Establishment of a Rabbit Liver Metastasis Model by Percutaneous Puncture of the Spleen and Implantation of the VX2 Tumor Strain under CT Guidance

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Research Article

Keywords: CT guidance, Spleen, Rabbit VX2 tumor model, Liver metastasis

Posted Date: May 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1624580/v1

Abstract

Objective

This study aimed to compare the feasibility, success rate, and safety of establishing a rabbit VX2 liver metastasis model by percutaneous splenic implantation under CT guidance and open splenic implantation of the VX2 tumor strain.

Methods

Fifty New Zealand white rabbits were randomly divided into group A (the percutaneous puncture group) (n = 25) and group B (the laparotomy group) (n = 25). In group A, 25 New Zealand white rabbits were implanted with tumor strains by percutaneous splenic puncture under CT guidance. In group B, 25 New Zealand white rabbits were implanted with tumor strains in the spleen by laparotomy. After 2–3 weeks of implantation, both group A and group B underwent MRI to confirm tumor growth in the spleen and metastasis to the liver. Two experimental rabbits randomly selected from groups A and B were killed for pathological examination. The success rate, complication rate, and operation time in groups A and B were compared and analyzed.

Results

A total of 22 rabbits in group A were successfully modeled, and the success rate was 88% (22/25). The average time of tumor operation was 10.0 ± 3.4 minutes. A total of 21 rabbits in group B were successfully modeled, and the success rate was 85% (21/25). The average time of tumor operation in group B was 23.0 ± 6.2 minutes. There was no significant difference in the success rate of modeling between the two groups ($P > 0.05$). The MRI manifestations of liver metastases were multiple nodular and punctate abnormal signal shadows in the liver. Hematoxylin-eosin (HE) staining showed a large number of tumor cells in the tumor area.

Conclusion

CT-guided percutaneous splenic implantation of the VX2 tumor strain to establish a rabbit liver metastasis model is a minimally invasive and feasible modeling method. The success rate of this technique is not lower than that of open splenic implantation, with low mortality, low incidence of complications, and short operation time.

Introduction

The liver is one of the organs most prone to malignant tumor metastasis. To better understand the mechanism and to improve the treatment effect of liver metastases, many studies have been performed

using experimental animal models[1]. At present, there are two methods to establish a rabbit liver metastasis model using the VX2 tumor strain[2, 3]: (1) implanting the VX2 tumor strain directly in the rabbit liver; (2) implanting the tumor strain in the spleen to establish subsequent liver metastasis[2, 3]. Research shows that, compared with the method that implants the VX2 tumor strain directly in the rabbit liver, the biological characteristics of the rabbit liver metastasis model formed by splenic implantation are closer to the biological characteristics of human liver metastasis[4]; the aforementioned model can better mimic the whole process of tumor growth and metastasis; thus, it is a more ideal animal model[4]. However, to date, as reported in studies, almost all the modeling methods of splenic implantation have used a laparotomy[4]. This method has disadvantages, such as large trauma and higher mortality. The method of implanting tumor strain into target organ by an image-guided percutaneous puncture to establish the tumor model has been reported. This method is associated with less injury and a low complication rate[5]. However, as the rabbit spleen is relatively small and difficult to puncture, there is no report on percutaneous splenic implantation under CT guidance.

This study intends to explore the feasibility of CT-guided percutaneous splenic implantation of the VX2 tumor strain to establish a rabbit liver metastasis model, and to compare its feasibility, success rate, and safety with open splenic implantation of the VX2 tumor strain.

Materials And Methods

Animals

This research was approved by the Animal Use and Care Committee of Affiliated Hospital of North Sichuan Medical College. A total of 50 healthy New Zealand white rabbits, aged from four to six months and weighing from 2.5 to 3.5 kg, were included in this study, and they were provided by the Experimental Animal Center, North Sichuan Medical College. The rabbits were randomly divided into group A (the percutaneous puncture group) (n = 25) and group B (the laparotomy group) (n = 25).

Preparation of VX2 tumor suspension

A total of 3 mL/Kg of 3% pentobarbitone sodium was injected into a VX2 tumor-bearing rabbit through the auricular vein. The tumor was removed in a sterile environment; the necrotic tissue, fascia, blood vessels, and other tissues were also removed; only the white and transparent fish like tumor tissue was selected; the tissue was ground and placed in normal saline to make a VX2 tumor suspension (Fig. 1).

CT guided percutaneous splenic implantation

After general anesthesia, the rabbits were fixed on the CT scanning table in a prone position. A self-made metal positioner was used for positioning. Then, the puncture path, direction, and depth were determined. Under sterile conditions, a 22G coaxial puncture needle was used to puncture the rabbit spleen step by step under CT guidance. After confirmation of the position of the needle tip by CT scanning, the VX2 tumor suspension was injected into the spleen. After dropping the suspension, the needle path was

blocked with a gelatin sponge. The needle was pulled out and the puncture point was pressed for 1–2 min to confirm that there was no bleeding (Fig. 2).

Open splenic implantation

After general anesthesia, the rabbits were fixed on the experimental table in a supine position and they were depilated through the lower edge of the left costal arch. The depilated area was then disinfected locally, and a cut was made with a scalpel to expose the spleen. The VX2 tumor suspension was then injected into the exposed spleen. The abdominal wall was closed after no fluid and blood outflow from the spleen was confirmed.

Magnetic resonance scanning and sequence parameters

After 2–3 weeks of implantation, both groups A and B underwent Magnetic Resonance Imaging (MRI) (SIEMENS AG, Aero) to confirm tumor growth in the spleen and metastasis to the liver. A 15-channel knee phase-controlled front coil was used. After general anesthesia, rabbit liver metastasis models were placed in the channel coil for a conventional MRI scan, and the scan sequence included 3D-Vibe-T1WI, FS-TSE-T2WI, diffusion-weighted imaging (DWI), and 3D-Vibe-T1WI enhanced-MRI scan (Table 1).

Table 1
MR scanning and sequence parameters

MR sequence	Patient position	TE (ms)	TR (ms)	Slice thickness (mm)	Matrix	FA	FoV (mm)
fs-tse-T2WI	axial	110.0	3000.0	3.0	256 × 256	140.0	180 × 180
3D-Vibe-T1WI	axial	5.0	10.0	1.2	320 × 195	10.0	260 × 211
DWI	axial	77.0	6000.0	3.0	112 × 112	90.0	230 × 230
3D-Vibe-T1WI + C	axial	5.0	10.0	1.2	320 × 195	10.0	260 × 211
TE: echo time; TR: repetition time; FA: flip angle; FoV: field of view.							

Histological observation

Two experimental rabbits were randomly selected from groups A and B, and they were killed by injecting 20 ml of air along the ear vein. The spleen and liver were removed under sterile conditions. The spleen tumor and liver metastasis were identified, and the location, shape, size, color, and boundary of the tumor were observed. The tumor was peeled out, cut into small pieces, and soaked in 10% neutral formaldehyde fixation solution; then the fixed tumor was dehydrated and sliced, and the pathological changes were observed.

Statistical analysis

Statistical Package for Social Sciences software (version 23.0, IBM) was used for data statistical analysis. The mean ± standard deviation (x ± s) was used for all measurement data, and a chi-square test ($\chi 2$ test) was used for the count data. The success rate of modeling, operation time, and complication rate in the two groups were compared and analyzed. $P < 0.05$ was considered statistically significant.

Results

Modeling of rabbit liver metastasis

A total of 22 rabbits in group A were successfully modeled, and the success rate was 88% (22/25). One of the rabbits had no tumor growth, one had both needle track and peritoneal metastasis, and one had only peritoneal metastasis. The average time of tumor operation was 10.0 ± 3.4 minutes. A total of 21 rabbits in group B were successfully modeled, and the success rate was 85% (21/25). Two of the rabbits died within 3 weeks. After death, one rabbit was found to have multiple peritoneal metastases and a large amount of peritoneal effusion, but there was no obvious metastasis in the liver. The cause of death of another rabbit was not known, and the other two rabbits had peritoneal metastasis. The average time of tumor operation in group B was 23.0 ± 6.2 minutes. There were 4 cases of skin wound infection in group B. No obvious complications occurred in group A. There were significant differences in the operation time and complication rate between the two groups ($P < 0.05$). But there was no significant difference in the success rate of modeling between the two groups ($P > 0.05$) (Table 2).

Table 2
Comparison of rabbit VX2 liver metastasis modeling in groups A and B

Group	rabbit (No.)	success rate (%)	operation time (min)	complication rate (%)
percutaneous puncture (A)	25	88	10 ± 3.4	0
laparotomy (B)	25	85	23 ± 6.2	16
P value		0.691	0.000	0.038

MRI findings

MRI manifestations of liver metastases were multiple nodular and punctate abnormal signal shadows in the liver, low signal on T1WI, a slightly high or equal signal on T2WI, and a high signal on DWI. Circular enhancement was seen on enhanced scanning, and no enhancement area was seen in the center. The specimens showed that liver metastases were multiple round-like nodules, the edges were irregular, and the boundary with the normal liver tissue was still clear.

Pathological findings

The tumor was gray, transparent, and fish-like, with dotted milky white necrotic areas in the middle. The spleen tumor was massive or nodular, grayish-white and transparent fish-like, and the volume of the spleen was increased. Hematoxylin-eosin (HE) staining showed a large number of tumor cells in the tumor area, the tumor cells had large, dark-stained nuclei and an irregular morphology, and a large number of necrotic cells were located in the center of the tumor.

Discussion

The establishment of an animal liver metastasis model has great significance for clinical and scientific research[6]. In this study, a rabbit liver metastasis model was established by percutaneous splenic implantation of the VX2 tumor strain under CT guidance, and it was compared with the establishment of open splenic implantation. The results showed that CT guided percutaneous splenic implantation of the VX2 tumor strain to establish a rabbit liver metastasis model is a minimally invasive and feasible method, the success rate of this method is not lower than the method using open splenic implantation, and this method is associated with low mortality, low incidence of complications, and short operation time. At present, a similar method has not been reported.

The study showed that the biological characteristics of the rabbit liver metastasis model formed by splenic implantation are closer to the biological characteristics of human liver metastasis. Shi Bo et al.[3] reported that the model established by spleen inoculated with the VX2 tumor strain was found to be closer to human primary liver metastasis by using the CT scan and digital subtraction angiography (DSA). Our research also confirmed this finding. But laparotomy can cause large trauma, and then can lead to higher mortality[3]. One study has also reported the use of an image-guided percutaneous puncture target organ to establish a tumor model[7]. But the rabbit spleen is relatively small, and it is difficult to operate under ultrasound guidance. Therefore, we established a liver metastasis model by puncturing the spleen under CT guidance.

The results of this study showed that CT-guided percutaneous splenic implantation to establish a rabbit liver metastasis model is a feasible modeling method, and its success rate is similar to that of open splenic implantation. In group A, one rabbit had no tumor growth, one had both needle track and peritoneal metastasis, and one had only peritoneal metastasis. The reason for this occurrence may be that the needle tip did not enter the spleen during the drip of VX2 tumor suspension; thus, the VX2 tumor strain was implanted in the peritoneum and puncture path. There were 3 cases of peritoneal metastasis in group B, of which 2 rabbits died. The reason for this occurrence may be that the VX2 tumor suspension leaked along the puncture path after implanting into the spleen, resulting in extensive peritoneal implantation. On the comparison of complications, two cases of wound infection occurred in group B, as the laparotomy is more traumatic. No obvious complications occurred in group A. The result indicates that the method of CT-guided percutaneous puncture is associated with less trauma and a low incidence of complications.

Attention should be paid to the following aspects in the operation of CT-guided percutaneous splenic implantation. (1) Localization scanning: because the rabbit spleen is small, thin-layer continuous scanning with a thickness of 1–2 mm is required, and the largest slice of the spleen should be selected for puncture. In addition, the spleen is crescent-shaped; thus, we should formulate an oblique puncture angle from the inside to the outside, increase the length of the needle path in the spleen, and then reduce the probability of penetrating the spleen to damage the stomach and other structures. (2) Needle selection: due to the small volume and large mobility of the rabbit spleen, a 22G coaxial puncture needle should be selected, as the needle tip is inclined and sharp, which can reduce the probability that the use of a small force cannot puncture the spleen and use of a large force completely penetrates the spleen, and it can reduce the risk of spleen rupture and bleeding. (3) Puncture technology: when the needle tip approaches the spleen, it is necessary to quickly puncture the spleen, which can effectively reduce the repeated puncture and save the puncture time. (4) After dropping the suspension, the needle path should be blocked with a gelatin sponge to prevent the leakage of tumor suspension, which can reduce the incidence of peritoneal and needle path metastases.

Meanwhile, the operation time of CT-guided percutaneous splenic implantation is significantly lower than that of open splenic implantation. After mastering the key points of CT-guided percutaneous splenic implantation, the operator can complete the operation in a short time. However, the steps of laparotomy are relatively complex and cumbersome. If the laparotomy incision is small, it is difficult to identify the spleen; and if the incision is large, the injury to the rabbit is obvious and it also increases the risk of infection[8]. A skin suture and other operations are also required; thus, a relatively long time is needed.

MRI manifestations of the two groups of liver metastases were multiple nodular-like abnormal signal shadows in the liver, low signal on T1WI, a slightly high or equal signal on T2WI, high signal on DWI, and circular enhancement on enhanced scanning. They were typical imaging manifestations of liver metastasis, which were similar to those of human liver metastasis[9, 10]. In addition, HE stained sections from groups A and B showed that there were vigorous tumor cells in liver metastases. There was no difference in the imaging and pathological findings between the two groups. This finding is consistent with the research by Shi Bo et al[3].

Conclusion

In this study, CT-guided percutaneous splenic implantation of the VX2 tumor strain was used to establish a rabbit liver metastasis model, which is a minimally invasive and feasible modeling method. The success rate of the use of the aforementioned model was similar to that of open splenic implantation, and is associated with low mortality, low incidence of complications, and short operation time. Furthermore, the proposed method provides a new and minimally invasive modeling method for clinical use, which has important clinical and scientific research significance.

Declarations

Acknowledgments We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Author contributions Bing Li and Guiling Feng and Xu Feng wrote the main manuscript text, Qing Zhang and Chuan Zhang prepared figures 1-4. Xiaoxue Xu and Hanfeng Zhang carried out bioinformatic analyses and revised the manuscript. Yong Du conceived the study and revised the manuscript. All authors reviewed the manuscript.

Funding This work was supported by China Nanchong Municipal School Science and Technology Strategic Cooperation Special Project,The value of GD-Eob-DTPA enhanced magnetic resonance in evaluating the efficacy of CT-guided tumor ablation for VX2 liver metastases in rabbits. 2020-01-01 to 2021-12-31(NO.19SXHZ0257).

Data availability The raw data obtained and analyzed from this study are available from the corresponding author upon reasonable request.

Conflict of interest The authors declare that they have no competing interest.

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Figures

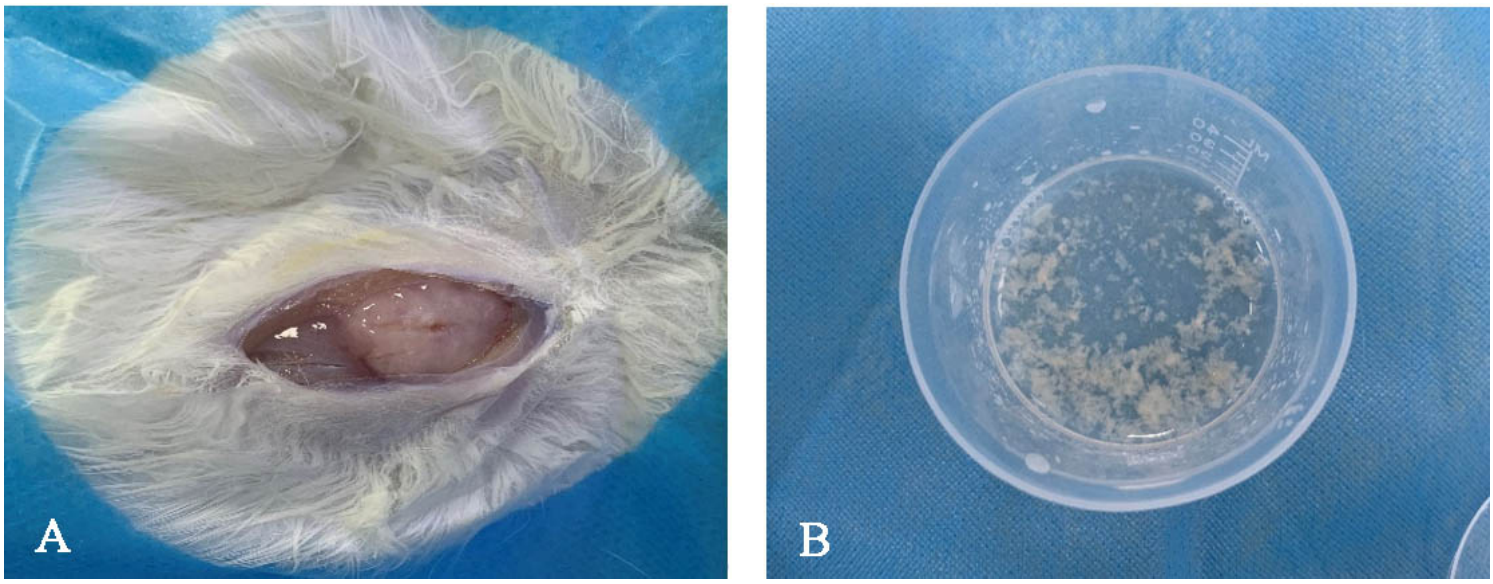

Figure 1

Preparation of VX2 tumor suspension. The tumor was removed (A); the VX2 tumor suspension was made (B).

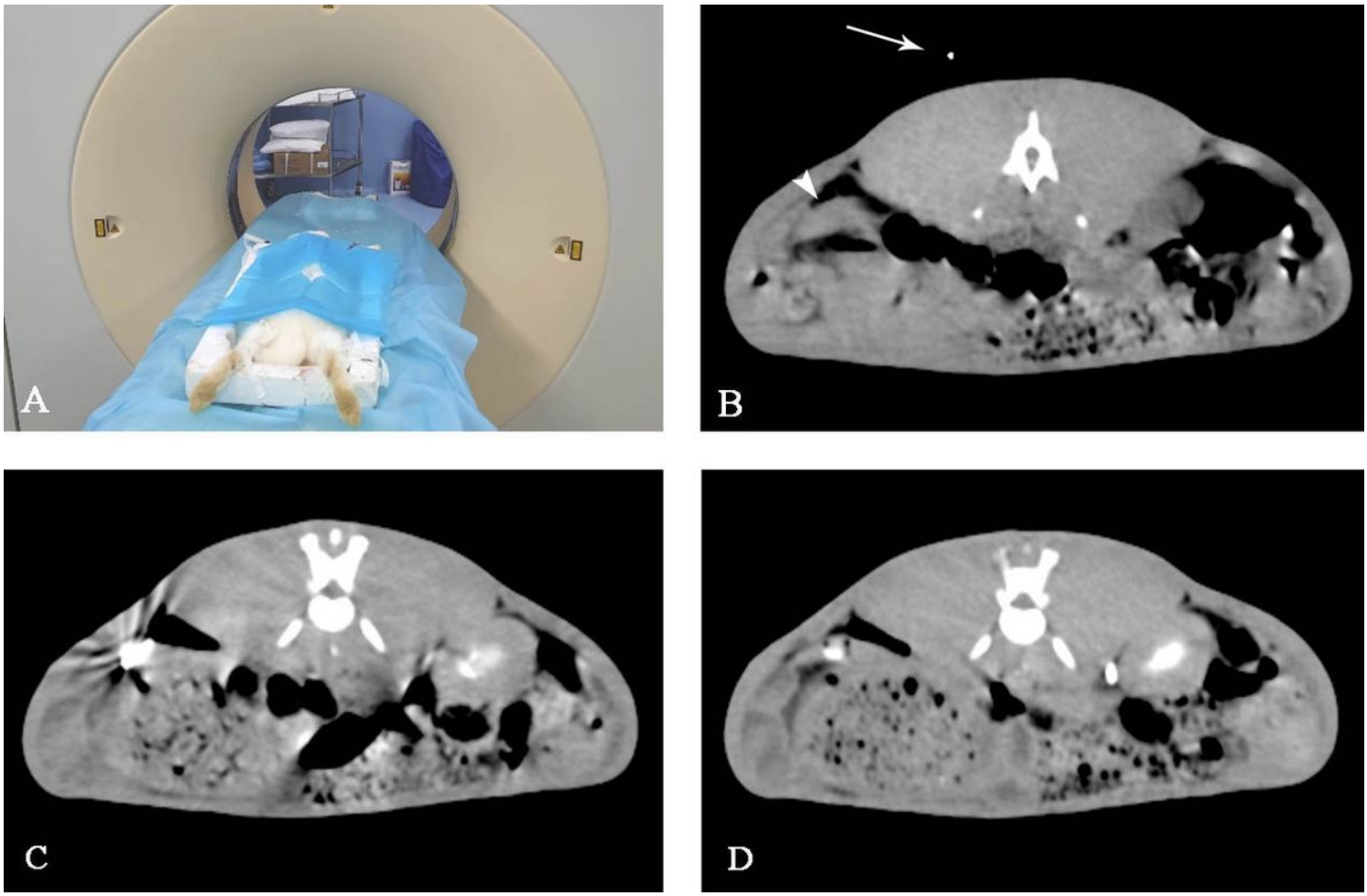

Figure 2

CT-guided percutaneous splenic implantation. The rabbits were fixed on the CT scanning table in a prone position (A); A self-made metal positioner (arrow) was used for positioning (B), spleen (arrowhead); a 22G coaxial puncture needle was used to puncture the rabbit spleen (C); The needle path was blocked with a gelatin sponge (D).

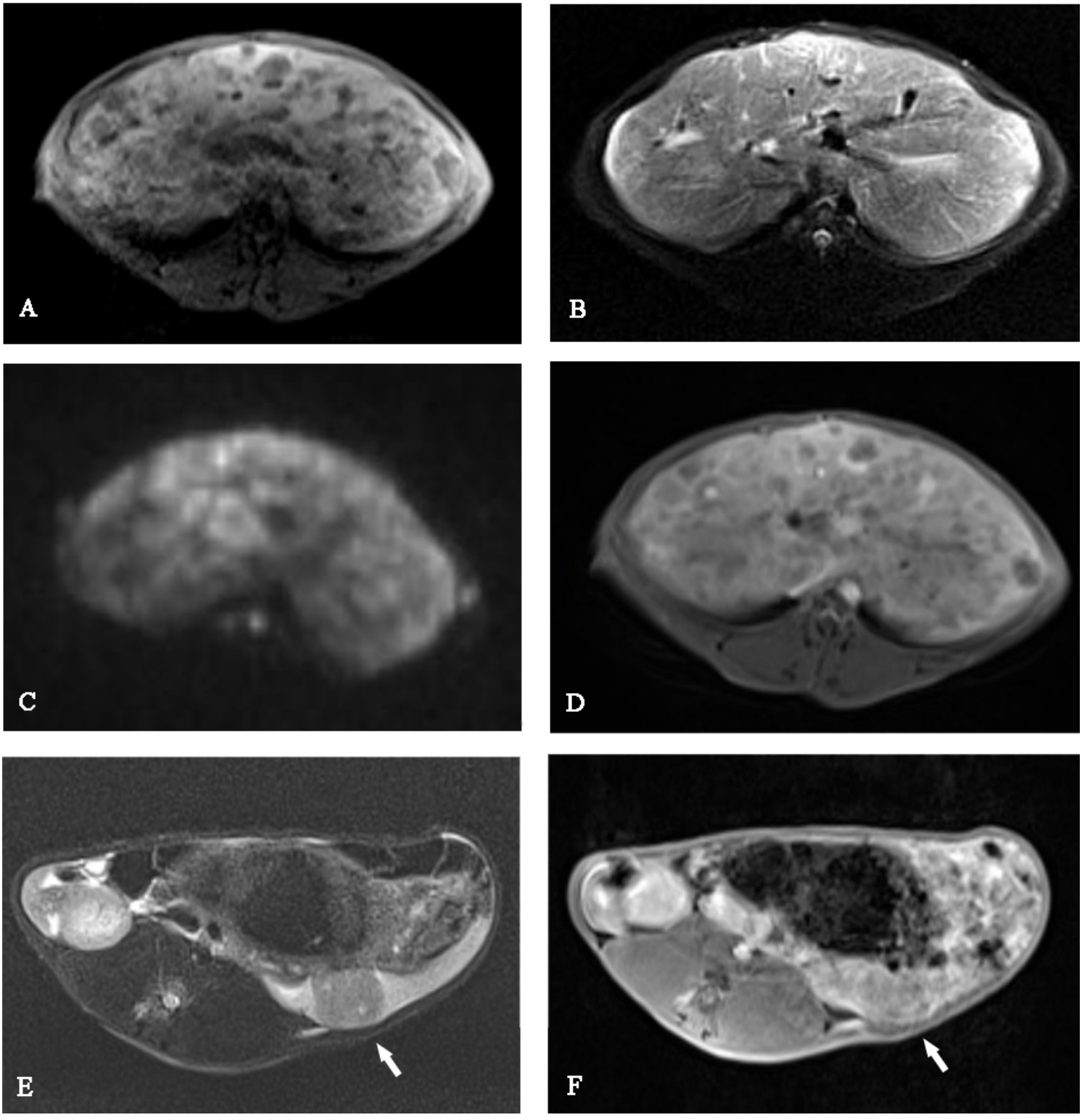

Figure 3

MRI manifestations of liver metastases were multiple nodular and punctate abnormal signal shadows in the liver, low signal on T1WI (A), slightly high or equal signal on T2WI (B), high signal on DWI (C), and circular enhancement on enhanced scanning (D); E, F: T2WI and T1WI sequences showed that the liver was enlarged and with a nodular shadow (white arrow).

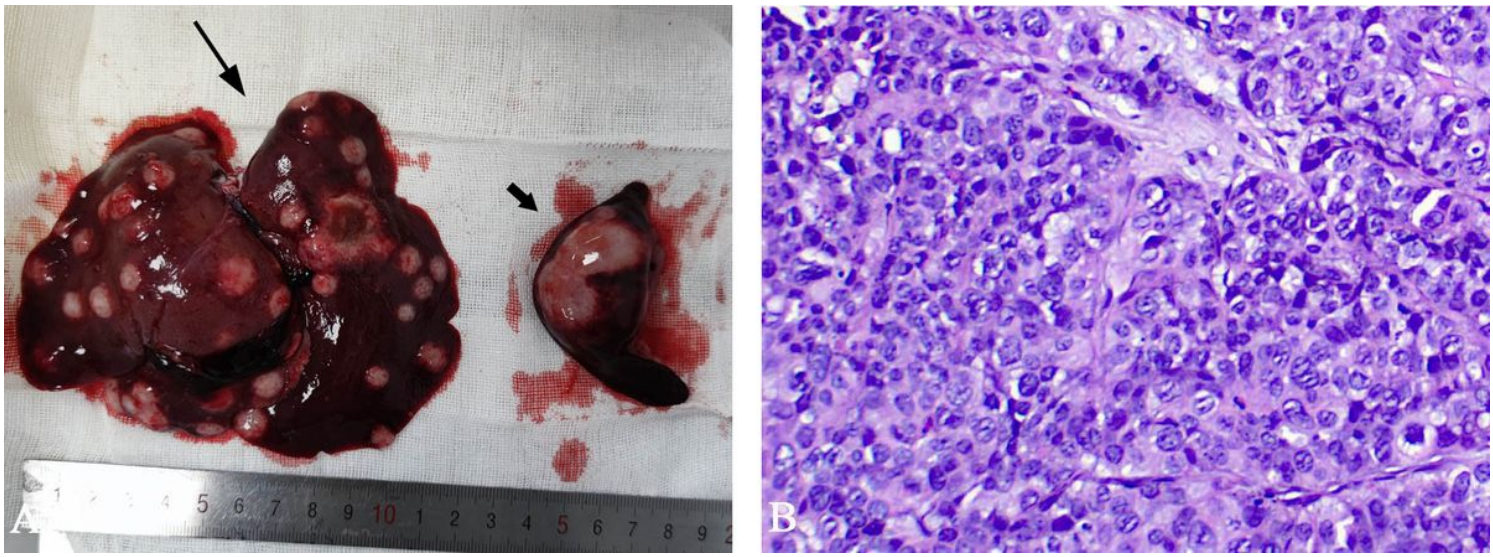

Figure 4

The specimens (A) showed multiple gray-white nodular shadows in the liver (long black arrow); The volume of the spleen (short black arrow) was increased, and a fish-like nodular tumor could be seen; B: HE staining showed that the nucleus was large and deeply stained, and the morphology was irregular.