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RESEARCH ARTICLE

# Comparative Study of CT and MR Guided Microwave Ablation in the Treatment of Para-vascular VX2 Liver Tumor Model in Rabbits

Ren Ziwang[1], Feng Guiling[1], Feng Xu[2], Liu Zhu[1], Li Bing[1] and Du Yong[1,*]

[1]*Radiology Department of Affiliated Hospital of North Sichuan Medical College, Nanchong 637000, China*
[2]*Radiology Department of Yibin Second People's Hospital, Yibin 644000, China*

**Abstract:**

***Objective:***

To analyze the efficacy of microwave ablation (MWA) guided by computed tomography (CT) and 1.5T magnetic resonance (MR) in the treatment of VX2 para-vascular liver tumor model in rabbits.

***Materials and Methods:***

Sixty para-vascular VX2 liver tumor models in rabbits were randomly divided into CT-guided microwave ablation group (CT group, n=35) and MR-guided microwave ablation group (MR group, n=35). The complete ablation rate, mean operation time, postoperative complication rate and mean survival time were compared between the two groups.

***Results:***

In the CT group, the rate of complete ablation was 68.6% (24/35), and the mean operation time was 42.1 ± 9.7 minutes. Three cases had ascites and one case had abdominal wall injury. In the MR group, the rate of complete ablation was 94.2% (33/35), and the mean operation time was 53.4 ± 10.9 minutes. One case was complicated with ascites. No serious complications such as pneumothorax, liver abscess, pleural effusion and diaphragm perforation were found in both groups. Between the two groups, the difference in complete ablation rate was statistically significant ($P=0.006 < 0.05$). A statistically significant difference can also be found in mean operation time ($P < 0.01$). The follow-up time was 21 days after the operation. As for the postoperative complication rate (11.4% in the CT group and 2.9% in the MR group, P=0.353) and mean survival time (16.9 ± 1.8 days in CT group, 18.3 ± 2.3 days in the MR group, P=0.925), the differences were not statistically significant.

***Conclusion:***

Compared with CT guidance, although the microwave ablation time under MR guidance was longer, the complete ablation rate under MR guidance was high, which proved that MR guidance was a more effective way of microwave ablation guidance and was worth promoting in the clinic. In this experiment, the postoperative complication rate was lower in the MR group, although the difference was not statistically significant, which may be related to the small sample size, and the subsequent study on the postoperative complication rate can increase the sample content.

**Keywords:** Computed tomography, Magnetic resonance imaging, Microwave ablation, Rabbit VX2 tumor, Therapeutic effect, Liver Tumor model.

| Article History | Received: February 28, 2023 | Revised: June 17, 2023 | Accepted: July 28, 2023 |
|---|---|---|---|

## 1. INTRODUCTION

The prevalence of hepatocellular carcinoma (HCC) is increasing year by year. In China, it has risen from the third largest cancer-related mortality rate in 2018 to the second in 2020 and is the cancer with the sixth highest incidence rateworldwide, making it a major issue for global public health [1 - 3]. At present, the main treatment for liver tumor has changed from a single surgical resection model to a multidisciplinary treatment model (MDT) based on surgical resection. For its minimally invasive, safe and reproducible features, microwave ablation has become the principal therapy for patients with small HCC or those who have lost the chance

* Address correspondence to this author at the Radiology Department of Affiliated Hospital of North Sichuan Medical College, Nanchong 637000, China; E-mail: duyong@nsmc.edu.cn

of surgery in recent years [45].

However, it is found in clinical work that the recurrence rate after microwave ablation of tumors located in the liver next to large vessels is high and residual unablated tumors are prone to occur [4 - 6]. In addition to the reason that the heat is carried away by large vessels adjacent to the tumor, resulting in incomplete ablation, the guidance and monitoring methods are also one of the reasons, such as ultrasound and CT are difficult to distinguish the inflammatory edema zone from the tumor residue after ablation [7, 8].

With high resolution for soft-tissue, clear imaging and real-time monitoring of the ablation range [9], MR guide can improve the ablation efficacy of hepatocellular carcinoma [8], and perhaps better for the para-vascular hepatocellular carcinoma [10, 11], but there is no relevant study to confirm this view. Therefore, in this study, the efficacy of CT- and MR-guided microwave ablation in the therapy of para-vascular hepatocellular carcinoma was comparatively studied by setting up a para-vascular VX2 liver tumor model in rabbits with complete ablation rate, mean operation time, postoperative complication rate and mean survival time.

## 2. MATERIALS AND METHODS

### 2.1. Experimental Animals and VX2 Holotype Rabbits

The experimental protocol was approved by the Animal Ethics Committee of North Sichuan Medical College [approval number: NSMC Animal Ethics Review (2022) No. 44]. Sixty healthy New-Zealand rabbits, weighing 2.5-3.5 kg, 4-6 months old, males only, with health quarantine certificates, were purchased from the Experimental Animal Center of North Sichuan Medical College. Two VX2 holotype rabbits, male, weighing 2.5 kg and 2.7 kg, were purchased from Hangzhou Huashu Biotechnology Co.

### 2.2. Experimental Equipment and Instruments

#### *2.2.1. Experimental Equipment*

MR scanning equipment: 1.5 T magnetic resonance scanner (Area, Siemens, Germany), special coils for animal experiments; MR microwave therapy instrument: MTC-3 microwave therapy instrument, National Instrument Note Approval 20153251978; MR scanning parameters are specified in Table **1**.

CT scanning equipment: 16-row spiral CT scanner (MX16, Philips, Philips Medical Ltd., Netherlands); CT microwave therapy instrument: a cooled-shaft microwave system (KY-2000, Kangyou Medical, Nanjing, China), with a 15-gauge antenna(KY-2450B-1). The length of the antenna is 18 cm, and the active tip of the antenna is 1.1 cm; CT scanning parameters are window width (WW) 250HU, window position (WL) 35HU, layer thickness 2.0mm.

**Table 1. MR scanning parameters.**

| Scan Sequence | Scanning Direction | Echo Time (ms) | Repeat Time (ms) | Slice Thickness (mm) | Matrix | Flip Angle | Field of View (mm×mm) |
|---|---|---|---|---|---|---|---|
| T1WI Vibe Dixon | Axial | 5.0 | 10.0 | 1.2 | 320×195 | 10.0 | 260×211 |
| Haste T2WI fs | Axial | 110.0 | 3000 | 3.0 | 256×256 | 140.0 | 180×180 |
| Diffusion-weighted imaging(DWI) | Axial | 77.0 | 6000 | 3.0 | 112×112 | 90.0 | 230×230 |
| T1WI Vibe Dixon C+ | Axial | 5.0 | 10.0 | 1.2 | 320×195 | 10.0 | 260×211 |

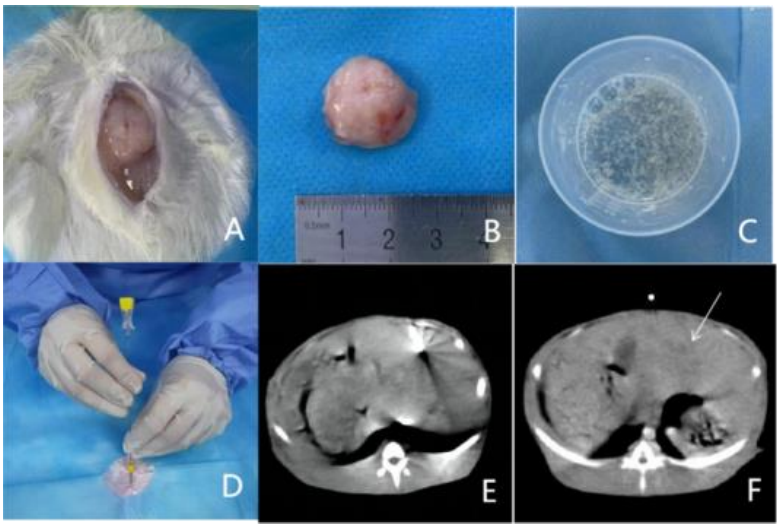


**Fig. (1).** Establishment of rabbit VX2 liver cancer model under CT guidance. A,B: VX2 tumor strain. C: VX2 tumor suspension. D: Puncture in the subxiphoid area of the rabbit. E: CT-guided puncture (puncture needle is of high density). F: Two weeks later, tumors formed in the left lobe of the liver (white arrow).

#### *2.2.2. Experimental Instruments*

Gelatin sponge, 5ml plastic syringe with 23G needle,10ml plastic syringe with 20G needle, hair removal cream, scalpel, tweezers, *etc*.

### 2.3. Preparation and Passaging of Rabbit VX2 Tumors

The skin of the hind leg of VX2 tumor-bearing rabbits was peeled off to expose the tumor tissues in the leg muscles, and the tumor tissue morphology, size, blood supply and the presence of necrosis were observed. After the tumor was peeled off, the tissue was cut into small pieces with a surgical blade, and selected homogeneous, fish-like tissues were placed in physiological saline. The selected tissues were further chopped, ground and filtered to prepare a suspension, which was injected into the hind leg muscle of healthy New Zealand rabbits, and new tumor tissues were formed after 2-3 weeks to complete the transmission of VX2 tumors (Fig. **1**).

### 2.4. CT-guided Preparation of Para-vascular VX2 Liver Tumor Model in Rabbits

Healthy New Zealand rabbits were randomly selected and weighed, fasted for 12 hours before surgery, not restricted to drinking water. General anesthesia by intravenous injection of 3% pentobarbital sodium (1 mL/kg) through the ear margin. The rabbits were placed supine and fixed on all four limbs on a homemade experimental operating table after general anesthesia. Preoperatively, routine CT scans were performed, and no significant variation in liver anatomy was observed in all experimental rabbits. The left lobe of the rabbit liver was selected as the intended implantation site next to the large vessels (vessel diameter ≥3 mm). Skin preparation in the subxiphoid area of the rabbit, and routinely disinfected and toweled before surgery. After the puncture path was formulated on the CT scan image, the needle was inserted along the puncture path with a 20G coaxial puncture needle, and after the tip of the needle reached the intended site, a 5 ml empty syringe was used to aspirate without returning blood, and the suspension (prepared in the same way as 1.2) was dropped into the needle sheath and pushed into the liver with repeated insertion of the needle core, followed by placing a small piece of gelatin sponge into the needle sheath and pushing it into the liver with the needle core to seal the needle tract and then withdrawing the needle. After the removal of the needle, external pressure was applied to the puncture site for 2-3 min, and after confirming that there was no bleeding, the towel was removed and the puncture site was routinely bandaged. A postoperative CT scan showed no obvious liver injury and bleeding. Intramuscular injection of Penicillin (400,000 U/d) for 3 consecutive days after operation to prevent infection. The CT and MR scans were repeated with intravenous contrast administration 2 weeks after surgery to observe the growth of intrahepatic tumors and prepare for the MWA process. Successful preparation of rabbit hepatic para-vascular hepatocellular carcinoma model was defined as: formation of a single tumor lesion next to the large vessels in the left lobe of the liver, no obvious lesions in the rest of the liver, no obvious metastases in the peritoneum and distant organs, no obvious scites, *etc*. [12] (Fig. **1F**).

Seventy successful models were numbered sequentially, according to the pre-set sample size of the experimental group, selecting the corresponding samples in random numbers and allocating themto two groups: CT group (N = 35) and MR group (N = 35). There were no significant differences between the two groups in the diameter of tumors (CT group 1.55 ± 0.31 *vs* MR group 1.58 ± 0.30; P=0.666).

### 2.5. Process of MWA

#### *2.5.1. CT-guided MWA Procedure for Rabbit Para-vascular VX2 Liver Tumor*

After general anesthesia (same as 1.4), the rabbits in the CT group were placed supine and fixed on the homemade experimental table. CT was routinely scanned to determine the tumor location and route of ablation. The skin of the operating area was prepared and disinfected, then the ablation needle was inserted gradually along the predetermined insertion point and path. Because the tumors were all smaller than 3.0 cm in diameter, only a single antenna was used to form ablation dimensions to cover it. When the needle tip is punctured to the tumor site, CT scan and coronal sagittal reconstruction are performed to confirm the relationship between the needle tip and the tumor site, ensure that the tip was located at the tumor site, and judge the relationship between the ablation needle and the surrounding tissue, so as to minimize the damage to the surrounding tissue and reduce the occurrence of complications while ensuring complete ablation (Fig. **2**). The output power of MWA was set between 40 and 50 W and the time was between 3—5min, according to tumor location and diameter. The ablation area shall cover at least 5mm beyond the edge of the original tumor. When withdrawing the needle, the needle tract is heated to avoid tumor needle tract metastasis and needle tract bleeding. After pulling out the ablation needle, a CT scan was performed to confirm whether the ablation range covered the tumor lesion and observe the presence of bleeding, pneumothorax, hemopneumothorax, abdominal bleeding and other complications (Fig. **2**).

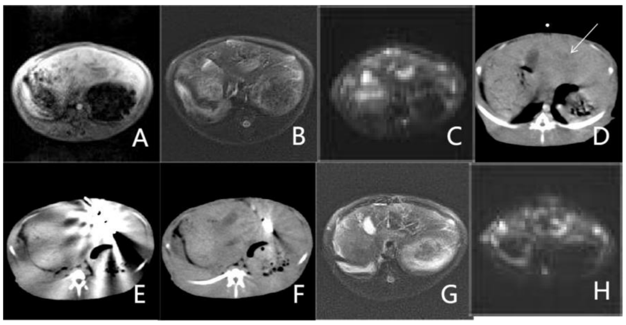


**Fig. (2).** CT guided microwave ablation. **A**, **B**, **C**: Preoperative T1WI, T2WI, DWI images show that the lesion is located at the left lobe of liver. **D**: Preoperative CT image, white arrow shows the tumor lesion, which is of slightly low density. **E**, **F**: Intraoperative CT images show that the high density ablation needle tip passes through the focus. **G**, **H**: Postoperative T2WI and DWI images show that T2WI and DWI signal of lesion changed immediately from high signal in Figure B,C to low signal.

#### *2.5.2. MR-guided MWA Procedure for Rabbit Para-vascular VX2 liver Tumor*

After general anesthesia (same anesthesia as 1.4), the experimental rabbits in the MR group were routinely prepared with skin in the operative area, a homemade localizer was placed in the operative area, and the animal loop coil was placed in the subxiphoid puncture area, and MR scans (T1WI, T2WI and DWI sequences, see Table **1** for specific parameters) were performed in parallel. After the preoperative scan, the needle path was formulated according to each sequence, and the surgical area was routinely disinfected and toweled. The MR-compatible microwave puncture needle was used to gradually enter the needle along the predetermined path, and the T1WI scan was performed during the puncture process for timely adjustment until the needle reached the tumor site. The output power of MWA was set between 40 and 50 W and the time was between 3—5min, according to tumor location and diameter. The ablation area shall cover at least 5mm beyond the edge of the original tumor. The needle tract was heated to avoid tumor needle tract metastasis and needle tract bleeding when the needle was withdrawn. After pulling out the ablation needle, MR scan was performed to confirm whether the ablation range covered the tumor lesion and observe the presence of bleeding, pneumothorax, hemopneumothorax, abdominal bleeding and other complications (Fig. **3**).

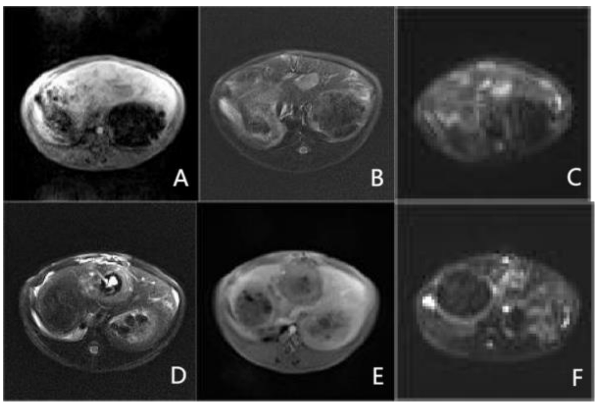


**Fig. (3).** MR guided microwave ablation. **A**, **B**, **C**: Preoperative T1WI, T2WI and DWI images show the tumor lesion in the left lobe of the liver. The lesion is of low signal intensity on T1WI sequence, high signal intensity on T2WI sequence, and high signal intensity on DWI sequence. **D**: Intraoperative T2WI sequence shows that the ablation needle tip passes through the lesion and the ablation area is basically inclusive of the focus. **E**: Postoperative T1WI shows a low signal in the ablation area. **F**: Postoperative DWI sequence shows reduced signal intensity in the lesion.

### 2.6. MWA Postoperative Evaluation Criteria

All experimental rabbits were scanned by MR with intravenous contrast administration to observe whether the tumor lesions were completely ablated and the progress of the residual unablated tumors, distant metastasis and complications. The follow-up time was 14 days and the review time point was set at 3, 7 and 14 days after ablation. Whether the ablation was complete or not was referred to the response evaluation criteria in solid tumor (RECIST) [13]: Complete ablation is defined as no enhancement in the arterial phase of the ablated lesions and no diffusion limitation showed in DWI. If, during the follow-up period, enhanced nodules in the arterial phase were observed at the original site or significantly enhanced lesions with diffusion limitation in DWI were observed at the edge of the ablated area, it is defined as incomplete ablation. The ablative margin is defined as the shortest distance in the space between the preoperative tumor contour and post-ablation necrosis. The measurement of ablative margin is achieved through registration in the images before and after MWA treatment and is measured by experienced radiologists, and the minimum ablative margin must exceed 5mm. New lesions that appeared elsewhere in the liver during follow-up time were called recurrence. The operation time was defined as the time from general anesthesia till the extraction of ablation needle. Postoperative complications include thoracoabdominal effusion, liver abscess, diaphragm injury, abdominal wall injury, and hemorrhage.

## 3. STATISTICAL PROCESSING

Evaluation was performed by SPSS 22.0 statistical. Quantitative data were expressed as mean ± standard deviation ($\bar{x}$ ± s). Independent sample t-test and rank sum test were used to compare the difference of continuous quantitative data. Counting data were expressed as frequency(%) and compared with the Chi-square test. Survival time was estimated with the Kaplan-Meier method and compared with the log-rank test. $P < 0.05$ means a significant difference.

## 4. RESULTS

### 4.1. Complete Ablation Rate and Mean Operation Time Evaluation

Microwave ablation is performed by a doctor with intermediate or higher professional titles and more than five years of experience in interventional surgery, who is unaware of the process of establishing the model. 24/35 (68.6%) were completely ablated in the CT group and 33/35 (94.2%) were completely ablated in the MR group. The difference in complete ablated rate was statistically significant ($P = 0.006 < 0.05$). The operation time of the CT group was 42.1 ± 9.7 min, while that of MR group was 53.4 ± 10.9 min (Table **2**). The difference in mean operation time was statistically significant ($P < 0.01$).

### 4.2. Evaluation of Postoperative Complication Rate

There were 3 cases of postoperative complications of ascites and 1 case of abdominal wall injury in the CT group (4/35,11.4%) and 1 case of postoperative complications of ascites in the MR group (1/35, 2.9%), $P=0.353>0.05$, there was no significant difference. No complications such as pneumothorax, liquid pneumothorax, liver abscess, diaphragm injury, or abdominal wall injury were seen in either groups (Table **2**).

**Table 2. Comparison of CT and MR guided microwave ablation of VX2 para-vascular liver tumor in rabbits.**

| Guiding Equipment | Completely Ablated Rabbits/Experimental Rabbits | Complete Ablation Rate | Operation Time (min) | Postoperative Complication Rate | Mean Survival Time (d) | Maximum Diameter of Tumors (cm) | Minimum Diameter of Tumors (cm) |
|---|---|---|---|---|---|---|---|
| CT | 24/35 | 68.6% | 42.1±9.7 | 11.4% | 16.9±1.8 | 2.2 | 1.2 |
| MR | 33/35 | 94.2% | 53.4±10.9 | 2.9% | 18.3±2.3 | 2.3 | 1.2 |
| P | 0.006 | | < 0.01 | 0.353 | 0.925 | 0.666 | |

### 4.3. Mean Survival Time Evaluation

At follow-up until 21 days after surgery, the survival time was 16.9 ± 1.8 days (95% CI, 9.51–18.49) for the CT group and 18.3 ± 2.3 days (95% CI, 10.53–17.47) for the MR group ($P = 0.925 > 0.05$), there was no significant difference.

## 5. DISCUSSION

In this study, the rabbit paravascular VX2 liver tumor was prepared by percutaneous puncture implantation. After pulling out the puncture needle, the path could not be observed on images, and the tiny traces on the body surface caused by the puncture could not be distinguished after 2 weeks. In addition, the doctor who performed MWA was unaware of the process of model establishing and took the conventional ablation technology roadmap. Therefore, the model generated in this way only produces small deviations when simulating the clinical ablation situation. In this study, the microwave ablation for rabbit para-vascular liver tumor was performed by both CT and MR guidance modalities in a group experiment, and the complete ablation rate, mean operation time, postoperative complication rate and mean survival time were compared and analyzed. The complete ablation rate was higher in the MR group than in the CT group, although the MR group took a longer time. There were no significant differences in the postoperative complication rate and mean survival time. This study explored a safer and more effective ablation guidance method and provided an experimental basis for subsequent studies related to ablation treatment of liver tumor, which has certain scientific and clinical values.

Para-vascular hepatocellular carcinoma is a major challenge in the clinical therapy of hepatocellular carcinoma [14, 15]. According to Han *et al*. [16], the tumor is located in a difficult site (para-vascular, on the surface of the liver or next to the bile duct) is an independent risk factor for recurrence one year after ablation, and the tumor location is closely related to both the complete rate of microwave ablation and postoperative recurrence for the following two main reasons [17]: first, due to the proximity of large blood vessels during ablation, the angle and position of the ablation needle entry is restricted to ensure that the blood vessels and tissues surrounding the tumor are not damaged, and the coverage of the ablation range is limited; second, the blood flow takes away some of the heat leading to uneven heat distribution, resulting in uneven and incomplete ablation and residual unablated tumors.

In addition, the choice of imaging guidance equipment is also one of the important factors for recurrence after liver tumor ablation [17 - 19]. However, ultrasound and CT make it difficult to distinguish the inflammatory edema zone from tumor residue after ablation [5], and the guidance devices are required to clearly display the tumor lesion, accurately formulate the needle path, and quickly evaluate the ablation scope and effect during and after microwave ablation. Compared with ultrasound or CT guidance, MR guidance has the advantages of no ionizing radiation, high soft tissue resolution, and clear display of the stress edema zone around the ablation, and can be multi-directional and angular, multi-sequence imaging, and has the ability to combine morphological functions [20] (including DWI, perfusion-weighted imaging (PWI) and spectral analysis, *etc* .)to provide a more accurate direction for guided ablation therapy in clinical practice.

In this study, MR guidance showed a higher rate of complete ablation, which may be related to the fact that MR guidance has a high soft tissue resolution, can clearly display and distinguish the postoperative inflammatory edema zone and tumor residue, has better visualization of the peritumor vessels and bile ducts than CT, and has the characteristics of multi-directional, multi-angle and multi-sequence imaging, which shows the lesions clearly. We only use intravenous contrast agents during CT and MR scans before and after MWA, but not during MWA surgery. The absence of intravenous contrast may lead to a lack of clear visualization of the tumor edge on CT, thereby reducing the complete ablation rate of the CT group. Although the differences in mean survival time and postoperative complication rate were not significant, the rate of postoperative complication in the CT group was higher, which was similar to the results of Li *et al*. [21], showing that the safety of the two groups was consistent.

The complete ablation rate under MR guidance was 93%, and the complete ablation rate under CT guidance was 70%, both of which were lower than 92.5%~100% in the previously reported literature [22 - 25]. We analyzed the following reasons: first, microwave ablation of hepatocellular carcinoma at special sites was the main cause of incomplete ablation rate, and second, the rabbit's liver was highly mobile with heavy respiratory artifacts, which required higher puncture technique and microwave ablation technique for the operator [26].

Although MR guidance has the above-mentioned advantages, MR guidance has disadvantages such as expensive, long scanning time, and the corresponding equipment requiring MR compatibility. Although CT guidance has the disadvantages of large ionizing radiation, metal artifacts of ablation needles interfering with image quality, and difficulty in showing the effect of ablation on the surrounding tissues of the lesion, it has the advantages of rapid imaging, mature technology, and simple operation, so it is commonly used in clinical practice. With the increasing popularity of MR equipment, the improvement and promotion of MR ablation technology, and the development and popularization of magnetically compatible devices, MR is expected to become the main force of MR-guided ablation of liver tumor treatment in the future [27].

There are some limitations of this experiment: 1. further research on large sample size is needed, because the experimental sample size is relatively small; 2. the size of the lesion varies during microwave ablation, and the microwave power and time set during ablation vary, so whether the tumor is completely ablated is mainly judged by the operator, and there may be some bias; 3. no comparative study with ultrasound guidance was conducted.

## CONCLUSION

In conclusion, MR-guided microwave ablation of para-vascular hepatocellular carcinoma of the rabbit liver is more effective than CT guidance. Although MR guidance has disadvantages such as long scanning time and expensive, with the progress of technology, MR technology is becoming more and more mature and can be better applied to the diagnosis and treatment of para-vascular hepatocellular carcinoma. Overall, researchers and clinical workers can choose the appropriate guidance modality according to the actual situation.

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| **MWA** | = | Microwave Ablation |
| **CT** | = | Computed Tomography |
| **MR** | = | Magnetic Resonance |
| **HCC** | = | Hepatocellular Carcinoma |
| **MDT** | = | Multidisciplinary Treatment Model |

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental protocol was approved by the Animal Ethics Committee of North Sichuan Medical College [approval number: NSMC Animal Ethics Review (2022) No. 44].

## HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All animal research procedures were followed in accordance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals (published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

## FUNDING

None.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

Declared none.

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