

Forcible intra-arterial injection of a non-adhesive liquid embolic agent under micro-balloon  
occlusion: experimental study in swine liver

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## ABSTRACT

**PURPOSE:** To evaluate the feasibility and effectiveness of transcatheter embolization by forcible intra-arterial injection of ethylene vinyl alcohol copolymer (EVAL)/ethanol mixture under micro-balloon occlusion in comparison with conventional transcatheter arterial embolization methods in non-tumoral swine liver.

**MATERIALS AND METHODS:** Nine swine were divided into three groups: embolization with EVAL/ethanol mixture (EVAL group, n=5), with ethiodized oil (ethiodized oil group, n=2) and with microspheres (microspheres group, n=2). Embolization was performed at the subsegmental hepatic artery. EVAL/ethanol mixture was injected forcibly through a microcatheter with a balloon, which was inflated to prevent backflow of the mixture during the injection. Ethiodized oil or microspheres were injected into the artery using a microcatheter without balloon occlusion. Two animals of the EVAL group were euthanized immediately after embolization, and the distribution of EVAL was assessed microscopically. The remaining seven animals were euthanized four weeks after embolization, and the histopathological changes were assessed.

**RESULTS:** All procedures were technically successful. EVAL occupied greater than 80% of the embolized hepatic arterial, portal venous and sinusoidal lumens. Four weeks after embolization, ischemic coagulation necrosis was observed in the EVAL group. In the ethiodized oil and microspheres groups, parenchymal necrosis was not observed.

**CONCLUSION:** Transcatheter embolization by forcible intra-arterial injection of EVAL/ethanol mixture under micro-balloon occlusion was feasible and achieved the simultaneous embolization of hepatic artery, portal vein and sinusoids in swine liver, resulting in complete necrosis of the embolized segment.

## INTRODUCTION

Transcatheter arterial embolization (TAE) is an effective therapeutic option for hepatocellular carcinoma (HCC)(1, 2, 3). However, local recurrence often occurs due to insufficient embolization of tumor and surrounding vessels (2, 3, 4). In hypervascular HCCs, the blood supply from feeding hepatic arteries drains into the surrounding portal venules and hepatic sinusoids which communicate with tumor blood sinusoids (5, 6, 7). It has been speculated that particulate TAE often allows the peripheral portion of the HCC to survive probably due to reversed blood flow from the hepatic sinusoids and/or portal venules surrounding the tumor. A theoretical advantage of using a liquid agent such as ethiodized oil (Lipiodol, Andre Guerbet, Aulnay-sous-Bois, France) for embolization of hypervascular HCCs is its tendency to flow into the peritumoral portal vein or surrounding hepatic sinusoids through the peribiliary plexus (PBP) (3, 8, 9, 10) and drainage routes from the tumor (7). However, the embolization effect of ethiodized oil is often temporary and thus insufficient (3, 11).

Ethylene vinyl alcohol copolymer dissolved in dimethyl sulfoxide (Onyx LES, ev3 endovascular, Inc., Plymouth MA) represents a widely used permanent liquid embolic agent (12). However, because dimethyl sulfoxide has shown vascular toxicity when injected rapidly (13), a mixture of ethylene vinyl alcohol copolymer and ethanol (EVAL/ethanol mixture) was developed by Hamada et al. and has been used for the embolization of arteriovenous malformations with favorable clinical results (14, 15). This EVAL/ethanol mixture has a nonadhesive property and is a completely liquid material that can therefore be injected forcibly through a microcatheter. Because of this unique feature, it is theoretically possible to embolize the hepatic artery, tumor blood sinusoids, portal vein and hepatic sinusoids simultaneously by forcible intra-arterial injection of the EVAL/ethanol mixture using balloon occlusion, possibly resulting in complete infarction of the tumor and surrounding parenchyma (transcatheter ablation). The purpose of this study was to experimentally evaluate the feasibility and effectiveness of transcatheter embolization by forcible intra-arterial injection of EVAL/ethanol mixture under micro-balloon occlusion in comparison with conventional TAE in non-tumoral swine liver.

## MATERIALS AND METHODS

### Animals

Nine swine (Landrace Large White and Duroc) that weighed 38-45 kg each were used in this study. All experiments were performed after permission was obtained from the Animal Experimentation Ethical Committee of Kanazawa University and according to the Animal Care Guidelines of the Committee.

### Embolic agents

EVAL/ethanol mixture is comprised of 4 g ethylene vinyl alcohol copolymer (EVAL) dissolved in 60 g iopamidol with an iodine concentration of 300 mg/ml (Iopamiron, Bayer Yakuhin, Osaka, Japan) and 36 g of 96 % ethanol as described previously (14). The EVAL consists of a 0.68-mol fraction of vinyl alcohol and a 0.32-mol fraction of ethylene, and its degree of polymerization is 1100. The EVAL can be dissolved easily in the iopamidol/ethanol mixture by heating the components to 80 degrees centigrade for 30 minutes. After the EVAL is completely dissolved, the mixture is sterilized for 20 minutes in a steam autoclave (121 °C at 11 Pa). Before use, the EVAL/ethanol mixture is re-warmed to 80 degrees centigrade for at least five minutes because EVAL precipitates at room temperature. Once the precipitate dissolves, EVAL remains in a dissolved state for at least two hours. The mixture has a low viscosity of 33.2 to 35.1 centipoise at 37 °C. When the EVAL/ethanol mixture contacts with a larger amount of blood (in larger vessels), EVAL precipitates rapidly and forms a sponge-like cast, while in smaller vessels precipitation

occurs only gradually (14). The EVAL/ethanol mixture was provided by Hamada et al. (14) who originally developed this material. It was reheated immediately before embolization.

In this study, two other embolic agents were used, namely ethiodized oil, and microspheres (Bead Block 100-300  $\mu$ m, Terumo Europe Interventional Systems, Leuven, Belgium). The microspheres were mixed with a contrast agent (Imagenil 300, Terumo) in accordance with the instructions for use. The purpose of this study was to evaluate the embolization effect of forcible intra-arterial injection of EVAL/ethanol mixture under micro-balloon occlusion in comparison with conventional TAE methods, and was not to explore the most effective embolic agents to evoke segmental infarction in the liver by the forcible injection of various kinds of embolic agents under micro-balloon occlusion. Therefore, simple intra-arterial injection of ethiodized oil and microspheres was employed as control TAE methods in this study.

### Embolization procedure

The animals were divided into three groups according to the TAE method used: embolization with EVAL/ethanol mixture (EVAL group, n=5), in which TAE was performed by forcible intra-arterial injection of EVAL/ethanol mixture under micro-balloon occlusion; embolization only with ethiodized oil (ethiodized oil group, n=2) and only with microspheres (microspheres group, n=2), both without balloon occlusion.

All animals were placed under general anesthesia after sedation with an intramuscular injection of azaperone (4 mg/kg), atropin sulfate (0.05 mg/kg), and ketamine hydrochloride (15 mg/kg). Anesthesia was induced with a nitrous oxide (2 L), oxygen (2 L), and 5 % sevoflurane mixture by inhalation. An endotracheal tube was inserted to maintain the anesthesia. Pancuronium bromide (0.1 mg/kg) was administered intravenously for muscle relaxation. Monitoring consisted of electrocardiography, invasive blood pressure measurements, and percutaneous recording of oxygen saturation.

The right femoral artery was surgically exposed, and a 6-F guiding sheath (Destination, Terumo, Tokyo, Japan) was introduced by direct puncture. Baseline arteriography of the celiac trunk, common hepatic artery and arterial portography via the superior mesenteric artery was performed with a 5-F Cobra shaped catheter (Terumo). The tip of the guiding sheath was then placed into the celiac trunk.

In all five animals of EVAL group, a 2.8-F tip microcatheter with a silicone balloon (IIGUMAN type C, Fuji Systems Corp, Tokyo, Japan) was placed into the subsegmental hepatic artery. A 0.012 inch guidewire (GT Wire, Terumo) was used to aid the microcatheter insertion. The silicone balloon of the microcatheter was inflated to prevent backflow during the forcible injection of the EVAL/ethanol mixture. The main lumen of the microcatheter was flushed with 30 % ethanol to prevent premature solidification of the EVAL/ethanol mixture. EVAL/ethanol mixture filling a 2.5 ml syringe was injected forcibly through the main lumen of the microcatheter. The progress of the embolization procedure was controlled under fluoroscopic guidance. The embolization endpoint was complete filling of the hepatic artery distal to the balloon microcatheter. In this study, an average of  $1.4 \pm 0.1$  ml of 30% ethanol was injected before the mixture injection and with the mixture.

In all animals of the ethiodized oil and microspheres groups, a 2.3-F tip microcatheter (Progreat, Terumo) was placed into the subsegmental hepatic artery. A 0.018 inch guidewire (GT Wire, Terumo) was used to aid its insertion. In the ethiodized oil group (n=2), ethiodized oil filling a 2.5 ml syringe was injected through the microcatheter. In the microspheres group (n=2), microspheres filling a 2.5 ml syringe were injected through the microcatheter. The progress of the embolization procedure was controlled under fluoroscopic guidance. The embolization endpoint was determined as the point at which stasis of arterial blood flow or the portal vein was observed.

### Handling after embolization

After embolization, celiac arteriography and arterial portography via the superior mesenteric artery were performed in all animals. Two animals of the EVAL group were euthanized immediately after the embolization procedure, and the livers were retrieved. The remaining seven animals were allowed to recover from anesthesia. The animals were monitored for fever and malaise during the follow-up period. Four weeks after embolization, the animals were placed under general anesthesia, and follow-up angiography was performed. In clinical practice, the impairment of liver function induced by TACE for HCC usually subsides around one month after TACE, and therefore four weeks' follow up period was chosen in this experimental study. The animals were euthanized and their livers were retrieved. All specimens were fixed in 10% formaldehyde for at least a week. Sections were cut into thin slices and embedded in paraffin, and stained with hematoxylin and eosin for histopathological evaluation. It has been clarified that EVAL does not dissolve during graded alcohol dehydration for the staining (14).

### Histopathological evaluation

One section from the central portion of the embolized segment including the proximal portion of the embolized segmental artery was subjected to the analysis. The numbers of hepatic arteries and portal veins filled with embolic materials were counted on the microscopic sections of four square centimeters near the embolized subsegmental hepatic artery at a magnification of 100 in the two animals of EVAL group euthanized immediately after embolization. Furthermore, the number of hepatic lobules in which embolic materials filled more than 80% of the sinusoid area was determined at the same site. The percentages of the embolized vessels and hepatic lobules were calculated by dividing the number of vessels and hepatic lobules filled with EVAL by the total number of those in the region of interest. In the four-week follow up animals (EVAL group: n=3, ethiodized oil group: n=2, microspheres group: n=2), the distribution of embolic material and the presence of tissue necrosis were assessed by light microscopy.

## RESULTS

### Overall Procedural Success

All procedures were successfully completed without technical difficulties. The microcatheter with a balloon was successfully introduced into the subsegmental hepatic artery in all EVAL group animals. No complications occurred during the procedure. No backflow of the EVAL/ethanol mixture through the balloon or extravasation of the mixture was observed. The blood pressure and oxygen saturation were stable during the embolization procedures in all animals. All animals survived the observation period (four weeks) in good health.

### Angiographic findings

The angiographic findings are summarized in Table 1. On the angiography performed immediately after TAE, both hepatic artery and portal vein in the embolized segments were occluded in the EVAL and ethiodized oil groups (Fig. 1, Fig. S1; available online). In contrast, in the microspheres group, only the hepatic artery was occluded with the portal vein remaining patent (Fig. S2; available online). On the angiography performed four weeks after TAE, recanalization of the embolized hepatic artery was not observed in the EVAL or microspheres groups but was in the ethiodized oil group. The portal vein in the embolized hepatic segment remained occluded in the EVAL group (Fig. 1), whereas it was restored in the ethiodized oil group (Fig. S1; available online). In the microspheres group, no change in portal vein visualization was observed either immediately or four weeks after embolization (Fig. S2; available online).

### Histopathological analysis

Macroscopically, the changes in the central portion of the embolized segments were homogeneous in the specimens of the two animals of the EVAL groups euthanized immediately after embolization. The EVAL was observed as a light gray material by light microscopy on hematoxylin-eosin staining. In the specimens of the two animals of EVAL group, more than 80 % of the entire lumens of the embolized hepatic arteries and portal veins were filled with EVAL (Fig. 2). Furthermore, the EVAL material was observed in 80 % or more of the area of the sinusoids in the embolized segment (Table 2, Fig. 2). There was no evidence of perivascular hemorrhage or damage to the vessels wall.

Four weeks after embolization, in all of the three animals of EVAL group, the embolized segments were severely atrophic and capsular fibrosis was observed on gross pathology (Fig. 3). Microscopically, massive ischemic coagulation necrosis was observed in the embolized segment (Fig. 3). In one of these cases, EVAL was seen to fill the hepatic arteries, portal veins and sinusoids (Fig. 3), but it was difficult to identify in the other cases because of marked parenchymal necrosis and degeneration in the embolized segments. Parenchymal necrosis was not observed in any animals of the ethiodized oil or microspheres groups (Fig. S3; available online). Microspheres were observed only in the hepatic arteries, but not in the portal vein or sinusoids (Fig. S3; available online). Ethiodized oil was not observed in any specimens. Since the numbers of animals studied were small, a statistical analysis was not performed because of the reliability lowered. However, the difference between EVAL group and the other two groups was obvious.

## DISCUSSION

Nakao et al. previously reported that combined direct embolization of both hepatic artery and portal vein induced almost complete infarction of the obstructed segment of the liver and necrosis of HCC within it (16). However, this procedure has not been widely employed because of its extreme invasiveness. To achieve embolization of both hepatic artery and portal vein less invasively, superselective (subsegmental) TACE with ethiodized oil and gelatin sponge was developed in Japan but local HCC recurrence still occurs (3, 11). To overcome this problem, introduction of a new TAE technique for relatively small HCC was intended. The results of this study showed that the forcible intra-arterial injection of EVAL/ethanol mixture into a subsegmental hepatic artery under balloon occlusion was technically feasible, and caused marked parenchymal necrosis with simultaneous embolization of the hepatic arteries, portal veins and hepatic sinusoids. Generally speaking, to induce infarction in the liver is dangerous for clinical application, but it is well known that infarction in a limited area (subsegment, etc.) of the liver can be tolerated even in cirrhotic patients (3, 11, 16).

Microspheres have been used for transcatheter arterial chemoembolization (TACE) or bland embolization to treat advanced HCC (17, 18, 19). The previous study (20) and this study showed no evidence of obstruction of the portal vein and hepatic sinusoids or parenchymal necrosis after embolization with microspheres. Microspheres travel to the distal artery and embolize the peripheral arterioles with a diameter corresponding to the particle size with out portal vein obstruction as confirmed in this study. For this reason, insufficient embolization of HCC and surrounding liver mainly due to the remaining portal venous supply from the surrounding liver is expected resulting in a relatively high local recurrence rate of HCC following TAE therapy with microspheres (17, 18).

On the other hand, as mentioned above, ethiodized oil injected into the hepatic artery can flow into the portal venules and hepatic sinusoids through PBP but cannot remain in them sufficiently long to induce complete infarction of the liver (10). In this study, this was also reconfirmed. In clinical practice, during TAE therapy, ethiodized oil is very often observed to flow into the portal vein but gradually disappears on fluoroscopic observation. When a particulate embolic agent is injected followed by ethiodized oil injection, the speed of the disappearance of ethiodized oil from the portal vein becomes slow and may induce more severe infarction. However,

in subsegmental TAE for HCCs, the inflow of ethiodized oil into the portal veins is not always adequate probably depending on individual differences in the microangioarchitecture of the liver and tumors (11).

In this study, the EVAL/ethanol mixture was injected forcibly into the vessels under balloon occlusion to achieve embolization of all intrahepatic vessels including hepatic arterioles, portal venules and hepatic sinusoids resulting in almost complete necrosis of the embolized liver parenchyma. The results obtained in this study confirmed that this was achieved. The EVAL/ethanol mixture has low viscosity, and has the advantage of having a non-adhesive property as opposed to N-butyl-2-cyanoacrylate (NBCA). Because of these features, the EVAL/ethanol mixture was considered to be able to flow into the portal vein and even into the sinusoids through the PBP. Moreover, by injecting while blocking the hepatic artery flow, the amount of blood mixed with the EVAL/ethanol mixture was decreased, and it was considered that there was an effect of prolonging the solidification time of EVAL. However, it could not be analyzed how much of the EVAL/ethanol mixture actually passed through hepatic sinusoids and reached the pulmonary capillary vessels. The oxygen saturation level was monitored and no significant deterioration was observed during the procedure, but further investigation is needed before embarking on clinical application.

Technically, by forcible injection, it was assumed that the risk of vessel rupture would increase resulting in insufficient embolization. However, the EVAL/ethanol mixture was visible by fluoroscopy, making it possible to control the injection pressure with safe injection of the embolic agent. In addition, no adhesion of the catheter to the arterial wall was observed, and it was pulled out after TAE without any problems in all animals. However, for future clinical application, it would be desirable to develop a more easily controllable way of forcible injection, as compared to manual, to achieve more consistent results and avoid under- or over-embolization.

As a fluid embolic agent, absolute ethanol has also been used in TAE for the treatment of some renal conditions, arteriovenous malformations and other pathologies in clinical practice (21, 22). It may also flow into the portal veins and hepatic sinusoids following injection into the hepatic artery and damage them, resulting in hepatic segmental infarction. The mixture of ethiodized oil and absolute ethanol was previously used in subsegmental TAE for HCCs, but the embolic effect was not superior to that of conventional TAE with ethiodized oil followed by particulate embolus without consistent segmental infarction (3). The reason why segmental infarction was not achieved might be the immediate capillary blockage induced in PBP and terminal arterioles by absolute ethanol. Therefore, TAE with absolute ethanol was not employed in this study. A small amount of ethanol was contained as a solvent in the embolic material and a small amount of 30% ethanol was injected immediately before the EVAL/ethanol mixture injection in this study. However, no definite changes typically seen in ethanol embolization such as severe endothelial damages or perivascular inflammatory changes were observed, as also reported by Hamada et al. (14, 21). Therefore, the embolic effect of ethanol in this study was considered to be minimal.

This study has a number of limitations. The sample population was relatively small and the livers were normal rather than cirrhotic or tumorous. Passage of EVAL into the pulmonary circulation was not directly evaluated. It should be noted, EVAL/ethanol was not commercially available to the investigators and its use was limited under permission of individual institutional ethics committees. However, this embolic material is essentially the same as commercially available Onyx which was however unavailable in Japan where this study was performed. Despite these limitations, this preliminary study successfully demonstrated the feasibility of the technique and the need for further evaluation.

In conclusion, the transcatheter embolization by forcible intra-arterial injection of EVAL/ethanol mixture under micro-balloon occlusion was feasible and achieved the simultaneous embolization of hepatic artery, portal vein and hepatic sinusoids in swine liver for a prolonged period (more than 4 weeks), resulting in complete infarction (coagulation necrosis) of the embolized segment.

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## FIGURE LEGENDS

Figure 1. Representative angiogram from EVAL group. (a) Base line angiogram of the portal vein shows subsegmental branches (I, II, III). (b) Common hepatic arteriography also shows three major subsegmental branches corresponding to I, II and III. (c) The subsegmental hepatic arteriogram corresponds to II. Forcible intra-arterial injection of EVAL/ethanol mixture under micro-balloon occlusion was performed from this subsegmental hepatic artery. (d) Arterial portography performed immediately after embolization shows that the portal vein in the embolized segments (II) is occluded (arrow). (e) Arterial portography performed four weeks after embolization shows that the portal vein in the embolized segments (II) remains occluded. (f) Common hepatic arteriography performed four weeks after embolization shows that the embolized hepatic artery (II) is not re-canalized.

Figure 2. Photomicrograph (Hematoxylin-eosin stain, original magnification x200) of the embolized segment from EVAL group euthanized immediately after embolization. (a) EVAL is visualized as pale pink in this staining and fills the hepatic arteries (long arrow) and portal veins (short arrows). (b) The hepatic sinusoids are diffusely dilated with diffuse EVAL filling (arrows).

Figure 3. (a) Gross specimen from the same EVAL case as seen in Figure 1 shows marked atrophy of the embolized subsegment with capsular retraction due to fibrotic changes (arrows). (b) Photomicrograph (Hematoxylin-eosin stain, original magnification x100) from the embolized segment shows a ghostly appearance of the hepatocytes indicating coagulation necrosis. EVAL fills the hepatic artery, portal vein, and hepatic sinusoids (arrows).

Figure S1. Representative angiogram from ethiodized oil group. (a) Base line angiogram of the portal vein shows two subsegmental branches (I, II). (b) Arterial portography performed immediately after embolization of the subsegmental artery corresponding to II shows that the portal vein in the embolized segments is occluded (arrow). (c) Four weeks after embolization, arterial portography shows that the embolized portal vein (II) is restored (arrow).

Figure S2. Representative angiogram from microspheres group. (a) Base line angiogram of the portal vein, shows two subsegmental branches (I, II). (b, c) Arterial portography performed immediately after embolization (b) and four weeks after embolization (c) of the subsegmental artery corresponding to II shows no obliteration of II.

Figure S3. (a) Gross specimen from the same microspheres case as seen in Figure S2 shows no evidence of atrophy or fibrosis. (b) Photomicrograph (Hematoxylin-eosin stain, original magnification x100) shows microspheres filling only the hepatic artery (short arrows), not the portal vein (long arrow), and there is no definite abnormality in the liver parenchyma.

**Table 1. Summary of angiographic findings and parenchymal necrosis**

Group	Animal No.	Follow up	Angiographic findings of embolized segment				Parenchymal necrosis
			Immediately after embolization		After follow up		
			Hepatic artery	Portal vein	Hepatic artery	Portal vein	
EVAL	1	0	Occluded	Occluded	N/A	N/A	N/A
	2	0	Occluded	Occluded	N/A	N/A	N/A
	3	4wk.	Occluded	Occluded	Occluded	Occluded	Diffuse
	4	4wk.	Occluded	Occluded	Occluded	Occluded	Diffuse
	5	4wk.	Occluded	Occluded	Occluded	Occluded	Diffuse
ethiodized oil	6	4wk.	Occluded	Occluded	Re-canalized	Re-canalized	None
	7	4wk.	Occluded	Occluded	Re-canalized	Re-canalized	None
microspheres	8	4wk.	Occluded	No change	Occluded	No change	None
	9	4wk.	Occluded	No change	Occluded	No change	None

EVAL; transcatheter arterial embolization (TAE) with a mixture of ethylene vinyl alcohol copolymer and ethanol mixture (EVAL/ethanol mixture) under micro-balloon occlusion  
ethiodized oil; TAE with simple intra-arterial injection of ethiodized oil without balloon occlusion  
microspheres; TAE with simple intra-arterial injection of microspheres without balloon occlusion

**Table 2. Vascular distribution of EVAL in microscopic specimens**

Location	Number of vessels and lobules	
	Total	EVAL materials were present
animal No.1		
Hepatic artery	348	297 (85%)
Portal vein	348	282 (81%)
Hepatic lobules	820	820 (100%)
animal No.2		
Hepatic artery	183	174 (95%)
Portal vein	177	160 (90%)
Hepatic lobules	274	274 (100%)

Number of vessels; the number of vessels where visible embolic materials were present on the microscopic sections of four square centimeters near the proximal portion of the embolized subsegmental artery  
Number of lobules; the number of hepatic lobules where the embolic materials were present more than 80% of the sinusoid area at the same site

Figure 1.

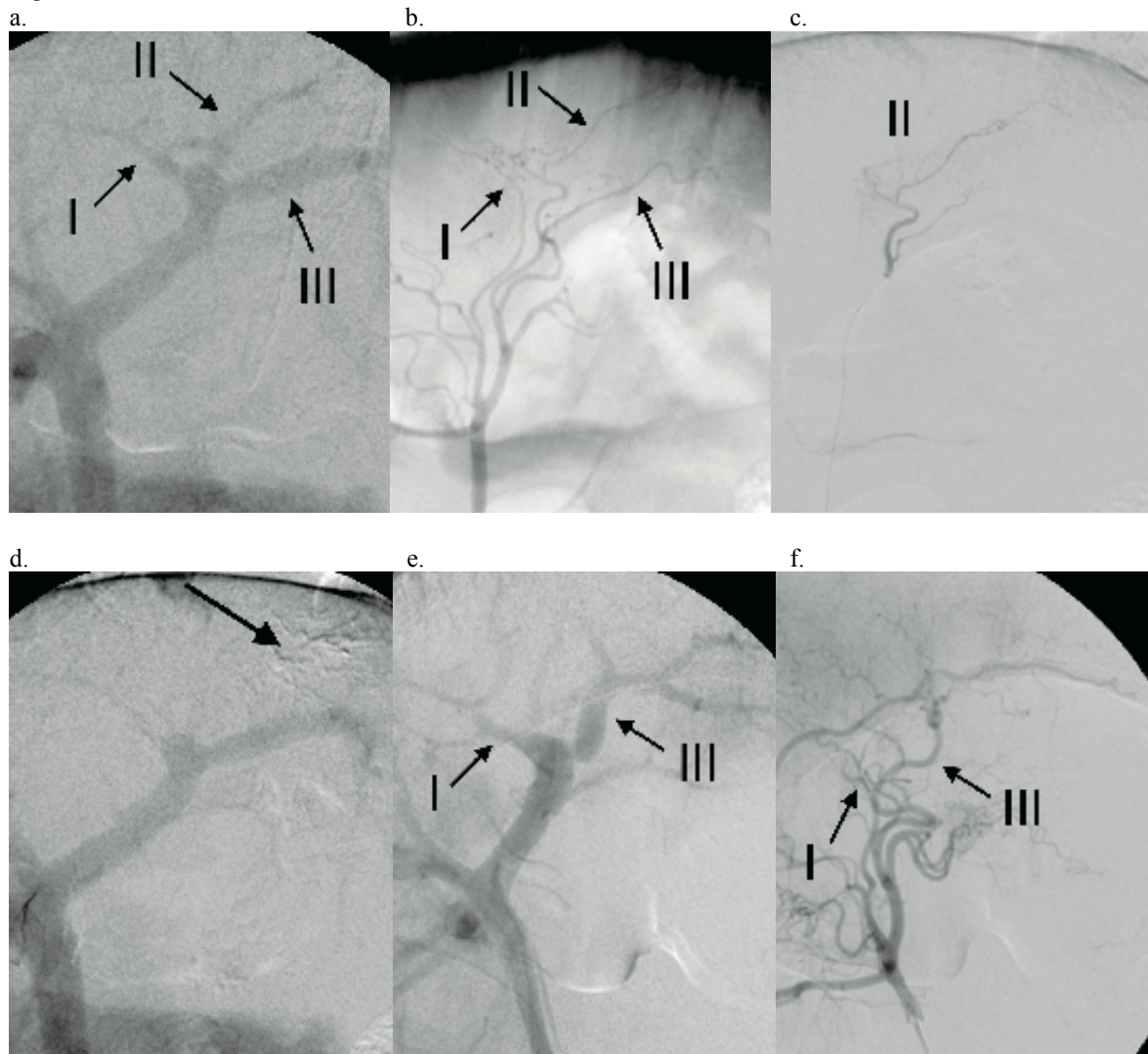


Figure 2.

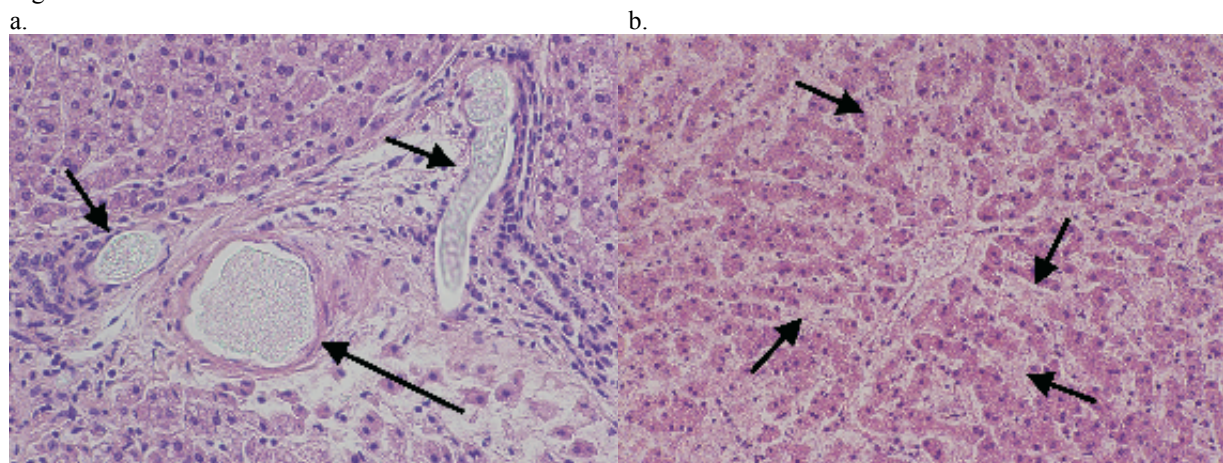


Figure 3.

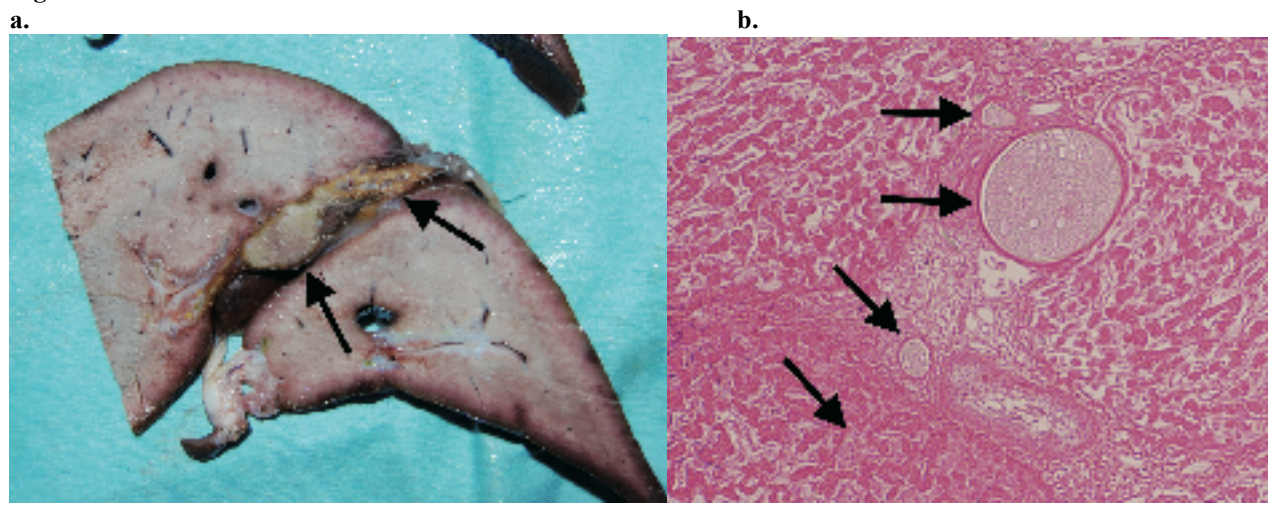


Figure S1.

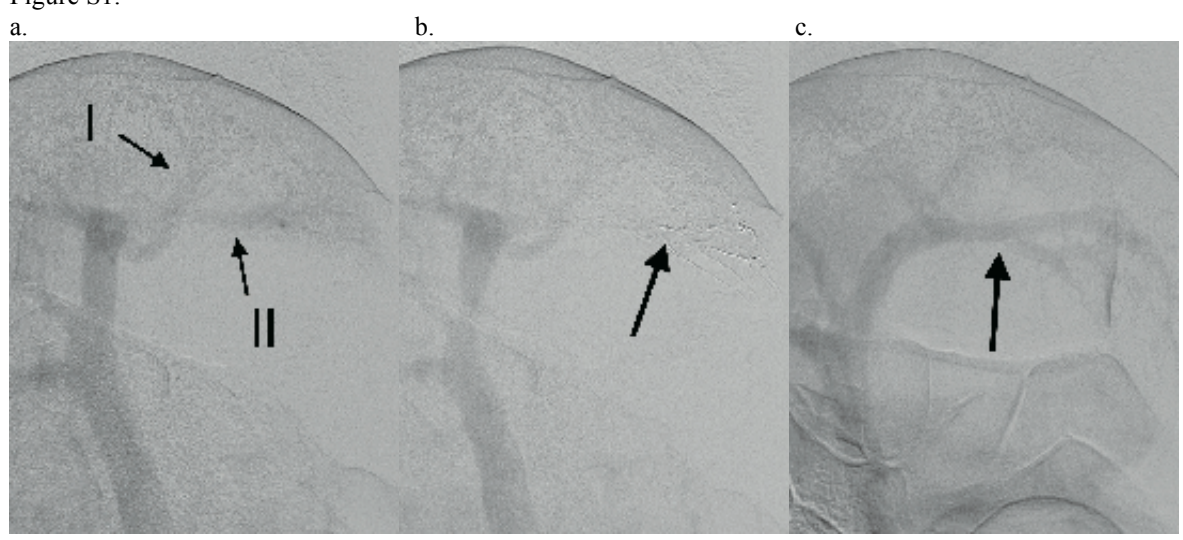


Figure S2.

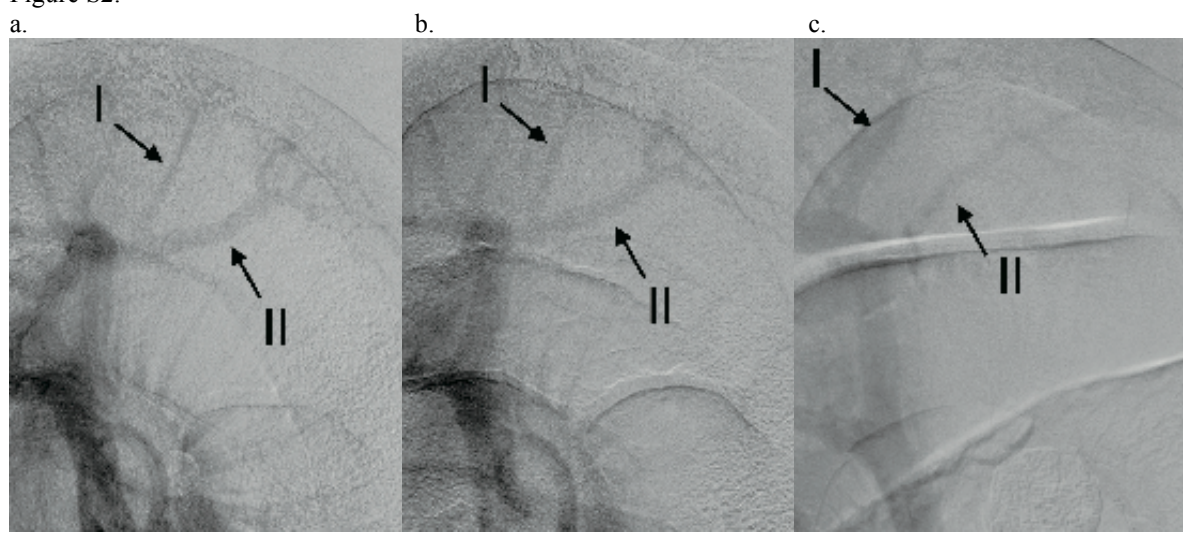


Figure S3.

a.



b.

