

# Prognostic role of lipids, apolipoproteins in patients with resectable hepatocellular carcinoma

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## Research

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## Abstract

**Background:** To further clarify the association between abnormal levels of serum lipid components as the main features of dyslipidaemia and hepatocellular carcinoma, which remains unclear.

**Methods:** We examined the serum level of lipids and apolipoproteins pattern in 471 patients undergoing curative resection for HCC, 193 patients with chronic liver disease, and 104 patients with benign liver diseases. We performed uni- and multivariate analyses to evaluate the predictive roles of lipids and apolipoproteins for recurrence and survival of HCC in a training cohort of 242 patients and then validated in a cohort of 229 patients.

**Results:** The majority circulating lipid and apolipoprotein levels such as ApoA1, HDL, LDL in chronic liver disease and HCC were slight to significantly decreased as compared with those in benign lesion. But no significant differential expression patterns of lipids and apolipoproteins were observed between chronic liver hepatitis and HCC. Multivariable analysis identified ApoA1 as a key parameter related to recurrence and survival in both training and validation cohorts. Moreover, we further demonstrated that low ApoA1 was an independent prognostic factor of poor early recurrence in two cohorts.

**Conclusions:** Although the alterations of circulating lipids and apolipoproteins were observed in HCC, none of lipids and apolipoproteins could serve as a diagnostic marker. Serum ApoA1 merits consideration as a novel prognostic marker for patients with HCC undergoing surgery, since it predicts early recurrence and survival, especially for early stage patients and may improve the prognostic stratification of patients for clinical management and promote HCC clinic outcomes.

## Introduction

Hepatocellular carcinoma (HCC) is the most prevalent cancer with a dismal prognosis worldwide[1]. Until now, surgical resection has remained the primary treatment to cure this disease, but the prognosis of patients with HCC remains poor, mainly because most patients are diagnosed at advanced stages and because of a high incidence of tumor recurrence post-surgery[2]. Although HCC patients who receive curative therapy are at a very early/early disease stage, early recurrence is considered an important adverse prognostic factor after curative therapy for HCC[3, 4]. Therefore, sensitive and effective biomarkers are urgently needed for early diagnosis and the accurate prognostic prediction of patients undergoing curative resection. The identification of patients who are at high risk of early recurrence after curative surgery allows clinicians to provide active surveillance to detect recurrent HCC at its earliest stage and to improve the prognosis of this population when curative therapy may still be feasible.

Many epidemiological studies have consistently linked dyslipidemia with increased incidence and death from diverse types of cancer[5–7]. The liver plays a key role in the metabolism of lipids and lipoproteins. The majority of patients with HCC are frequently affected by liver cirrhosis, and chronic hepatitis B and C are the major etiological agents[8]. Therefore, liver function is clearly impaired in HCC, which leads to a distinctly abnormal pattern of serum lipid and lipoprotein expression. It has been shown that high-density lipoprotein (HDL) and its major apolipoproteins, ApoA1 and ApoA2, are frequently reduced in patients with HCC[9, 10]. Studies have also demonstrated that plasma triglycerides (TG) and total cholesterol (TC) are decreased in patients with HCC[11]. In contrast, Alsabti[12] reported that serum TG and TC were increased in HCC patients compared with serum TG and TC in patients with cirrhosis. Ooi et al[13] reported that the plasma TG levels in HCC patients were not significantly different compared with those in controls. A potential explanation for these conflicting results could be the sole focus on the level of lipids or lipoproteins without an extensive list of potential confounders that are not always controlled such as body mass index (BMI) and glucose metabolism agents.

Ahaneku et al[14] analyzed lipid profiles in cirrhosis patients with or without HCC and found that plasma HDL-C, HDL-PL and HDL-C/HDL-PL were significantly lower in HCC patients than in controls. It is suggested that variations in the level of plasma lipids and apolipoproteins may assist in describing the nature of these two forms of liver disease. More striking is the discovery, by mass spectrometric analysis, that ApoA1 was differentially expressed between chronic hepatitis C virus (HCV) infection and HCC patients, which suggested that ApoA1 might be a candidate biomarker for early diagnosis, prognosis, and monitoring of HCC[15]. A similar result was found by Steel et al[16] in that ApoA1 had a diagnostic role in patients with HCC compared with hepatitis B virus-positive individuals. These results suggested that the plasma lipid profile might be a potential candidate that can be used to differentiate HCC from other liver diseases and from normal liver. The plasma lipid profile also acted as a diagnostic biomarker of HCC. However, the limitations of the studies mentioned above are obvious, such as the small number of cases enrolled and the techniques used, which are unsuitable for large-scale analysis in clinical application. Therefore, the accuracy and reliability of the experimental results mentioned above have yet to be verified.

Thus far, few studies have investigated the use of serum lipids as biomarkers for diagnosing HCC and predicting the recurrence or survival of patients with HCC. In this study, we investigated the expression pattern of blood lipids and apolipoproteins including ApoA1, ApoB, ApoE, TC, TG, HDL, and low-density lipoprotein (LDL) in HCC and other liver diseases including chronic hepatitis, cirrhosis and benign disease. We also explored their plausibility as novel diagnostic biomarker candidates for HCC. Furthermore, we analyzed the associations of the circulating lipid and apolipoprotein levels with the clinicopathological characteristics and prognosis of patients with HCC.

## Materials And Methods

### Study population

In all, 471 patients with primary HCC who underwent curative liver resection between December 2010 and January 2012 at the Liver Cancer Institute, Zhongshan Hospital, Fudan University were included in this retrospective study. All patients had histologically confirmed HCC. None of the patients had received presurgical cancer treatment and none had a recurrence of HCC or any other known malignancy. The patients were randomly divided into a training cohort(n=242) and a validation cohort(n=229). In addition, 140 patients with chronic hepatitis, 53 with cirrhosis, and 104 with benign liver diseases, including

hepatic hemangioma, hepatic cyst and focal nodular hyperplasia, were also enrolled in this study. In this cohort, patients with benign diseases had normal liver function relative to normal, age-matched donors.

Lipid and apolipoprotein profiles were studied descriptively in relation to potential confounders of metabolism including BMI, blood concentration of C reactive protein (CRP) as a biomarker for systemic inflammation, fasting blood glucose (GLU), hemoglobin A1C as well as insulin resistance (C peptide, insulin). As an index of insulin resistance, we used the homeostatic model assessment of insulin resistance (HOMA2-IR). Clinical tumor stages were determined according to the TNM classification system of the International Union Against Cancer. Tumor differentiation was assigned according to the Edmondson-Steiner classification.

Follow-up procedures were described in our previous study[17]. Overall survival (OS) was defined as the interval between surgery and either the time of death or the last follow-up. Disease-free survival (DFS) was defined as the interval between surgery and the time of recurrence or the date of the last follow-up without recurrence. The overall median follow-up time was 70 months (range, 7-79months).

### **Laboratory measurements**

All baseline blood samples were collected prior to surgery from patients who underwent surgery and from nonsurgery participants during the first week after admission. TC and TG were quantitatively determined by a colorimetric method, while HDL and LDL were measured using enzymatic methods. ApoA1, ApoB and ApoE were determined by turbidimetric methods. All the above measurements were performed in an automated biochemistry analyzer (Hitachi 7600). C-peptide and insulin were examined by an electrochemiluminescent immunoassay using an Roche Cobas 602 autoanalyzer.

### **Statistical analysis**

SPSS version 20 (SPSS Inc, Chicago, IL) was used to analyze the data. Continuous variables were compared using the Mann-Whitney U-test or One-way ANOVA. Categorical variables were compared using the  $\chi^2$  test or Fisher's exact test. The Spearman rank correlation test was performed to determine any associations between variables. The overall survival and disease-free survival were computed using the Kaplan-Meier method and were calculated using the log-rank test. A Cox regression hazard model was utilized for the multivariate analysis. To avoid colinearity bias, the lipids and apolipoproteins were preliminarily tested in a multivariate model that included the individual variables comprising the TNM stage. All statistical tests were two-sided, and p-values <0.05 were interpreted as significant.

## **Results**

### **Measurement of the expression pattern of serum lipids and apolipoproteins in liver disease**

We first compared the serum lipid and apolipoprotein levels in the four different groups. In patients with HCC, the serum level of ApoA1 was statistically lower than that in patients with benign liver disease but was higher than that in the liver cirrhosis group. No significant difference was observed between HCC and the hepatitis group. In addition, the expression pattern of HDL was similar to that of ApoA1. The serum ApoE level was significantly higher in the HCC group compared with the chronic liver hepatitis group, whereas the level did not differ significantly between the HCC group and the other two groups. Compared with the liver cirrhosis group, the TG and LDL levels were significantly elevated in the HCC, liver hepatitis and benign liver disease groups, whereas no significant differences were shown in the expression levels among these three groups. Moreover, we did not find any significant differences in the serum ApoB and TC levels among these four groups (Figure 1 and Figure S1).

### **Correlations between the lipid and apolipoprotein levels and clinical characteristics**

As shown in Table 1, no significant differences were found in the relationships between lipid and apolipoprotein levels and clinical background factors such as age, liver cirrhosis, alanine aminotransferase (ALT), albumin (ALB), alpha-fetoprotein (AFP), and serum total bilirubin (TB). However, ApoA1, ApoE and HDL were closely associated with gender. With regard to metabolic profiles, TG and HDL had positive correlations with BMI, whereas LDL and ApoB were associated with glycometabolic indexes such as blood glucose and hemoglobin A1C (HbA1C). TG had a close relationship with HbA1C and the insulin resistance index HOMA2-IR. However, most lipids and apolipoproteins were not closely related to metabolic parameters in HCC patients. Finally, with respect to tumor-related covariates, we revealed that ApoA1 was significantly related to vascular invasion and that LDL was associated with tumor size. However, no significant relationships were observed between most lipids and tumor-related factors.

### **Prediction of Tumor Recurrence and Patient Survival in the training cohort**

We next explored the prognostic significance of lipids and apolipoproteins in patients with HCC. The baseline characteristics of the patients were not significantly different between the training and validation cohorts (Table S1). Using X-tile 3.6.1 software, the optimal cutoff values of each lipid and apolipoprotein were established to separate patients in the training cohort into low and high groups[18]. Divisions of the validation cohort were also based on these numerical standards. A univariate analysis indicated that a lower ApoA1 level was significantly correlated with poor prognosis of HCC patients. The cumulative OS and DFS rates of patients in the low ApoA1 group were significantly lower than those of patients in the high ApoA1 group (5-year OS rate: 65 vs. 78.8%, P=0.02; 5-year DFS rate: 45.5 vs. 59.6%, P=0.01) (Figure 2). In addition, an elevated baseline level of LDL was significantly associated with reduced OS and DFS, while other lipids were not significant predictive factors in the univariate analysis (Figure S2 and S3). A multivariate survival analysis performed with the Cox regression model confirmed ApoA1 as an independent predictor of both OS and DFS (OS: Hazard ratio (HR): 0.587; 95% confidence interval (CI): 0.352-0.979; P=0.04; DFS: Hazard ratio (HR): 0.607; 95% confidence interval (CI): 0.413-0.893; P=0.01). Then, a multivariate Cox regression model was used to further assess the independent prognostic ability of TNM stage. The analysis showed that low ApoA1 remained an independent unfavorable factor that influenced the OS and DFS (OS: HR: 0.517; 95% CI: 0.309-0.866; P=0.01; DFS: HR: 0.537; 95% CI: 0.363-0.793; P=0.002) (Table 2 and Table S2).

## Prognostic value of lipid and apolipoprotein levels in the validation cohort

Lipids and apolipoproteins were further assessed for their prognostic power in an independent cohort of 229 patients. We found that HCC patients with lower serum ApoA1 levels had a significantly worse prognosis than those with higher serum ApoA1 levels. In the univariate analysis of OS, ApoA1 and ApoE were univariate predictors of OS ( $P=0.01$ ;  $P=0.02$ , for ApoA1 and ApoE, respectively)(Figure 2 and Figure S4). A Cox regression analysis demonstrated that a lower serum ApoA1 level was an independent indicator of OS (HR: 0.418; 95% CI: 0.234-0.747;  $P=0.003$ ). In addition, a higher serum ApoE level was also an independent risk factor for OS (HR:1.897; 95% CI: 1.123-3.204;  $P=0.02$ ). In the univariate analysis of DFS, a lower ApoA1 level was a significant predictor of poor prognosis ( $P=0.007$ ), and a Cox regression analysis showed that ApoA1 remained an independent prognostic factor for DFS (HR:0.609; 95% CI: 0.409-0.908;  $P=0.02$ )(Table S3 and Table S4). However, other lipids and apolipoproteins including TC, TG, HDL, LDL and ApoB did not show any statistically significant associations with OS and DFS(Figure S4 and S5).

## Serum ApoA-1 levels are correlated with early recurrence in the training and validation cohorts

Furthermore, the prognostic significance of the serum ApoA-1 level in patients with AFP levels  $\leq 400$  ng/mL and/or in patients with early recurrence was investigated. Among patients with an AFP concentration  $\leq 400$  ng/mL, the OS and DFS rates in low ApoA1 patients were significantly lower than those in high ApoA1 patients in the training cohort (OS: 68.3% vs. 83.1%,  $P=0.006$ ; DFS: 49% vs. 66.7%,  $P=0.01$ , respectively, for low ApoA1 and high ApoA1 patients). In accordance with the prognostic value of ApoA1 in the training cohort, ApoA1 remained associated with the OS and DFS rates in the lower AFP subgroup in the validation cohort (OS: 72.2% vs. 86.4%,  $P=0.025$ ; DFS: 47.4% vs. 62.1%,  $P=0.04$ ) (Figure S6).

In addition, it is worth noting that, for early recurrence in the training cohort, the serum ApoA-1 level was significantly associated with recurrence in the univariate analysis. In the multivariate analysis, the serum ApoA-1 level was an independent indicator of early recurrence (HR: 0.51; 95% CI: 0.297-0.875;  $P=0.02$ ). Similar results were confirmed in the validation cohort: lower ApoA-1 was an independent predictor of early recurrence (HR, 0.478; 95% CI: 0.278-0.881;  $P=0.008$ )(Figure 3, Table 3 and Table S5). Previous studies have shown that predictive factors for early recurrence are mainly tumor-related clinicopathologic factors (i.e., tumor size, tumor number, tumor markers, microscopic vascular invasion)[19,20]. The results of the multivariate analysis in the present study also show that early recurrence was associated with larger tumor size, vascular invasion, and advanced TNM stage. Further stratification analyses were conducted in the subgroups based on AFP, tumor size, number, vascular invasion, and TNM stage. We found that low ApoA1 was remarkably related to early recurrence in patients with an AFP concentration  $\leq 400$   $\mu\text{g/L}$ , a tumor size ranging from 2cm to 5cm, TNM stage I and solitary tumor in both the training and validation cohorts. By contrast, no significant associations were observed between the ApoA1 level and the early recurrence rate in the vascular invasion-negative subgroup (Figure S7 and Figure S8).

## Discussion

Until now, the association between lipids and apolipoproteins and HCC has not been thoroughly investigated, and limited data concerning their clinical significance in relation to HCC have been comprehensively reported. In this study, we analyzed the serum lipid and apolipoprotein levels in patients with benign lesions, chronic hepatitis, liver cirrhosis and HCC, and found that most circulating lipid and apolipoprotein levels in chronic liver disease and HCC were slightly to significantly decreased compared with those in benign lesions. This result was in accordance with some previous studies[9, 21–23].

As far as we know, the currently available serological biomarkers, such as AFP, are insufficiently sensitive or specific for use in diagnostic assays[24, 25], and thus novel serum markers capable of precisely detecting HCC at an early stage as well as accurate monitoring of HCC over a long period are needed. Proteomic technology has revealed that certain apolipoproteins, such as ApoA1, can distinguish serum from patients with cancer and healthy control subjects with high sensitivity and specificity. Mustafa et al[15] discovered proteomic alterations in ApoA1 in HCC-derived serum samples and a decreased ApoA1 level relative to HCV. Similar to the result described above, Liu et al[26] found a great discriminatory value of ApoA1 between the two patient populations with HCC and liver cirrhosis and suggested that ApoA1 had diagnostic ability in detecting HCC. According to the above mentioned results, it seems that the conclusion that ApoA1 is an effective diagnostic biomarker for HCC is valid. Although our results showed that the levels of most lipids and apolipoproteins are apparently different between HCC and the normal benign groups, no significant differential expression patterns of lipids and apolipoproteins were observed between chronic liver hepatitis and HCC in our study. Furthermore, the circulating ApoA1, HDL and LDL levels were substantially lower in the liver cirrhosis group compared with the HCC group, which did not contradict results of previous studies[10, 22, 28]. A possible explanation for this is that most previous studies included patients with more advanced stages of liver cancer accompanied by more severe liver dysfunction, which inevitably led to a lower level of lipids and apolipoproteins compared with our patients. Collectively, we considered that ApoA1 was not a sufficiently sensitive or specific biomarker to separate HCC from other chronic liver diseases. In addition, some data have suggested that ApoA1 could provide diagnostic utility to distinguish patients with bladder cancer, gastric cancer and breast cancer from healthy controls. The more important concern is that these results emphasize that changes in lipid and apolipoprotein profiles may imply a relationship with the presence and/or risk of cancer, although the underlying biological mechanisms are not fully understood.

The majority of HCC patients have an underlying chronic liver disease, and therefore, hepatic function is obviously damaged, which significantly influences lipid and apolipoprotein metabolism[27]. Consequently, it has been suggested that plasma levels of lipids and apolipoproteins in HCC reflect the hepatic cellular impairment status. We revealed that the majority of lipids and apolipoproteins were positively correlated with prealbumin and gamma-glutamyl transferase, two hepatic protein synthetic ability indices, and suggested that the pattern of changes in lipid and apolipoprotein levels may be a good indicator of the hepatic protein synthetic ability and liver injury during the perioperative period after hepatectomy. Many studies have demonstrated that liver injury parameters are closely related to poor prognosis in HCC[28, 29], and hence, we hypothesize that circulating lipids and apolipoproteins could serve as indicators of prognosis after curative hepatectomy in routine clinical practice. In fact, the correlation of lipid and apolipoprotein levels in peripheral blood with the prognosis of HCC has never been investigated. Our study has revealed that although most of the lipids and apolipoproteins were not significantly associated with overall survival and tumor recurrence in either the training or validation cohorts, a lower serum ApoA1 level was a negative prognostic factor

for OS and DFS in patients with HCC in two independent cohorts. Therefore, serum ApoA1 might be a powerful and noninvasive biomarker that can predict the prognosis of HCC patients who undergo curative surgery. Our results also suggest that ApoA1 could also serve as a prognostic indicator in patients with lower AFP concentrations. Therefore, this marker may help surgeons identify surgical candidates at high risk of recurrence and poor prognosis before surgery. Furthermore, this would be a clinical monitoring indicator for patients with lower AFP concentrations after surgery since AFP is not feasible as a monitoring indicator, while ApoA1 is significantly correlated with the prognosis of these patients.

Notably, the current study demonstrated no progressive relationship between ApoA1 expression and postoperative survival in patients with an AFP concentration  $\geq 400$   $\mu\text{g/L}$ , which is generally associated with advanced stage and tumor progression. This suggests that ApoA1 may be an efficient predictor of early prognostic status for patients. Various reports have suggested that patients with early recurrence generally have a worse prognosis than those with late-phase recurrence[30, 31]. Since the clinical outcome of patients who experience early recurrence is extremely dismal[32], identifying patients at high risk for early recurrence may help improve the prognosis of this population by rational adjuvant treatment. Therefore, we next investigated the prognostic value of ApoA1 to identify its correlations with the early recurrence of HCC. Here, we further demonstrated that low ApoA1 was an independent prognostic factor for early recurrence according to the multivariate analysis in two cohorts. As we know, HCCs larger than 5 cm always have a poor prognosis[33, 34], while small HCCs less than 2 cm are always associated with a good prognosis. We then stratified patients into three subgroups according to tumor size. We observed a statistically significant difference in the curves for early HCC recurrence-free survival: patients with low ApoA1 had a higher recurrence rate when their tumor size ranged from 2 cm to 5 cm. It is also worth noting that in patients with an AFP concentration  $< 400$   $\mu\text{g/L}$  and TNM stage I, Kaplan-Meier survival curves showed that patients with lower ApoA1 levels in both cohorts had a poor early recurrence rate. Consequently, we consider that ApoA1 might be utilized to identify a certain potentially manageable portion of HCC patients at high risk for early recurrence who are good candidates to receive more intensive surveillance and aggressive treatment post-surgery, especially for those with early-stage disease and an negative AFP concentration.

To date, the precise mechanisms by which serum lipids and apolipoproteins contribute to the initiation and/or progression of cancer are not completely understood. Dyslipidemia is known to be associated with the induction of oxidative stress as well as the chronic inflammatory response[35, 36] and is closely related to insulin resistance[37], all of which have certain relationships with tumorigenesis[38, 39]. In vitro studies have confirmed that several types of prostate, colon and breast cancer cell lines contain high levels of cholesterol-rich lipid rafts in the plasma membrane, which enhance cholesterol anabolism by reducing the expression of ABCA1; this in turn leads to cholesterol efflux from these cells and helps meet the increasing requirements of cell proliferation. In addition, HDL and ApoA1 have attracted much interest due to their protective roles in cancer, as suggested by several large clinical studies[40, 41]. Furthermore, in mouse tumor models[42], ApoA1 not only prevented further tumor development but also led to tumor shrinkage. The anti-tumor effect of ApoA1 is profound and manifold and results in large part from the modulation of the anti-tumor immune response. ApoA1 promotes the accumulation of anti-tumor macrophages of the M1 phenotype, increases levels of tumor cell-killing macrophages and recruits CD8 T cells within the tumor microenvironment. However, the way in which ApoA1/HDL triggers these effects is not known and is currently under investigation. Notably, the current study demonstrated a progressive relationship among low serum ApoA1 levels, postoperative early recurrence and poor outcomes in HCC patients and illustrated that ApoA1 might be a protective agent against liver cancer. Moreover, high density lipoprotein as a potential carrier for the delivery of anti-tumor drugs into hepatoma cells[43] and the provision that human ApoA1 is therapeutic against established tumors, seem to provide a new treatment method for liver cancer.

Our study has some limitations to consider. First, all enrolled participants with HCC underwent surgery. Since most patients who underwent hepatic resection had well-compensated liver function, the serum lipid and apolipoprotein levels might not have been significantly influenced by HCC compared with those with chronic liver diseases. This partly explained the lack of a significant difference in the lipid level that was revealed between the HCC and chronic liver disease groups according to our data. Second, the number of participants with chronic liver disease was relatively small compared with the number of participants with HCC, and thus the power to detect a statistical difference might be weakened. Last, the present study was a retrospective single-centric study, and further multicentric prospective studies are warranted to confirm our results.

## Conclusions

The current study showed alterations in lipid and apolipoprotein patterns in patients with resectable HCC, chronic infection and benign liver disease. Among these factors, although ApoA1 was not a suitable diagnostic biomarker for HCC, it was confirmed to be an independent prognostic factor for recurrence and survival in HCC. Finally, ApoA1 might indicate a poor prognosis in patients with early-stage HCC. Further trials are therefore necessary to clarify the mechanisms underlying the anti-tumor effects of ApoA1.

## Abbreviations

HCC Hepatocellular carcinoma

HDL High-density lipoprotein

TG Triglycerides

TC Total cholesterol

HCV Hepatitis C virus

LDL Low-density lipoprotein

CRP C reactive protein

GLU Glucose

HOMA2-IR Homeostatic model assessment of insulin resistance

OS Overall survival

DFS Disease-free survival

ALT Alanine aminotransferase

ALB Albumin

AFP Alpha-fetoprotein

TB Serum total bilirubin

HbA1C Hemoglobin A1C

## Declarations

### Acknowledgements

Not applicable

### Authors' contributions

Conception/Design: Xiao-Chun Ni, Jie Xu, Shuang-Jian Qiu

Provision of study material or patients: Yong Yi, Yi-Peng Fu, Xiao-Chun Ni

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Xiao-Chun Ni and Yong Yi contributed equally to this work.

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### Availability of data and materials

The datasets that were analyzed during the current study are available from the corresponding author upon reasonable request.

### Ethics approval and consent to participate

This study was performed in accordance with the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of Zhongshan Hospital of China Fudan University.

### Consent for publication

Not applicable

### Competing interests

The authors declare that they have no competing interests.

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## Tables

Table 1 Relationship between lipids and apolipoproteins level and clinical variables of HCC patients

Variables	ApoA1		p Value	ApoB		p Value	ApoE		p Value	TC		p Value	TG		p Value	HDL		p Value		
	low	high		low	high		low	high		low	high		low	high		low	high			
	n=282	n=189	n=345	n=126	n=253	n=218	n=420	n=51	n=377	n=94	n=279	n=192	n=166							
Gender	Male	258	140	0	290	108	0.66	222	176	0.04	316	82	0.41	222	176	<0.001	135			
	Female	24	49		55	18		31	42		65	8		61	12		57	16	31	
Age(years)	<55	152	87	0.09	182	57	0.15	130	109	0.77	214	25	0.79	192	47	0.87	132	107	0.07	86
	≥55	130	102		163	69		123	109		206	26		185	47		147	85		80
liver cirrhosis	absence	76	51	1	86	41	0.1	62	65	0.21	109	18	0.18	93	34	0.03	68	59	0.14	39
	present	206	138		259	85		191	153		311	33		284	60		211	133		127
Hepatitis history	absence	92	47	0.08	98	41	0.43	74	65	0.92	125	14	0.75	103	36	0.04	80	59	0.68	51
	present	190	142		247	85		179	153		295	37		274	58		199	133		115
BMI	<23.5	141	90	0.64	175	56	0.25	132	99	0.17	207	24	0.77	202	29	<0.001	149	82	0.03	87
	≥23.5	141	99		170	70		121	119		213	27		175	65		130	110		79
GLU	<5.6	228	146	0.35	284	90	0.01	209	165	0.07	338	36	0.14	302	72	0.48	224	150	0.64	141
	≥5.6	54	43		61	36		44	53		82	15		75	22		55	42		25
APLB	<0.25	212	130	0.13	265	77	0	200	142	0	313	29	0.01	296	46	<0.001	208	134	0.26	136
	≥0.25	70	59		80	49		53	76		107	22		81	48		71	58		30
ALT	≤50	224	146	0.57	270	100	0.8	210	160	0.01	333	37	0.27	301	69	0.17	212	158	0.1	132
	>50	58	43		75	26		43	58		87	14		76	25		67	34		34
TB	≤20	258	171	0.71	314	115	0.93	233	196	0.41	380	49	0.19	344	85	0.8	255	174	0.77	149
	>20	24	18		31	11		20	22		40	2		33	9		24	18		17
AFP	≤400	201	138	0.68	244	95	0.32	181	158	0.82	304	35	0.57	262	77	0.02	193	146	0.1	117
	>400	81	51		101	31		72	60		116	16		115	17		86	46		49
ALB	≤40	90	61	0.94	119	32	0.06	77	74	0.42	136	15	0.67	123	28	0.6	98	53	0.09	59
	>40	192	128		226	94		176	144		284	36		254	66		181	139		107
GGT	≤60	175	108	0.29	219	64	0.01	165	118	0.01	255	28	0.42	239	44	0	157	126	0.04	114
	>60	107	81		126	62		88	100		165	23		138	50		122	66		52
HbA1C	<6	232	154	0.83	298	88	<0.001	212	174	0.26	348	38	0.14	317	69	0.02	231	155	0.57	146
	≥6	50	35		47	38		41	44		72	13		60	25		48	37		20
HOMA2-IR	<26.9	262	175	0.9	318	119	0.4	237	200	0.42	390	30	0.86	355	82	0.02	261	176	0.44	155
	≥26.9	20	14		27	7		16	18		47	4		22	12		18	16		11
CRP	<3	198	144	0.15	267	75	<0.001	188	154	0.37	306	36	0.73	274	68	0.95	206	136	0.47	134
	≥3	84	45		78	51		65	64		114	15		103	26		73	56		32
Tumor size	≤5cm	184	133	0.27	237	80	0.32	180	137	0.06	284	33	0.86	250	67	0.39	182	135	0.27	124
	>5cm	98	56		108	46		73	81		136	18		127	27		97	57		42
Tumor number	Single	245	162	0.72	303	104	0.14	222	185	0.36	367	40	0.08	328	79	0.45	237	170	0.26	138
	Multiple	37	27		42	22		31	33		53	11		49	15		42	22		28
Tumor capsule	complete	180	123	0.78	219	84	0.52	160	143	0.6	269	34	0.71	240	63	0.54	176	127	0.5	98
	None	102	66		126	42		93	75		151	17		137	31		103	65		68
Vascular invasion	absence	198	152	0.01	260	90	0.39	190	160	0.67	314	36	0.52	274	76	0.11	204	146	0.48	130
	present	84	37		85	36		63	58		106	15		103	18		75	46		36
Edmondson grade	I-II	202	133	0.77	239	96	0.14	178	157	0.69	295	40	0.22	263	72	0.19	188	147	0.03	112
	III-IV	80	56		106	30		75	61		125	11		114	22		91	45		54
TNM stage	I	230	154	0.98	286	98	0.45	211	173	0.26	346	38	0.39	308	76	0.4	222	162	0.39	133
	II	28	18		31	15		25	21		39	7		34	12		31	15		20
	III	24	17		28	13		17	24		35	6		35	6		26	15		13

Abbreviations: BMI: body mass index; GLU: glucose; APLB: prealbumin; ALT: alanine aminotransferase; TB: total bilirubin; AFP: alpha-fetoprotein; ALB: prealbumin; GGT: glutamyl transpeptidase; HbA1C: hemoglobin A1C; CRP: C reactive protein; TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein;

Table 2 Univariate and multivariate analyses of prognostic factors in HCC in training cohort

Variables	OS			DFS		
	Univariate	Multivariate		Univariate	Multivariate	
	P	HR (95% CI)	P	P	HR (95% CI)	P
Gender	0.93			0.57		
Age(years)	0.46			0.08		
liver cirrhosis	0.59			0.45		
Hepatitis history	0.81			0.15		
BMI	0.18			0.11		
GLU	0.59			0.7		
APLB	0.08			0.12		
ALT	0.89			0.55		
TB	0.25			0.61		
AFP	0.002	1.687(1.028-2.771)	0.04	0.004		
ALB	0.56			0.71		
GGT	0.01			0.004		
CHOL	0.56			0.51		
TG	0.24			0.32		
HDL	0.36			0.57		
LDL	0.01	1.868(1.036-3.368)	0.04	0.045		
Tumor size	<0.001	2.216(1.379-3.561)	0.001	<0.001	1.974(1.363-2.862)	<0.001
Tumor number	0.08			0.09		
Tumor capsule	0.04			0.26		
Vascular invasion	<0.001	2.502(1.542-4.061)	<0.001	<0.001	1.997(1.336-2.984)	0.001
Edmondson grade	0.054			0.007	1.458(0.98-2.17)	0.06
TNM stage	0.003			0.001		
ApoA1	0.02	0.587(0.352-0.979)	0.04	0.01	0.607(0.413-0.893)	0.01
ApoB	0.36			0.83		
ApoE	0.18			0.39		

Univariate analysis: Kaplan-Meier method; multivariate analysis: Cox proportional hazards regression model;

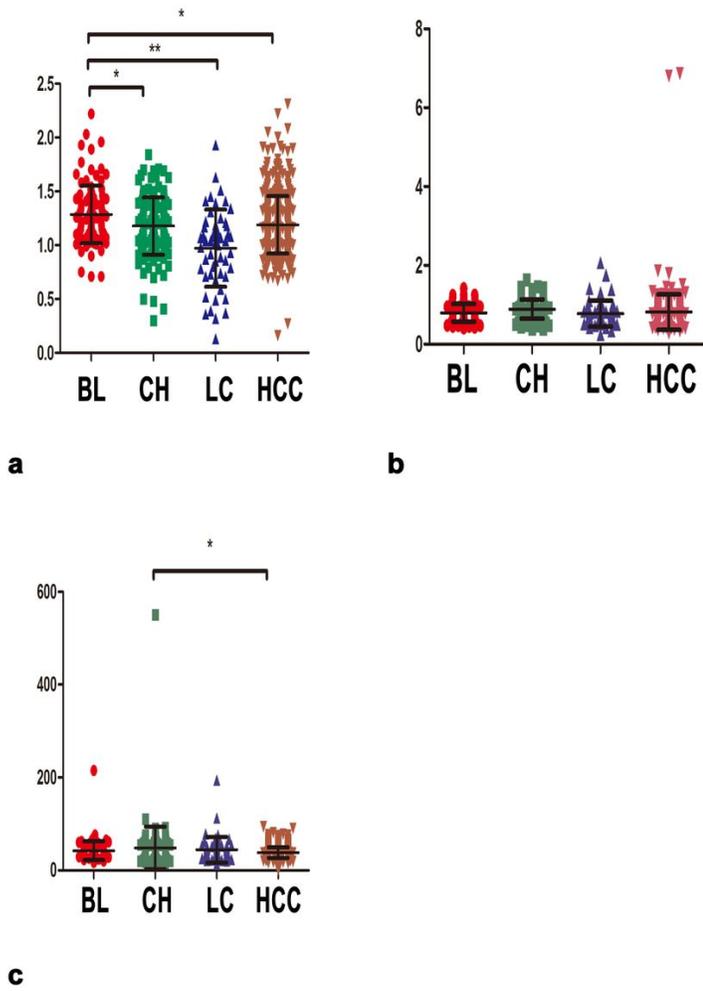
Abbreviations: BMI: body mass index; GLU: glucose; APLB: prealbumin; ALT: alanine aminotransferase; TB: total bilirubin; AFP: alpha-fetoprotein; ALB: prealbumin; GGT: glutamyl transpeptidase; HbA1C: hemoglobin A1C; CRP: C reactive protein; TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL:low-density lipoprotein; OS: overall survival; DFS: disease free survival; HR: Hazard Ratio; CI: confidence interval.

Table 3 Univariate and multivariate analyses for independent variables associated with HCC early recurrence

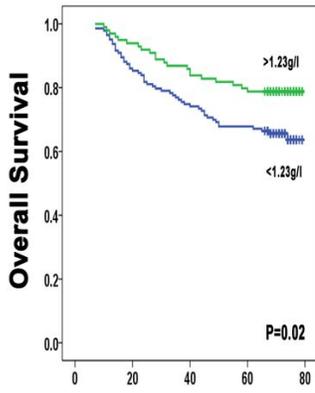
Variables	early recurrence (training cohort)			early recurrence (validation cohort)		
	Univariate	Multivariate		Univariate	Multivariate	
	P	HR (95% CI)	P	P	HR (95% CI)	P
Gender	0.45			0.005		
Age(years)	0.28			0.44		
liver cirrhosis	0.46			0.17		
Hepatitis history	0.45			0.18		
BMI	0.1			0.14		
GLU	0.78			0.11		
APLB	0.08			0.106		
ALT	0.87			0.01	1.747(1.026-2.975)	0.04
TB	0.87			0.41		
AFP	0.002			0.03		
ALB	0.67			0.7		
GGT	0.01			<0.001	1.663(1.004-2.752)	0.048
CHOL	0.47			0.98		
TG	0.4			0.57		
HDL	0.86			0.88		
LDL	0.18			0.22		
Tumor size	<0.001	2.869(1.761-4.673)	<0.001	<0.001	2.084(1.275-3.407)	0.003
Tumor number	0.04			0.15		
Tumor capsule	0.01			0.44		
Vascular invasion	<0.001	2.862(1.769-4.631)	<0.001	<0.001	1.897(1.159-3.106)	0.01
Edmondson grade	0.003			0.15		
TNM stage	<0.001			0.04		
ApoA1	0.002	0.51(0.297-0.875)	0.02	0.003	0.478(0.278-0.821)	0.008
ApoB	0.57			0.25		
ApoE	0.03	0.574(0.353-0.935)	0.03	0.87		

Abbreviations: BMI: body mass index; GLU: glucose; APLB: prealbumin; ALT: alanine aminotransferase; TB: total bilirubin; AFP: alpha-fetoprotein; ALB: prealbumin; GGT: glutamyl transpeptidase; HbA1C: hemoglobin A1C; CRP: C reactive protein; TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HR: Hazard Ratio; CI: confidence interval.

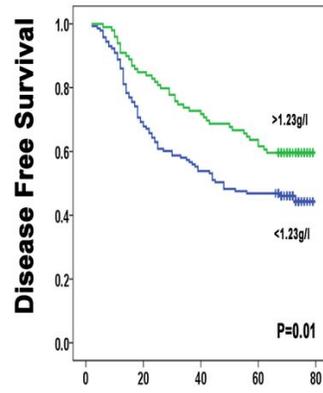
## Figures



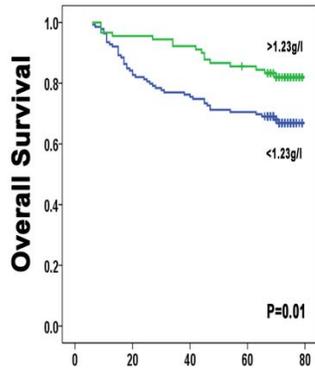
**Figure 2**  
 The blood expression levels of ApoA1, ApoB, and ApoE in benign liver disease (BL), chronic hepatitis (CH), liver cirrhosis (LC), and heparocellular carcinoma (HCC) groups. A, ApoA1. B, ApoB. C, ApoE. \* indicates  $P < 0.05$ , \*\* indicates  $P < 0.01$



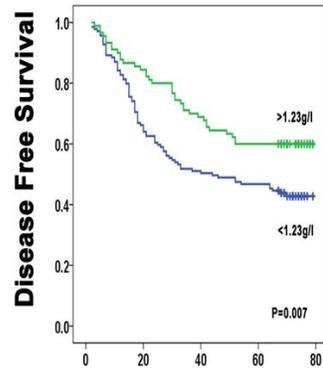
**a** Time(month)



**b** Time(month)



**c** Time(month)



**d** Time(month)

**Figure 4**  
 Low ApoA1 level is significantly associated with poorer patient survival. Low ApoA1 level is significantly associated with poorer overall survival (A) and disease-free survival (B), in a test cohort of 242 HCC patients. Low ApoA1 level is significantly associated with poorer overall survival (C) and recurrence-free survival (D), in a validation cohort of 229 HCC patients.

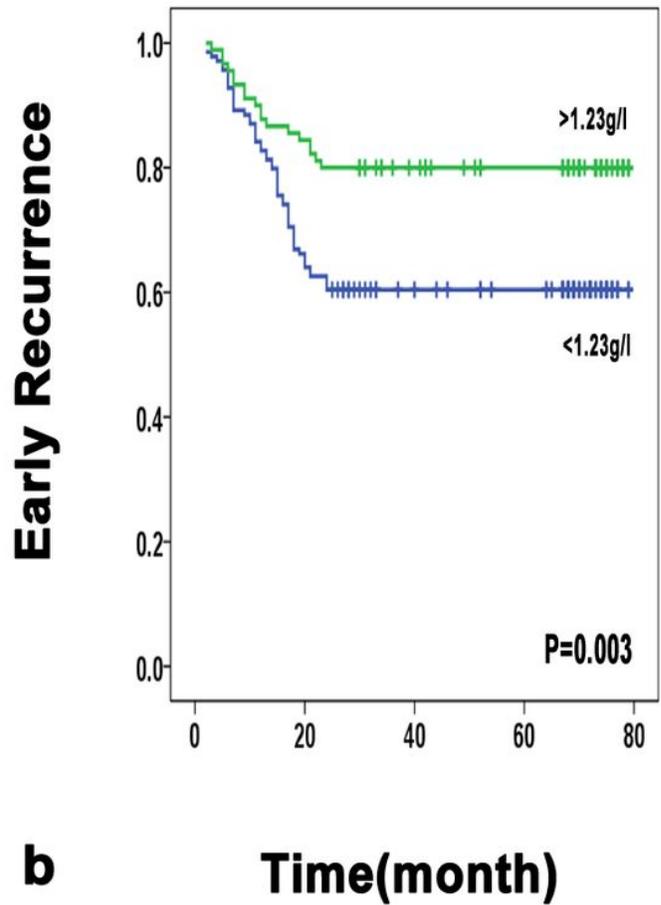
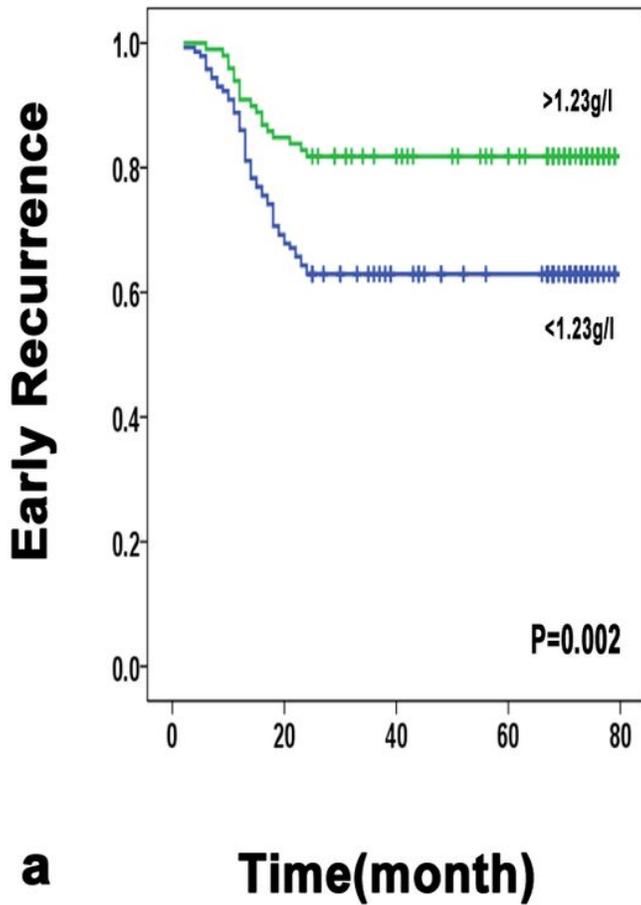


Figure 6

Early recurrence curves in the training and validation cohorts according to ApoA1  $\leq 1.23\text{g/l}$  or ApoA1  $>1.23\text{g/l}$ . (A) training cohort. (B) validation cohort.

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