Influence of AFP On Surgical Outcomes in Non-B Non-C Patients With Curative Resection For Hepatocellular Carcinoma

Xiao-ping Tan

Guangzhou Medical University Second Affiliated Hospital

Qing-li Zeng

nan chang university

Kai Zhou

Jiangxi Provincial People's Hospital

Wei Chen (✉ chenw47@mail.sysu.edu.cn)

Sun Yat-Sen University <https://orcid.org/0000-0002-6444-1724>

Research Article

Keywords: Hepatocellular carcinoma, Non-B non-C-HCC, AFP, Prognosis

Posted Date: December 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1170033/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Clinical and Experimental Medicine on March 15th, 2022. See the published version at <https://doi.org/10.1007/s10238-022-00813-4>.

Abstract

Objective: To study the clinical and prognostic features of non-B non-C alpha-fetoprotein (AFP) (-)-hepatocellular carcinoma (HCC) (NBNC-AFP(-)-HCC), and the relationship between the prognostic features of HCC and hepatitis B virus surface antigen (HBsAg) status and AFP.

Methods: We enrolled 227 patients underwent hepatic resection for HCC between January 1998 and December 2007 in Sun Yat-Sen University Cancer Center, all of them were diagnosed with HCC by pathology. All patients were stratified into one of four groups (B-AFP(+)-HCC, B-AFP(-)-HCC, NBNC-AFP(+)-HCC, and NBNC-AFP(-)-HCC) according to AFP levels and HBsAg status. The clinicopathologic and survival characteristics of NBNC-AFP(-)-HCC patients were compared with all other three groups.

Results: Out of the 105 NBNC-HCC patients, 43 patients (40.9%) were AFP-negative HCC. There were some differences in factors between the B-AFP(+) and NBNC-AFP(-) patients, such as age, body mass index (BMI), diabetes, and ALT ($P < 0.05$). On univariate analysis, tumor size, secondary tumor, and portal invasion were prognostic factors for overall survival (OS) and disease-free survival (DFS) ($P < 0.05$). Cox multivariate regression analysis suggested that tumor size and tumor number ($P < 0.05$) were independent predictors. In addition, compared with that in the B-AFP(+)-HCC, B-AFP(-)-HCC, and NBNC-AFP(+)-HCC groups, the NBNC-AFP(-)-HCC patients had the best DFS ($P < 0.05$). Compared with that in the B-AFP(+)-HCC and NBNC-AFP(+)-HCC groups, the NBNC-AFP(-)-HCC patients had better OS ($P < 0.05$), and survival rates were similar to those of B-AFP(-)-HCC patients.

Conclusion: NBNC-AFP(-)-HCC patients had a relatively favorable prognosis. It can serve as a useful marker in predicting the risk of tumor recurrence in the early stages.

Background

Hepatocellular carcinoma (referred to as liver cancer or HCC) is the 3rd most common cancer worldwide, as well as the third leading cause of cancer-related death^[1–3]. In China, the number and proportion of cases of non-B, non-C hepatocellular carcinoma (NBNC-HCC) have been increasing gradually^[4]. The characteristics and prognosis of NBNC-HCC in these patients differ from those in patients with chronic hepatitis virus infection. Its related epidemiological factors include aging, sex, alcoholism, diabetes, and metabolic syndrome^[5, 6]. In addition, the diabetes and obesity have been suggested as risk factors for NBNC-HCC in large cohort and case-control studies both with and without pre-existing non-alcoholic fatty liver (NAFLD), especially in terms of NBNC-HCC^[7–10].

So far, the prognostic risk factors of NBNC-HCC have not been completely determined yet. The previous studies showed that the prognosis of NBNC-HCC is associated with some factors, such as AFP level, liver fibrosis, tumor size and ductal invasion^[11, 12]. Therefore, investigating the clinical symptoms and prognosis of NBNC-HCC is helpful to understand the intrinsic mechanism of HCC progression. In this study, we selected 105 patients with NBNC-HCC and randomly selected 122 patients who were hepatitis B

surface antigen (HBsAg)-positive and hepatitis C virus antibody (HCVAb)-negative (B-HCC). Our current study investigated the clinical characteristics and prognosis of NBNC-AFP(-)-HCC and explored the association between expression of HBsAg and AFP and survival prognosis.

Patients And Methods

Materials

This study selected cases from 2591 patients who underwent liver cancer resection at Sun Yat-sen University Cancer Center from January 1998 to December 2007. Inclusion criteria were that all cases should be patients who underwent liver tumor resection and postoperative pathological examination revealed HCC. All of patients were tested for HBV and HCV at admission. A total of 105 NBNC-HCC patients were screened, as well as 122 patients from the remaining 2486 non-NBNC-HCC patients were randomly selected as controls (Figure 1). According to their expression of AFP and HBsAg, we divided the 227 patients into four groups (the negative standard was set as $\text{AFP} \leq 20 \text{ ng/ml}$), the NBNC-AFP(-)-HCC group (who were HBsAg-negative, HCVAb-negative, and AFP-negative, $n=43$), NBNC-AFP(+)-HCC group (who were HBsAg-negative, HCVAb-negative, and AFP-positive, $n=62$), B-AFP(+)-HCC group (who were HBsAg-positive, HCVAb-negative, and AFP-positive, $n=93$), and B-AFP(-)-HCC group (who were HBsAg-positive, HCVAb-negative, and AFP-negative, $n=29$). The study was approved by the Ethics Committee of Jiangxi Provincial People's Hospital (No.2018-362).

Definition of clinical pathological characteristics

The patients' vital signs or assay index were recorded according to their test results after their admission to hospital and before surgery. Influencing factors included age, body mass index (BMI), metabolic syndrome (pathoglycemia, abnormal blood pressure, and dyslipidemia), ALT, AST, S/T, size of tumor, secondary tumor, incisal edge, portal invasion, and pathological grade.

In this study, the diagnosis of metabolic syndrome was based on the 2005 International Diabetes Federation Standard and patients who met three of the following five conditions would be diagnosed as metabolic syndrome: (1) obesity: waistline $> 90 \text{ cm}$ (men) or $> 80 \text{ cm}$ (women), and/or $\text{BMI} > 25 \text{ kg/m}^2$; (2) serum triglyceride (TG) $\geq 1.7 \text{ mmol/L}$ or previously diagnosed with hypertriglyceridemia; (3) high density lipoprotein cholesterol (HDL-C) $< 1.03 \text{ mmol/L}$ (men) or $< 1.29 \text{ mmol/L}$ (women); (4) arterial blood pressure $\geq 130/85 \text{ mmHg}$ or previously diagnosed with hypertension; and (5) fasting plasma glucose (FPG) $\geq 5.6 \text{ mmol/L}$ or previously diagnosed with type II diabetes.

Follow-up and statistical analysis

Patients were followed up until January 2012. The interval of recurrence was calculated in months while survival time was calculated in years. The cut-off point for disease-free survival (DFS) time was tumor recurrence or metastasis confirmed on initial postoperative imaging, such as computed tomography (CT), B-ultrasound, or magnetic resonance imaging (MRI). The overall survival (OS) was calculated as the interval between the date of surgery and date of death or date of the last follow-up. Survival rates were calculated using Kaplan-Meier analysis and the log-rank test was used for univariate analysis. The

variables that were found to be significant in the univariate analysis were introduced into the Cox model for further multivariate analysis. $P < 0.05$ was considered statistically significant. We used SPSS13.0 to perform our statistical analysis.

Results

Clinical pathological characteristics of NBNC-AFP(-)-HCC patients

As is shown in Figure 1, there were 43 NBNC-AFP(-)-HCC patients and 62 NBNC-AFP(+)-HCC patients, which accounts for 1.6%(43/2591) and 2.4% (62/2591) of all patients, respectively. The chi-squared test was used to compare differences in clinical pathological characteristics between the NBNC-AFP(-)-HCC group and other three groups (Table 1). Consequently, there are statistically significant differences in age, BMI, diabetes, metabolic syndrome, ALT, AST, S/T, size of tumor, secondary tumor, portal invasion, and pathological grade ($P < 0.05$) between first groups (B-AFP(+)-VS.NBNC-AFP(-)). The age, ALT, portal invasion, and pathological grade were shown statistically significant differences in the third group (B-AFP(-)-VS.NBNC-AFP(-)) ($P < 0.05$). In addition, there were statistical differences in AST, S/T, size of tumor, portal invasion, and pathological grade between NBNC-AFP(-)-HCC patients and NBNC-AFP(+)-HCC patients.

Table 1
Comparison of clinicopathological features of NBNC-AFP(-)-HCC patients

Characteristic	NBNC-AFP(-)	B-AFP(+)	P	NBNC-AFP(+)	P	B-AFP(-)	P
Age (years)			0.040		0.097		0.024
≥50	32	45		53		14	
<50	11	48		8		15	
Drink			0.111		0.695		0.440
Yes	9	10		15		4	
No	34	83		47		25	
BMI			0.015		0.156		0.612
>25kg/m ²	11	9		9		9	
≤25kg/m ²	32	84		53		20	
Diabetes			0.040		0.097		0.109
Yes	11	7		8		3	
No	32	85		54		26	
Metabolic syndrome			0.019		0.684		0.650
Yes	6	3		7		3	
No	37	90		55		26	
ALT			<0.001		0.305		0.002
>40 U/L	12	62		12		19	
≤40 U/L	31	31		50		10	
AST			0.001		<0.001		0.104
>40 U/L	14	60		59		15	
≤40 U/L	29	33		3		14	
S/T			0.100		<0.001		0.396
>1	28	46		58		16	

There were statistically significant differences in age, BMI, diabetes, metabolic syndrome, ALT, AST, tumor size, secondary tumor, portal violation and pathological grade between NBNC-AFP(-) and B-AFP(+). In addition, the portal invasion and pathological grade were found to be statistically significant differences in other two groups.

Characteristic	NBNC-AFP(-)	B-AFP(+)	P	NBNC-AFP(+)	P	B-AFP(-)	P
≤1	15	46		4		13	
Tumor size (cm)			0.011		0.006		0.664
<5	20	23		13		15	
≥5	23	70		49		14	
Secondary tumor			0.026		0.015		0.506
Yes	5	27		20		2	
No	38	66		42		27	
Portal violation			0.025		<0.001		<0.001
Yes	0	10		15		8	
No	43	83		47		21	
Pathological grade			0.007		0.001		0.022
I	7	3		0		0	
II-IV	36	90		62		29	
There were statistically significant differences in age, BMI, diabetes, metabolic syndrome, ALT, AST, tumor size, secondary tumor, portal violation and pathological grade between NBNC-AFP(-) and B-AFP(+). In addition, the portal invasion and pathological grade were found to be statistically significant differences in other two groups.							

DFS of NBNC-AFP(-)-HCC patients

Univariate analysis of all 227 HCC patients in this study showed that factors influencing postoperative DFS included AST, S/T, size of tumor, secondary tumor, and portal invasion ($P<0.05$) (Table 2). After introducing these statistically significant indexes in the univariate analysis to the Cox model for multivariate analysis, it was shown that size of tumor, secondary tumor, and portal invasion were all independent influencing factors for postoperative DFS ($P=0.008$, $P<0.001$, and $P=0.006$, respectively) (Table 3). Univariate analysis of 43 NBNC-AFP(-)-HCC patients revealed that secondary tumor was a factor that could influence postoperative DFS ($P=0.016$) (Table 4).

Table 2 Univariate analysis of DFS and OS after liver cancer resection

Characteristic	DFS[P]	OS[P]
Age	0.294	0.863
BMI	0.897	0.917
Drink	0.408	0.478
Diabetes	0.925	0.174
Metabolic syndrome	0.156	0.923
ALT	0.708	0.204
AST	0.049	0.216
S/T	0.003	0.216
Tumor size	0.001	0.001
Secondary tumor	<0.001	0.001
Portal violation	0.011	0.001
Pathological grade	0.230	0.193

Table 3 Multivariate analysis of disease-free survival rate and overall survival rate after liver cancer resection

Characteristic	DFS		OS	
	95.0% CI for Exp(B)	P	95.0% CI for Exp(B)	P
AST	0.761-1.820	0.464	0.902---2.142	0.136
S/T	-----	-----	0.934---2.237	0.099
Tumor size	0.294-0.831	0.008	0.365---0.981	0.042
Secondary tumor	1.712-4.162	<0.001	1.076---2.398	0.020
Portal violation	1.260—3.821	0.006	0.775---2.020	0.359

A total of five clinical variables were screened as prognostic factors to predict OS and DFS using a multivariate analysis.

Table 4
Univariate analysis of postoperative DFS and OS in patients with NBNC-AFP(-)-HCC

Characteristic	DFS(<i>P</i>)	OS(<i>P</i>)
Age	0.057	0.985
BMI	0.822	0.419
Drink	0.408	0.397
Diabetes	0.118	0.711
Metabolic syndrome	0.467	0.635
ALT	0.668	0.649
AST	0.695	0.292
S/T	0.848	0.249
Tumor size	0.162	0.528
Secondary tumor	0.016	0.959
Portal violation	0.500	0.510
pathological grade	0.165	0.365
A total of twelve clinical variables were screened as prognostic factors to predict OS and DFS using a univariate analysis in NBNC-AFP(-)-HCC group.		

The Kaplan-Meier analysis showed that NBNC-AFP(-)-HCC patients had longer DFS than the other three groups (Figure 2). With the follow-up time ranging from 1 month to 132 months, the median DFS of the four groups (NBNC-AFP(+)/(-)-HCC groups and B-AFP(+)/(-)-HCC groups) was 15 months, 90 months, 10 months, and 24 months, respectively, and their corresponding 1-year DFS was 50.3%, 84.6%, 47.3%, and 50.1%, while their 2-year DFS was 43.1%, 72.9%, 42.3%, and 42.9%. The 3-year DFS was 38.3%, 68.8%, 35.3%, and 21.5%, respectively; 5-year DFS was 23%, 51%, 29%, and 21.5%; and 10-year DFS was 0%, 40.8%, 9.7%, and 0%. Among these three groups of case controls, the NBNC-AFP(-)-HCC group was found to be statistically different from the B-AFP(+)-HCC, NBNC-AFP(+)-HCC, and B-AFP(-)-HCC groups in terms of patients' DFS duration ($P=0.001$, $P=0.011$, and $P=0.015$, respectively). The above data show that the 1-year, 2-year, 3-year, 5-year, and 10-year DFS of NBNC-AFP(-)-HCC patients were 84.6%, 72.9%, 68.8%, 51%, and 40.8% respectively, and the patients' median DFS duration was 90 months. After 10 years, 40.8% of patients had no tumor recurrence, which outperformed the other three groups in terms of DFS (Table 5).

Table 5
The comparison between groups in terms of the DFS and OS

DFS(year)	1	3	5	10
NBNC-AFP(-)	84.6	68.8	51	40.8
B-AFP(+)	47.3	35.3	29	9.7
NBNC-AFP(-)	84.6	68.8	51	40.8
NBNC-AFP(+)	50.3	38.3	23	0
NBNC-AFP(-)	84.6	68.8	51	40.8
B-AFP(-)	50.1	21.5	21.5	0
OS(year)				
NBNC-AFP(-)	80.1	76.1	65.1	52.1
B-AFP(+)	66.4	44.6	40.5	29.7
NBNC-AFP(-)	80.1	76.1	65.1	52.1
NBNC-AFP(+)	83.9	34.2	28.9	25.7
NBNC-AFP(-)	80.1	76.1	65.1	52.1
B-AFP(-)	82.2	66.5	51.5	51.5
NBNC-AFP(-)-HCC patients had significantly better DFS and OS than NBNC-AFP(+)-HCC and B-AFP(+)-HCC patients				

OS of NBNC-AFP(-)-HCC patients

The univariate analysis showed that some factors were associated with OS ($P < 0.05$), such as size of tumor, secondary tumor, and portal vein invasion (Table 2), while Cox multivariate analysis indicated that size of tumor ($P = 0.042$) and secondary tumor ($P = 0.020$) were both independent influencing factors for postoperative OS (Table 3). In this study, no relevant factors affecting postoperative OS in NBNC-AFP(-)-HCC patients were found (Table 4).

Kaplan-Meier analysis revealed that NBNC-AFP(-)-HCC patients had significantly better OS than NBNC-AFP(+)-HCC and B-AFP(+)-HCC patients, but there was no statistical difference compared with that in B-AFP(-)-HCC patients. The median survival time of patients in these four groups (NBNC-AFP(+)/(-)-HCC groups and B-AFP(+)/(-)-HCC groups) was 1 year, 10 years, 2 years, and 4 years, and their 1-year OS was 83.9%, 80.1%, 66.4%, and 82.2% respectively. The 3-year OS was 34.2%, 76.1%, 44.6%, and 66.5%; the 5-year OS was 28.9%, 65.1%, 40.5%, and 51.5%; and the 10-year OS was 25.7%, 52.1%, 29.7%, and 51.5% (follow-up ranged from 1 to 11 years). The median survival time of NBNC-AFP(-)-HCC patients was 10 years, and 52.1% of postoperative patients survived for at least 10 years (Table 5).

Discussion

HCC is the most common malignant tumor and the leading cause of cancer-related deaths worldwide^[13]. The HBV and HCV are the main cause of HCC in China and Japan respectively^[14–16]. With the widespread use of HBV vaccine, the number of childhood and those carriers of HCV significantly decline in recent years^[17]. There is about 5%-20% of HCC patients, who are negative for both HBsAg and HCVAb, so-called “NBNC-HCC”, has recently tended to increase^[18, 19].

So far, the underlying causes of NBNC-HCC are not yet completely understood. Our research identified that the etiology of NBNC-AFP(-)-HCC in patients in China was related to obesity, diabetes, metabolic syndrome, and NAFLD, which is consistent with existing reports^[20]. Some factors are associated with the non-alcoholic steatohepatitis (NASH) and liver fibrosis, such as age, obesity, hypertension, type II diabetes, ALT, S/T >1, and decreased platelet count^[21, 22]. A series of studies confirmed that obesity and diabetes were independent risk factors for HCC^[23]. The etiological basis of such findings was insulin resistance (IR), which was the shared pathogenic factor for obesity, diabetes, hypertension, and dyslipidemia for causing fatty liver, as well as a factor during the progression of liver disease from steatohepatitis, liver fibrosis, and cirrhosis to liver cancer^[24]. In addition, this study did not exclude alcoholics patients. There was no statistical difference between the three groups of patients who were included according to the standard of excessive drinking (drinking alcohol >280 g/week [men] or >140 g/week [women]), which indicated that there may be no strong correlation between NBNC-AFP(-)-HCC and alcohol.

Meanwhile, no statistical difference existed in age, BMI, history of drinking, metabolic syndrome, or diabetes between NBNC-AFP(-)-HCC and NBNC-AFP(+)-HCC, indicating that these two types shared a similar etiology. However, patients with NBNC-AFP(-)-HCC had special clinical characteristics, which can be seen in the existence of statistical differences in size of tumor, pathological type, secondary tumor, and portal invasion. The pathological mechanism might be that the lack of expression of AFP in the absence of hepatitis B and C virus infections could lead to good clinical pathological characteristics. Shian et al reported that HCC patients who expressed high levels of AFP (>200 ng/ml) had larger tumor diameters (>5 cm), increased pathological grading (II–IV), and increased portal invasion (IIIA–IV HCC), compared with those who expressed low levels of AFP (<200 ng/ml). The high expression of AFP would also lead to the increment of the recurrence rate in HCC patients^[25]. This was consistent with our findings and further confirmed that AFP was an important factor affecting survival in HCC^[26, 27]. Meanwhile, multivariate analysis showed that size of tumor, secondary tumor, and portal invasion were all independent influencing factors for DFS, while size of tumor and secondary tumor were independent influencing factors for OS. Hence, we believe that the special clinical characteristics of NBNC-AFP(-)-HCC patients directly lead to their good survival prognosis.

The univariate analysis showed that secondary tumor was the factor that influenced DFS, while no factors were found to affect OS. Our research suggests that being AFP- and HBsAg-negative at the same

time could indicate a low recurrence rate and there was a partial difference in terms of the OS. At the same time, NBNC-AFP(-)-HCC patients had lower recurrence rates and higher OS than NBNC-AFP(+)-HCC patients. The pathological mechanism of the good prognosis for NBNC-AFP(-)-HCC might be the negative expression of AFP in the absence of hepatitis B and C virus infections. In China, most HCC progress on the basis of chronic hepatic damage, hepatitis virus could not only induce the occurrence of tumors, but also promote the recurrence and metastasis of HCC [28, 29]. Meanwhile, the high expression of AFP also plays an important role in the progression of HCC and could serve as a useful symbol for predicting the risk of early HCC recurrence postoperatively.

Conclusions

To sum up, NBNC-AFP(-)-HCC was correlated with aging, obesity, diabetes and metabolic syndrome, suggesting that the etiology of NBNC-AFP(-)-HCC was related to NAFLD. Patients with NBNC-AFP(-)-HCC were those with the lowest recurrence rate, which was associated with their special clinical characteristics and serological features. However, there are some limitations of our study, such as a retrospective single-center study, and lack of analysis of hepatitis C-related liver cancer patients in the control group.

Abbreviations

AFP: Alpha-Fetoprotein;

ALD: Alcoholic Hepatitis;

ALT: Alanine aminotransferase;

AST: Aspartate aminotransferase;

B-HCC: Hepatitis B virus related hepatocellular carcinoma;

DFS: Disease-free survival;

FPG: Fasting plasma glucose;

HBV: Hepatitis B virus;

HCC: Hepatocellular carcinoma;

HCV: Hepatitis C virus;

HDL-C: High-density lipoprotein cholesterol

IR: Insulin resistance;

NAFLD: Non-alcoholic fatty liver;

NASH: Nonalcoholic steatohepatitis;

NBNC- HCC: non-B non-C HCC;

OS: Overall survival;

S/T: Aspartate aminotransferase/Alanine aminotransferase;

TG: Triglyceride.

Declarations

Acknowledgements

None

Authors' contributions

XPT study design, data collection, data analysis and interpretation, draft of the manuscript, approval of final manuscript, supervision. WC study design, data collection, data analysis and interpretation, draft of the manuscript, approval of final manuscript, supervision. QLZ study design, data analysis and interpretation, revision of the manuscript, approval of final manuscript. KZ study design, data collection, data analysis and interpretation, revision of the manuscript, approval of final manuscript. All authors approved the final version of the manuscript.

Funding

This study was supported by the Guangdong Natural Science Foundation (2014A030310021) National Natural Science Foundation Project, Regional Science Foundation Project (Grant Number: 81260368).

Availability of data and materials

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was conducted in compliance with all national and international ethical standards for research with humans. All study procedures were approved by the Ethics Committee of Jiangxi Provincial People's Hospital and patients gave written informed consent before being enrolled.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

¹*Department of Emergency, The Second Affiliated Hospital of Guangzhou medical university, Guangzhou, 510655, China.*

²The 334 Hospital Affiliated to Nanchang University, Nanchang, 330024, Jiangxi, China.

³Jiangxi Provincial People's Hospital, Nanchang, 330006, Jiangxi, China.

⁴*Department of Colorectal Surgery, The Six Affiliated Hospital, Sun Yat-sen University, Guangzhou, 510655, China.*

⁵Guangdong Provincial Key laboratory of Colorectal and Pelvic Floor Disease, The Sixth Affiliated Hospital of Sun Yat-sen University, 510655, Guangzhou, China.

⁶Guangdong Research Institute of Gastroenterology, The Sixth Affiliated Hospital of Sun Yat-sen University, 510655, Guangzhou, China.

References

1. Nie W, Yan L , Lee YH , et al. Advanced mass spectrometry-based multi-omics technologies for exploring the pathogenesis of hepatocellular carcinoma[J]. *Mass Spectrometry Reviews*, 2016, 35(3):331-349.
2. Jou J, Diehl AM. Epithelial-mesenchymal transitions and hepatocarcinogenesis[J]. *Journal of Clinical Investigation*, 2010, 120(4):1031-1034.
3. Hennemig T, Venkatesh SK. Imaging of hepatocellular carcinoma: diagnosis, staging and treatment monitoring[J]. *Cancer Imaging the Official Publication of the International Cancer Imaging Society*, 2013, 12(3):530-547.
4. Zhang W, Tan Y, Jiang L, et al. Prognostic nomogram for patients with non-B non-C hepatocellular carcinoma after curative liver resection[J]. *International Journal of Surgery*, 2017, 44:160-165.
5. Nishio T, Hatano E, Okuno M, et al. Clinical Characteristics of Patients with Non-B Non-C Hepatocellular Carcinoma Undergoing Hepatic Resection[J]. *Journal of Surgical Research*, 2014, 186(2):632-633.
6. Ting CF, Huang WH, Feng CL, et al. Clinical factors associated with the survival of patients with intrahepatic cholangiocarcinoma[J]. *Advances in Digestive Medicine*, 2016, 3:11-17.
7. Younossi ZM, Otgonsuren M, Henry L, et al. Association of Non-alcoholic Fatty Liver Disease (NAFLD) with Hepatocellular Carcinoma (HCC) in the United States from 2004-2009[J]. *Hepatology*, 2015,

62(6):1723-1730.

8. El-serag HB, Tran T, Everhart J E. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma[J]. *Gastroenterology*, 2004, 126(2): 460-468.
9. Kawamura Y, Arase Y, Ikeda K, et al. Large-scale long-term follow-up study of Japanese patients with non-alcoholic fatty liver disease for the onset of hepatocellular carcinoma[J]. *American Journal of Gastroenterology*, 2012, 107(2): 253-261.
10. Patton HM, Yates K, Unalp-Arida A, et al. Association between metabolic syndrome and liver histology among children with nonalcoholic fatty liver disease[J]. *The American journal of gastroenterology*, 2010, 105(9): 2093-2102.
11. Ochiai T, Ogino S, Ishimoto T, et al. Prognostic impact of hepatectomy for patients with non-hepatitis B, non-hepatitis C hepatocellular carcinoma[J]. *Anticancer Research*, 2014, 34(8): 4399-4410.
12. Iida H, Aihara T, Ikuta S, et al. Clinicopathological characteristics of hepatitis B surface antigen-negative and hepatitis C antibody-negative hepatocellular carcinoma[J]. *Oncology letters*, 2015, 10(4): 2233-2238.
13. Siegel R L, Miller K D, Jemal A. Cancer statistics, 2019[J]. *CA: a cancer journal for clinicians*, 2019, 69(1): 7-34.
14. Niederau C. Chronic hepatitis B in 2014: great therapeutic progress, large diagnostic deficit[J]. *World journal of gastroenterology:WJG*, 2014, 20(33): 11595-11617.
15. Utsunomiya T, Shimada M, Kudo M, et al. Nationwide study of 4741 patients with non-B non-C hepatocellular carcinoma with special reference to the therapeutic impact[J]. *Annals of Surgery*, 2014, 259(2): 336-345.
16. Chang M H, Chen C J, Lai M S, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children[J]. *New England Journal of Medicine*, 1997, 336(26): 1855-1859.
17. Nishikawa H, Osaki Y. Non-B, non-C hepatocellular carcinoma[J]. *International Journal of Oncology*, 2013, 43(5): 1333-1342.
18. Utsunomiya T, Shimada M, Kudo M, et al. A comparison of the surgical outcomes among patients with HBV-positive, HCV-positive, and non-B non-C hepatocellular carcinoma: a nationwide study of 11,950 patients[J]. *Annals of surgery*, 2015, 261(3): 513-520.
19. Wu ZF, Xu Z, Li WS, et al. Impact of occult hepatitis B virus infection on outcome after resection for non-B non-C hepatocellular carcinoma[J]. *journal of surgical research*, 2015, 193(1): 153-160.

- 20.Fan JG, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. J Hepatol, 2009, 50(1):0-210.
- 21.Shinkawa H, Uenishi T, Takemura S, et al. Risk factors for postoperative recurrence of non-B non-C hepatocellular carcinoma[J]. Journal of Hepato-Biliary-Pancreatic Sciences, 2010, 17(3):291-295.
- 22.Farrell GC, Chitturi S, Lau GK, et al. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. J Gastroenterol Hepatol, 2007, 22(6):775-777.
- 23.Morzyglod L, Caüzac M, Popineau L, et al. Growth factor receptor binding protein 14 inhibition triggers insulin-induced mouse hepatocyte proliferation and is associated with hepatocellular carcinoma[J]. Hepatology, 2017, 65(4):1352-1368.
- 24.Farrell G. Insulin resistance, obesity, and liver cancer[J]. Clinical Gastroenterology & Hepatology, 2014, 12(1):117-119.
25. Kim SK, Marusawa H, Eso Y, et al. Clinical characteristics of non-B non-C hepatocellular carcinoma: a single-center retrospective study. Digestion, 2011, 84: 43-49.
- 26.Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management. Hepatology, 2009, 49(1): 306-317.
- 27.Torres DM, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. Gastroenterology, 2008, 134(6): 1682-1698.
28. Amini M, Khedmat H, Taheri S. Hepatitis C Virus Infection, Metabolic Disorders and Non-alcoholic Fatty Liver Disease: A Literature Review[J]. British Journal of Medicine & Medical Research, 2015, 5:1396-1402.
29. Marchesini G, Marzocchi R, Agostini F, et al. Nonalcoholic fatty liver disease and the metabolic syndrome. Curr Opin Lipidol, 2005, 16(4): 421-427.

Figures

Figure 1

Consort diagram of the study selection process. A total of 227 patients were selected from 2591 HCC patients treated at Sun Yat-sen University Cancer Center between January 1998 and December 2007.

Figure 2

Comparison of clinical outcomes between the NBNC-AFP(-)-HCC patients and other groups.