

RESEARCH ARTICLE

Analysis of Prognostic Factors of Colorectal Cancer Liver Metastasis after Microwave Ablation

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Abstract: Objective: To investigate the risk factors associated with intrahepatic recurrence and survival after microwave ablation (MWA) for colorectal cancer with liver metastases. **Methods:** Retrospective analysis of clinical, pathological and follow-up data of 60 patients who underwent ultrasound-guided microwave ablation for treating liver metastases from January 2013 to December 2015 at the Sixth Affiliated Hospital, Sun Yat-Sen University, with Cox univariate and multifactorial regression analyses of risk factors affecting intrahepatic recurrence and overall survival after ablation of liver metastases. **Results:** After follow-up for 12.5 to 47.4 months, intrahepatic recurrence occurred in 26 cases, of which 6.1% (7/114) showed local tumor progression at the original ablation site; 11 cases died for tumor-related reasons. Multi-factor Cox regression analysis showed that age ≥ 60 years and maximum diameter of liver metastases ≥ 2 cm were independent risk factors for patients to develop intrahepatic recurrence after surgery. HBsAb positivity and chemotherapy after ablation until intrahepatic recurrence are independent protective factors for patients developing intrahepatic recurrence after surgery. Multi-factor Cox regression analysis showed that the number of liver metastases ≥ 3 was an independent risk factor for overall survival. **Conclusion:** After microwave ablation for patients with colorectal cancer liver metastases, patients aged ≥ 60 years, HBsAb negative and with maximum liver metastases ≥ 2 cm in diameter were more likely to have recurrent intrahepatic metastases, while aggressive chemotherapy after ablation was effective in reducing recurrent intrahepatic metastases, and the number of liver metastases ≥ 3 indicated a poor overall prognosis.

Keywords: Colorectal cancer, Liver metastases, Microwave ablation, Progression-free survival

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Colorectal cancer (CRC) is highly susceptible to liver metastases, with about 25% of CRC patients having concurrent liver metastases at the time of initial diagnosis and 20%–30% of CRC patients also developing heterochronic liver metastases after resection of the primary tumor^[1]. Liver metastases are one of the most important factors that affect the prognosis of CRC patients. Complete resection of liver metastases is the standard of treatment for colorectal cancer liver metastases (CRLM), but less than 20% of CRC patients are treated with complete resection

of liver metastases^[1]. Previously palliative chemotherapy was the standard of treatment for unresectable CRLM. However, with the development and use of imaging-guided thermal ablation techniques, particularly radiofrequency ablation (RFA) and microwave ablation (MWA) brought new options for patients with unresectable liver metastases. Studies have shown that RFA has a good safety, initial and adjuvant efficacy in small CRLM and significantly improves survival outcomes in patients with liver metastases^[2-4]. Compared with RFA, MWA has the advantages of greater ablation energy, shorter treatment time, no charring effect and lower complication rate, but MWA still lacks sufficient data from multicenter trials in the treatment of CRLM and it is still somewhat controversial. In this study, we retrospectively analyzed the clinical data of CRLM patients treated with ultrasound-guided percutaneous MWA from January 2013 to December 2015 at the Sixth Hospital of Sun Yat-sen University to investigate the factors affecting their intrahepatic recurrence and prognosis, aiming to provide reference for the comprehensive treatment of CRLM patients.

1. Materials and methods

1.1. Clinical information

Criteria for inclusion: (1) age from 18 to 85 years old; (2) pathologically confirmed colorectal adenocarcinoma at the primary site of the tumor, and the primary site of the tumor is amenable to radical surgical resection; (3) the presence of liver metastases is confirmed by any two detection methods, including Contrast-Enhanced Ultrasound (CEUS), Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography-Computed Tomography (PET-CT) or the liver lesion puncture biopsies; (4) no extra-hepatic distant metastases according to CT, MRI or PET-CT; (5) high number and distribution of scattered of liver metastases, physical condition and other aspects that make them unsuitable for surgical liver resection; (6) patients who refuse surgery in favor of ultrasound-guided percutaneous MWA treatment; (7) no history of hepatic resection or other local treatment of the liver prior before MWA treatment; (8) liver metastases can be treated completely with a single MWA treatment; (9) no local tumor residue after MWA treatment; (10) normal function of main organs; (11) no history of other malignancies; (12) no serious comorbidities (e.g. severe infection, gastrointestinal bleeding, coagulation disorders); (13) ultrasound allows precise localization of the tumor at least 0.5 cm away from major vessels and major bile ducts.

According to the above criteria, clinical data of 60 patients with simultaneous or heterochronic CRLM treated with ultrasound-guided percutaneous MWA in

the Sixth Affiliated Hospital, Sun Yat-Sen University from January 2013 to December 2015 were collected. If patients had multiple MWA treatments, only the data of the first MWA treatment of each patient were taken in this study.

1.2. Treatment methods

1.2.1 Development of treatment strategies

The treatment strategy for patients with CRLM is developed in collaboration with the unit's multi-disciplinary colorectal cancer team (MDT), which consists of specialists in colorectal surgery, hepatobiliary surgery, diagnostic radiology, interventional ultrasound, vascular intervention, urology, thoracic surgery, radiation oncology, pathology and medical oncology. The patient and family are then informed in detail of the treatment strategy and sign an informed consent form for the treatment. Patients receiving MWA treatment need to meet the following criteria: ≤ 5 liver metastases; liver metastases ≤ 5 cm in diameter; liver metastases at least 0.5 cm away from major vessels and major bile ducts. Chemotherapy regimens are administered according to protocols developed by the MDT, including preoperative chemotherapy (neoadjuvant and conversion chemotherapy, including FOLFOX, FOLFOXIRI and Degramant) and postoperative chemotherapy (adjuvant and palliative chemotherapy after relapse, including FOLFOX, FOLFOXIRI, XELODA, FOLFIRI and XELOX). Surgery for primary colorectal cancer is performed in accordance with the standard procedure of total mesenteric excision (TME) or complete mesenteric excision (CME) for rectal cancer, either open or laparoscopic surgery.

1.2.2. Microwave ablation

All patients sign an informed consent form before undergoing ultrasound-guided percutaneous MWA treatment. For concurrent CRLM, MWA treatment is usually given 1 week before or 1–4 weeks after the primary site surgery; For heterochronic CRLM, MWA treatment is usually given after diagnosis or 2 to 4 weeks after the last chemotherapy session. CEUS to check tumor size and number before MWA and again the day after MWA or on day 2 to rule out bleeding, bile leaks and incomplete ablation. In this study, the KY2000 microwave therapy system (Nanjing Canyon Medical Inc.) was used for MWA treatment. For pain management during percutaneous MWA, pethidine hydrochloride (50–75 mg) is given intramuscularly approximately 30 min prior to the procedure; in addition, local lidocaine (10–15 mL) was injected locally at the beginning of the MWA to provide local anesthesia. The patient underwent percutaneous MWA under real-time

ECG monitoring and low-flow oxygenation. Percutaneous MWA is performed with a 1.0 to 5.0 MHz probe under the guidance of real-time ultrasound (LOGIQ E9, GE). The microwave radiation from the probe causes rapid oscillation of the water molecules, which causes frictional heating and leads to coagulation and necrosis of the tissue around the probe. MWA frequency is 2,450 MHz, and the power is 30–100 W; a No.14-spec (200 mm) cooled shift electrode (KY2450A, Nanjing Canyon Medical Inc.) is used for 5–20 min.

1.3. Method of follow-up visits

CEUS will be reviewed 1 month after MAW treatment and followed up every 2–3 months thereafter, including physical examination, serum markers (including tumor-related markers, liver and kidney function, coagulation function, and blood count) and one or more imaging tests (CT, MRI, CEUS, PET–CT) including liver. The deadline for follow-up visits is 31 December 2017. Overall survival (OS) after ablation is defined as the time from surgery of the primary site and treatment of the liver metastases with MWA until the patient's death. Disease progression defined as a new tumor or local recurrence outside or within the liver. Progression-free survival (PFS) is defined as the time from surgery of the primary site and treatment of the liver metastases with MWA until the patient develops a recurrence or death. Liver progression-free survival (LPFS) defined as the time from the start of MWA treatment until the patient develops intrahepatic recurrence or death.

1.4. Statistical processing

SPSS 19.0 software was applied for statistical analysis. The KaPlan-Meier method was used to calculate patients' OS and LPFS, and a Cox proportional risk model was used for univariate analysis of clinical, pathological and therapeutic measures that might affect postoperative recurrence; Factors with $P < 0.1$ were subjected to multifactorial prognostic analysis using a Cox proportional risk model. $P < 0.05$ indicates that the difference is statistically significant.

2. Results

2.1. General information

Among 60 patients with CRLM, there were 41 men and 19 women, aged from 29–85 years old, with a median age of 55.0 years old. The number of liver metastases per patient ranged from 1 to 5, with a mean of (1.90 ± 0.14) , for a total of 114 lesions treated with MWA; the maximum diameter of liver metastases ranged from 0.5 to 4.5 cm, with an average of (1.70 ± 0.97) cm. The 60 patients were followed up for 12.5 to 47.4 months,

with a median follow-up time of 27.2 months, and none of them had the ablation-related complications such as intrahepatic abscess, biliary obstruction, intestinal fistula, bile leak, bleeding, pneumothorax or tumor spread. During the follow-up period, intrahepatic recurrence occurred in 26 patients, of which 6.1% (7/114) had local tumor progression at the original ablation site and 11 patients had tumor-related deaths. LPFS rates of 67.2%, 56.1% and 38.0% at 6, 12 and 24 months respectively. The 24-month OS rate is 87.2%.

2.2. Analysis of factors associated with the prognosis of patients

Univariate analysis showed that hepatitis B virus surface antibody (HBsAb), site of liver metastases, maximum diameter of liver metastases and chemotherapy after ablation until intrahepatic recurrence were associated with LPFS ($P < 0.05$ or 0.01), and only the number of liver metastases was associated with postoperative OS of patients ($P = 0.032$). HBsAg-positive patients had a higher 24-month OS rate than HBsAg-negative patients (100.0% vs 68.9%), but the difference was not statistically significant ($P > 0.05$). See **Table 1** for details. Cox regression multifactor analysis was performed on age, HBsAb, liver metastasis site, maximum diameter of liver metastases, and chemotherapy after ablation until intrahepatic recurrence at $P < 0.1$ in **Table 1**. As shown in **Table 2**, age ≥ 60 years and maximum diameter of liver metastases ≥ 2 cm were independent risk factors for patients to develop intrahepatic recurrence after surgery ($P < 0.05$ or 0.01). The survival curves for both are shown in **Figures 1** and **2**. HBsAb positivity and the presence of chemotherapy after ablation until intrahepatic recurrence were independent protective factors for patients developing intrahepatic recurrence after surgery ($P < 0.05$ or 0.01), and the survival curves for both are shown in **Figures 3** and **4**. The number of liver metastases ≥ 3 (HR: 3.685, 95% CI: 1.122–12.097, $P = 0.032$) was an independent risk factor for overall patient survival, and its survival curve is shown in **Figure 5**.

3. Discussion

The current focus of CRLM treatment is the complete elimination of all lesions, including colorectal primary foci and liver metastases, while still preserving sufficient liver parenchyma. Removal of both primary tumors and liver metastases improves patient survival, and laparoscopic CRLM resection and open CRLM resection have the similar effects^[5]. However, only 10%–20% of CRLMs are suitable for hepatic resection^[1]. OS of unresectable CRLM is 6–12 months, with the systemic chemotherapy extends OS to 20–24 months for unresectable CRLM^[2]. The development of imaging-

guided. guided thermal ablation techniques such as RFA and MWA has expanded the potential for surgical eradication of CRLM. Thermal ablation has the advantages of being repeatable, does not interfere with the conditions of local liver surgery, does not require prolonged chemotherapy interruptions and maintains the patient's quality of life, meeting the needs of CRLM, which is highly prone to relapse and requires repeated treatment. A randomized clinical trial by Ruers *et al.*^[6] compared the efficacy of RFA in combination with systemic chemotherapy versus systemic chemotherapy

alone as first-line treatment for CRLM, with median PFS extending to 16.8 months in the combination group compared to 9.9 months in the chemotherapy alone group; overall survival at 30 months in the combination therapy group was 61.7% compared to 57.6% of the chemotherapy alone group. The wide variation in local recurrence rates after RFA is an important limitation to the widespread use of RFA^[7].

Table 1. Univariate analysis of factors affecting LPFS and OS after MWA in patients with CRLM

Influencing factors	n	LPFS			LPFS HR (95% CI)	P-value	24months OS		
		6 months	12 months	24 months			OS	HR (95% CI)	P-value
Sex					1.705 (0.771– 3.770)	0.188		0.870 (0.254– 2.979)	0.824
Female	19	72.2%	66.2%	52.6%			89.2%		
Male	41	64.9%	51.6%	31.5%			86.3%		
Age					1.851 (0.936– 3.661)	0.077		1.773 (0.541– 5.814)	0.344
<60	37	75.3%	69.5%	44.4%			88.3%		
≥60	23	64.9%	51.6%	31.5%			86.1%		
Primary focal site					1.141 (0.579– 2.249)	0.703		2.288 (0.600– 8.719)	0.225
Colonic	28	66.7%	63.0%	39.9%			88.4%		
Rectum	32	67.4%	49.2%	36.4%			86.3%		
Level of differentiation					0.843 (0.441– 1.952)	0.843		4.581 (0.585– 35.871)	0.147
Highly differentiated	17	76.5%	57.4%	35.7%			93.8%		
Low to medium differentiated	41	64.3%	56.3%	39.3%			84.4%		
Situation unknown	2	50.0%	50.0%	50.0%			100.0%		
Regional lymph node metastasis					1.768 (0.839– 3.727)	0.134		3.378 (0.679– 16.801)	0.137
No	22	86.1%	66.1%	47.1%			94.1%		
Yes	37	54.4%	48.3%	33.2%			82.8%		
Situation unknown	1	100.0%	0.0%	0.0%			100.0%		
Cancer nodules					1.619 (0.806– 3.249)	0.176		2.399 (0.689– 8.353)	0.169
No	32	77.9%	64.5%	44.9%			89.7%		
Yes	26	54.5%	45.4%	31.8%			83.8%		
Situation unknown	2	50.0%	50.0%	0.0%			100.0%		
Carcinoembryonic antigen CEA					1.684 (0.855– 3.318)	0.132		2.252 (0.476– 10.646)	0.306
<5 µg/L	34	69.3%	56.7%	50.4%			89.3%		
≥5 µg/L	26	64.5%	55.6%	23.8%			75.0%		
HBsAg					1.847 (0.756– 4.513)	0.179		0.338 (0.099– 1.158)	0.084
Negative	52	67.8%	59.2%	40.8%			68.9%		
Positive	8	62.5%	37.5%	25.0%			100.0%		
HBsAb					0.422 (0.214– 0.833)	0.013		0.902 (0.266– 3.056)	0.868
Negative	25	54.2%	41.7%	16.7%			91.5%		

Table 1. Continued.

Influencing factors	<i>n</i>	LPFS			LPFS HR (95% CI)	<i>P</i> -value	24months OS		
		6 months	12 months	24 months			OS	HR (95% CI)	<i>P</i> -value
Positive	35	76.1%	66.3%	53.1%			84.4%		
Transfer time					1.450 (0.717– 2.934)	0.301		0.956 (0.433– 2.109)	0.911
Heterochronous	24	68.9%	59.7%	45.5%			87.5%		
Simultaneity	36	65.5%	53.4%	32.2%			73.7%		
Liver metastasis site					2.433 (1.217– 4.867)	0.012		1.423 (0.425– 4.761)	0.567
Right liver metastasis only	33	75.4%	68.8%	55.1%			93.7%		
with left liver metastasis	27	56.9%	39.9%	15.5%			78.9%		
Number of liver metastases					1.306 (0.568– 3.001)	0.530		3.685 (1.122– 12.097)	0.032
≤2	49	68.8%	55.7%	41.3%			90.3%		
≥3	11	58.3%	58.3%	23.3%			61.4%		
Maximum diameter of liver metastases					2.727 (1.311– 5.673)	0.007		0.907 (0.192– 4.282)	0.902
<2 cm	47	73.4%	61.8%	46.8%			86.2%		
≥2 cm	13	44.9%	35.9%	0.0%			90.9%		
Chemotherapy before discovery of liver metastases				0.552 (0.257– 1.185)	0.127		0.205 (0.026– 1.604)	0.131	
No	40	63.6%	52.2%	29.8%			83.6%		
Yes	20	74.1%	63.5%	52.4%			95.0%		
Discovery of liver metastases to pre- ablation chemotherapy			1.504 (0.730– 3.097)	0.268		2.422 (0.674– 8.709)	0.176		
No	44	67.7%	57.8%	44.4%			90.4%		
Yes	16	65.5%	50.9%	19.4%			76.9%		
Chemotherapy after ablation and before intrahepatic recurrence				0.242 (0.121– 0.484)	0.000		0.380 (0.110– 1.313)	0.126	
No	18	31.7%	19.0%	9.5%			77.0%		
Yes	42	82.4%	72.1%	49.6%			91.7%		

Table 2. Multifactorial analysis of LPFS after MWA affecting CRLM patients

Influencing factors	Regression factor	HR (95% CI)	<i>P</i> -value
Age ≥60 years	0.861	2.365 (1.161–4.819)	0.018
HBsAb positive	–0.825	0.438 (0.217–0.884)	0.021
Site of liver metastases (with left hepatic metastases/right hepatic metastases only)	0.590	1.805 (0.838–3.886)	0.131
Liver metastases up to ≥2 cm in diameter	1.168	3.214 (1.505–6.865)	0.003
Chemotherapy after ablation and before intrahepatic recurrence	–1.455	0.233 (0.113–0.481)	0.000

Microwave is an electromagnetic wave with a frequency of 900–2,450 MHz. MWA uses a microwave antenna to bring high frequency electromagnetic fields into the tumor, when the oscillating charge from the microwave radiation interacts with water molecules, it causes the molecules to flip and rotate, generating

friction and heat, resulting in high temperatures in a very short period of time, thus taking advantage of the heat-insensitive properties of the tumor tissue to cause coagulative necrosis of the cells and destroy the blood supply to the tumor^[7-9]. MWA is less sensitive in the “radiator” phenomenon compared to RFA, resulting in a

larger ablation area in a relatively short period of time^[7]. MWA is thought to be more effective in treating larger lesions^[10]. However, MWA treatment for CRLM still lacks data from large multicenter studies. The results of this study showed that 6.1% (7/114) of the original ablation sites showed local tumor progression, in general agreement with the literature^[11]. Shibata *et al.*^[12] showed that the average survival time in the MWA group was 27 months, as compared to 25 months in the surgery group; the average disease-free survival time was 11.3 months in the MWA group and 13.3 months in the surgery group, the difference was not statistically significant ($P > 0.05$). The incidence of surgery-related complications was similar in both groups, but blood transfusion was significantly higher in the surgery group^[12]. These results all suggest that MWA is safe and reliable for treating CRLM.

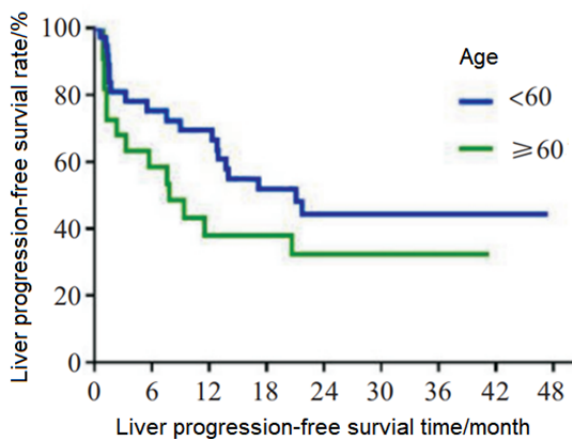


Figure 1. Comparison of liver progression-free survival curves of CRLM patients aged <60 years and ≥60 years after MWA.

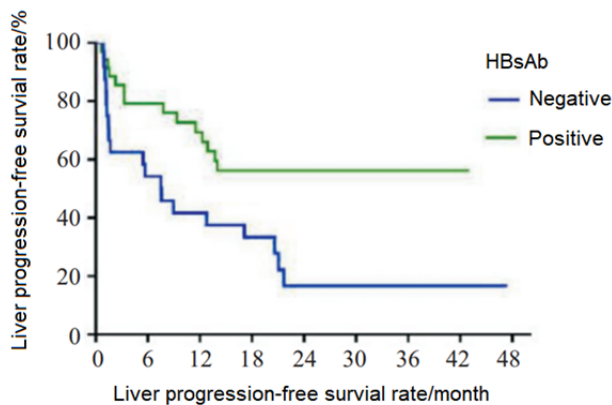


Figure 2. Comparison of liver progression-free survival curves of HBsAb negative and positive CRLM patients after MWA.

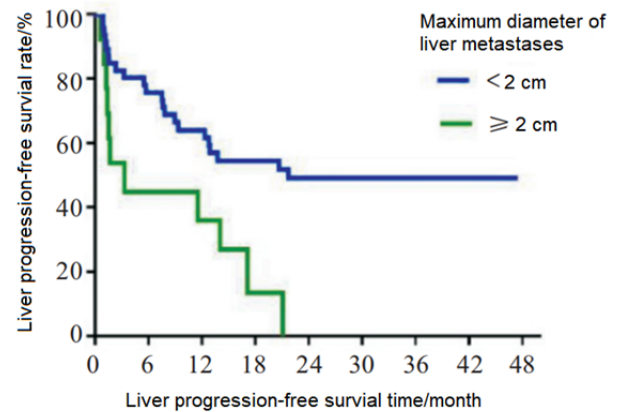


Figure 3. comparison of liver progression-free survival curves of CRLM patients with maximum diameter of liver metastases <2 cm and ≥2 cm after MWA.

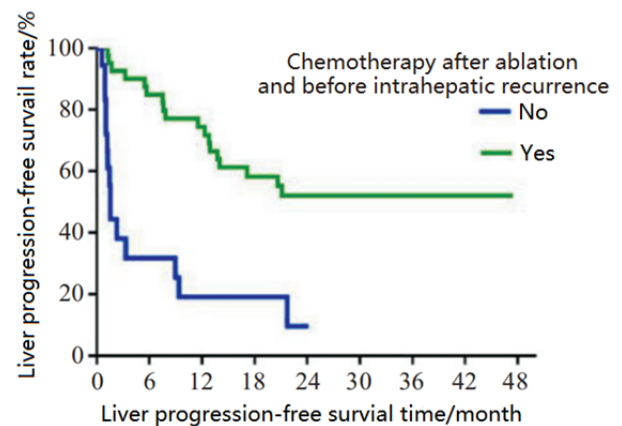


Figure 4. Comparison of liver progression-free survival curves of CRLM patients with and without chemotherapy after MWA and before intrahepatic recurrence.

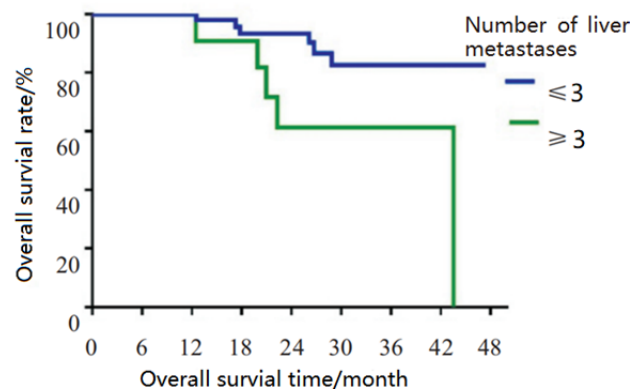


Figure 5. Comparison of overall survival curves between CRLM patients with ≤2 and ≥3 liver metastases after MWA.

However, regardless of whether CRLM is treated with surgical resection, RFA or MWA, there is a risk of recurrent liver metastases after surgery. The results of this study showed that the LPFS rates at 6, 12 and 24 months after MWA treatment for CRLM were 67.2%,

56.1% and 38.0% respectively. Therefore, there is a need to find clinical biomarkers to predict different prognosis of patients for individual postoperative interventions. However, there are few studies on the factors influencing intrahepatic recurrence after MWA treatment. The results of this study showed that age ≥ 60 years and maximum diameter of liver metastases ≥ 2 cm were independent risk factors for recurrence of intrahepatic metastases after MWA treatment, it shows that the diameter of the initial metastases of CRLM not only affects the local effect of the ablation site, but also affects the occurrence of metastases elsewhere in the liver after ablation.

Hepatitis B virus (HBV) infection has been shown to affect the survival of some malignancies more or less. The impact of HBV on CRLM is still controversial. Huo *et al.*^[13] reported that the incidence of concurrent liver metastases was significantly higher in the HBsAg-positive group than in the HBsAg-negative group (15.57% vs. 8.60%, $P < 0.01$). Reduced liver metastases and increased survival in HBV-infected CRC patients compared to non-infected patients^[14,15]. Our results showed that the 24-month OS for HBsAg-positive and negative patients was 100.0% and 68.9% respectively, with a trend towards increased overall survival for HBsAg-positive patients, but not yet reaching a statistical difference ($P = 0.084$), probably due to the small sample amount of this study. Zhao *et al.*^[16] showed that after hepatectomy in patients with CRLM alone, 3-year OS and PFS were higher in the HBV-infected group than in the uninfected group. In a multifactorial Cox analysis, HBV infection was identified as an independent factor for better 3-year OS, but not an independent factor for 3-year PFS. In contrast, our analysis of intrahepatic recurrence after MWA treatment showed that LPFS was worse in HBsAg-positive patients, but the difference between the two groups was not statistically significant ($P > 0.05$). Our results only showed that the number of liver metastases was a factor in OS, the patients with ≥ 3 liver metastases having worse 24-month OS, but HBsAb-positive patients had significantly higher LPFS than negative patients, in contrast to the result of Zhao *et al.*'s study^[16]. Most of the previous studies on the relationship between HBV and liver metastases have focused on the analysis of antigens and rare on the patient's antibody production. The mechanism of the effect of HBV infection on liver metastasis of different malignancies is still unclear, and the altered liver microenvironment and activation of liver-associated immunity caused by HBV infection may lead to its anti-tumor effects in different malignancies. The antigenic and antibody profile of patients after HBV exposure is a dynamic process, the analysis of the effect of one point in time on a long-term tumor lesion alone seems to be incomplete, and perhaps this is the reason why different studies have produced contrasting results, which need to be verified by further

research. In addition, further studies are needed to be done in different local liver treatments, especially in thermal ablation affecting the mode of action of HBV infection.

This study analyzed the patients' chemotherapy at different periods and showed that chemotherapy exposure before the appearance of liver metastases and neoadjuvant chemotherapy after the appearance of liver metastases had no effect on LPFS after MWA treatment for CRLM, in contrast, the presence or absence of timely adjuvant chemotherapy after MWA treatment significantly affected intrahepatic recurrence, with a 24-month LPFS of 49.6% and 9.5%, respectively, after ablation and before intrahepatic recurrence with or without chemotherapy, suggests that adjuvant chemotherapy should be administered as early as possible after MWA treatment, also should reduce the interval and it is essential to reduce the rate of intrahepatic recurrence.

In conclusion, after MWA treatment for CRLM patients, those age ≥ 60 years, HBsAb negative and with maximum liver metastases ≥ 2 cm in diameter were more likely to have recurrent intrahepatic metastases, while aggressive chemotherapy after ablation was effective in reducing recurrent intrahepatic metastases, and the number of liver metastases ≥ 3 suggested a poor overall prognosis.

Conflict of interest

No conflict of interest was reported by all authors.

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