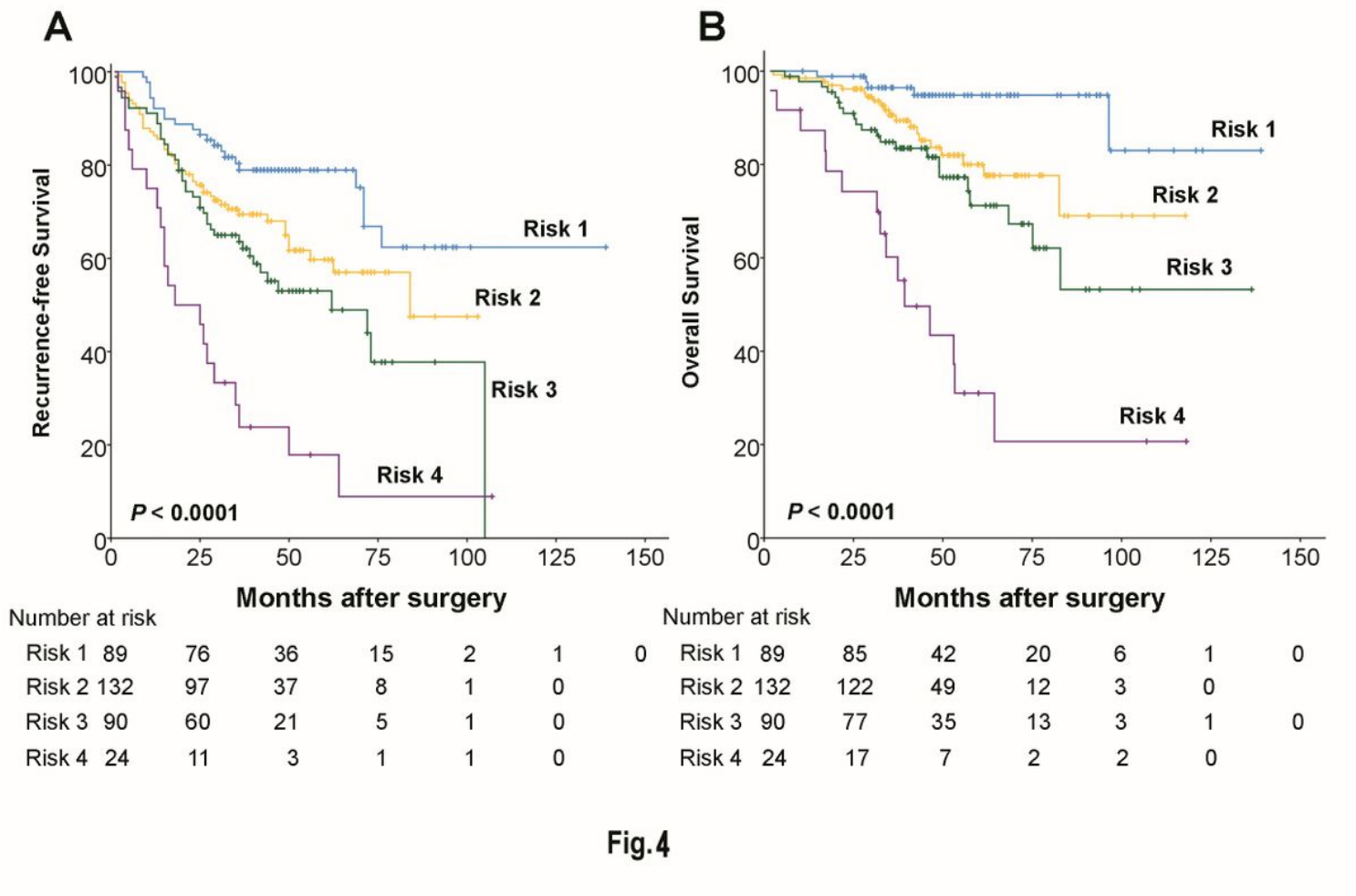

Fig. 4

Figure 4

The proposed prognostic model successfully stratified risk for survival prediction of patients with sHCC (log-rank test). Using this model, these sHCC patients were stratified into four groups: risk 1, n = 89; risk 2, n = 132; risk 3, n = 90; risk 4, n = 24. A, The RFS curves of the three groups were significantly different (P < 0.0001). B, The OS curves of the three groups were significantly different (P < 0.0001).