

High serum serotonin as a favorable prognostic factor for patients with hepatocellular carcinoma

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Abstract

Background

Serotonin contributes to liver cancer progression; however, it also plays a pivotal role in liver regeneration after partial resection. This study aims to elucidate whether high serum serotonin is a prognostic factor for patients with hepatocellular carcinoma (HCC).

Methods

We measured the pre-operation serum serotonin level of patients with HCC. Based on the median value of serum serotonin, patients were divided into the high serotonin group and the low serotonin group. The survival rates after operation were compared between the two groups.

Results

The median serum serotonin level for 176 patients was 90.5 ng/mL (range, 1.0 – 399.5); 88 patients with serum serotonin level ≥ 90.5 were allocated to the high serotonin group and 88 patients to the low serotonin group. Histological findings showed that tumor stage was not different between the two groups. After a median follow-up period of 3.2 years (range, 0.9 – 7.4), median overall survival was 6.6 years (95% confidence interval [CI], 5.7–NA) and 5.7 years (95% CI, 3.5–NA) in the high and the low serotonin groups, respectively ($P = 0.042$). The median recurrence-free survival of patients in the high serotonin group (2.2 years [95% CI, 1.7–3.2]) tended to be longer than that of patients in the low serotonin group (2.0 years [1.0–2.2]) ($P = 0.091$). The independent variables for overall survival were the high serum serotonin level ($P = 0.049$) and des-gamma-carboxy prothrombin level ($P = 0.032$).

Conclusions

High serum serotonin could be a favorable prognostic factor for HCC patients undergoing liver resection.

Trial registration

The study was approved (protocol number: 131) by the institutional review boards of Nihon University on December 15 in 2011.

Background

Serotonin is produced in the enteric nervous system of the gastrointestinal tract, stored in the platelets, and has multifunctional roles such as modulating blood coagulation, vasoconstriction, and pain threshold [1, 2]. This molecule is also involved in diverse types of pathological conditions of the liver; it works as a mediator of liver regeneration after partial resection or ischemic injury [3]. On the other hand, serotonin contributes to the development of liver diseases such as fibrosis [4], cholestasis [5], non-alcoholic steatohepatitis [6], and viral hepatitis [7].

It was reported that serotonin receptors are overexpressed in the advanced stages of hepatocellular carcinoma (HCC) and the blocking the receptors consistently decreased proliferation of liver cancer cells [8]. Serotonin was also shown to facilitate tumor growth of primary liver cancers such as cholangiocarcinoma and HCC via signaling pathway activation [9, 10]. Finally, serotonin was shown to be available as a diagnostic biomarker for HCC in clinical practice [11, 12] and in a mouse model [13]. However, the assertion that high serum serotonin can affect the surgical outcomes in patients undergoing resection for HCC is still controversial [14 – 16], in part due to the multifunctional physiology of serotonin in the liver.

In this study, we measured the serum serotonin level and platelet count of patients who underwent resection for HCC, and investigated whether serotonin released from the platelets affected the surgical outcomes of patients with HCC.

Methods

Patients

Patients with HCC, who underwent liver resection between 2011 and 2014 in the Department of Digestive Surgery, Nihon University School of Medicine, were recruited in this study. Each participant provided written informed consent, and the study was approved (protocol number: 131) by the institutional review boards of Nihon University. All the clinical investigations were conducted according to the principles mentioned in the Declaration of Helsinki. Surgical specimens were immediately cut into small pieces after resection, snap frozen in liquid nitrogen, and stored at – 80 °C.

Sample preparation

Peripheral blood was preoperatively obtained after induction of anesthesia, put in a vacutainer tube without anti-coagulants coated, and centrifuged at 1,000 g at room temperature for 15 min. The resulting supernatant was labeled as serum and stored at – 20 °C.

Quantification of serum serotonin

Serum serotonin level was measured via Serotonin ELISA Kit (ENZO Life Sciences; reference range, 0.5 – 500 ng/mL) according to the manufacturer's protocols. Based on the median value of serum serotonin, patients were divided into the high serotonin group and the low serotonin group.

Inclusion and exclusion criteria

Only the patients who were histologically diagnosed as HCC were included. The following patients were excluded; patients without HCC by pathological findings after operation, those who had received treatments for other types of cancer within 3 years, or those who underwent non-curative liver resection were excluded from this study.

Tumor stage based on the General Rules for the Clinical and Pathological Study of Primary Liver Cancer was determined by the pathological diagnosis after operation [17].

Surgical procedures

Open liver resection was performed in all patients according to the criteria based on liver function [18]. Curative resection was defined as the complete removal of recognizable viable HCC diagnosed preoperatively or intraoperatively with macroscopically tumor-free surgical margins. Postoperative complications were stratified according to the Clavien-Dindo classification [19], which defines morbidities as complications with a score of $\geq 3a$.

Follow-up after operation

All patients were followed for postoperative recurrence as described previously [20]. Briefly, tumor marker levels, including the levels of alpha-fetoprotein and des-gamma- carboxy prothrombin (DCP), were measured, and imaging studies including computed tomography and ultrasonography were performed every three months in all patients. Recurrence was diagnosed by dynamic computed tomography and/or magnetic resonance imaging. The date of recurrence was defined as the date of examination when the recurrent HCC was noted.

Statistical analysis

Fisher's exact test, Student's t-test, and Wilcoxon rank-sum test were used to assess the statistical significance of the data collected from the patients. The correlation coefficient was calculated using the Spearman test. Survival curves were generated using the Kaplan–Meier method and compared by the log-rank test. Prognostic factors for survival were identified using the Cox proportional hazards regression model. A P-value of less than 0.10 was set as the cut-off value for elimination. The following 18 variables, considered as potential confounders, were examined: age (≥ 70 versus < 70 years), sex, positive for hepatitis B or C virus, alcohol abuse, diabetes mellitus, platelet count ($\geq 15 \times 10^4$ versus $< 15 \times 10^4$ /mL), serotonin (≥ 90.5 versus < 90.5 ng/mL), Child-Pugh score (5 vs ≥ 6), indocyanine green clearance rate at 15 minutes (ICGR15) (≥ 15 versus $< 15\%$), esophageal varices, serum alpha-fetoprotein level (≥ 100 versus < 100 ng/mL), serum DCP level (≥ 100 versus < 100 mAU/mL), tumor size (≥ 3.0 versus < 3.0 cm), tumor number (single versus multiple), poorly differentiated, tumor thrombus, and liver cirrhosis [21, 22]. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using the JMP 12.0.1 statistical software package (SAS Institute Inc, Cary, NC).

Results

Serum serotonin level

The median of the preoperative serum serotonin level and platelet count from 176 HCC patients were 90.5 ng/mL (range, 1.0 – 399.5) and 12.5×10^4 /mL (range; 3.2×10^4 – 68.6×10^4), respectively (Fig. 1a). Furthermore, 88 patients with serum serotonin ≥ 90.5 ng/mL were defined as the high serotonin group

and other 88 patients as the low serotonin group (Table 1). Patient background was not different between the two groups. The serum serotonin level was not correlated with the platelet count (coefficients of determination [R^2] between the serum serotonin level and the platelet count was 0.013) (Fig. 1b).

Table 1
Patient background

	High serotonin (n = 88)	Low serotonin (n = 88)	P value
Age, years	66 (31 – 85)	69 (40 – 84)	0.136
Sex, male	70 (79.5)	69 (78.4)	1.000
Hepatitis B	20 (22.7)	19 (21.5)	1.000
Hepatitis C	32 (36.3)	45 (51.1)	0.067
Alcoholic	30 (34.0)	28 (31.8)	0.872
Diabetes mellitus	29 (32.9)	25 (28.4)	0.624
Platelet count	13.6 (5.4 – 54.0)	12.9 (3.2 – 68.6)	0.255
Child-Pugh, 5	68 (77.2)	60 (68.1)	0.236
ICGR15	14.4 (3.3 – 38.7)	13.9 (3.1 – 38.7)	0.883
Varices	25 (28.4)	19 (21.5)	0.446
Alpha-fetoprotein	15 (1 – 31,723)	11 (1 – 154,356)	0.572
DCP	48 (1 – 75,000)	73 (6 – 75,000)	0.368
Data are presented as n (%) or median (range). ICGR15, indocyanine green clearance rate at 15 minutes; DCP, des-gamma-carboxy prothrombin.			

Operative data

Operative data and complication rates were not different between the two groups (Table 2). Eight patient (4.5%) in the two groups underwent reoperation for intra-abdominal bleeding (four patients), portal venous thrombus (two patients), bile leakage (one patients), and intra-abdominal abscess (one patient). There was no operative death in this series. Histological findings showed that tumor stage including tumor number, size, differentiation grade, vascular invasion, and tumor stage and liver status were also not different between the two groups.

Table 2
Operative data

	High serotonin (n = 88)	Low serotonin (n = 88)	P value
Operative data			
Operative time, min	314 (131 - 685)	317 (154 - 722)	0.517
Clamp time, min	58 (0 - 385)	64 (0 - 227)	0.226
Bleeding, mL	278 (22 - 1,593)	318 (26 - 3,887)	0.435
Transfusion	2 (2.2)	4 (4.5)	0.682
Complications			
Overall	34 (38.6)	23 (26.1)	0.106
Morbidity	23 (26.1)	19 (21.5)	0.596
Re-operation	4 (4.5)	4 (4.5)	1.000
Operative death	0	0	1.000
Hospital stay, days	10 (6 - 32)	10 (7 - 20)	0.976
Pathology			
No. of tumor, multiple	25 (28.4)	20 (22.7)	0.166
Tumor size	3.1 (1.2 - 18.0)	3.0 (1.2 - 16.5)	0.371
Differentiation grade, Poorly	6 (6.8)	10 (11.3)	0.188
Vascular invasion	23 (26.1)	27 (30.6)	0.867
Tumor stage, I / II	66 (75.0)	67 (76.1)	1.000
Cirrhosis	31 (35.2)	38 (43.1)	0.188
Data are presented as n (%) or median (range).			

Survival

During a median follow-up period of 3.2 years (range, 0.9 - 7.4), a total of 108 patients (61.3%) had recurrence: 96 patients (88.8%) in the remnant liver, and 7 patients (6.4%) in distant sites, and 5 patients (4.6%) in both intra and extrahepatic sites) (Table 3). Furthermore, 55 patients (31.2%) died after HCC recurrence. Treatments for recurrent HCC were not different between the two groups.

Table 3
Treatment for recurrence

	High serotonin (n = 52)	Low serotonin (n = 56)	<i>P</i> value
Recurrent sites			
Intrahepatic	46 (88.4)	50 (89.2)	0.904
at stump	14 (30.4)	11 (22.0)	
Distant sites	4 (7.6)	3 (5.3)	
Both	2 (3.8)	3 (5.3)	
Treatments			
Second resection	18 (34.6)	16 (28.5)	0.910
TACE/TAI	31 (59.6)	37 (66.0)	
Chemotherapy	1 (1.9)	2 (3.5)	
Radiation therapy	1 (1.9)	1 (1.7)	
None	1 (1.9)	1 (1.7)	
Data are presented as n (%). TACE, transcatheter arterial chemoembolization; TAI, transcatheter arterial infusion.			

The median overall survival periods were 6.6 years (95% confidence interval [CI], 5.7–NA) and 5.7 years (95% CI, 3.5–NA; $P=0.042$) in the high and the low serotonin groups, respectively (Fig. 2a). The median recurrence-free survival of patients in the high serotonin group tended to be longer than that of the patients in the low serotonin group (2.2 years [95% CI, 1.7–3.2] vs. 1.7 years [1.0–2.2], $P=0.091$) (Fig. 2b). The 5-year overall survival rates were 71.7% and 53.9%, and the 5-year recurrence-free survival rates were 27.1% and 19.8% in the high and the low serotonin groups, respectively.

The overall survival of patients with advanced tumor (stage III/IV) (hazard ratio [HR], 1.85, 95% CI, 1.04–3.18, $P=0.034$) and/or liver cirrhosis ($P=0.014$) were significantly shorter. The independent variables for overall survival were the high serum serotonin level (HR, 0.58, 95% CI, 0.34–0.99, $P=0.046$) and DCP level ($P=0.032$) (Table 4). High serotonin level (HR, 0.31, 95% CI, 0.07–0.98, $P=0.042$) and DCP level ($P=0.037$) were significant variables by univariate analysis when treated as a continuous variable.

Table 4
Prognostic factors for survival

Variables	Univariate		Multivariate	
	Hazard ratio	<i>P</i> value	Hazard ratio	<i>P</i> value
Age	1.13 (0.67 - 1.94)	0.630		
Sex	0.84 (0.46 - 1.64)	0.610		
Hepatitis B	1.21 (0.61 - 2.22)	0.563		
Hepatitis C	1.25 (0.74 - 2.10)	0.391		
Alcohol	1.50 (0.87 - 2.55)	0.137		
Diabetes Mellitus	0.94 (0.52 - 1.65)	0.853		
Platelet count	0.67 (0.37 - 1.16)	0.160		
Serotonin	0.56 (0.32 - 0.95)	0.032	0.58 (0.34 - 0.99)	0.046
Child-Pugh	2.19 (1.28 - 3.72)	0.004	1.72 (0.95 - 3.14)	0.072
ICGR15	2.19 (1.28 - 3.83)	0.003	1.36 (0.69 - 2.68)	0.370
Varices	1.95 (1.11 - 3.34)	0.020	1.41 (0.70 - 2.86)	0.328
Alpha-fetoprotein	1.50 (0.80 - 2.65)	0.194		
DCP	1.75 (1.03 - 3.00)	0.037	1.84 (1.05 - 3.25)	0.032
No. of tumor	1.77 (1.01 - 3.03)	0.044	1.64 (0.92 - 2.84)	0.090
Tumor size	1.45 (0.84 - 2.59)	0.174		
Differentiation grade	0.79 (0.35 - 1.53)	0.520		
Vascular invasion	1.03 (0.55 - 1.82)	0.916		
Liver cirrhosis	1.92 (1.13 - 3.29)	0.014	1.39 (0.71 - 2.70)	0.328
Data are presented with 95% confidence Interval. ICGR15, indocyanine green clearance rate at 15 minutes; DCP, des-gamma-carboxy prothrombin.				

Discussion

Our data showed that surgical outcomes of HCC patients with high serum serotonin level were favorable. Therefore, preoperative serum serotonin could be available as the prognostic marker for patients undergoing resection for HCC.

Consistent with the present data, both serum serotonin and intra-platelet serotonin levels were lower in patients with early recurrence of HCC, although the cohort of patients was small ($n = 40$) [14]. Conversely,

recurrence-free survival of patients with high intra-platelet serotonin levels (> 134 ng/mL) was significantly shorter, despite severe morbidities being associated with low intra-platelet serotonin levels (< 73 ng/mL) [15]. Furthermore, another study reported that lower serum and intra-platelet serotonin levels were negatively correlated with both overall survival and recurrence-free survival [16]. This discrepancy of relations between survival and the serotonin levels could be attributed to the multifunctional roles of serotonin [23, 24].

Serotonin was reported to contribute to liver cancer progression via serotonin receptors, HTR1B and HTR2B, in tumors [8], by activating Wnt/ β -catenin pathways [9], or by regulating YAP gene expression [10], and serotonin receptors could be potential therapeutic targets for HCC [8 – 10]. In other types of cancer such as prostate, lung, colorectal, and bile duct cancer, serotonin was involved in cancer cell migration, invasiveness, and metastatic processes through 5HT1A receptor [25]. In human, serotonin in the tumor may act on the cell growth, differentiation of neighboring cells through paracrine mechanisms, and may facilitate tumor recurrence and metastasis, correlated with shorter survivals [26].

On the other hand, platelets support regeneration of the remnant liver after resection [27, 28], and platelet-derived serotonin is a potent mitogen for hepatocytes, and plays a critical role in liver regeneration after partial resection, both in clinical practice [29] and in mouse models [3, 30, 31]. Furthermore, serotonin administration with hexoses or ionizing radiation synergistically and negatively affect the tumor growth [32, 33]. Thus, the effect of serotonin on tumor progression was controversial even in the experimental level.

Owing to the contradictory effects of serotonin, which balance between liver regeneration and liver cancer progression, it is still controversial whether high serum serotonin or intra-platelet serotonin levels are favorable or unfavorable for the survivals of patients after resection for HCC. Tumor stage including number, size, differentiation grade, and vascular invasion were not different between the two groups. Given that liver regeneration was prompted by high serotonin content even under cirrhotic conditions [31] and that most patients had liver cirrhosis or fibrosis in this cohort, the functional role of serotonin for liver regeneration was superior to that for cancer progression in this study.

We previously reported that high platelet count ($\geq 20 \times 10^4$ /mL) was a poor prognostic marker for HCC patients without cirrhosis. Given that most of serotonin is synthesized in the enterochromaffin cells of the gastrointestinal tract and is subsequently stored and transported by platelets [34], we hypothesized that high serum serotonin was associated with poor survival rates of HCC patients. However, serum serotonin concentrations were not correlated with platelet count and the present study showed opposite results. Therefore, we assumed that poor prognosis with high platelet count in HCC patients without cirrhosis was due to the other growth factors such as platelet-derived growth factor, transforming growth factor- β , and hepatocyte growth factor which are secreted by platelets, and not due to serotonin.

Serotonin level and DCP were the independent factors for overall survival in this study. Both liver function and tumor status are the prognostic factors for HCC, and consistent with the many previous reports,

Child-Pugh and number of tumor had the marginal P value in the multivariate analysis. Therefore, serotonin which might have pivotal role in liver regeneration and lead to the preservation of liver function was acceptable as one of independent factors for overall survival.

There were several limitations in this study. First, this cohort was limited to the patients who underwent liver resection, and serotonin plays a pivotal role in liver regeneration and favorably affected the patient survivals. Therefore this result does not apply to all HCC patients undergoing other types of treatments such as radiofrequency ablation, transcatheter arterial chemoembolization, and chemotherapies, which were not accompanied with liver regeneration. Second, serotonin receptors of HCC were not estimated in this series. Given that increased expression of serotonin receptors were negatively associated with the survivals of HCC patients [8, 9], the effects of serotonin in those patients should have been evaluated by serum serotonin level and expression of its receptors in the tumor. Third, only the serum serotonin level, not plasma or intra-platelet serotonin, was measured in this study. However, serum, plasma, and intraplatelet serotonin levels were nearly same [16], only one type of serotonin level from blood may predict the prognosis of HCC patients. Finally, serotonin level of healthy patients were not measured in this study. Compared to the healthy patient data using Serotonin ELISA Kit which was used in this study, however, we found that serotonin level of HCC patients in this study were apparently higher.

Conclusions

A high serum serotonin level was associated with longer survival of patients undergoing resection for HCC. This result was probably due to the positive role of serotonin in liver regeneration, and it showed that serotonin is available to predict the prognosis of the patient after surgery. However, serotonin plays a pivotal role in activating liver cancer progression via its receptors; therefore, two different aspects of this molecule should be estimated, both at the serum level as well as at the receptor level in the future studies.

Abbreviations

HCC
hepatocellular carcinoma
DCP
des-gamma- carboxy prothrombin
ICGR15
indocyanine green clearance rate at 15 minutes

Declarations

Ethics approval and consent to participate

Each participant provided written, informed consent, and this study was approved by the institutional review board of Nihon University (protocol number: 131).

Consent for publication

Not applicable

Availability of data and materials

Data and materials analyzed in this study are not available due to the protection of individual privacy, but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TK and YM acquired the clinical data and wrote the initial draft of the manuscript; YM designed the study; TT conceived of the study, and participated in its design and coordination and helped to draft the manuscript; AN, MS, and GN acquired the experimental data; ST performed statistical analysis. All authors have read and approved the final version of the manuscript.

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Not applicable

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Figures

Fig. 1

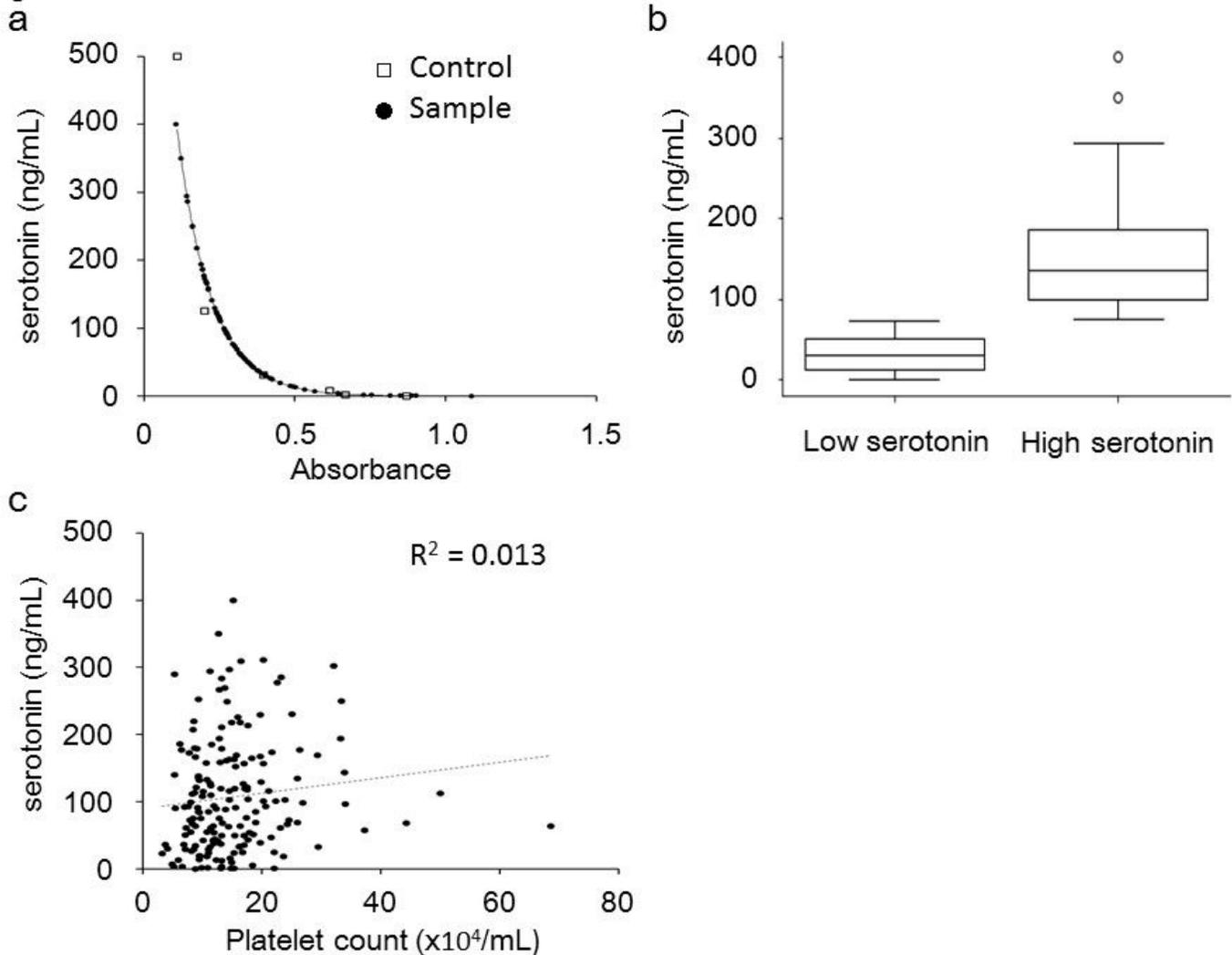


Figure 1

Serum serotonin levels of HCC patients. (a) Serotonin levels measured by ELISA of 88 HCC patients. (b) Box plot of serotonin levels of 176 HCC patients with median and interquartile range. The average of serotonin levels were 107.1 ± 84.1 ng/mL, 172.7 ± 69.5 ng/mL, and 41.4 ± 25.9 ng/mL in total, the high serotonin group, and the low serotonin group, respectively. (c) Serum serotonin levels and platelet count were not correlated ($R^2 = 0.013$).

Fig. 2

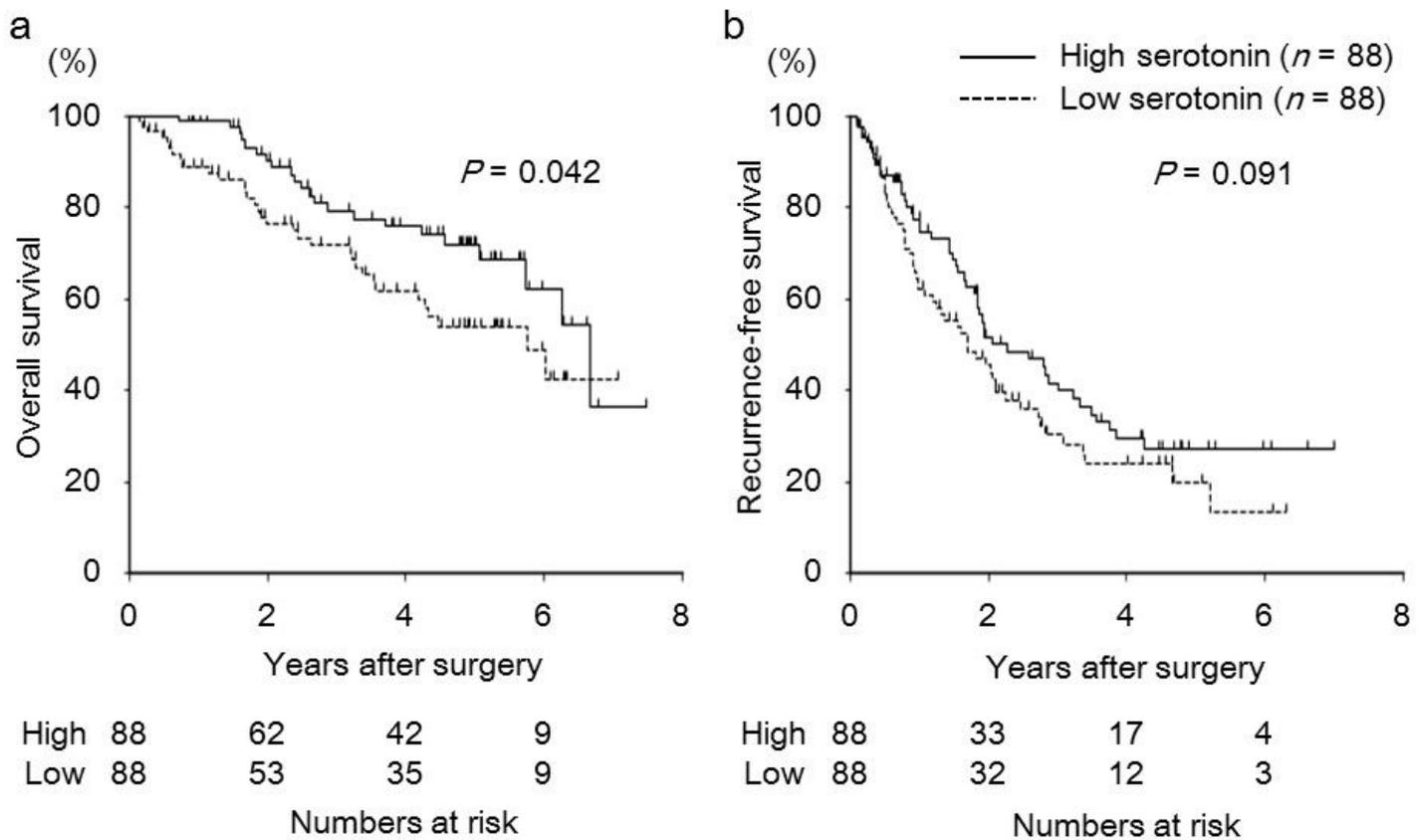


Figure 2

Survival outcomes following resection in HCC patients. (a) The overall survival of patients of the high serotonin group was significantly longer compared to that of patients of the low serotonin group ($P = 0.042$). (b) The recurrence-free survival of patients of the high serotonin group tended to be longer compared to that of patients of the low serotonin group ($P = 0.091$).