HBV Infection Statuses Indicate Different Risks of Synchronous and Metastasis Liver Metastasis in Colorectal Cancer

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Abstract

Background: Previous studies on the effect of HBV infection on CRLM are contrary. This study identified more specific and different impacts of HBV on CRLM.

Methods: A total of 3132 CRC patients were analyzed retrospectively and followed up for five years. All patients were divided into three groups: group A, HBsAg positive with HBV infection; group B, HBsAg negative with HBV infection; and group C, no HBV infection. The risk factors for SYN-CRLM, MET-CRLM, 5-year OS, and LDFS were analyzed.

Results: A total of 829 patients had CRLM. SYN-CRLM was found in 425 patients. The incidence of SYN-CRLM was 16.95%, 11.60% and 13.50% (P <0.03) in groups A, B, and C, respectively. HBsAg-positive HBV infection increased the risk of SYN-CRLM (P<0.01), with a worse prognosis in group A (P<0.05). MET-CRLM was found in 404 patients. The incidence of MET-CRLM in groups A, B, and C, was 16.51%, 11.53% and 16.50% (P=0.02). HBsAg-negative HBV infection decreased the incidence of MET-CRLM (P=0.02) with a better 5-year LDFS (P=0.01), but was not related to 5-year OS (P=0.15).

Conclusion: HBsAg positivity infection increased the risk of SYN-CRLM with poor prognosis. HBsAg-negative infection reduced the risk of MET-CRLM with better LDFS after surgery.

Introduction

In recent years, the global incidence of colorectal cancer (CRC) has continued to rise. CRC is the third most common malignant tumor with the second-highest mortality rate [1]. In China, the incidence and mortality of CRC are ranked fourth and fifth [2]. The majority of CRC patients who died had distant metastasis, and liver metastasis was the most common. Synchronous CRC liver metastasis (SYN-CRLM) was shown in 14–20% of CRC patients at the time of diagnosis [3–5]. In patients without SYN-CRLM, nearly 17% of them suffered from metachronous CRC liver metastasis (MET-CRLM), even after resection of the primary tumor [6,7]. According to statistics, the SYN-CRLM rate of colorectal cancer in China is about 25–30%, and the MET-CRLM rate is about 12–21%.

Hepatitis B virus (HBV) infection is the most common chronic liver disease in humans. It is estimated that approximately 2 billion people worldwide have been infected with HBV, and more than 350 million people have chronic hepatitis B (CHB) [8]. Approximately 120 million people in China are chronic HBV carriers, accounting for approximately one-third of the world's total population, and 30 million of them have HBV virus replication throughout their life [9,10]. Hepatitis B is one of the causes of hepatocellular carcinoma, increasing the risk of intrahepatic metastasis [11–13]. In addition, it has been reported that HBV infection also increases the risk of liver metastasis in pancreatic cancer, B-cell lymphoma, and other malignant tumors [14–16].

Several previous studies have reported the risk of HBV for CRC liver metastasis, but there are two different opinions. Utsunomiya et al. suggested that the risk of CRLM is lower in patients with HBV and HCV infections [17]. Song et al. suggested that HBV replication can reduce the incidence of CRLM [18]. Augustin et al. and Cai et al. reported in a meta-analysis that the risk of liver metastasis in CRC patients with CHB was reduced [19,20]. Zhao et al. suggested that HBV infection is a good predictor of prognosis after liver metastasis resection [21]. However, in recent years, scholars have raised different objections. Huo et al. indicated that hepatitis B virus surface antigen (HBsAg) positivity is an independent risk factor for SYN-CRLM [22]. All of the above studies have some limitations. There are no analyses of CRLM in different periods based on different HBV results and HBV infection statuses. At present, there is still a lack of large samples, comprehensive retrospective studies, and studies with support from laboratory data. Therefore, this study reviewed the data of 3040 patients treated at the Sixth Affiliated Hospital of Sun Yat-sen University from 2013 to 2015 and analyzed the relationship between HBV infection and CRLM.

Patients And Methods

Patients

From January 1, 2013, to December 31, 2015, a total of 3914 newly admitted and confirmed CRC patients were analyzed retrospectively. The inclusion criteria were as follows: (1) pathological diagnosis of colorectal adenocarcinoma; (2) no current or previous history of other malignant tumors; (3) no viral coinfection of the liver, except HBV; and (4) absence of drug hepatitis, alcoholic liver disease, fatty liver disease, autoimmune liver disease, and pregnancy with CHB. Patients who died within 30 days after surgery were excluded. Patients without hepatitis B virus serological marker (HBVM) or liver examination results at the first diagnosis of CRC were excluded. A total of 3132 patients were enrolled in this study. The medical ethics committee of the Sixth Affiliated Hospital of Sun Yat-sen University approved this study (Ethics approval number 2020ZSLYEC-101). Research was performed in accordance with the relevant guidelines and regulations. All participants provided written informed consent according to the declaration of Helsinki. See Fig1.

Data collection

The data included sex, age, tumor location, differentiation degree, HBVM results at first admission, carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), aspartate aminotransferase (AST), alanine aminotransferase (ALT), fibrosis index based on the four factors (FIB-4), chemotherapy (including neoadjuvant or adjuvant chemotherapy), Tumor, Node, Metastasis (TNM) stage (according to American Joint Committee on Cancer (AJCC), version 7 of CRC), liver disease-free survival (LDFS) and overall survival (OS) during follow-up.

Judgment of CRLM

There are variable definitions of synchronous and metachronous CRLM reported in the literature. According to the statements from the Expert Group on OncoSurgery management of Liver Metastases group (EGOSLIM), SYN-CRLM should be termed “synchronously detected liver metastases”[23]. We diagnosed the intrahepatic nodule as SYN-CRLM at the first diagnosis of CRC and MET-CRLM at any time after that.

In this study, for the intrahepatic nodule, after the first diagnosis of CRC, we examined the patient's radiology images, detected the serum CEA and alpha-fetoprotein (AFP) levels, and performed ultrasound-guided biopsy and pathological diagnosis when necessary. After excluding primary liver cancer, hemangioma, and liver cyst, all the results were judged by two radiologists who had more than 5 years of experience in specifically diagnosing CRLM.

Judgment of HBV infection

All patients were tested for HBV using HBVM (five items of hepatitis B) at the first diagnosis of CRC. According to the results of HBsAg, hepatitis B surface antibody (anti-HBs), hepatitis B e antigen (HBeAg), hepatitis B e antibody (anti-HBe), and hepatitis B core antibody (anti-HBc). We divided patients into three groups: groups A, B, and C. Group A included patients with HBsAg-positive HBV infection. Group B included patients with HBsAg-negative HBV infection who were positive for HBeAg, anti-HBe, and anti-HBc. Group C included patients who were negative for HBVM or were only anti-HBs-positive.

Follow-up

The time between the first diagnosis of CRC and the diagnosis of MET-CRLM was defined as LDFS, and the time between the first diagnosis of CRC and death was defined as OS. CT or B-ultrasound examinations were performed every 6-12 months after the operation. If abnormal nodules in the liver were found, the diagnosis of CRLM was made by contrast-enhanced ultrasonography or MRI. If necessary, a biopsy was performed for pathological diagnosis. The follow-up office of the Sixth Affiliated Hospital of Sun Yat-sen University followed up all the patients. We collected and recorded the follow-up data through the patient's return visit. For the patients who are examined in a local hospital due to living in a remote area, if there was suspected tumor recurrence or progress, they were required to return to our hospital for further diagnosis.

Statistical analysis

Categorical variables are shown as the number and percentage of patients. For binary variables, baseline categorical clinical parameters were compared by χ^2-test or Fisher’s exact test, and numerical values were compared using Student’s t-test. Significant risk factors for SYN-CRLM and MET-CRLM were analyzed by logistic regression analysis. A log-rank test was used to determine OS and LDFS in each group. The

hazard ratio (HR) and the corresponding 95% confidence interval (CI) were estimated with a mixed effects Cox regression model. Statistical analysis was performed by SPSS 22 (IBM company) and GraphPad Prism 8. A two-tailed P-value less than 0.05 was considered a significant difference.

Results

Baseline characteristics

From January 1, 2013, to December 31, 2015, 3914 CRC patients were confirmed of having CRLM in our hospital. 782 patients were excluded because of the lack of HBVM results and the inability to evaluate preoperative liver metastasis. A total of 3132 patients were included in final analysis, which included 1922 men and 1210 females. The median age was 56 years (±11.17, range from 17 to 95). There were 1526 patients with rectal cancer and 1606 patients with colon cancer. According to the HBVM results, patients were divided into group A, B or C. Group A was the HBsAg-positive group, with a total of 413 (13.19%) patients. Group B was the HBsAg-negative infection group, with a total of 638 (20.37) patients. Group C was the non-infection group, with a total of 2081 (66.44%) patients. 2893 patients received surgery of primary tumor resection during their treatment, while the other 239 did not.

SYN-CRLM were found in 425 (13.57%) patients at their first diagnosis, while the other 2707 were not. In those who without SYN-CRLM, MET-CRLM happened in 404 (12.90%) patients after surgery, while CRLM was not found in the remaining 2211 (70.59%) patients during follow-up. 92 patients did not have surgery and lost connection. See Table 1

Differences between HBV infection groups

The statistical results showed that there was no difference in tumor location, tumor differentiation, T stage, N stage, CEA and CA19-9 in different HBV infection groups. In the male proportion, there was a significant difference between group A (62.95%), group B (65.20%) and group C (59.88%) ($P = 0.04$). The proportion of patients younger than 56 years old in group A (56.90%) was higher than that in group B (43.89%) and group C (45.65%) ($P < 0.01$). The abnormal proportion of AST ($P < 0.01$), ALT ($P < 0.01$) and FIB-4 ($P < 0.01$) in group A was significantly higher than that in group B and group C, with statistical differences. See Table 2

Among the 3132 patients with CRC (before operation), the incidence of SYN-CRLM in group A, group B and group C was 16.95% (70 / 413), 11.60% (74 / 638) and 13.50 (281 / 1800), respectively, with statistical difference(P=0.05). See Table 3. Of the 2707 patients without SYN-CRLM, 92 were lost because of refusing surgery or transferring to other hospitals. 2615 patients underwent primary tumor resection and were followed up. The incidence of MET-CRLM in group A, B and C was 16.51% (54 / 327), 11.53% (64 / 555) and 16.50% (286 / 1733), respectively, with statistical difference(P=0.02). See Table 4.

Risk factors for SYN-CRLM

In this study, a total of 425 people was found to have SYN-CRLM at the first diagnosis of CRC before surgery. Significant differences in tumor location, pathology differentiation, T stage, N stage, HBV infection, CEA, CA19-9, AST, ALT between patients with and without SYN-CRLM were found. There were no significant differences in sex, age or FIB-4 among the SYN-CRLM group and the non-SYN-CRLM groups. In multivariate analysis, high or moderate differentiation (P=0.01, HR = 0.62 (0.44-0.87)) was an independent protective factor for SYN-CRLM. The independent risk factors for SYN-CRLM were T stage of 3 or 4 (P<0.01, HR = 3.94 (2.08-7.48)), N stage of 1 or 2 (P<0.01, HR = 1.83 (1.38-2.46)), CEA>5 ng/ml (P<0.01, HR=3.85 (2.84-5.22)), CA19-9>37 U/ml (P<0.01, HR=2.18 (1.59-2.98)), AST>40 (P<0.01, HR=3.85 (2.84-5.22)), and ALT>40 U/L (P<0.01), HR=2.15 (1.49-3.10)). In addition, HBsAg positive was an independent risk factor for SYN-CRLM (P < 0.01). Compared with HBsAg positive group, the relative risk of SYN-CRLM in HBsAg negative group was 0.19 (0.11-0.34), while that of non-infection group was 0.29 (0.18-0.47). See Table 5.

Risk factors for MET-CRLM

After excluding SYN-CRLM, 92 patients were lost because of refusal further treatment or transfer to other hospital. A total of 2615 patients had surgery for primary tumor resection and were followed up; of these patients, 404 developed MET-CRLM, accounting for 12.90%. In patients with MET-CRLM and those without MET-CRLM, there were no differences in sex, age, AST level, ALT level, or FIB-4 level. There were significant differences in the location of the primary tumor, degree of tissue differentiation, T stage, N stage, M stage, CEA level, CA19-9 level and chemotherapy (P<0.01).

In the Cox regression analysis, results showed that better pathological differentiation (P=0.04, HR=0.77 (0.61-0.99)) were independent protective factors. The presence of extrahepatic organ metastasis (P<0.01, HR=10.12 (7.90-12.95)), initial CA19-9 level at the first diagnosis of CRC (P<0.01, HR=1.50 (1.19-1.89)) and chemotherapy (P<0.01, HR=3.11 (1.91-5.04)) were independent risk factors.

There were also differences in the HBV infection groups (P=0.02). What was different from SYN-CRLM, we found that HBsAg negative infection in group B was an independent protective factor to MET-CRLM (relative to group A, P=0.04, HR= 1.47 (1.02-2.10) and group C, P<0.01, HR=1.49 (1.14-1.95)). See Table 6.

5-year OS and LDFS between different HBV infection groups

In this study, the follow-up period ended on January 1, 2020. A total of 2893 patients were followed up with a median follow-up time of 43 months (range from 0-73). 239 patients were lost. A total of 587 people died, 2306 survived during the follow-up. The 5-year survival rate was 79.71%. The mortality of patients in groups A, B, and C was 24.87% (93/374), 19.26% (115/597), and 19.72% (379/1922),

respectively. There was a significant difference in the 5-year OS rate among the three groups (P=0.05). See Fig2.

During the follow-up, in 2615 patients who had surgery and were without SYN-CRLM, 404 MET-CRLM patients were found, and the 5-year LDFS was 84.55% (2211/2615). The MET-CRLM rates of groups A, B, and C were 16.51% (54/327), 11.53% (64/555), and 16.50% (286/1733), respectively. There was a significant difference in the 5-year LDFS (P=0.01). See Fig3. In 2615 patients without SYN-CRLM, 450 died, and the OS rate was 82.79%. There were no significant differences among the groups (P=0.15). See Fig4.

Discussion

There have been several previous studies on the relationship between HBV and CRLM, but the conclusions are contradictory. According to Qi et al., the risk of MET-CRLM in patients with CHB is 14.2%, which is significantly lower than that in control group (28.2%)[24]. Li et al. suggested that the chance of CRLM in patients with CHB or chronic Hepatitis C (CHC) decreased 3.2%, comparing with 9.4% in no infected group[25]. Wang et al. showed that the risk of CRLM in patients with CHB or CHC was 2.86%, significantly lower than 16.9% in no infected group[26]. On the contrary, Huo et al. indicated that patients who are HBsAg-positive are more likely to have SYN-CRLM[22]. None of the above studies considered that HBV infection might have different effects on CRLM before and after surgery, and these studies did not analyze the effects caused by different HBV infection statuses independently. We believe that is the reason why they have different conclusions.

In this study, the incidence of CRLM was 26.47% (829/3040), and the incidence in groups A, B and C were 30.02% (124/413), 21.63% (138/629) and 27.25% (567/2081), respectively. There were significant differences among the three groups (P<0.01), suggesting that three different HBV infection statuses have different effects on CRLM.

According to the examination at the first diagnosis of CRC, SYN-CRLM was found in 425 patients, accounting for 13.57%, 2707 patients did not have SYN-CRLM. The incidence of SYN-CRLM in group B (P<0.01, HR=0.19, 95% CI (0.10-0.33)) and group C (P<0.01, HR=0.28, 95% CI (0.17-0.46)) was significantly lower compared to group A. This indicates that the active replication of HBV may increase the risk of SYN-CRLM, and this result is the same as that found by Huo et al. [22]. Abnormal elevation of AST and ALT were independent risk factors for SYN-CRLM, suggesting that SYN-CRLM may be related to HBV-induced hepatocyte damage.

After resection of primary tumor in 2615 patients, MET-CRLM was found in 404 patients (15.45%) during the follow-up. However, different from the effect on SYN-CRLM, HBV infection (including groups A and B) was an independent protective factor relative to group C in the Cox regression analysis. The 5-year LDFS rate of group B was 88.47%, which was significantly better than that of group A (83.49%) and group C (83.50%) (P=0.01). This conclusion is similar to that from many previous studies [17-20]. This result

suggested that the liver's immune status may reduce the risk of CRLM in patients who do not have HBV replication or have recovered from HBV infection.

When the cancer cells from the primary sites in the colon escape into the bloodstream, the most likely location where they are lodged is the liver. Kelly et al. suggested that micro-metastasis occurred when cancer cells from the primary CRC escape from the primary location into the portal circulation[27]. The liver immune system removes the tumor cells at the beginning of metastasis [28]. However, in HBsAg-positive patients, the liver immune function was deficient. Peng et al. observed upregulation of the PD-1/PD-L1 pathway in patients with CHB, thereby inhibiting the function and expression of interferon-r (INF-r) and CD8+ T cells, resulting in sustained liver cell damage [29]. Previous studies have suggested that the pathogenesis and metastasis of CRC are related to the upregulation of the PD-1/PD-L1 negative regulatory signaling pathway, which leads to immune escape [30-32]. Therefore, we believe that in HBsAg positive CRC patients, the liver immune function is impaired. Before surgery, when the primary tumor continues to enter the liver through blood flow, the consumption of the immune system in the liver is intensified, and the incidence of SYN-CRLM is increased. Similar phenomena were observed in primary liver cancer with HBV infection [33,34].

When the primary tumor is removed surgically, the process of tumor cells continuously entering the liver through the blood is terminated. However, MET-CRLM is still present in about 15% of patients. This is because the liver's immune system is unable to effectively remove residual tumor cells. In this study, the incidence of MET-CRLM in group B was significantly lower than that of group A and C. It indicates that there may be some immune response related to HBV infection, which enhances the clearance of tumor cells in the liver.

A number of studies have pointed out that HBV related immune function has been enhanced in patients with HBV infection, and its antiviral function is better than that of patients with chronic hepatitis B infection. Wu et al. noted that PD-1 expression in HBeAg-negative HBV patients was significantly lower than that in HBeAg-positive HBV patients [35]. Jung et al. found that the frequency of CD8+ CD45R0+ memory T lymphocytes increased in acute HBV infection [36]. Penna showed that the frequency of HBV-specific T cells in self-limited acute hepatitis B was comparable to that observed in the acute stage of infection and, usually, was higher than that in patients with chronic HBV infection [37]. Zhang et al. pointed out that PD-1 expression was significantly upregulated on HBV-specific CD8 T cells in the early phase of acute HBV infection, and successful viral clearance correlated with a subsequent decrease in PD-1 expression [38].

The results showed that HBsAg-positive HBV infection increased the risk of SYN-CRLM before surgery, probably by damaging hepatocytes and the liver immune system. HBsAg-negative HBV infection decreased the risk of MET-CRLM after surgery, probably by a good liver immune response. Compared to previous studies, our results can better explain the relationship between HBV and CRLM in the same cohort. However, this study is a single center retrospective analysis, and lack of HBV-DNA data. The

occurrence of CRLM may be related to the primary tumor, HBV infection statues, and liver immunology, but the mechanism remain unknown. Therefore, we will carry out a multi-center prospective observational study in the future work, collect complete clinical and experimental data, and further study the mechanism of how HBV infection affects the occurrence of CRLM.

Abbreviations

CRC——colorectal cancer

CRLM——colorectal liver metastasis

SYN-CRLM——synchronous colorectal cancer liver metastasis

MET-CRLM——metachronous colorectal cancer liver metastasis

HBV——hepatitis B virus

HBVM——hepatitis B virus serological marker

HBsAg——hepatitis B virus surface antigen

anti-HBs——hepatitis B surface antibody

HBeAg——hepatitis B e antigen

anti-HBe——hepatitis B e antibody

anti-HBc——hepatitis B core antibody

CEA——carcinoembryonic antigen

CA19-9——carbohydrate antigen 19-9

AST——aspartate aminotransferase

ALT——alanine aminotransferase

FIB-4——fibrosis index based on the four factors

OS——overall survival

LDFS——liver disease-free survival

HR——hazard ratio

CI——confidence interval

Declarations

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Competing interests

The authors declare no competing interests.

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Tables

Table 1. Baseline characteristics of patients		
Factors	Number	Percentage (%)
Gender		
Male	1922	61.37
Female	1210	38.63
Age (years old)		
Median 56±11.17, range from 17 to 95		
Tumor Location		
Rectum	1526	48.72
Colon	1606	51.28
HBV infection statues		
HBsAg (+) infection (group A)	413	13.19
HBsAg (–) infection (group B)	638	20.37
Non-infection (group C)	2081	66.44
Surgery for primary tumor resection		
Presence	2893	92.37
Absence	239	7.63
CRLM*		
SYN-CRLM	425	13.57
MET-CRLM	404	12.90
No CRLM	2211	70.59
Lost during follow-up	92	2.94

Table 2. Difference between HBV infection groups				
	Group A HBsAg(+) infection N=413（%）	Group B HBsAg(-) infection N=638（%）	Group C No infection N=2081（%）	
Gender				0.04
Male	260（63）	416（65）	1246（60）	
Female	153（37）	222（35）	835（40）	
Age				<0.01
>=56	178（43）	358（56）	1131（54）	
<56	235（57）	280（44）	950（46）	
Location				0.12
Rectum	186（45）	329（52）	1011（49）	
Colon	227（55）	309（48）	1070（51）	
Differentiation				0.08
High or middle	353（85）	571（89）	1797（86）	
Low	60（15）	67（11）	284（14）	
T stage				0.23
3 or 4	300（80）	452（76）	1469（76）	
0, 1 or 2	74（20）	145（24）	453（24）	
N stage				0.32
1 or 2	165（44）	234（39）	794（41）	
0	209（56）	363（61）	1128（59）	
M stage				0.01
1	148（36）	152（24）	641（31）	
0	265（64）	486（76）	1440（69）	
CRLM				0.01
SYN-CRLM	70（18）	74（12）	281（14）	
MET-CRLM	54（14）	64（10）	286（14）	
No CRLM	273（68）	491（78）	1447（72）	

CEA				0.29
>5ng/ml	140（34）	195（31）	624（30）	
<=5ng/ml	273（66）	443（69）	1457（70）	
CA19-9				0.74
37U/ml	77（19）	107（17）	365（18）	
<=37U/ml	336（81）	531（83）	1716（82）	
AST				<0.01
40 U/L	337（82）	130（20）	454（22）	
<=40 U/L	76（18）	508（80）	1627（78）	
ALT				<0.01
>40 U/L	267（65）	126（20）	427（21）	
<=40 U/L	146（35）	512（80）	1654（79）	
FIB-4 index				<0.01
>3.25	119（29）	104（16）	336（16）	
<=3.25	294（71）	534（84）	1745（84）	

Table 3. Incidence rate of SYN-CRLM in different HBV infection statues N=3132

	SYN-CRLM	No SYN-CRLM (%)	Total
Group A	70 (16.9%)	343 (83.1%)	413
Group B	74 (11.6%)	564 (88.4%)	638
Group C	281 (13.5%)	1800 (86.5%)	2081
P=0.05			

Table 4. Incidence rate of MET-CRLM in different HBV infection statues N=2615			
	MET-CRLM	No MET-CRLM	Total
Group A	54 (16.5%)	273 (83.5%)	327
Group B	64 (11.5%)	491 (88.5%)	555
Group C	286 (16.5%)	1447 (83.5%)	1733
P=0.02			

Table 5. Risk factors of SYN-CRLM N=3132						
Factor	SYN-CRLM N=425(%)	No SYN-CRLM N=2707(%)	Univariate	Multivariate	HR	95%CI
Gender			0.71			
Male	257 (60)	1665 (62)				
Female	168 (40)	1042 (38)				
Age			0.08	0.13	0.81	0.61-1.07
>=56	209 (49)	1458 (54)				
<56	216 (51)	1249 (46)				
Location			0.02	0.28	0.86	0.65-1.13
Rectum	185 (44)	1341 (50)				
Colon	240 (56)	1366 (50)				
Differentiation			<0.01	0.01	0.64	0.45-0.89
High or middle	312 (73)	2409 (89)				
Low	113 (27)	298 (11)				
T stage			<0.01	<0.01	4.04	2.12-7.67
3 or 4	267 (96)	1954 (75)				
0, 1 or 2	11 (4)	661 (25)				
N stage			<0.01	<0.01	1.82	1.36-2.43
1 or 2	186 (67)	1007 (39)				
0	92 (33)	1608 (61)				
M stage			<0.01	<0.01	22.4	16.1-35.2
1	425 (100)	470 (17)				
0	0 (0)	2237 (83)				
HBV infection			0.05	<0.01		
group A	70 (16)	343 (13)		Ref		
group B	74 (17)	564 (21)		<0.01	0.19	0.11-0.34
group C	281 (67)	1800 (66)		<0.01	0.29	0.18-0.47
CEA			<0.01	<0.01	3.91	2.88-5.30

>5ng/ml	304 (72)	655 (24)				
<=5ng/ml	121 (28)	2052 (76)				
CA19-9			<0.01	<0.01	2.17	1.59-2.98
37U/ml	211 (50)	338 (12)				
<=37U/ml	214 (50)	2369 (88)				
AST			<0.01	<0.01	0.277	0.18-0.42
40 U/L	85 (20)	836 (31)				
<=40 U/L	340 (80)	1871 (69)				
ALT			<0.01	<0.01	0.49	0.35-0.72
>40 U/L	83 (20)	737 (27)				
<=40 U/L	342 (80)	1970 (73)				
FIB-4 index			0.45			
>3.25	70 (16)	489 (18)				
<=3.25	355 (84)	2218 (82)				

Table 6. Risk factors of MET-CRLM N=2615						
Factors	MET-CRLM N=404(%)	No MET-CRLM N=2211(%)	Univariate	Multivariate	HR	95%CI
Gender			0.08	0.15	1.16	0.95-1.43
Male	266 (66)	1351 (61)				
Female	138 (34)	860 (39)				
Age			0.44			
>=56	210 (52)	1195 (54)				
<56	194 (48)	1016 (46)				
Location			<0.01	0.48	0.93	0.76-1.14
Rectum	169 (42)	1141 (52)				
Colon	235 (58)	1070 (48)				
Differentiation			<0.01	0.04	0.77	0.61-0.99
High or middle	319 (79)	2017 (91)				
Low	85 (21)	194 (9)				
T stage			<0.01	0.05	1.54	1.01-2.36
3 or 4	378 (94)	1576 (71)				
0, 1 or 2	26 (6)	635 (29)				
N stage			<0.01	0.60	1.06	0.85-1.32
1 or 2	263 (65)	744 (34)				
0	141 (35)	1467 (66)				
M stage			<0.01	<0.01	10.12	7.90-12.95
0	124 (31)	2062 (93)				
1	280 (69)	149 (7)				
HBV infection			0.02	0.01		
group A	54 (13)	273 (12)		0.04	1.47	1.02-2.10
group B	64 (16)	491 (22)		Ref	-	-
group C	286 (71)	1447 (65)		<0.01	1.49	1.14-1.95
CEA			<0.01	<0.01	1.90	1.52-2.38

>5ng/ml	223 (55)	399 (18)				
<=5ng/ml	181 (45)	1812 (82)				
CA19-9			<0.01	<0.01	1.50	1.19-1.89
37U/ml	135 (33)	182 (8)				
<=37U/ml	269 (67)	2029 (92)				
AST			1.00			
40 U/L	124 (31)	682 (31)				
<=40 U/L	280 (69)	1529 (69)				
ALT			0.58			
>40 U/L	104 (26)	601 (27)				
<=40 U/L	300 (74)	1610 (73)				
FIB-4 index			0.18			
>3.25	83 (21)	392 (18)				
<=3.25	321 (79)	1819 (82)				
Chemotherapy			<0.01	<0.01	3.11	1.91-5.04
Presence	382 (95)	1280 (58)				
Absence	22 (5)	931 (42)				