



## MINI-ABSTRACT

A focused ultrasound toroid-shaped device was used non-invasively in the liver to create large coagulated volumes rapidly (7 cm<sup>3</sup> in 40s). Ablation was guided in real-time using an integrated ultrasound imaging probe and was feasible through large vessels (5 mm).

## SUMMARY

### Objective

To demonstrate in a porcine model that high intensity focused ultrasound (HIFU) with toroid-shaped emitters may have a role in treating unresectable colorectal liver metastases.

### Summary Background Data

Surgical resection is the only curative option for colorectal hepatic metastases. Only 20% of patients are suitable for surgery. Many ablative techniques have been assessed but several limitations have been documented: traumatic puncture of the parenchyma, limited size of lesions and inability to monitor the treatment in real time.

### Methods

A HIFU device with 256 toroid-shaped emitters and integrated ultrasound imaging probe was used. Single lesions, induced in 40 seconds, and juxtaposition of 6 single lesions were created under ultrasound guidance on 13 pigs. The lesions were studied on sonograms, macroscopically and microscopically up to 30 days after the treatment.

### Results

Ninety percent of the HIFU lesions were immediately hypoechoic on ultrasound imaging. The average coagulated volume obtained from a 40 s total exposure in the liver was  $7.0 \pm 2.5 \text{ cm}^3$  (1.5 – 20.0), average diameter:  $19.5 \pm 3.8 \text{ mm}$  (10.0 – 29.0). Using the real-time visualisation of the treated region, single lesions were easily juxtaposed in order to produce larger lesions up to 6 cm in diameter without any major complication.

### Conclusions

This toroid HIFU device allows short treatment times, non-invasiveness regarding the liver and real time ultrasound guidance. It appears to be simpler and more reliable to use than current ablative methods. Additionally, lesions through large vessels (up to 5 mm) being feasible, treatment of some juxta-vascular metastases should be possible.

High Intensity Focused Ultrasound ablation for the treatment of colorectal liver  
metastases during an open procedure. Study on the pig.

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## INTRODUCTION

Hepatic resection is the gold standard in the treatment of colorectal liver metastases, and currently is the only curative treatment. Patients are eligible for hepatic resection if complete resection of colorectal metastases is possible with tumor free margins. Additionally, the remaining functional hepatic volume must be sufficient to ensure adequate postoperative liver function. However, only 20% of patients with liver metastases are suitable for resection. In most patients the number, localization and/or size of the liver metastases or poor hepatic reserve preclude radical hepatic resection<sup>1</sup>. Post-operative 5-year survival in patients having had a hepatic resection is 20 to 35%. As a result, many regional ablative techniques have been assessed to provide an alternative to liver resection. The use of radiofrequency or cryotherapy is widespread and makes it possible to increase the number of patients treated with promising results<sup>2-4</sup>. These treatments can be employed via laparotomy with or without surgical resections or by percutaneous route. Nevertheless, regional destruction techniques present several common limitations. The first is hepatic trauma induced when electrodes or cryoprobes penetrates the liver. The second is the limitation of the size of the tissue destruction due to the decay of energy moving away from the probe axis. A reduction in treatment quality is observed when the zone to be coagulated is in contact with a large size vessel because blood flow decreases the therapeutic effectiveness by thermal convection effect<sup>5</sup>. Moreover, the hepatic local recurrence rate is between 30 and 40% at two years for cryotherapy<sup>6</sup> and was estimated at 15% (2 to 60%) for radiofrequency at a minimal follow-up of six months<sup>7</sup>. Additionally, monitoring destruction in real time is impossible<sup>8</sup>. Post-therapeutic evaluation (scanner and MRI) does not allow treatment to be adjusted during the intervention<sup>8,9</sup>.

We have studied a new form of treatment by high intensity focused ultrasound (HIFU), which could represent a promising alternative for treating colorectal liver metastases. The principal interest in HIFU lies in the possibility of treating metastases for which the use of local destruction techniques is contra-indicated, notably for metastases located near a large vascular structure<sup>10</sup>. Moreover, an ultrasound imaging system can be coupled with HIFU to visualize the coagulated zone after each ultrasound exposure accurately and in real time. This reliable ultrasound guidance makes it possible to envisage better therapeutic efficacy than current regional destruction techniques. The medical device presented in this study could be used in conjunction with resection. Therefore the approach selected is surgical laparotomy and consequently makes it possible to reach all regions of the liver without penetrating the hepatic capsule. Furthermore, a peroperative approach enables the protection of organs in the vicinity while eliminating the risk of secondary lesions.

Here we report a validation for this medical device by a preclinical animal study under conditions similar to those in man. The three objectives of this work were (i) to evaluate the feasibility and reproducibility of HIFU lesions on the livers of pigs *in vivo*, (ii) to study the lesions on ultrasound images, gross pathology and microscopy and (iii) to evaluate the local and general tolerance of the treatment.

## **MATERIALS AND METHODS**

### **Animals**

There is no established liver metastase model in pigs due to the absence of porcine liver cancer cell lines. However, the pig is an ideal animal to study the different treatment options available for liver metastases, due to its size and similar physiology to humans. The pig is the animal the most frequently used for experimental hepatic surgery. The study was carried out

on 13 Landrace pigs, 12-14 weeks old, with an average weight of  $31.3 \pm 4.2$  kg (23.0 – 37.0). The experiments were conducted at the Institute of Experimental Surgery of the Léon Bérard Center after local institutional review board approval. These experiments conformed to the requirements of the local Office of Animal Experimentation and were in accordance with the legal conditions of the National Commission on Animal Experimentation. The animals were kept in boxes on-site seven days before the start of the experiments. Total hepatectomy took place one hour (3 pigs), 4 days (4 pigs), 7 days (2 pigs), 15 days (2 pigs) and 30 days (2 pigs) after treatment. Two initial feasibility animals were studied at D0 and D4 to adjust the exposure parameters before continuing the research on the 11 following animals. The hepatectomies were carried out on different dates to study local and general treatment tolerance and the evolution of the sizes of the lesions over a period of time.

### **Animal preparation and anesthesia**

Twenty-four hours before and after HIFU, the food intake of the animal was restricted with free access to water. Premedication was performed 30 minutes before anesthesia using an intramuscular injection of ketamine (Kétalar®, Parke-Davis, Courbevoie, France) at a rate of 20 mg/kg. A 6.5 French venous catheter (Willy Rusch, Waiblingen, Germany) was placed in an auricular vein. Induction was made by an intravenous injection of propofol (Diprivan®, AstraZeneca, Rueil Malmaison, France). Tracheal intubation was carried out in the dorsal decubitus position. Ventilation was ensured by a Servo 900B® respirator (Siemens Life Support Services, Solna, Sweden) at 10 l/min and at a frequency of 18 to 20 respiratory movements per minute with a 70% air/oxygen mixture.

Anesthesia was maintained by a 1% propofol pressure infuser (Diprivan®, AstraZeneca, Rueil Malmaison, France) at a dose of 20 mg/kg/h, and sufentanil at a dose of 5 to 10 µg/kg/h (Sufenta®, Janssen-Cilag, Berchem, Belgium). For each animal, plasma samples were

withdrawn from a central venous catheter before surgery. Hepatocellular injury was assessed by standard laboratory assays of aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Other recorded serum parameters were haemoglobin level, haematocrit, thrombocyte count, prothrombin time (PT), activated partial thromboplastin time (APTT), bilirubin and ammonia. Animals were revived using infrared lamps, and clinically monitored before being taken back to the animal quarters. Post-operative analgesia was administered by a fentanyl patch at a 100- $\mu$ g dosage every three days (Durogesic®, Janssen-Cilag, Berchem, Belgium).

### **Ultrasound equipment**

The HIFU device was composed of a driving equipment similar to that previously reported<sup>11</sup> and a sterilizable treatment probe (Figure 1a). The ultrasound fields were generated with eight ultrasound emitters operating at a frequency of 3 MHz and distributed according to a toroidal geometry with a diameter of 70 mm. Each of the eight emitters was divided into 32 transducers so the location and intensity of the pressure field created can be controlled electronically by modulating the amplitude and phase applied to each separate individual transducer. Compared with conventional HIFU treatments this toroidal geometry associated with alternative and consecutive activation of the eight emitters makes it possible to treat large volumes over short periods of time<sup>12</sup>. With this technology, the destruction zone induced in 40 seconds has a theoretical diameter of 2 cm, a major axis of 2 cm, and is placed at 7 cm from the emitting surface to enable treatment of the deepest regions of the liver (Figure 1b). Therefore, the focal zone of the device is geometrically located at 7 cm from the emitters in order to treat deep seated liver metastases and spare superficial liver tissues. A HIFU lesion begins in the focal zone and slightly propagates toward the emitters. Due to the thickness of the porcine liver (2 – 4 cm), the coupling sterile envelope was filled with sterile water such

that approximately 50 – 60 mm of liquid was present between the device and tissues in order to place the focus in the centre of the liver. Nevertheless, it is possible to change the location of the focal zone by adjusting the quantity of water present between the device and tissues or by electronic focusing using the 256 transducers of the device. Under these conditions, the location of the focal zone can be placed from the surface of the liver to 9 cm in depth. Such parameter may permit to treat superficial or deep liver metastases in human without any restriction. A 7.5 MHz ultrasound imaging probe (Vermon, Tours, France) was placed in the centre of the device and connected to a BK HAWK 2102 EXL scanner (B-K Medical, Herlev, Denmark) to guide the treatment (Figure 1a). The HIFU probe was brought into contact with the liver using an ultrasound sterile cooling and coupling liquid (Ablasonic®, Edap-Technomed, Vaulx en Velin, France) contained in a sterile polyurethane envelope (Civco, Kaloma, Iowa, USA). A software developed in our laboratory made it possible to define the treatment zone by HIFU from a two-dimensional ultrasound image. The user interface allowed visualization by sonogram of the region to be coagulated by ultrasound exposure. The position of the HIFU focal region was superimposed on the ultrasound image. In this way, the user can position the treatment zone within the hepatic tissue precisely.

### **Surgical procedure**

A 25 cm median laparotomy was performed from the xyphoid process after classical surgical asepsis. Treatments were performed under sterile conditions. The HIFU probe was held by hand at the surface of the liver. The region to be treated was located using the integrated ultrasound imaging probe. Each HIFU exposure was performed during apnea to avoid liver movement. Apnea periods always began 5 seconds before sonication and lasted 45 seconds. The purpose of this maneuver was to limit the movements of the liver, which could have been deleterious to the quality of the treatment. The ultrasound exposures were performed using an

acoustic power of 50 watts, and the exposure time was set at 5 seconds for all ultrasound emitters. Each of the eight transducers was activated alternatively and consecutively. Therefore, the total exposure time for one elementary lesion was 40 seconds. These exposure conditions come from preliminary numerical studies performed using a software previously described<sup>13</sup>, and from preliminary *in vitro* studies. Two types of lesions were produced: single lesions and multiple lesions. Single lesions were produced by a 40-second exposure except when a vessel larger than 5 mm was visualized with the ultrasound. In this case, two successive 40-second exposures were performed without displacing the probe. Multiple lesions were produced by juxtaposition of six lesions (2 rows of 3 exposures) with a displacement of one centimeter between the exposures (Figure 2). This juxtaposition of lesions was made possible by the reliability of the ultrasound images previously described. Adjacent organs were carefully isolated from the liver using pads after the fourth pig. Additionally, cholecystectomy was done routinely after the sixth pig in order to eliminate any lesion at the vesicular level. The entire HIFU procedure was recorded on a support DVD. At the end of the treatment all lesions were observed on ultrasound images and palpated to verify their presence. Possible effects on adjacent organs, burns in particular, were carefully sought throughout the entire abdominal cavity. The laparotomy was closed in two planes.

### **Postsurgical monitoring**

Twice-daily clinical examinations were made up to the hepatectomy. Plasma samples were withdrawn from a central venous catheter on the first, fourth, seventh, fifteenth and thirtieth postoperative days. Hepatocellular injury was assessed by standard laboratory assays of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Other recorded serum parameters were haemoglobin level, haematocrit, thrombocyte count, prothrombin time (PT),

activated partial thromboplastin time (APTT), bilirubin and ammonia. The normal values allowed were those given by the Canadian Council on Animal Care<sup>14</sup>.

### **Hepatectomy, intermediate analysis and histology**

The pigs were euthanized under general anesthesia by a single intravenous 0.3 ml/kg injection containing embutramide, mebezonium and tetracaine (T61®), Intervet, Beaucouze, France). A total hepatectomy was then done in order to visually inspect ultrasound effects. The lesions were cut along the shot axis. Each lesion was then sliced into thin sections of 5 mm thick to determine whether thermal damages were homogeneous. The examiner measuring the dimensions of the lesions on gross pathology was different from the examiner measuring dimensions on sonograms. Representative portions of treated tissue were then immersed in 10% formalin for fixation and set in paraffin, or frozen in liquid nitrogen. Staining was done with hematoxylin-phloxine-saffron (HPS) or with NADH ( $\beta$ -nicotinamide adenine dinucleotide, reduced disodium salt hydrate, Sigma-Aldrich, Saint Louis, Missouri, USA) and Nitro blue tetrazolium (Sigma-Aldrich, Saint Louis, Missouri, USA). NADH staining has been used as an adjunct to routine hematoxylin and eosin (H & E) staining to study the effects of renal and liver RF ablation.<sup>5, 15</sup> NADH diaphorase has been shown to be active only in viable cells and its activity ceases immediately after cell death.<sup>16</sup> The NADH staining method permits the evaluation of tissue ablation based on cell viability rather than on histological characteristics, which may only be variably altered after intervention. The samples were treated and analyzed by the Anipath Laboratory (Lyon, France).

### **Data analysis**

Data is given as mean values  $\pm$  standard deviation (minimum value – maximum value). The criteria studied were the feasibility of the exposures, the sonication time to produce multiple

lesions (defined as the time from the first to the last sonication), local and general tolerance, ultrasound and gross pathologic appearance and dimensions of the HIFU lesions, and the microscopic appearance of the lesions. Possible anomalies in the treatment conditions were sought on the support DVD for each procedure.

The parameters studied were entered in their entirety into a 4D® database (ACI, San Jose, California, USA). Wilcoxon tests were performed to compare the evolution of the lesions over time (D4, D7, D15 and D30). The significance level for all tests was fixed at  $p = 0.05$ . The statistical analyses were performed using R® software (The R Foundation for Statistical Computing, Vienna, Austria).

## **RESULTS**

### **Preliminary study on 2 pigs**

This study made it possible to adjust the treatment parameters that were then applied for the remainder of the study. Thirteen lesions were produced including 2 multiple lesions. Hepatectomies were performed at D0 and D4. The lesions obtained with a single 40-second exposure were inhomogeneous when a vessel greater than 5 mm in diameter was located in the focal zone of ablation. Therefore it was decided to perform two 40-second exposures consecutively, without any displacement of the probe, to produce single lesions when a vessel of 5 mm or more was present in the focal zone.

### **Feasibility of HIFU treatment**

A total of 77 lesions were produced (15 at D0, 31 at D4, 13 at D7, 10 at D15 and 8 at D30). 36 single lesions were created by one 40-second exposure, 27 single lesions were created by two 40-second exposures and 14 multiple lesions were created by juxtaposing six single

lesions. All were palpable or visible on ultrasound images. On average,  $5.8 \pm 1.7$  (5 – 9) HIFU lesions were produced per animal. 16.9% of the lesions were created on the lateral right lobe, 29.9% on the medial right lobe, 26.0% on the medial left lobe, and 27.3% on the lateral left lobe. Two multiple lesions were excluded from the statistical study, due to the DVD recording of the procedure having shown a bad coupling between the ultrasonic emitters and the liver. The presence of bubbles in front of the probe surface preventing the propagation of the ultrasounds was then carefully checked before each HIFU treatment. In this study, it was not planned to evaluate the time required to create multiple lesions. Time was taken between two single lesions for collecting data about acoustical power, ultrasound images... Under these conditions the sonication time, defined as the time from the first to the last sonication for multiple lesions was on average  $21 \pm 8$  minutes (9 – 42).

### **Imaging**

92% of HIFU lesions in the liver were seen as a hypoechoic region with a central hyperechoic zone (Figure 3). In 8% of cases, lesions were masked by boiling and a hyperechoic region was visible. A good correlation was noted between the sizes of lesions measured macroscopically and the sizes of the same lesions measured by ultrasound ( $R^2 = 0.90$ ). The mean difference between the ultrasound and gross pathological diameters was  $2.4 \pm 2.1$  mm (0 – 11.4), or  $10.9 \pm 7.6$  % (0 – 36).

### **Gross pathological appearance and dimensions**

The gross pathology examination showed homogeneous necrosis for 12 multiple lesions out of 14 (86%) and for 26 single reference lesions out of 27 (96%). The inhomogeneity of the lesions was due to the presence of vessels with a diameter greater than 8 mm at their center and related exclusively to the feasibility study. All the lesions created after the feasibility

study were homogeneous even when a vessel of 5 mm in diameter was present in the ablated region (see histological results). The mean diameter of the single lesions was  $19.3 \pm 4.0$  mm (min. 12.0 – max. 29.0 mm, 95% confidence interval [CI] = 17.7 to 20.9 mm), which corresponded ideally to a destruction speed of  $5 \text{ cm}^3$  per minute. Analysis of single lesions showed, on several specimens, that necrosis could circumscribe completely vessels up to 5 mm in diameter (Figure 4). Bile stains were equally noted in many lesions indicating a rupture of minor intrahepatic bile duct. The sizes of the lesions did not change over time as shown in Figure 5. The diameter of the lesions observed at D0 was not significantly different as compared with the diameter at D4 ( $p=0.5501$ ), D7 ( $p=0.8583$ ), D15 ( $p=0.1336$ ) or D30 ( $p=0.1103$ ). A slight decrease was observed at D15 and D30 since the fibrous tissue response around the coagulated region was not taken into account in the measurement of the diameter. The mean diameter of the multiple lesions was  $33.5 \pm 13.5$  mm (min. 14.0 – max. 60.0 mm, 95% CI = 28.2 to 38.8 mm), which was close to the anticipated theoretical diameter (40 mm). The reliability of ultrasound location (Figures 6a and 6b) made it possible to juxtapose the lesions without leaving untreated spaces. In a particular case, two side by side multiple lesions which were close from each other formed a lesion of 7 cm on its major axis as shown in Figure 7.

### **General clinical and biological tolerance**

The local and biological tolerances of the treatment were excellent. The pigs remained hemodynamically stable during the procedure. They recovered from the procedure within two hours after termination of anesthesia, and quickly resumed eating and normal behavior. The mean weight gain was 14 kg in one month for the 2 pigs studied until D30. Immediately after resection, all serum parameters of hepatocellular damage were increased. Peak values were observed in all groups on the first postoperative day, and all parameters had normalized by

day four. Peak levels of AST ranged from 144 to 433 (preoperative range 26–42) units/l and ALT ranged from 54 to 98 (preoperative 27–64) units/l. No differences in peak values were observed as a function of time. Transient leucocytosis was noted in 42% of the cases (maximum 31 G/l at D7 for an average at 22 G/l). No alterations in haemoglobin, haematocrit, thrombocytes, PT, APTT, bilirubin or ammonia were observed (data not shown). There were no biological signs of renal insufficiency.

### **Local tolerance**

Five secondary lesions (6% of the total number of lesions) were noted due to the initial use of the device. All were related to sonications in zones where the pig liver was not thick enough, thus allowing ultrasounds to propagate into surrounding organs. Two gastric burns (on the first two pigs included in the feasibility study), a colonic burn (on the third treated pig) and a gall-bladder burn (on the fourth treated pig) were observed at the beginning of the experiment only. All these lesions were recognized at the time of the treatment. Regarding the two gastric burns, one was resected and no complication related to the resection was observed during the postsurgical monitoring (up to four days after the treatment). Nothing was done for the other gastric burn since hepatectomy was planned just after the HIFU procedure. The gall-bladder burn was treated by cholecystectomy and no related complication was observed during the postsurgical monitoring (up to four days after the treatment). The colonic burn seemed superficial. A resection was declined since the hepatectomy was planned four days after the treatment (at the time of autopsy the lesion has evolved but there was no perforation). From the fifth treated pig, it was decided to carefully isolate the liver using pads. Additionally, cholecystectomy was done routinely after the sixth pig in order to eliminate any lesion at the vesicular level. One case of biliary peritonitis (on the eighth treated pig) was detected when the liver was removed (one week after the treatment) and was related to repeated exposures on

the hepatic pedicle. After that, sonications were performed at distance from the hepatic pedicle (approximately 2 centimeters) and no other biliary complication was observed on the last five pigs.

In total, 18 single lesions created by one 40-second exposure, 26 single lesions created by two 40-second exposures and 12 multiple lesions were observed under the following conditions: cholecystectomy, careful isolation of the liver using pads and ablation at distance from the hepatic pedicle. None of these lesions was complicated by any burns in nearby organs.

### **Histology**

Using standard hematoxylin and eosin staining, histological evaluation of formalin fixed paraffin-embedded sections of excised HIFU ablation in liver tissues revealed evidence of tissue necrosis and cell death from D0 (Figure 8a and 8b). The presence of an edematous and congestive region at the periphery of the lesions was observed between D0 and D7 with complete focal necrosis inside the coagulated region. On microscopic observation, complete and homogeneous coagulation necrosis in the entire treated region was confirmed from D15. A conjunctive fibrous cap appeared at the periphery of the lesions at D15 and D30. NADH diaphorase staining which is a vital stain was used in order to more accurately evaluate local cell necrosis. In this study, a clear, non-stained zone was observed within the boundary of the ablation lesion, indicating a loss of cell viability in the entire treated region from D0 (figure 9a). Tissue damage was confined to regions that had been given HIFU exposure. Within these treated regions, there was no evidence of intact cells, whereas in the sharply demarcated surrounding tissue there was no evidence of cell damage. The margin between healthy and treated tissue was clearly demarcated, approximately 200  $\mu\text{m}$ , both in NADH and HPS staining from D0.

The ablated region was not reduced around blood vessels of less than 5 mm in diameter. Vessels up to 4mm in diameter presented alterations in their endothelium and thromboses. The endothelium of larger diameter vessels (5 mm) was preserved. There was no intervening healthy tissue surrounding vessels that were lower than or equal to 5 mm in diameter (Figure 9b).

## **DISCUSSION**

Current regional destruction techniques for treating colorectal liver metastases, radiofrequency and cryotherapy in particular, make it possible to increase the number of patients treated with curative intent. Although complete destruction of metastases has been demonstrated by histological examination of explanted liver, substantial recurrence rates of disease, between 30 and 40 percent at 2 years for cryotherapy<sup>6</sup> and 15% (2 to 60%) for radiofrequency<sup>7</sup>, have been documented. The lack of a reliable and immediate means of visualizing the induced lesions and dependence on these techniques with respect to perfusion are the source of these failures. High Intensity Focused Ultrasound is a treatment modality that presents many advantages over the above- mentioned local treatments. Focused beams pass harmlessly through superficial tissue and produce sufficiently strong heating in the focal zone to coagulate cells, even at distances relatively far from the transducer. Excellent results have been obtained both experimentally and clinically in inducing homogeneous and reproducible metastases destruction by thermal coagulation necrosis<sup>17</sup>. However, the dimensions of the lesions produced by conventional HIFU treatments are small, typically an ellipsoidal region of damage of 8-15 mm in length and 1-3 mm in diameter. Therefore, the main technical limitation to widespread clinical use of HIFU has been the long time required

to treat metastases of several cubic centimeters in volume. For example, HIFU thermal therapy requires significantly long treatment times; 149 minutes for prostate cancer<sup>18</sup> and 32 to 135 minutes for uterine leiomyomas<sup>19</sup>. In order to fill this therapeutic gap, we have designed a toroid geometry of HIFU transducers and developed a novel method of treatment that can rapidly induce large lesions. Irreversible thermal lesions of 2 centimeters in diameter can be induced in 40 seconds in the liver. Therefore, treatment duration is significantly shortened using this transducer geometry. The HIFU approach presented in this study is characterized by the intensity of the heat deposit (around 80°C in the targeted zone) and the brevity of the treatment (40 seconds for one lesion of 2 cm in diameter), which makes it possible to reduce treatment dependence from blood perfusion. Necroses induced by HIFU treatment circumscribe vessels with a maximum diameter of 5 mm. Thus, in contrast to current regional destruction techniques, treatment near large vascular structures is conceivable. In addition, the noninvasiveness of HIFU for the liver enables treatment without penetrating the hepatic capsule and thereby avoids the risks associated with introducing electrodes (radiofrequency) or cryoprobes (cryotherapy). As shown in this study, local and general tolerances were excellent; specifically, no morbidity was noted at the vascular level.

In addition, the ultrasound imaging probe placed in the center of the HIFU device provides real-time control of the treatment. Each lesion induced by HIFU is visible with high contrast on sonograms. We found a close correlation between the dimensions of the lesions measured on sonograms and those measured in gross pathology. This represents a major advantage in the improvement of treatment efficacy since the dimensions of the treatment zone can be enlarged by accurately juxtaposing elementary lesions. An array of these lesions can be built up in order to destroy metastases of several centimeters and perform conformal treatments. None of these features can be obtained using conventional local therapies. For example, during RF ablation procedures, there is an increase in the echogenicity of the ablated region in

the ultrasound images used to guide the treatment. This increase in echogenicity is due to the increasing presence of gas bubbles generated during temperature elevation. A clear demarcation of the thermal lesion from the metastases and from the surrounding normal tissues is difficult<sup>20,21</sup>.

In order to propose a therapeutic method by HIFU adapted to the treatment of colorectal liver metastases the treatment was performed during an open procedure. This choice allowed prevention of secondary lesions to nearby organs. Five secondary lesions were observed at the beginning of the study (two gastric burns, a colonic burn, a gallbladder burn and one case of biliary peritonitis) but were definitively eliminated when the treatment was performed under the following conditions: cholecystectomy, careful isolation of the liver using pads and ablation at distance from the hepatic pedicle. Under these conditions, all the lesions were created without any complication or secondary burns in nearby organs. Therefore the use of HIFU during an open procedure makes it possible to eliminate anatomical constraints and also to eliminate the risk of lesions in nearby organs. Indeed the liver is surrounded with anatomical features (ribs, air present in the lungs) that prevent linear propagation of the ultrasound. Furthermore, the main advantage of this treatment during an open procedure is to make it possible to associate the HIFU destruction technique with hepatic resection, which appears to be the best current strategy in curative treatment methods for clearing residual disease. Nevertheless, extracorporeal use of HIFU is possible as has been clinically demonstrated in the context of treatment for hepatocarcinomas<sup>22,23</sup>.

This study was performed on pigs due to their size and similar physiology to humans. To date, there is no porcine hepatic metastases model that enables us to evaluate the effect of HIFU on the destruction of liver metastases directly. Nevertheless, the efficacy of HIFU for malignant metastases is proven and non-specific to the soft biological tissue treated<sup>17</sup>. Furthermore, the histological study described in this paper confirms that the lesions observed are homogeneous

necroses. The results obtained in this study appear to us to be extrapolative to Humans. A biliary and vascular tolerance study will be conducted on the same animal model in order to better define the HIFU treatment indications before undertaking a clinical study.

In conclusion, the destruction of hepatic parenchyma by HIFU during an open procedure is feasible in a short time period and without organ penetration. Ultrasound guidance of the treatment makes it possible to envisage a better therapeutic efficacy due to objective real-time evaluation of the actual treated region. This method of local treatment could allow the treatment of juxta-vascular metastases inaccessible by other currently known therapeutic methods. Parenchymatous destruction by HIFU could supplant other ablative techniques of focused destruction of liver metastases of colorectal cancer because this technique is simple, reliable, adaptable to local conditions, powerful and non-invasive for hepatic parenchyma. The real-time control of placement and size of HIFU lesions endows this technique with excellent therapeutic safety. The approach described in this paper may have a role in treating unresectable colorectal liver metastases, and may also be used in conjunction with resection to extend its limits.

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**REFERENCES**

1. Manfredi S, Lepage C, Hatem C, et al. Epidemiology and management of liver metastases from colorectal cancer. *Ann Surg* 2006; 244(2):254-9.
2. Garcea G, Lloyd TD, Aylott C, et al. The emergent role of focal liver ablation techniques in the treatment of primary and secondary liver tumours. *Eur J Cancer* 2003; 39(15):2150-64.
3. Rivoire M, De Cian F, Meeus P, et al. Combination of neoadjuvant chemotherapy with cryotherapy and surgical resection for the treatment of unresectable liver metastases from colorectal carcinoma. *Cancer* 2002; 95(11):2283-92.
4. Curley SA, Marra P, Beaty K, et al. Early and late complications after radiofrequency ablation of malignant liver tumors in 608 patients. *Ann Surg* 2004; 239(4):450-8.
5. Tamaki K, Shimizu I, Oshio A, et al. Influence of large intrahepatic blood vessels on the gross and histological characteristics of lesions produced by radiofrequency ablation in a pig liver model. *Liver Int* 2004; 24(6):696-701.
6. Seifert JK, Morris DL. Indicators of recurrence following cryotherapy for hepatic metastases from colorectal cancer. *Br J Surg* 1999; 86(2):234-40.
7. Mulier S, Ni Y, Jamart J, et al. Local recurrence after hepatic radiofrequency coagulation: multivariate meta-analysis and review of contributing factors. *Ann Surg* 2005; 242(2):158-71.
8. Solbiati L, Ierace T, Tonolini M, Cova L. Guidance and monitoring of radiofrequency liver tumor ablation with contrast-enhanced ultrasound. *Eur J Radiol* 2004; 51 Suppl:S19-23.
9. Clasen S, Boss A, Schmidt D, et al. Magnetic resonance imaging for hepatic radiofrequency ablation. *Eur J Radiol* 2006; 59(2):140-8.

10. Melodelima D, Cathignol D. Cancer treatment by ultrasound: increasing the depth of necrosis. *Applied Physics Letters* 2004; 84:5365-7.
11. Melodelima D, Salomir R, Mougenot C, et al. 64-element intraluminal ultrasound cylindrical phased array for transesophageal thermal ablation under fast MR temperature mapping: an ex vivo study. *Med Phys* 2006; 33(8):2926-34.
12. Melodelima D, N'Djin A, Parmentier H, et al. Ultrasound surgery with a toric transducer allows the treatment of large volumes over short periods of time. *Applied Physics Letters* 2007; 91:193901.
13. Curiel L, Chavrier F, Gignoux B, et al. Experimental evaluation of lesion prediction modelling in the presence of cavitation bubbles: intended for high-intensity focused ultrasound prostate treatment. *Med Biol Eng Comput* 2004; 42(1):44-54.
14. Olfert ED, Cross BM, Mc William AA. *Guide to the Care and Use of Experimental Animals*. Vol. 1. Ottawa: Canadian council on animal care, 1993.
15. Scudamore CH, Lee SI, Patterson EJ, et al. Radiofrequency ablation followed by resection of malignant liver tumors. *Am J Surg* 1999; 177(5):411-7.
16. Neumann RA, Knobler RM, Pieczkowski F, Gebhart W. Enzyme histochemical analysis of cell viability after argon laser-induced coagulation necrosis of the skin. *J Am Acad Dermatol* 1991; 25(6 Pt 1):991-8.
17. Kennedy JE. High-intensity focused ultrasound in the treatment of solid tumours. *Nat Rev Cancer* 2005; 5(4):321-7.
18. Uchida T, Ohkusa H, Nagata Y, et al. Treatment of localized prostate cancer using high-intensity focused ultrasound. *BJU Int* 2006; 97(1):56-61.
19. Tempany CM, Stewart EA, McDannold N, et al. MR imaging-guided focused ultrasound surgery of uterine leiomyomas: a feasibility study. *Radiology* 2003; 226(3):897-905.

20. Varghese T, Daniels MJ. Real-time calibration of temperature estimates during radiofrequency ablation. *Ultrason Imaging* 2004; 26(3):185-200.
21. Minami Y, Kudo M, Chung H, et al. Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. *AJR Am J Roentgenol* 2007; 188(2):489-94.
22. Illing RO, Kennedy JE, Wu F, et al. The safety and feasibility of extracorporeal high-intensity focused ultrasound (HIFU) for the treatment of liver and kidney tumours in a Western population. *Br J Cancer* 2005; 93(8):890-5.
23. Kennedy JE, Wu F, ter Haar GR, et al. High-intensity focused ultrasound for the treatment of liver tumours. *Ultrasonics* 2004; 42(1-9):931-5.

## Figures caption

Figure 1. (a) Front view of the HIFU therapy hepatic imaging probe. (B) Geometry of the HIFU emitters and of the destruction zone.

Figure 2. Multiple lesions were produced using ultrasound guidance by the juxtaposition of six lesions (2 rows of 3 shots) with a displacement of approximately one centimeter between the shots using a hand-held probe.

Figure 3. User interface during treatment. (a) The user moves the HIFU probe with ultrasound guidance to position the treatment zone at the desired location. (b) After one 40-second insonification, the HIFU lesion appears on the ultrasound as a hypoechoic image.

Figure 4. HIFU single lesion produced in 40 seconds through a vessel 5 mm in diameter.

Figure 5. Evolution of the diameter of HIFU single lesions over 30 days. There is no significant difference between the lesions observed one hour after treatment and those observed 4 days, 7 days, 15 days and 30 days after treatment.

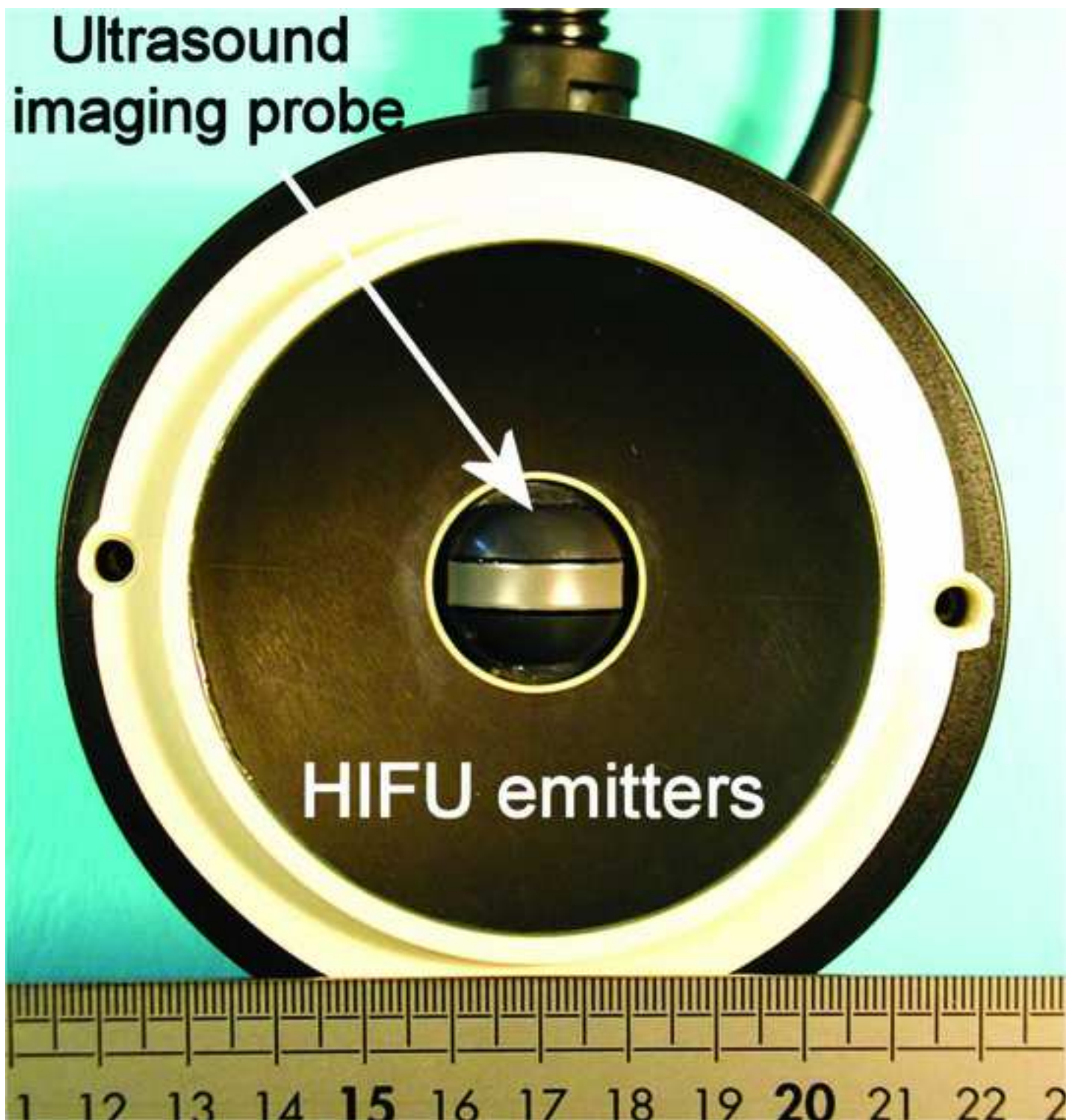
Figure 6. Juxtaposition of two HIFU lesions under ultrasonic guidance. a) Positioning of the exposure zone alongside the preceding HIFU lesion. b) Enlarged treated zone after the exposure.

Figure 7. Two side by side multiple lesions forming a lesion of 7 cm on its major axis.

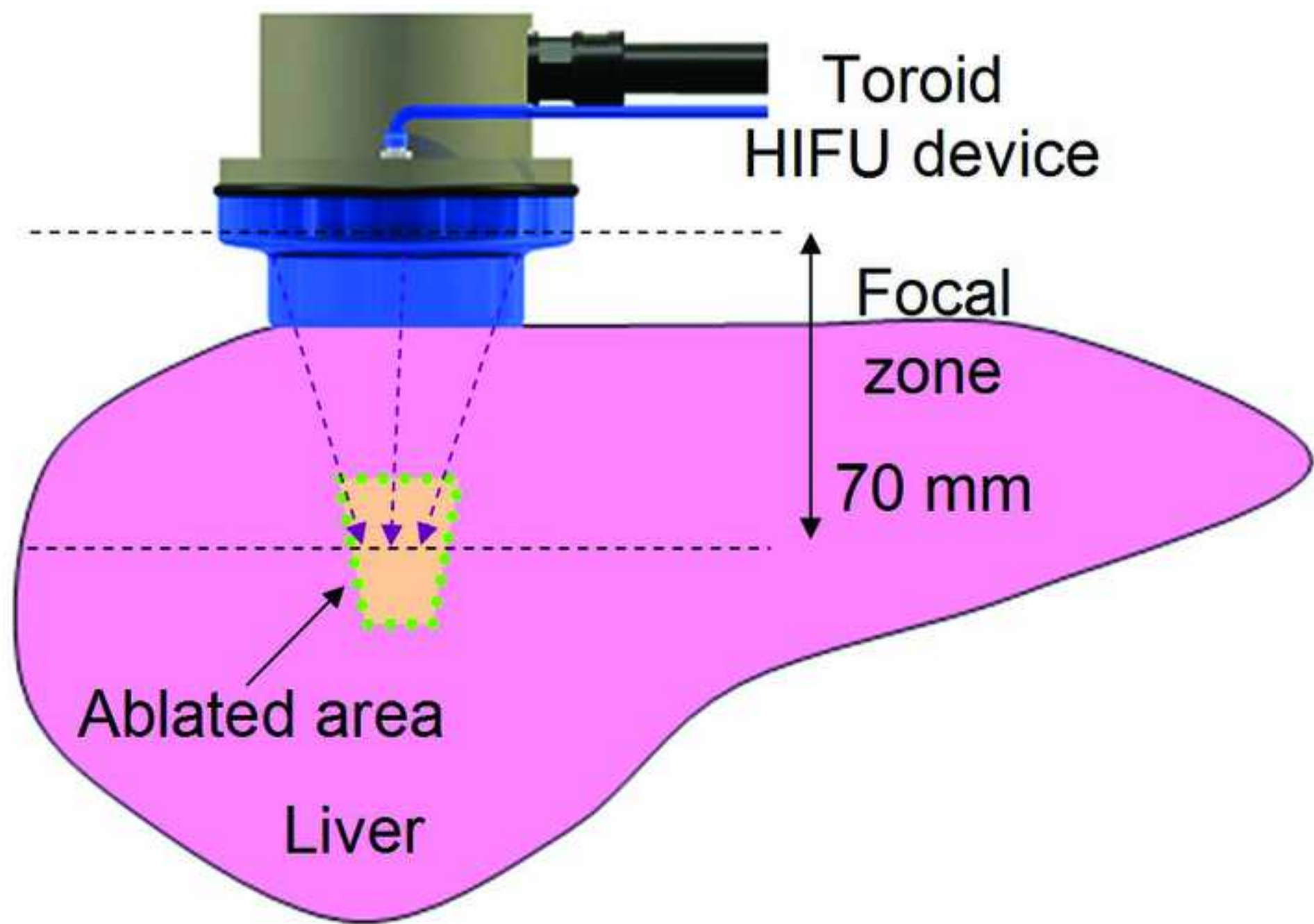
Figure 8. Vital staining by NADH of a liver zone treated by HIFU. (a) The HIFU lesion is homogeneous; no viable cells remain inside the destruction zone. (b) The margin between healthy and necrotic tissue is about 200  $\mu\text{m}$ . (\*) untreated region, (O) coagulated zone.

Figure 9: Standard HPS staining. (a) Blood vessel treated with the HIFU toroid probe. (\*) untreated region, (O) coagulated zone, ([]) blood vessel. b) The HIFU lesion extends until contact with blood vessels approximately 5 mm in diameter.

Figure 1a  
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**Figure 1b**  
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Figure2

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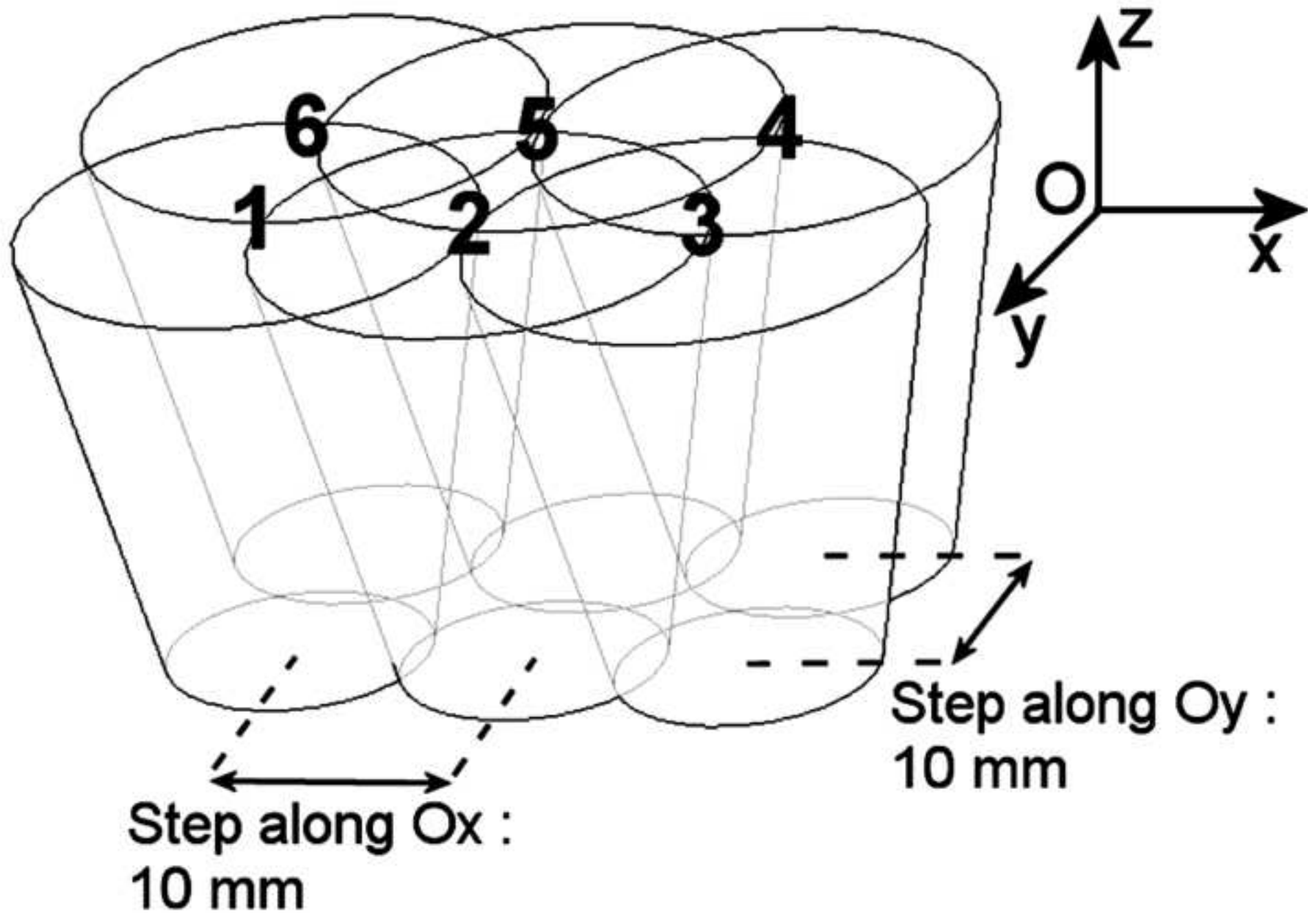


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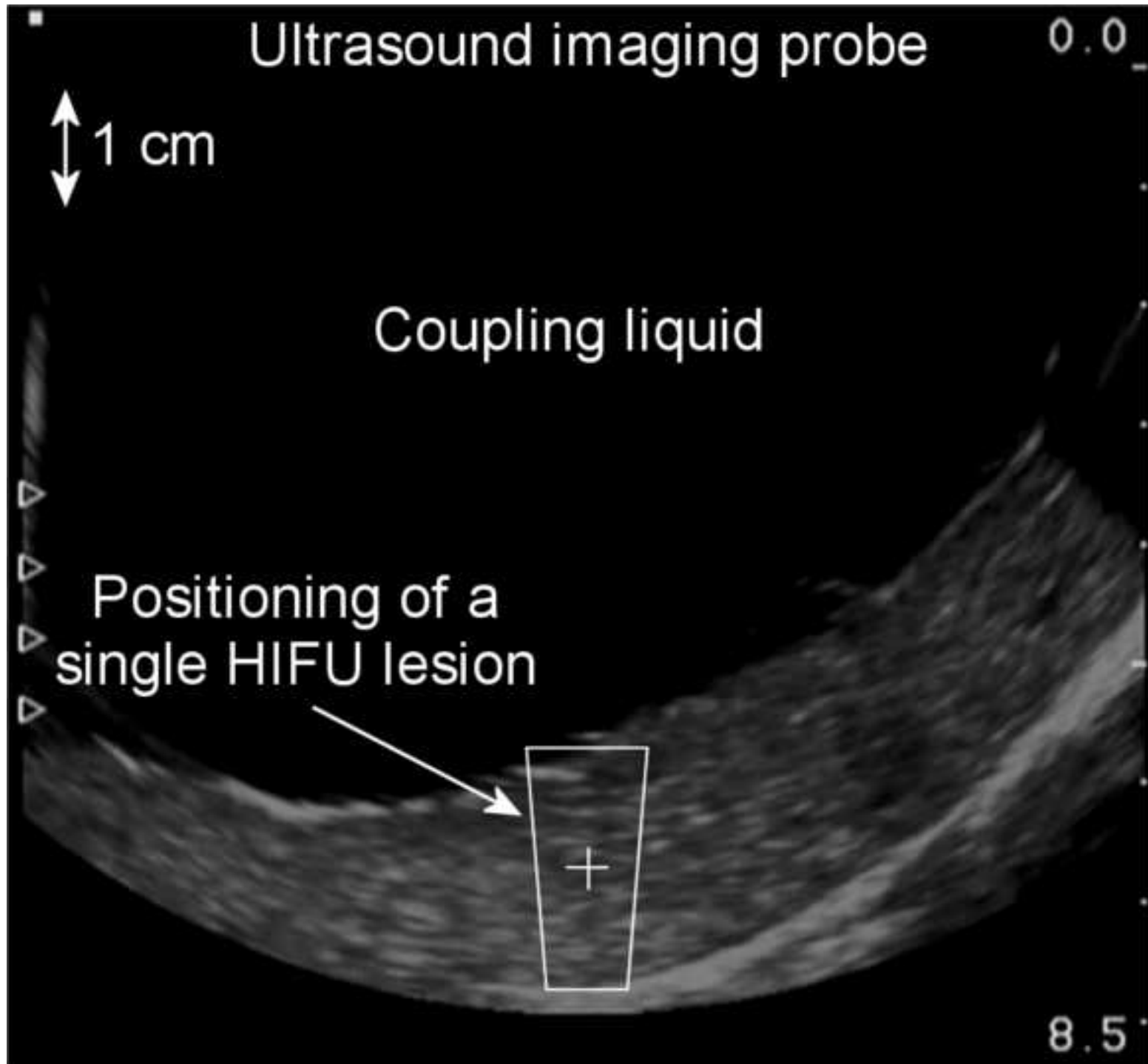


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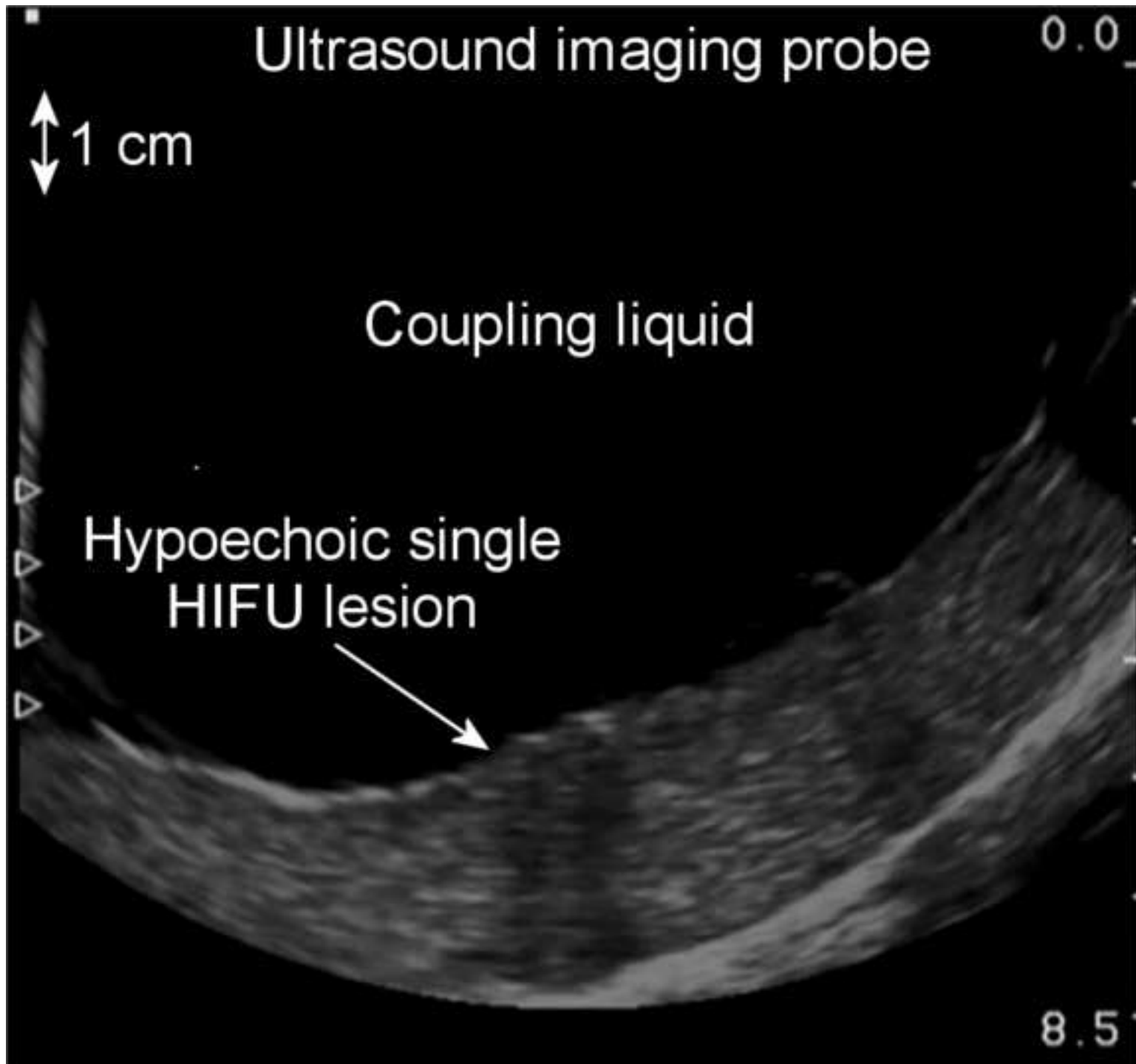


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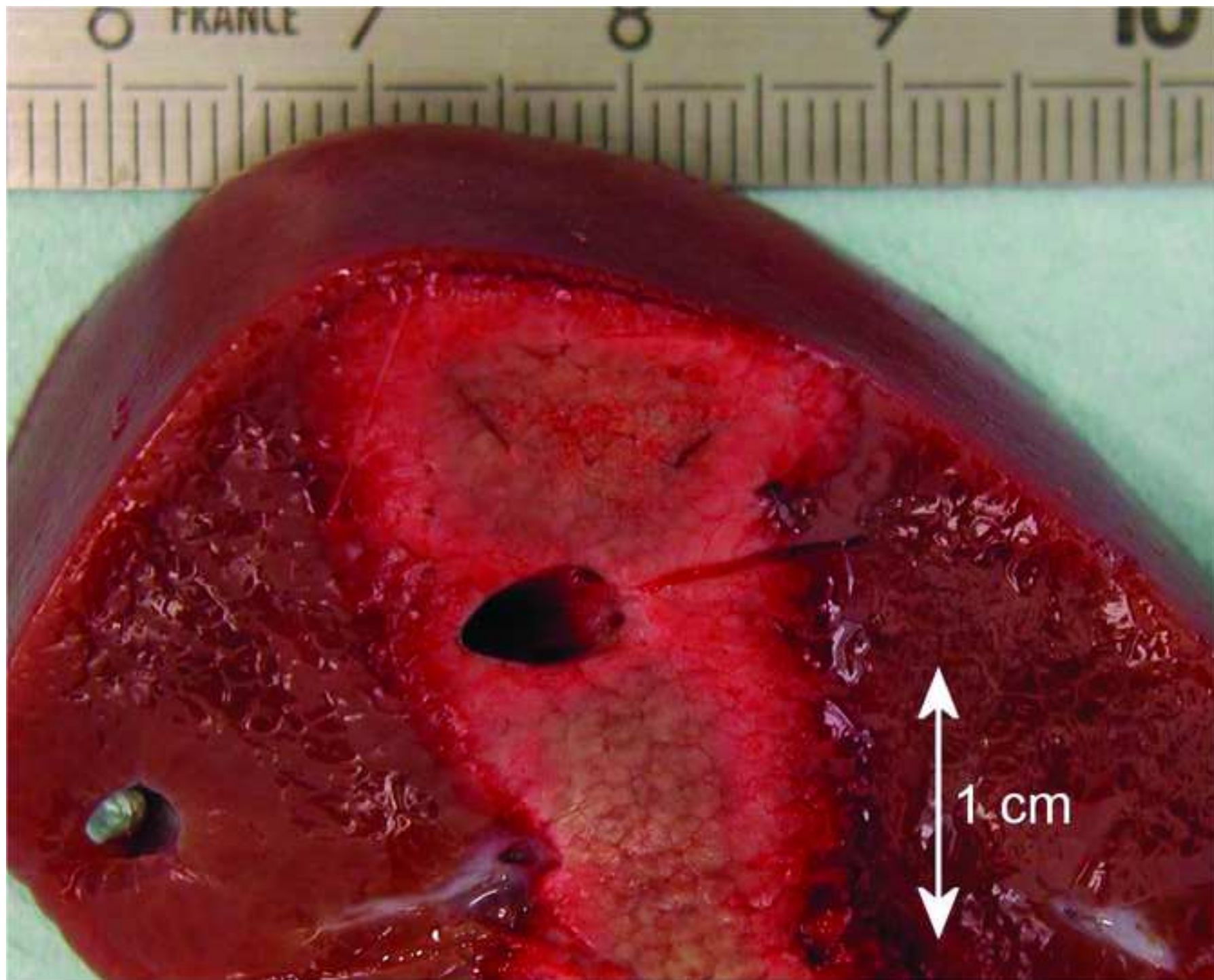
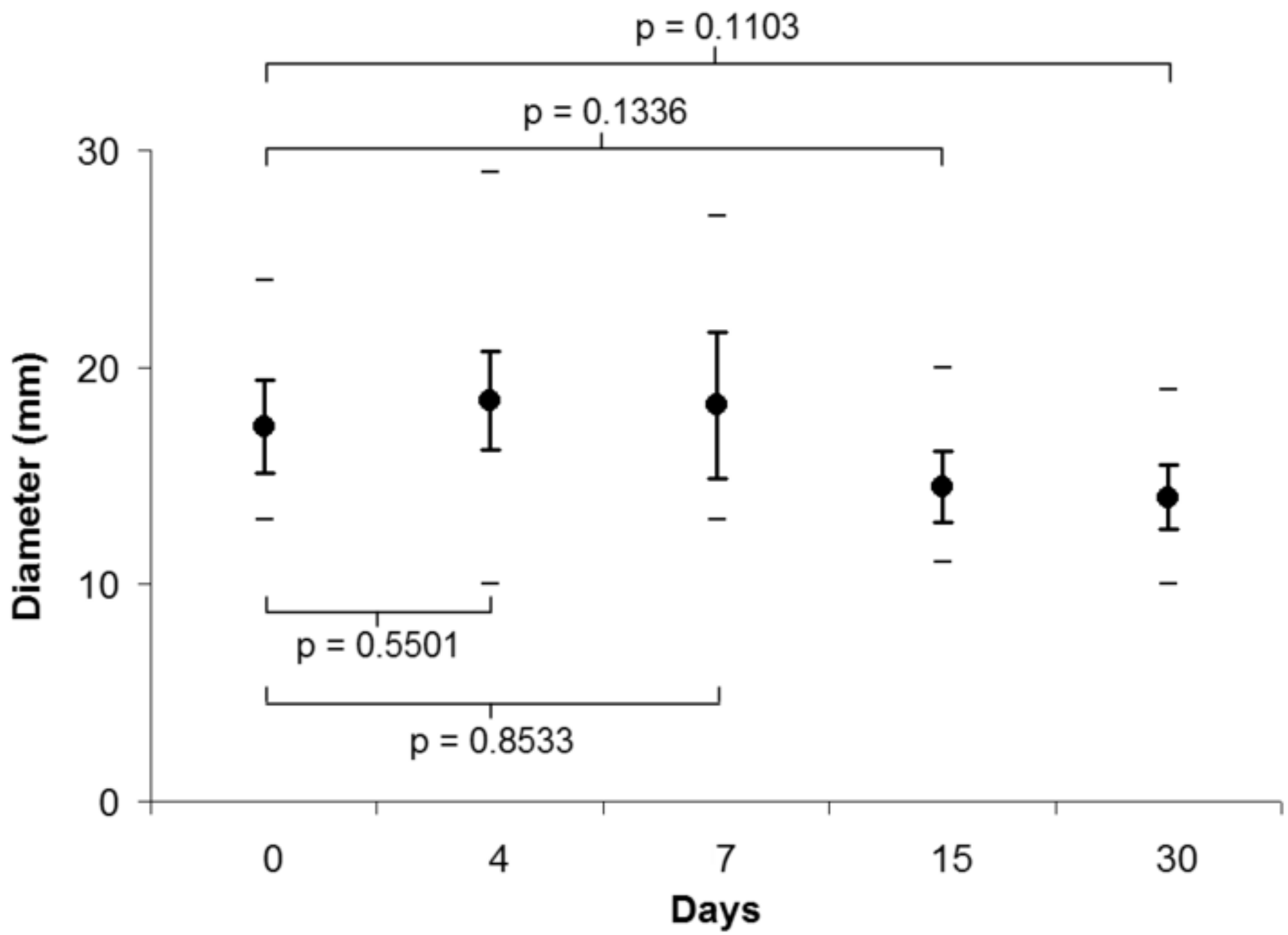


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Figure 6a  
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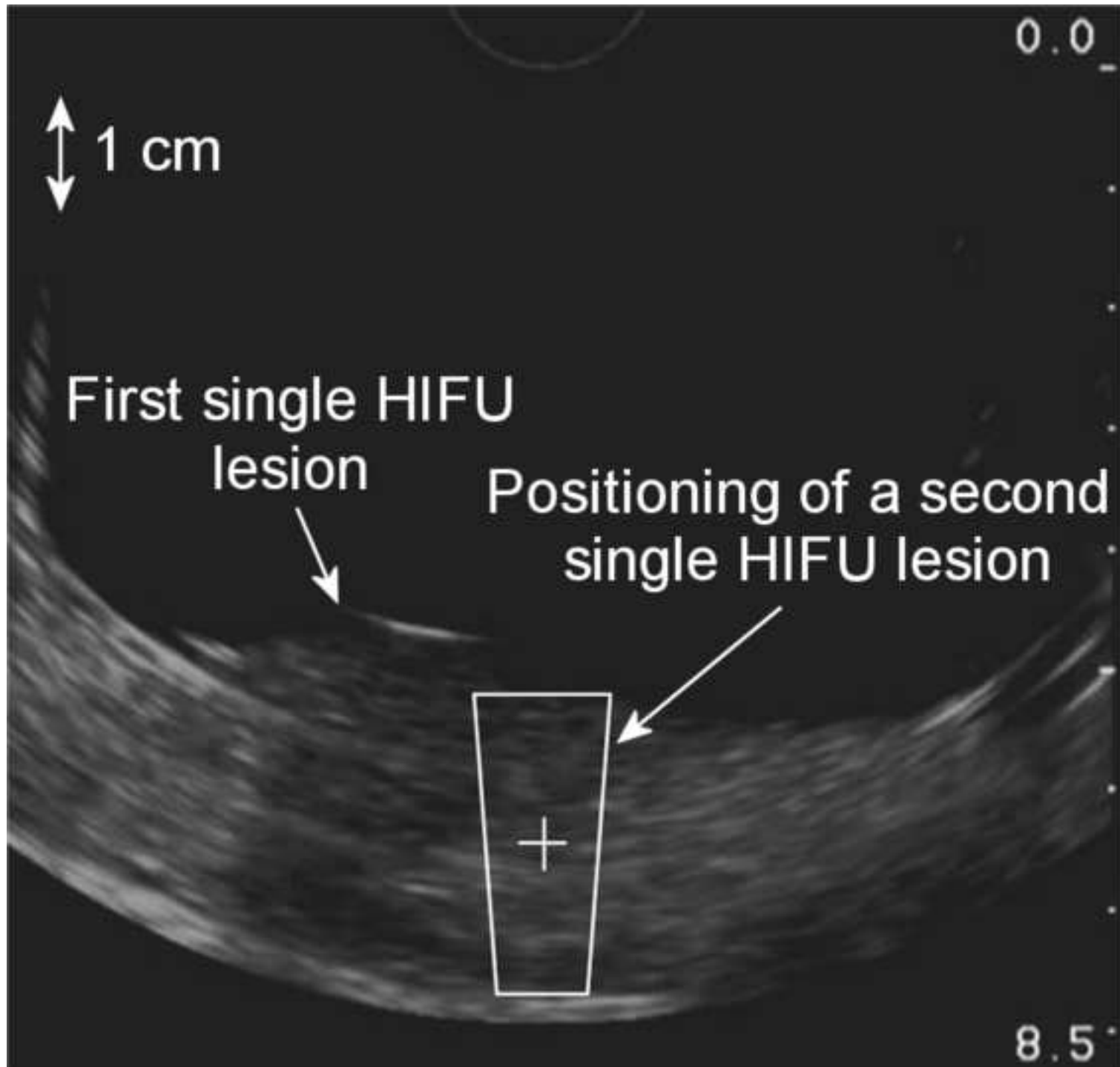


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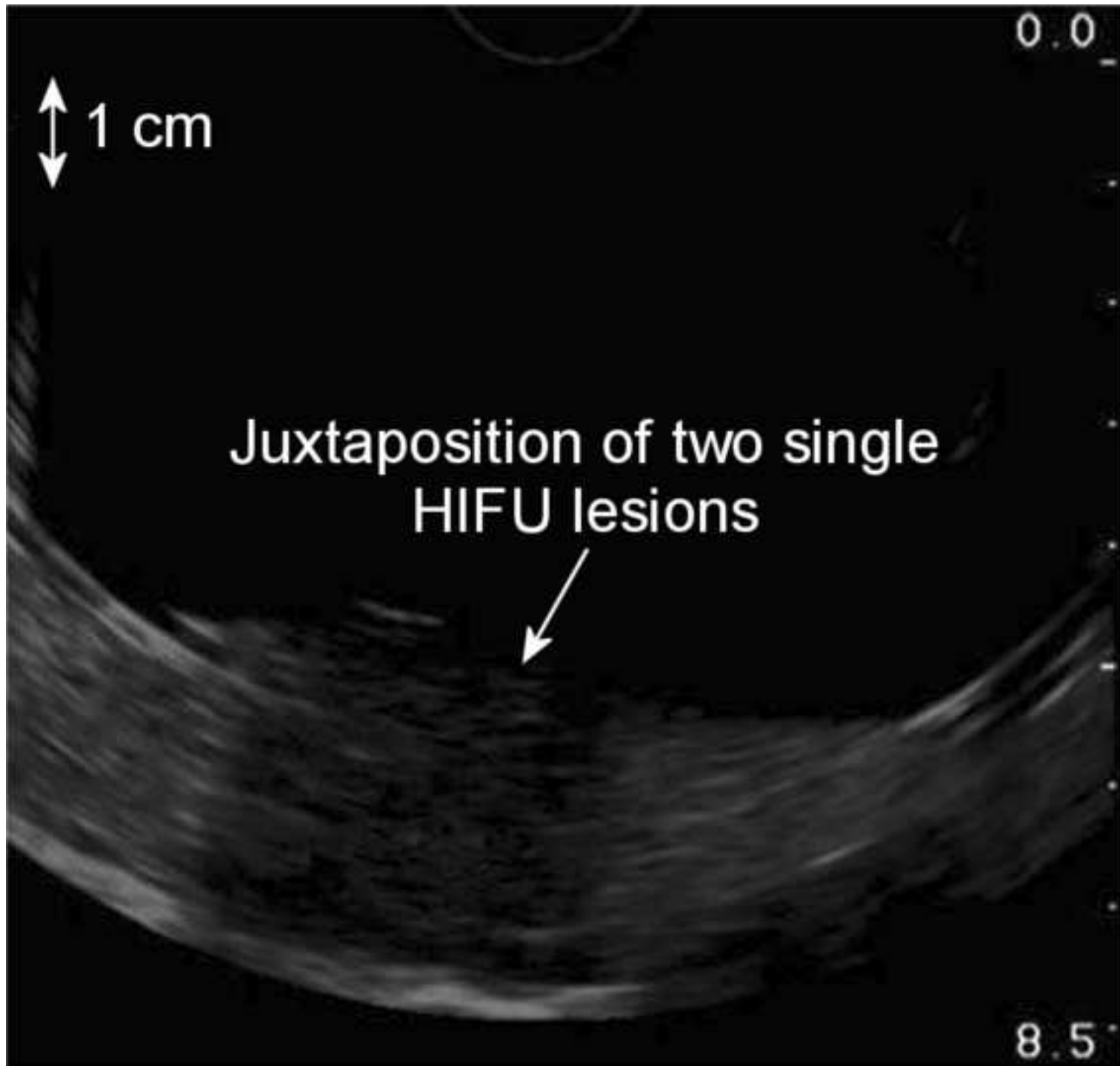


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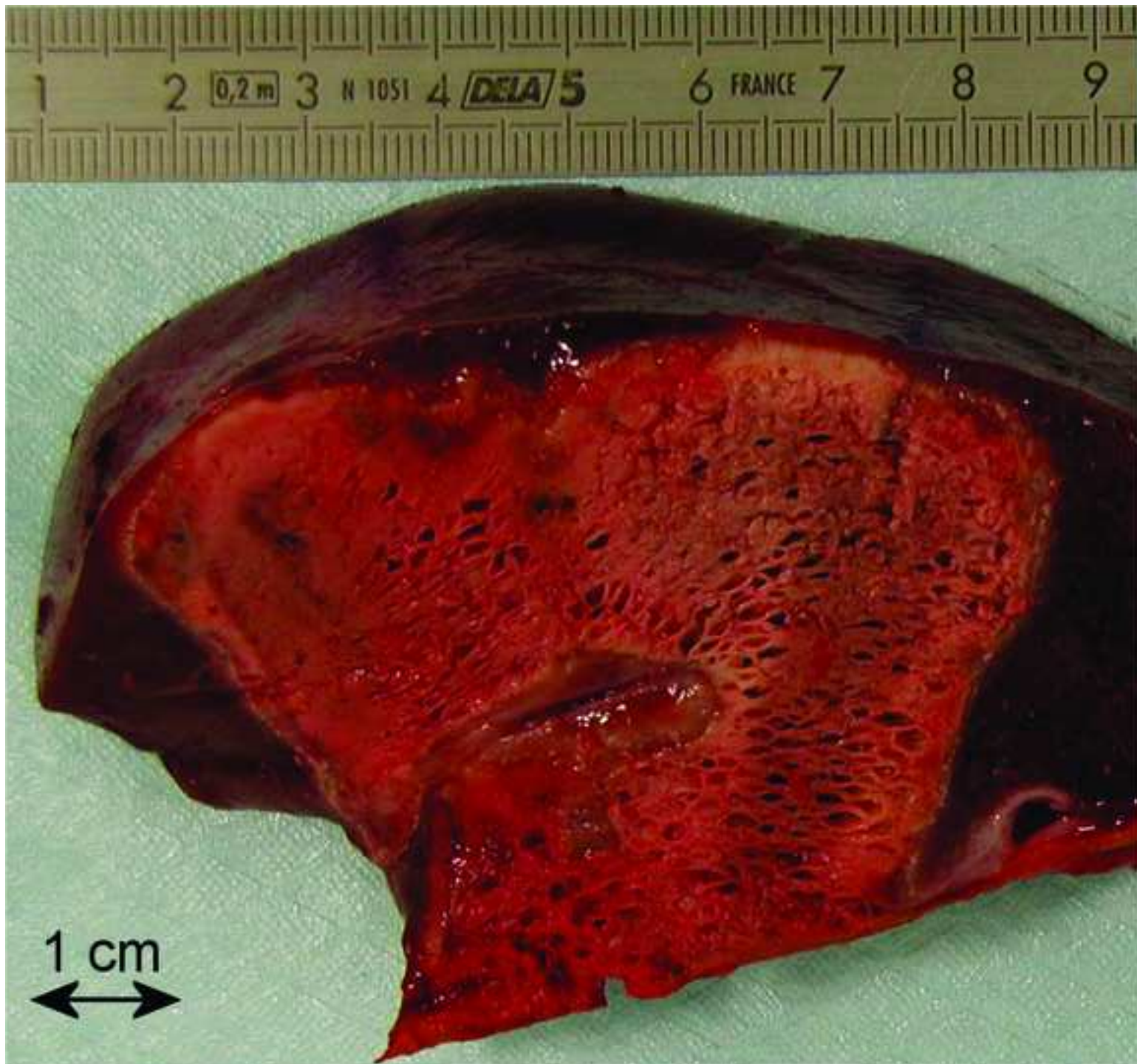


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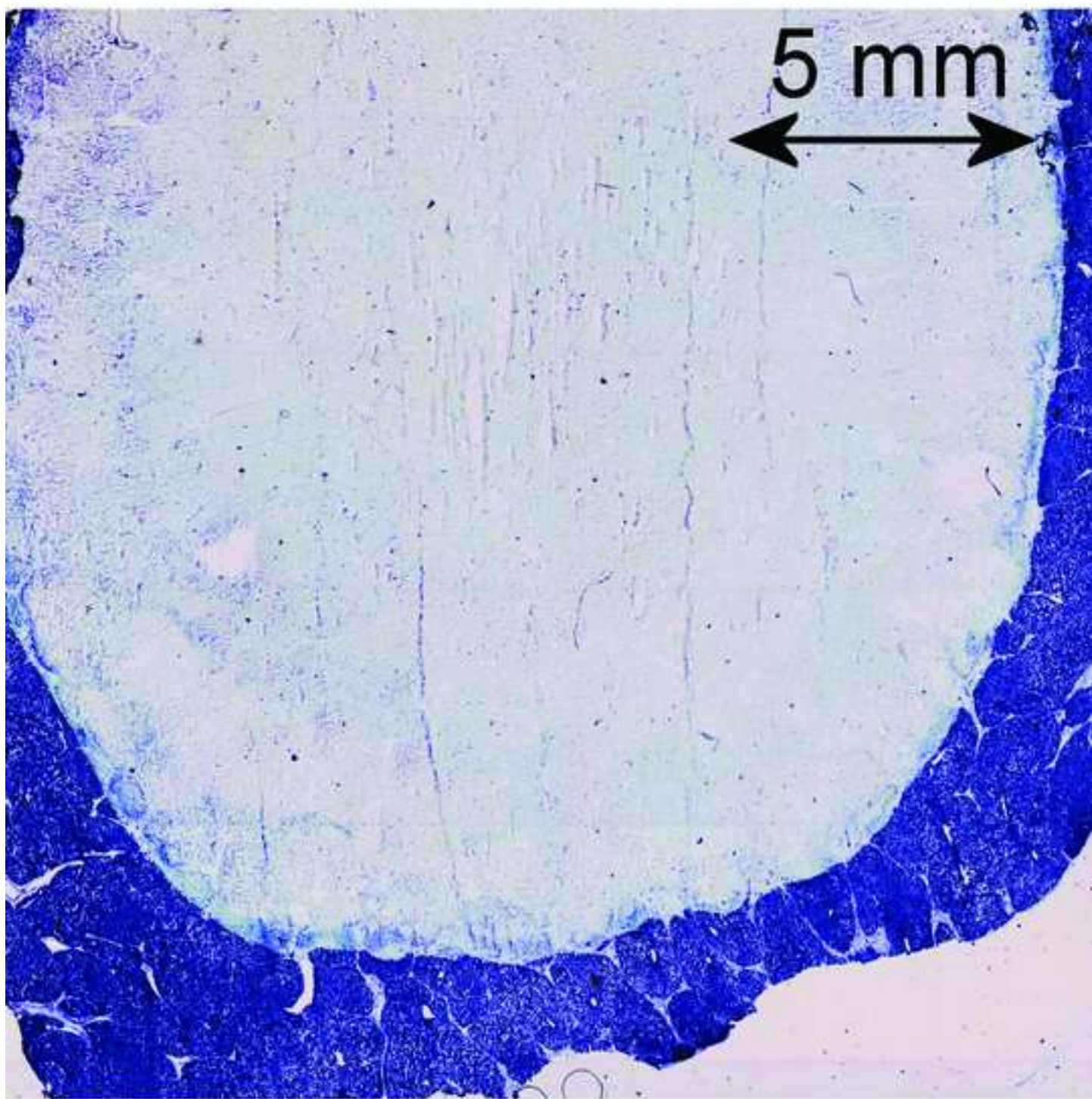
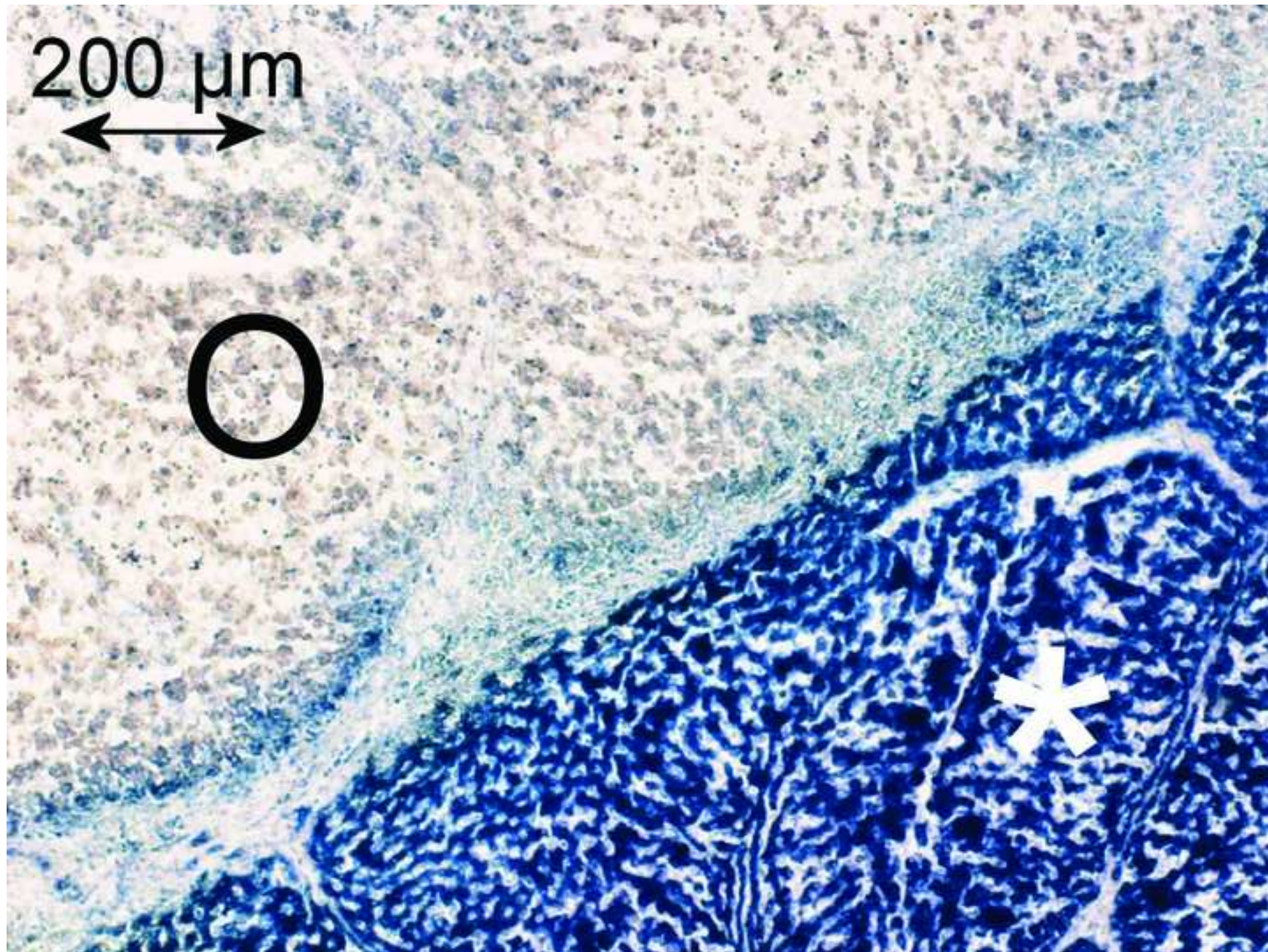


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**Figure 9a**  
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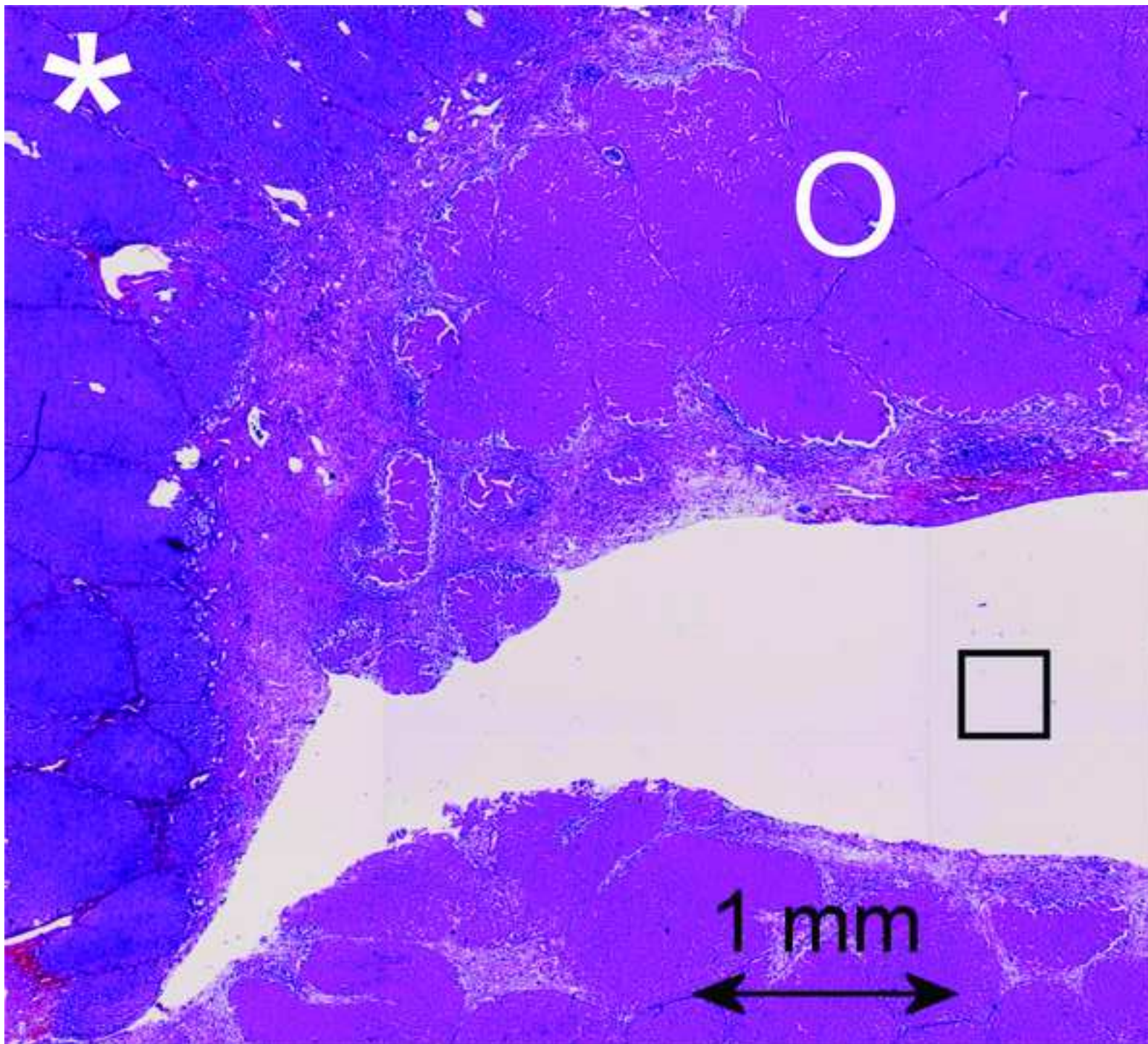
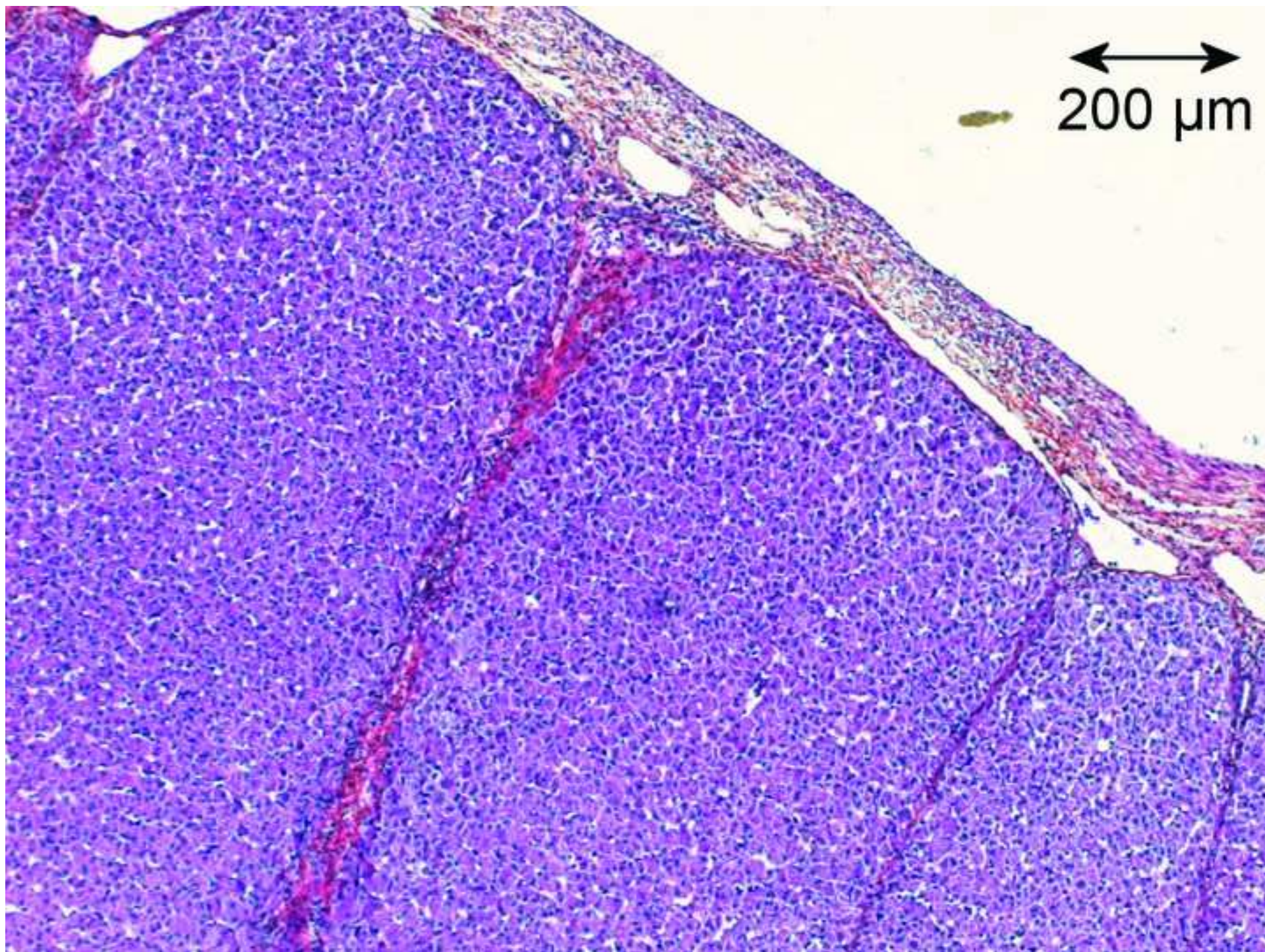


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