Ablative Liver Partition and Portal Vein Embolization (ALPPVE): Proof of Concept in a Rabbit Model

BY

Janesh Lakhoo
B.S., Northwestern University 2011
M.D., University of Illinois at Chicago College of Medicine, 2016

THESIS

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Defense Committee:

Jack Zwanziger, Chair and Advisor
Ron. C. Gaba, Department of Radiology, Division of Interventional Radiology
Charles E. Ray Jr., Chair of Department of Radiology

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LIST OF ABBREVIATIONS

ALPPS	Associated liver partition with portal vein ligation for staged hepatectomy
BRL	Biological Resources Laboratory
BSA	Body Surface Area
CLM	Colorectal cancer liver metastases
CRC	Colorectal cancer
FLR	Future liver remnant
H&E	Hematoxylin and Eosin
IR	Interventional Radiology
PHLF	Post-Hepatectomy Liver Failure
PVE	Portal vein embolization
RFA	Radiofrequency Ablation

SUMMARY

Partial hepatectomy allows definitive therapy for colorectal carcinoma liver metastases (CLM) and offers significant survival benefit. Unfortunately, not all CLM patients are candidates for hepatectomy due to inadequate remnant liver volumes that are incapable of supporting patient metabolic demands. Procedures such as portal vein embolization (PVE) and associated liver partition and portal vein ligation (ALPPS) have been successfully employed to increase the size of the remnant liver in order to expand patient candidacy for hepatectomy. However, these procedures are not without limitations. While PVE is minimally invasive and safe, it results in slow growth rates and limited growth volumes, and although ALPPS confers rapid growth rates and large regeneration volumes, it is limited by high morbidity and mortality rates by virtue of surgical invasiveness. Theoretically, a combination of these two procedures aimed at retaining the minimally invasive nature of PVE while exploiting the regenerative capacity of ALPPS may significantly enhance patient care by allowing high future liver remnant growth rates while maintaining low adverse events. This study examined the feasibility and effectiveness of a modified combination approach utilizing PVE and microwave ablation in a pre-clinical rabbit model.

In this Animal Care and Use approved study, New Zealand white rabbits were separated into 2 cohorts that underwent PVE alone or PVE with associated microwave ablation (ALP-PVE). Embolization of 3 of the 4 lobes of the rabbit liver was achieved utilizing 100-300 µm embolic spheres, and various coils while microwave ablation was achieved utilizing a commercially available device. Animals were sacrificed at 7 days post-procedure. The animal livers were harvested and massed immediately and after

drying for 4 weeks at 60 ºC and the corresponding masses were compared between groups. Immune histochemical analysis utilizing Ki-67 antibody staining for actively replicating hepatocytes was performed to compare hepatocyte hyperplasia between liver lobes between cohorts. Statistical comparison was performed using the one-tailed Student's t-test with p-value less than 0.05 considered significant.

The results of the current study showed that ALP-PVE results in larger growth of the FLR compared to PVE alone though not significantly, and significantly increased hyperplasia. As a proof of concept study, it provides evidence that the proposed procedure may have clinical benefit compared to PVE alone, allowing more patients to become eligible for hepatic resection for CLM. Further pre-clinical studies are required to further evaluate the feasibility of this procedure.

I. INTRODUCTION

A. Background

According to the American Cancer Society, approximately 136,830 Americans are diagnosed with colorectal cancer (CRC) annually, and 50,310 succumb to this disease yearly (1). Many such patients will develop metastatic liver disease, which is associated with significant mortality: 10-25% of CRC patients present with synchronous colorectal carcinoma liver metastases (CLM), 25-50% of CRC patients develop CLM during follow-up, and two-thirds of patients with CLM will die of metastatic liver disease (2, 3). The overall 5-year survival rate of individuals who undergo resection for colorectal liver metastases is around 25-40% (2) compared to approximately 11% for those treated with chemotherapy (4), making surgical resection the definitive treatment of choice. Unfortunately, only 10-20% of patients with CLM are candidates for surgical resection due to high disease burden, unfavorable operative anatomy, or insufficient liver volumetric reserve (5).

Patients with insufficient liver volumetric reserve are at high risk for developing post-hepatectomy liver failure (PHLF) (6), which is the result of inadequate capacity of remnant liver to support synthetic, excretory, detoxification, and other metabolic demands (7). Individuals with post-resection remnant liver volumes less than 20-30% have a higher incidence of significant post-surgical morbidity and mortality (8). In order to avert this complication and increase the number of patients eligible for safe resection, the future liver remnant (FLR) volume may be increased using one of two procedures: portal vein embolization (PVE), or more recently, associated liver partition and portal

vein ligation (ALPPS). PVE, the current standard of care for FLR growth (9), involves image-guided embolization of portal vein branches supplying the liver segment to be resected, spurring FLR growth between 8-27% over 2-60 days mediated by an increase in flow of blood and trophic factors (10). This growth effectively increases the number of individuals eligible for resection without worsening survival (5). There are multiple techniques for performing PVE using many different embolic materials, although none have demonstrated superiority (11).

ALPPS is a two-stage surgical procedure. Stage 1 involves dissection of the liver and vasculobiliary structures followed by hepatic parenchymal transection and ligation of the portal veins supplying the segments to be resected. Stage 2 involves hepatic resection after adequate FLR growth (12). Other authors have found that ALPPS results in rapid growth rate of between 40-80% over 6-9 days (13, 14). This rapid rate of growth has been credited to an increase in circulating growth factors as well as prevention of the formation of inter-lobar collateral blood vessels between the FLR and segment to be resected via complete liver transection during stage 1.

B. Study Rationale

While PVE is the current standard of care for preoperative FLR growth, not all patients who undergo PVE are candidates for resection either due to inadequate FLR growth or due to interim tumor growth because of the time required for FLR hypertrophy post-PVE (5). In a review of 1,791 patients from 44 studies between 1990 and 2011, van Lienden et al found that 51 (2.8%) patients had insufficient hepatic hypertrophy for resection, 6.1% had local tumor progression or newly developed metastases in the FLR

preventing resection despite adequate FLR growth, and 8.1% had extra-hepatic tumor spread after PVE precluding resection (5). An updated technical PVE approach that enhances liver hypertrophy over a shorter time frame may increase the number of patients who achieve or retain eligibility for subsequent hepatic resection.

ALPPS, on the other hand, results in greater FLR growth compared to PVE, but has high reported morbidity and mortality rates approximating 16-64% and 12-23%, respectively (10). The requirement for laparotomy for the first step may also render the second step of the operation more difficult secondary to peritoneal adhesions though this is limited due to earlier intervention. These drawbacks make ALPPS less favorable for use despite rapid growth rates. Furthermore, unlike PVE, there is also no long-term survival data available for ALPPS as most patients have not reached the 5-yr follow-up time point as of yet.

C. Study Objective

The broad, long-term objective of this research is to improve the survival of patients with metastatic colorectal carcinoma (CRC) by increasing the number of patients eligible for curative surgical resection. To achieve this, the current study aims to test the feasibility of a new procedure, ablative liver partition and portal vein embolization (ALP-PVE), that combines portal vein embolization with microwave ablation, and to compare the FLR mass after ALP-PVE compared to PVE alone in a rabbit model.

D. Significance and innovation

The approach to be employed in the current study is both significant and innovative. Development and growth of techniques that may allow definitive therapy for CLM are vitally important given the high incidence of CRC and CLM in the United States. The current proposal aims to utilize microwave ablation in a novel, previously unutilized fashion to provide a minimally invasive method of liver partition. It will theoretically have lower rates of morbidity and mortality compared to ALPPS because of its minimally invasive nature, while having improved rate of FLR growth compared to the current standard of therapy, PVE.

II. **CONCEPTUAL FRAMEWORK AND RELATED LITERATURE**

A. **Conceptual Framework**

This study is a proof of concept study looking to verify that the proposed procedure, ALP-PVE, achieves greater FLR growth than PVE alone within the same time period. The intervention, PVE vs. ALP-PVE, is the independent variable in this study and the outcome variable of interest is the mass of the embolized and non-embolized liver lobes. The difference in ratio of the mass of the non-embolized:whole liver between the two cohorts is the unknown. A higher ratio of non-embolized:whole liver lobes in the ALP-PVE cohort compared to the PVE cohort would favor the proposed procedure and provide evidence of its efficacy over PVE, while similar ratios, or a lower ratio would argue that PVE alone is sufficient. Of note, liver mass will be utilized as the primary outcome measure herein as opposed to liver volume given that it is a direct measure (in contrast, computed tomography measured liver volume is an indirect measure) that confers the ability control for edema as a cause of increased lobar size. Moreover, liver mass is directly related to liver volume given the known density of mammalian liver tissue measuring approximately 1.03 g/mL (15). Secondary outcomes such as immunohistochemical analysis would be performed to provide evidence of hypertrophy and hyperplasia and would serve to corroborate the primary outcomes.

B. Review of Related Literature

1. Future Liver Remnant and Post Hepatectomy Liver Failure

There is a not insignificant risk of developing PHLF with Mullen et al reporting rates of hepatic insufficiency up to 8% (6). One of the main determinants of developing PHLF is the presence of adequate FLR, which can be measured pre-operatively utilizing CT volumetry (16, 17). The size of the FLR required depends on the size of the individual (measured as body surface area (BSA)) as well as whether or not the individual has any underlying liver parenchymal disease such as cirrhosis, hepatitis or steatosis (16). The minimum safe FLR volume required to prevent PHLF in patients with normal underlying liver is 20% while those with a diseased liver require 40% of total liver volume (17). Any patient with an anticipated FLR below these cut-offs should be considered for preoperative FLR hypertrophy.

Similar to previous studies performed by van den Esschert et al. in which FLR hypertrophy was compared between PVE and portal vein ligation, liver mass between the two cohorts in this study will be used instead of CT volumetrics to determine differences in liver lobe size after intervention (18).

2. Portal Vein Embolization and Hypertrophy

Portal vein embolization is the current standard of therapy for preoperative future liver remnant hypertrophy with patients being selected based on preoperative CT volumetry (9). Portal vein embolization is performed by gaining access into the portal venous system either transvenously via the femoral vein or percutaneously through the

liver, selecting the portal vein branch to be embolized, and injecting embolic particles as well as larger embolic coils or plugs to occlude the vein. The strategy and location of PVE is determined based on the type of surgery planned and on the portal venous anatomy of the patient (19). Many different embolization materials including but not limited to n-butylcyanoacrylate, lipiodol, embospheres, and coils have been utilized for PVE however, there are no large clinical studies comparing the effect of different embolization materials on hypertrophy response in humans (5). Another factor that influences hypertrophy rates post embolization is liver parenchymal health. Patients with underlying liver disease tend to have decreased hypertrophy compared to those with healthy livers. Prior chemotherapy exposure, however, does not seem to influence hypertrophy rates (5).

3. Associated Liver Partition and Portal Vein Ligation (ALPPS)

ALPPS is an alternative surgical procedure compared to PVE that results in faster rates of FLR hyperplasia and hypertrophy allowing for earlier hepatectomy compared to PVE (12-14). Growth rates of between 40-80% over 6-9 days have been documented in the literature (10, 13, 14). It is performed as a two-stage procedure. The first stage involves dissection of relevant hepatic anatomy including the common bile duct, portal veins, and hepatic arteries followed by total or near total parenchymal dissection and subsequent ligation of the portal vein supplying the lobes to be resected. After appropriate FLR hypertrophy has occurred as measured by CT volumetry, the patient undergoes a second surgical procedure in which the liver lobe is then resected (12). ALPPS however has very high morbidity (16-64%) and mortality (12-23%)

incidence because it subjects patients to two surgical procedures (10). Denys et al and other authors have attributed the accelerated hypertrophy caused by ALPPS to the complete liver partitioning achieved, which prevents the formation of inter-lobar collateral blood vessels between the FLR and the liver segments to be resected. These collaterals are thought to limit the benefits of portal vein ligation or embolization by continuing to provide blood supply to the liver segments to be resected (20-22).

4. Microwave Ablation

Microwave ablation, which is commonly used to treat unresectable hepatic tumors (23, 24), is a thermal ablation technique that focally heats tissues to cause coagulative necrosis with eventual scar formation (25, 26). Approaches for treatment are flexible and include percutaneous, laparoscopic, and open surgical access. Once the optimal approach is determined, a microwave antenna is placed directly in the tumor and electromagnetic microwaves are emitted with a frequency between 900 and 2450 MHz at a power of up to 60W. The microwaves interact with water in the tissue resulting in rapid flipping of the molecules, which in turn results in a temperature increase (26). The size of the ablation zone can be controlled by adjusting the power, time of ablation, and number of antenna used.

Microwave ablation has many advantages compared to radiofrequency ablation (RFA), another thermal ablative technique. These include higher intratumoral temperatures within a shorter duration, larger tumor ablation zones, the ability to use multiple antennae simultaneously, an improved convection profile and because no

grounding pads are required (26). Microwave ablation also does not seem to be limited by charring and tissue desiccation.

The coagulative necrosis produced by microwave ablation has the potential to disrupt the inter-lobar collateral vascularization and could theoretically be used to create a non-invasive partition between the liver lobes similar to ALPPS, but without physical parenchymal separation.

5. Rabbit Model

New Zealand white rabbits represent a suitable preclinical model given useful attributes, including small size, easy maintenance, favorable anatomy, rapid hepatic hypertrophy post-PVE, and prior literature evidence of successful portal vein embolization (18, 27). Anatomically, the rabbit liver is divided into four lobes: the right lateral, left medial, left lateral, and caudal lobes (Fig. 1). The former 3 are collectively described as the cranial lobes. The caudal lobe is separated from the cranial lobes making it easily distinguishable and also has distinct portal venous branches that may be successfully isolated from embolization.

6. Ki-67: Immune Histochemistry

Ki-67 is a nuclear protein whose function has not been clearly elucidated but that is associated with ribosomal RNA transcription (28, 29). It is present in replicating cells during the active phases of the cell cycle (G_1, S, G_2, and mitosis), but is not present in resting cells (G_0) (30) and is thus an ideal marker for identifying

proliferating cells. Ki-67 has been utilized in the literature for tumor diagnostics, particularly for prostate and breast carcinomas (30). van den Esschert et al and other authors have also utilized it to evaluate differences in liver hyperplasia after PVE and portal vein ligation (18, 31).

III. **MATERIALS AND METHODS**

A. Design

This study was funded by an internal grant from the University of Illinois Hospital and Health Sciences System Department of Radiology, a grant from the Radiological Society of North America (RSNA) (Medical student grant RMS1525), and grant support from Neuwave Medical in the form of device transfer. Animal Care and Use Committee approval was obtained for this prospective study (Protocol 15-022). The experimental protocol employed disease free, 2.6-3.0 kg New Zealand white rabbits as subjects. These animals represent a suitable pre-clinical model given useful attributes including small size, easy maintenance, favorable anatomy and rapid hepatic hypertrophy post-PVE (27). Anatomically, the rabbit liver is divided into four lobes (Figure 1), with the caudal lobe being separated from the three cranial lobes making it easily distinguishable. The caudal lobe has its own distinct portal venous branch allowing it to be successfully catheterized, and excluded during embolization. The protocol included: (a) intervention, (b) animal sacrifice and liver explantation, and (c) liver histological and immunohistochemical analysis to qualitatively compare degree of cellular hyperplasia. The study consisted of two treatment arms: PVE alone vs. PVE with microwave ablation. Eight rabbits were included in each cohort, and were sacrificed at 7 days post-intervention.

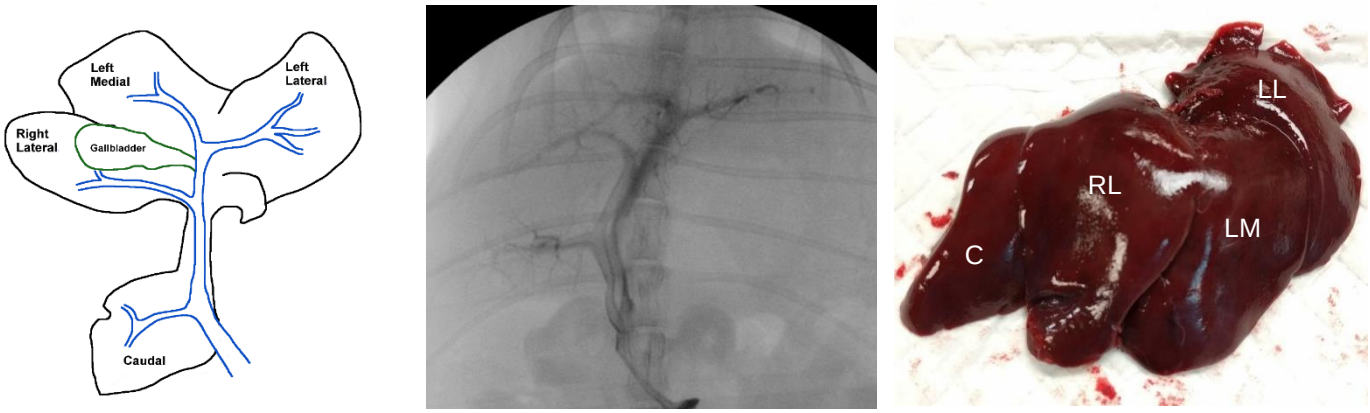

Figure 1. Rabbit Liver Anatomy. On the left is a pictorial diagram depicting rabbit liver anatomy. In the middle is a fluoroscopic image highlighting the portal venous supply to the liver. On the right is an explanted rabbit liver. C = caudal, RL = right lateral, LM = left medial, LL = left lateral.

Given that mean FLR growth post PVE and post ALPPS ranges from 8-27% and 40-80%, respectively (10), the maximum and minimum differences that could occur between the study groups is 70% and 13%, respectively. Using this information, and aiming for 80% power with significance defined as $P < 0.05$, a minimum of 4 and a maximum of 356 subjects would be required per group based on sample size calculation for proportion based data. The current study utilized 8 rabbits per group for a total of 16 animals. This study is underpowered as a difference in FLR growth in the range of 70% was not detected. However, this study was a feasibility and proof of concept study that has provided more information on the expected effect size for future studies.

B. Operative Procedures

Animals were placed under anesthesia and monitored during anesthesia by the trained veterinary staff of the Biological Resources Laboratory (BRL) at the University of Illinois in Chicago.

Operative procedures were performed according to the methodology described by van den Esschert et al (18). A mid-line subxiphoid laparotomy was performed under aseptic technique to access the peritoneal cavity. Loops of small bowel were then reflected out of the body and a branch of the inferior or superior mesenteric vein was cannulated with an 18-gauge catheter (B. Braun; Bethlehem PA) (Figure 2). Venography was performed at this point utilizing 1-2 cc of Omnipaque 300 (General Electric Healthcare; Chicago IL) under fluoroscopy utilizing a Phillips C-arm (Phillips BV Pulsera; Phillips; Andover MA) in order to delineate the portal venous system. A 3-French Renegade™ STC microcatheter (Boston Scientific; Natick MA) was inserted subsequently and advanced under fluoroscopy utilizing a guidewire (Covidien; Plymouth MN) into the portal vein branch to the cranial liver lobes (supplying approximately 80% of the total liver volume), bypassing the portal branch to the caudal liver lobe, which will serve as the FLR. Venography was performed (Figure 3) to evaluate positioning and was followed by injection under real-time fluoroscopic observation of 100-300 µm Embospheres (Merit Medical; Salt Lake City UT) followed by either 3-4 mm micronester coils (Cook Medical; Bloomington IN) or interlock coils (Boston Scientific; Marlborough MA) to an angiographic endpoint of no further antegrade blood flow (Figure 4). Coils were selected intraprocedurally based on the size and anatomy of the portal vein. Animals in the PVE group (n=8) underwent only PVE while animals in the ALP-PVE

cohort (n=8) then underwent microwave ablation immediately after embolization. The cranial liver lobes were retracted, and the caudal cranial liver lobe was identified utilizing location and appearance for guidance (see Figure 5). The portal vein supplying the caudal lobe was used as a landmark to positively identify the liver parenchymal bridge between the caudal and cranial lobes. A microwave ablation device (LK Microwave antenna, Neuwave Medical; Madison, WI) was then used to create a 1 cm ablation zone between the caudal liver lobe and the cranial liver lobes. Two ablations were performed on either side of the portal vein at 40W for a total of 1 minute each to ensure adequate ablation of the parenchymal bridge between the cranial and caudal lobes (Figure 6). Optimal ablation power and time was determined by bench test on fresh explanted chicken livers. After injection and ablation, all devices were removed and the canalized vessel was ligated with silk suture. After assessing for hemostatic control, the abdominal incision was closed in two layers using absorbable Vicryl suture (Ethicon, Somerville NJ) to seal the subcuticular tissue and non-absorbable Ethilon nylon suture (Ethicon, Somerville NJ) to seal the skin. The animals were aroused and recovered from anesthesia, returned to their cages, and followed-up daily until their respective time of sacrifice.

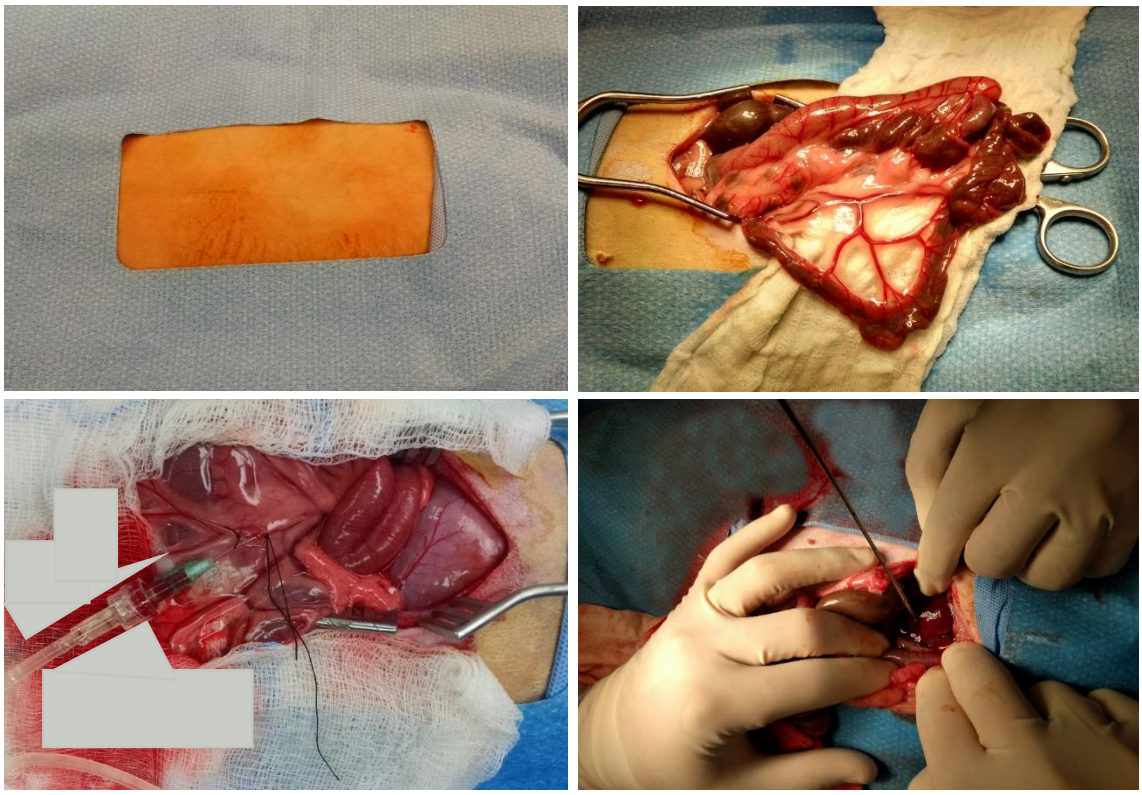

Figure 2. Procedural Overview. (A) Sterilely draped rabbit abdomen. (B) Midline incision with reflected bowel loops. (C) Reflected bowel with 18-gauge needle cannulating branch of mesenteric vein. (D) Ablation probe in liver parenchymal bridge between cranial and caudal lobes.

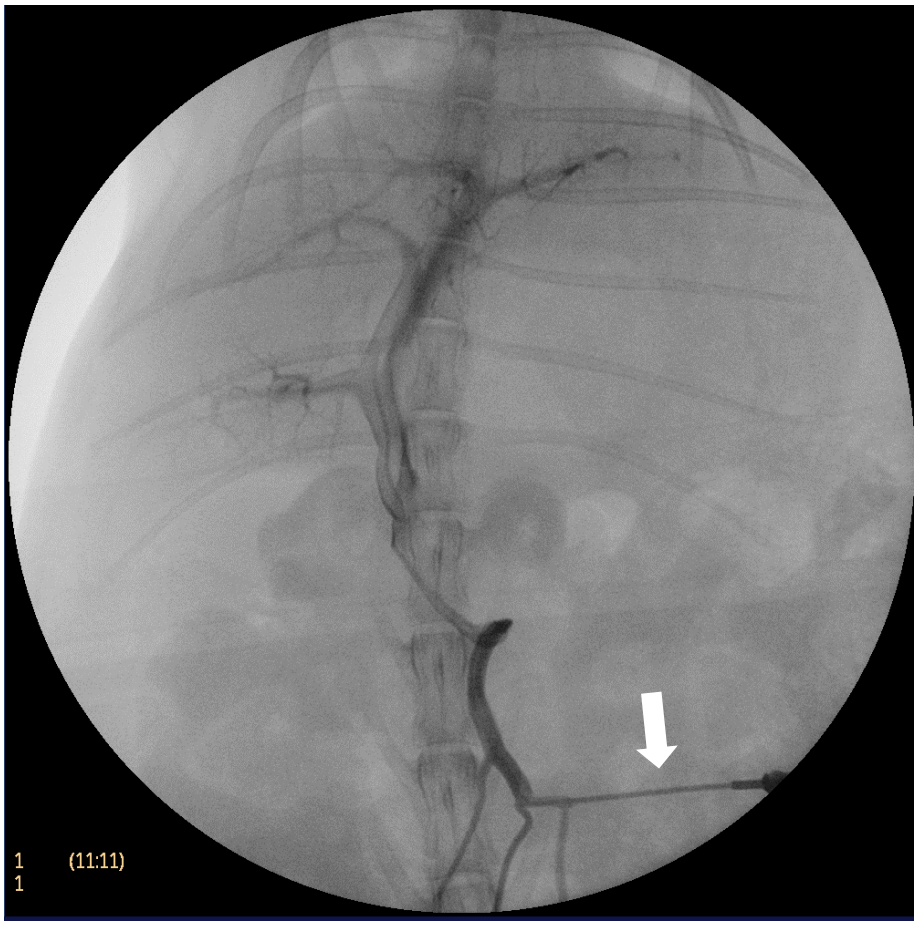

Figure 3. Venography of Portal Venous System. Venogram outlining the portal veins supplying the rabbit liver. Needle (white arrow) used to cannulate a branch of the mesenteric veins can be seen.

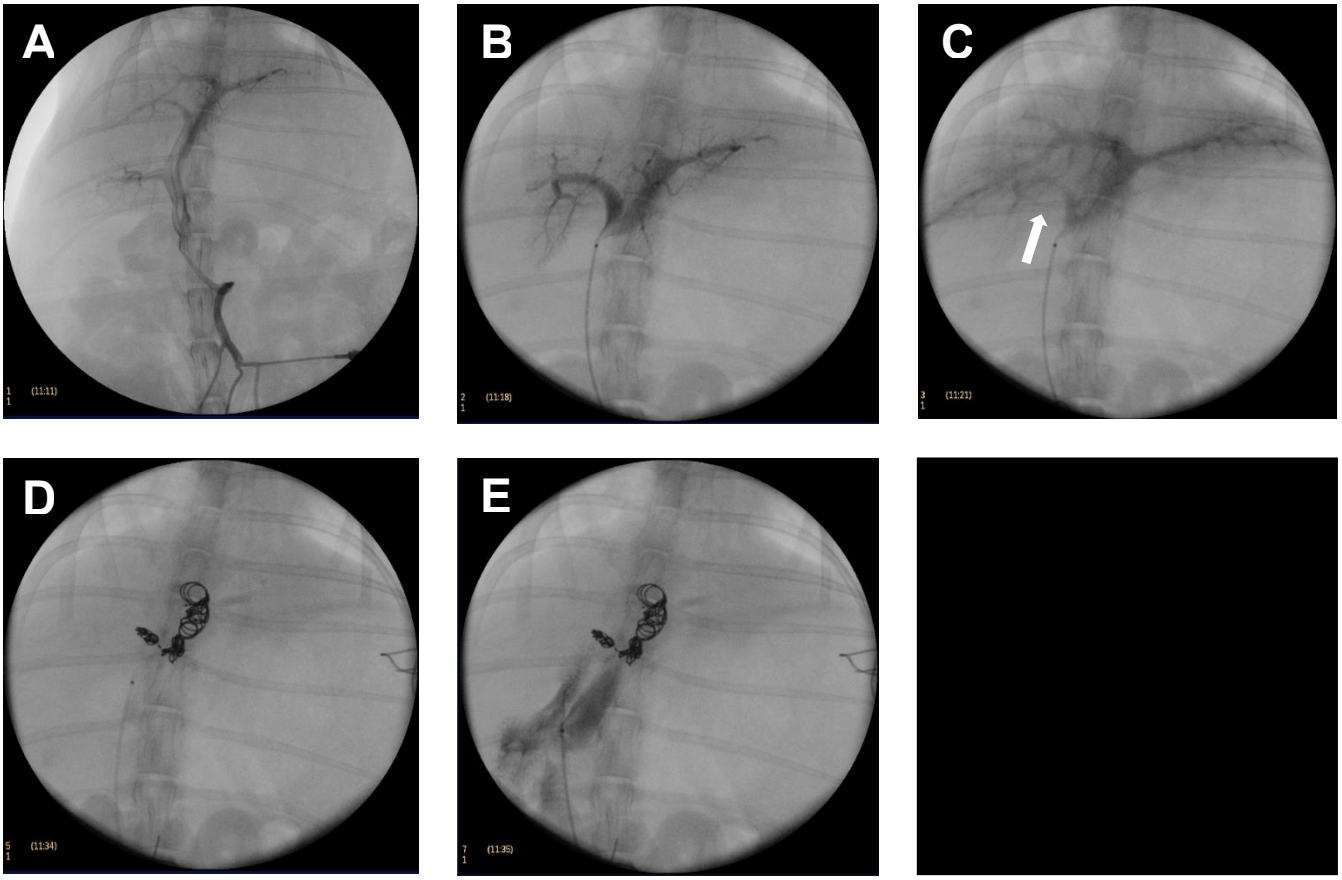

Figure 4. Embolization of Cranial Liver Lobes. (A) Venogram of portal veins. (B) Initial delivery of 100-300 µm Embospheres. Embosphere injection can be visualized as they were mixed with contrast material. (C) End of embosphere administration. Right lateral lobe (white arrow), which initially was not filling with spheres is now visualized. (D) Coils are placed in portal vein supplying cranial lobes to complete embolization. In this particular case, and additional coil was placed in the portal branch supplying the right lateral lobe to achieve complete embolization. (E) Endpoint of embolization was lack of antegrade flow past coils.

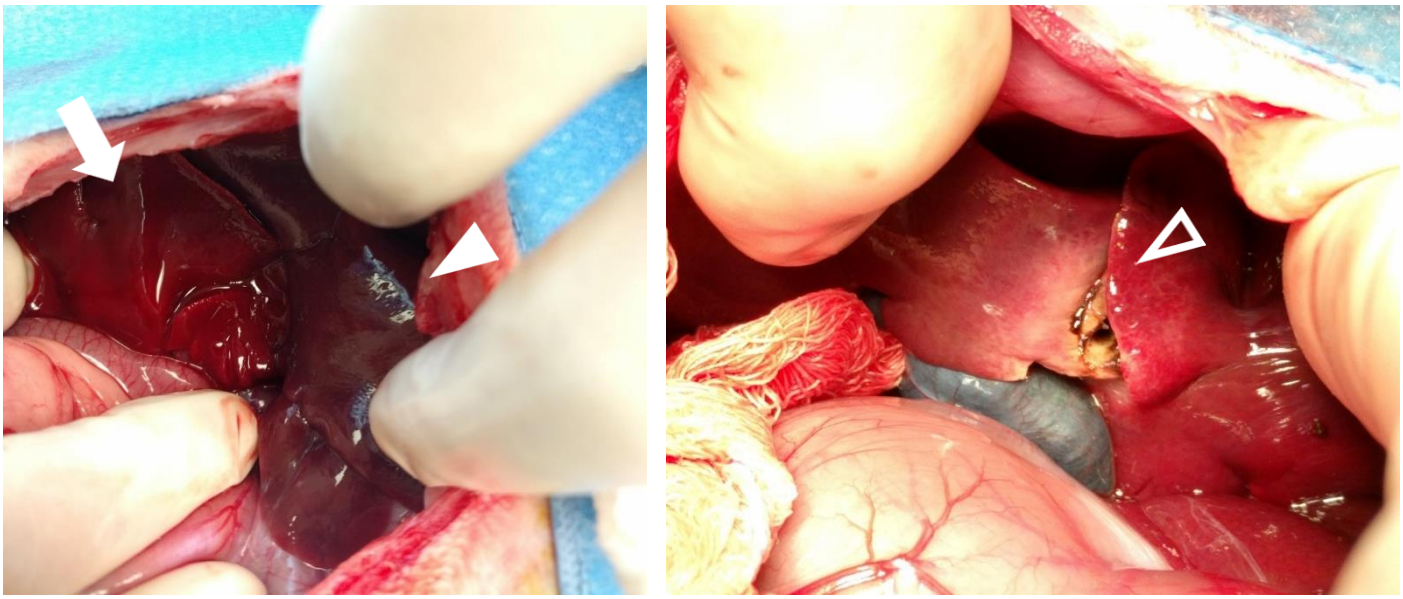

Figure 5.Caudal Lobe Post Embolization and Post Ablation. (Left) The vascular caudal lobe (white arrow) appears much redder compared to the now devascularized cranial lobes (arrowhead) post embolization. (Right) Post-ablation. Notice the area of discoloration (open arrowhead) around the entrance site of the ablation antenna. Images presented here are from different animals.

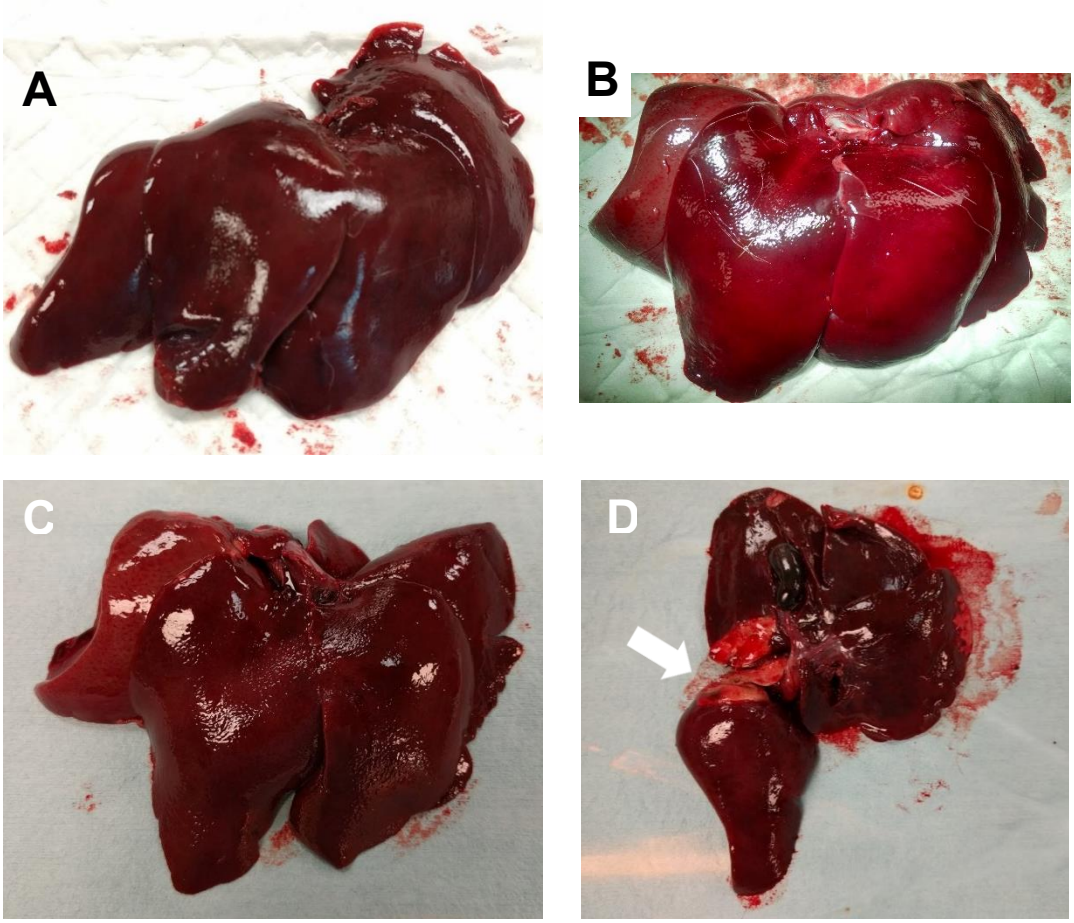

Figure 6. Liver Post-Intervention. (A) Control liver for comparison. (B) Gross liver post embolization. (C) Anterior surface of explanted liver post embolization and microwave ablation. Notice the difference in color between the vascular caudal lobe and nonvascular cranial lobes. (D) Explanted liver post embolization and microwave ablation with cranial lobes reflected to expose the ablated interlobar parenchymal bridge (white arrow).

C. Animal Necropsy and Tissue Harvest

Rabbits were euthanized at 7 days post-intervention using a lethal dose of 150mg/kg pentobarbital sodium solution (Schering-Plough, Kenilworth NJ). This was the earliest time point at which van den Esschert et al. found a difference in caudal liver

volume between experimental groups in their study comparing liver regeneration rates between PVE and portal vein ligation in a rabbit model (18). Necropsy was performed, rabbit livers were dissected and removed, and the caudal and cranial liver lobes were separated. The mass of the caudal and cranial liver lobes was measured immediately (wet mass) and a 1 mL sample of liver tissue was extracted from the caudal liver lobe and placed in a container containing 10% formaldehyde to be utilized for histologic assessment. The caudal and cranial liver lobes were then be dried at 60 °C for 4 weeks, at which time each was massed again (dry mass). Percentage of water was calculated utilizing the formula: (wet weight – dry weight)/(wet weight) x 100. The masses of each liver lobe was standardized to baseline preoperative body mass to obtain a liver-to-body weight index (LBWI). This was done to exclude the influence of body mass on liver mass. Animals used were limited to 2.6-3.0 kgs in overall mass in order to eliminate confounding from non-linearity between liver mass and overall animal mass. Dry mass was utilized to exclude the confounding effect of liver parenchymal edema on measured degree of hypertrophy. Of note, liver mass was utilized as the primary outcome measure herein as opposed to liver volume as it allowed exclusion of edema as a cause of increased lobar size.

D. Histologic Assessments

Samples of caudal and cranial liver were collected, fixed in buffered formalin, and then embedded in paraffin. Sections were immunostained with anti-Ki-67 antibodies (monoclonal mouse anti-human Ki-67 antigen, Dako, Carpinteria, CA). Ki-67 and hematoxylin-positive cells were manually counted in 2 medium power (20x

magnification) fields per section in a blinded fashion by a board-trained pathologist. The mitotic index, defined as the percentage of Ki-67 positive hepatocytes, was then calculated and compared between cohorts.

E. Statistical Methods

Statistical analysis was performed using commercially available statistics program (SPSS Statistics 23.0; SPSS Inc., Chicago IL). Shapiro-Wilk test was performed to test for normality of data and values between groups were compared using the one-tailed student's t-test or chi-square test where appropriate with p-value less than 0.05 considered significant. The one-tailed student's t-test was utilized as opposed to the two-tailed because FLR hypertrophy of the ALPPVE cohort was expected to be greater than that of PVE cohort, just as ALPPS results in greater FLR growth compared to PVE alone.

IV. RESULTS

Rabbit characteristics are summarized in Table I.

TABLE I
ANIMAL BASELINE CHARACTERISTICS

	PVE (n=8)	ALP-PVE (n=7)	p-value
Mass* (kg)	2.6 ± .17	2.7 ± .27	.234
Gender (F:M)	5:3	6:1	.310

*Mean

A. Procedure

15 of 18 rabbits underwent successful procedures. 8 rabbits underwent PVE alone and 7 underwent ALP-PVE. One rabbit in the ALP-PVE cohort died secondary to mesenteric ischemia on post-procedure day 1, one rabbit died during anesthesia administration prior to any intervention, and one rabbit had unsuccessful PVE. These animals were excluded from analysis. All animals required 1 vial of embospheres with an average of 3 coils for PVE. 71% (5/7) of animals in the ALP-PVE cohort underwent 2 serial ablations at 40W for 1 minute each while the first two animals only underwent 1 ablation at 40W for 1 minute. One animal had non-target ablation of the diaphragm and 2 animals had larger than expected blood loss from the liver capsule during ablation but with adequate control prior to closure. These complications were all clinically inconsequential.

B. Gross Liver

There were no obvious differences in gross liver appearance between cohorts. (Figure 6). Only 1/15 livers from the PVE cohort, showed signs of gross necrosis after explant (Figure 7). The animal showed no signs of illness prior to sacrifice. The caudal lobes subjectively appeared more plump and voluminous compared to the cranial lobes of the same liver in both cohorts.

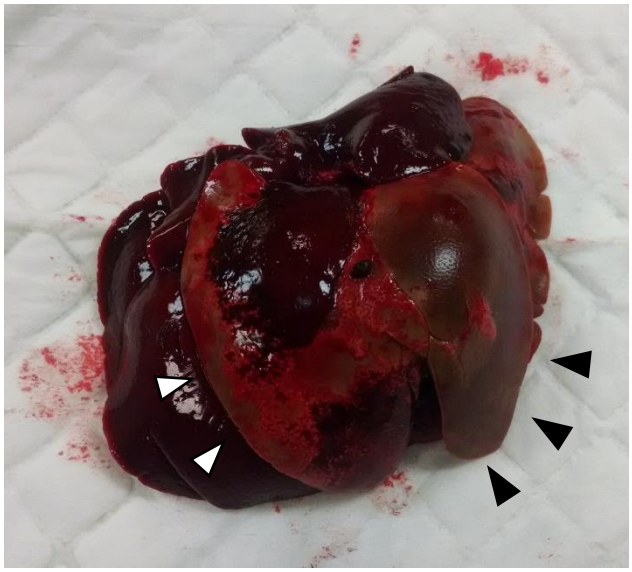

Figure 7. Infarcted Gross Liver. The discolored areas (arrow heads) depict areas of infarcted liver. This was the only liver that showed gross signs of infarction.

C. Liver Mass

Liver mass data can be found in Table II and the mean dry and wet mass of the cranial and caudal liver lobes as well as ratio of caudal to whole liver is summarized in Table III. The absolute wet or dry mass of the ALPPVE caudal lobes was larger than the PVE cohort but not significantly (26.1 ± 6.1 vs 23 ± 3.2; p = .113 and 5.87 ± .9 vs. 5.85 ± 1.5; p = .490).

TABLE II
DATA FOR EACH ANIMAL

RABBIT	COHORT	GENDER	WEIGHT (KG)	CAUDAL WET (G)	CRANIAL WET (G)	CAUDAL DRY (G)	CRANIAL DRY (G)	COMPLICATIONS
1	PVE	F	2.6	62	28	16	8	Minor non-target embolization
2	PVE	F	2.6	72	22	18	6	.
3	PVE	F	2.7	68	26	14	6	.
4	PVE	F	2.9	62	22	16	8	.
5	Excluded	M	2.5	48	12	12	2	Died during anesthesia
6	PVE	F	2.5	66	20	10	4	.
7	PVE	M	2.7	66	20	16	4	.
8	PVE	M	2.4	46	20	12	5	.
9	PVE	M	2.4	64	26	16	6	.
10	ALP-PVE	M	2.7	54	16	14	4	Non-target ablation of diaphragm
11	Excluded	F	2.8	64	22	12	4	Unsuccessful PVE and Ablation
12	ALP-PVE	F	2.4	48	26	12	6	.
13	ALP-PVE	F	2.4	52	32	10	6	.
14	ALP-PVE	F	2.7	55	24	14	6	.
15	Excluded	F	2.7	.	.	.	.	Died from mesenteric ischemia on day 2
16	ALP-PVE	F	3.0	64	35	14	7	.
17	ALP-PVE	F	3.0	59	26	14	6	.
18	ALP-PVE	F	3.0	64	24	14	6	.

TABLE III
ABSOLUTE LIVER MASSES BY COHORT

Liver Lobe	PVE (g)	ALP-PVE (g)	P-value(1-tailed)
Wet Cranial	63.2 ± 7.7	56.6 ± 6.1	.044
Dry Cranial	14.8 ± 2.6	13.1 ± 1.6	.09
Wet Caudal	23 ± 3.2	26.1 ± 6.1	.113
Dry Caudal	5.9 ± 1.5	5.9 ± .9	.490
Wet Caudal:Cranial	0.367 ±.061	0.464 ± .110	.025
Dry Caudal:Cranial	0.384 ± .073	0.453 ± .096	.072
Wet Caudal:Whole Liver	0.267 ± .032	0.313 ± .052	.029
Dry Caudal:Whole Liver	0.276 ± .039	0.309 ± .047	.081

The wet caudal liver by body mass index (LBWI) (Table IV) of the ALPPVE cohort was also larger than that of the PVE cohort but once again not significantly (9.6 ± 2.4 vs 8.8 ± 1.3, p = .240). The ratio of wet caudal:whole liver, however, was significantly larger in the ALPPVE cohort compared to the PVE cohort (.313 ± .052 vs. .267 ± .032; p = .029). After drying however, there is still a difference but it is no longer significant (.309 ± .047 vs. .276 ± .039; p = .081).

TABLE IV
LIVER BY BODY MASS INDEX BY COHORT

Liver Lobe	PVE ($x10^{-3}$)	ALP-PVE ($x10^{-3}$)	P-value(1-tailed)
LBWI Wet Cranial	24.3 ± 2.8	20.6 ± .8	.003
LBWI Dry Cranial	5.6 ± .9	4.8 ± .3	.020
LBWI Wet Caudal	8.8 ± 1.3	9.6 ± 2.4	.240
LBWI Dry Caudal	2.15 ± .5	2.16 ± .3	.335

The same data was also evaluated after excluding the first two animals in each cohort, which was considered the learning period for optimizing each procedure. The differences between the ALP-PVE and PVE cohorts found when excluding the initial 2 cases in each cohort were exaggerated compared to when all the animals are included as can be seen in Table V and VI. The absolute wet and dry caudal masses and ratio of wet and dry caudal:whole liver masses of the ALPPVE cohort when excluding the initial 2 animals was significantly larger compared to the PVE cohort (see Table V and VI). While the caudal LBWI was also larger in the ALPPVE cohort, it was not significantly so.

The cranial liver lobes (embolized liver lobes) were significantly smaller in the ALPPVE gr for both the wet and dry absolute masses and for both wet and dry cranial LBWIs.

TABLE V

ABSOLUTE LIVER MASSES BY COHORT EXCLUDING FIRST TWO CASES IN EACH COHORT

Liver Lobe	PVE (g)	ALP-PVE (g)	P-value (1-tailed)
Wet Cranial	62 ± 8.1	58.8 ± 5.4	.235
Dry Cranial	14.0 ± 2.5	13.2 ± 1.8	.020
Wet Caudal	22.3 ± 2.94	28.2 ± 5.0	.284
Dry Caudal	5.5 ± 1.5	6.2 ± .45	.175
Wet Caudal:Cranial	0.364 ±.054	0.482 ± .096	.015
Dry Caudal:Cranial	0.374 ± .065	0.477 ± .075	.019
Wet Caudal:Whole Liver	0.266 ± .029	0.323 ± .043	.014
Dry Caudal:Whole Liver	0.271 ± .036	0.321 ± .047	.020

TABLE VI

LIVER BY BODY MASS INDEX BY COHORT EXCLUDING FIRST TWO CASES IN EACH COHORT

Liver Lobe	PVE ($x10^{-3}$)	ALP-PVE ($x10^{-3}$)	P-value(1-tailed)
LBWI Wet Cranial	23.8 ± 2.9	20.9 ± .8	.030
LBWI Dry Cranial	5.4 ± .9	4.7 ± .4	.067
LBWI Wet Caudal	8.6 ± 1.3	10.1± 2.3	.106
LBWI Dry Caudal	2.1 ± .5	2.2 ± .2	.339

There was no difference in the mean water content between ALP-PVE and PVE cohorts (Table VII).

TABLE VII
WATER CONTENT

Liver Lobe	PVE (%)	ALPPVE (%)	P-value (1-tailed)
Cranial Water Content	76.5 ± 3.7	76.7 ± 2.4	.462
Caudal Water Content	74.6 ± 5.4	77.1 ± 2.5	.461

D. Ki-67 Immune Histochemistry

Figure 8 displays characteristic staining of actively replicating hepatocytes utilizing Ki-67 and compares the differences seen between liver lobes between cohorts. All collected samples displayed active staining. The percentage of Ki-67 positive hepatocytes was statistically significantly greater in the caudal lobes of both the PVE (9.3 ± 5.7% vs. 2.5 ± 0.7%, p = 0.002) and ALP-PVE (14.0 ± 6.3% vs. 3.7 ± 3.1%, p = 0.001) cohorts compared to the corresponding cranial lobes. The percentage of Ki-67 positive cells was also significantly greater in the ALP-PVE caudal lobes (14± 6.3%) compared to that of the PVE cohort (9.3± 5.7%, p = 0.078) (see Table VIII) and this almost reached statistical significance when excluding the initial 2 cases from each cohort (16.4± 4.5% vs. 10.6± 6.1%, p = 0.054). The number of Ki-67 cells was also greater in the PVE and ALP-PVE cohorts compared to two control livers though no statistical analysis can be performed because of small sample size in the control group

(n=2). No difference was found when comparing cranial to caudal lobes in the control cohort.

TABLE VIII
Ki-67 MITOTIC INDEX BY COHORT AND LIVER LOBE

Liver Lobe	PVE (%)	ALPPVE (%)	P-value (1-tailed)
Cranial	2.5 ± 0.73	3.8 ± 3.0	0.136
Caudal	9.0 ± 6.3	14 ± 5.7	0.078
Cranial Excl. Initial 2 Cases	2.5 ± 0.86	3.7 ± 3.5	0.210
Caudal Excl. Initial 2 Cases	10.6 ± 6.1	16.4 ± 4.5	0.054

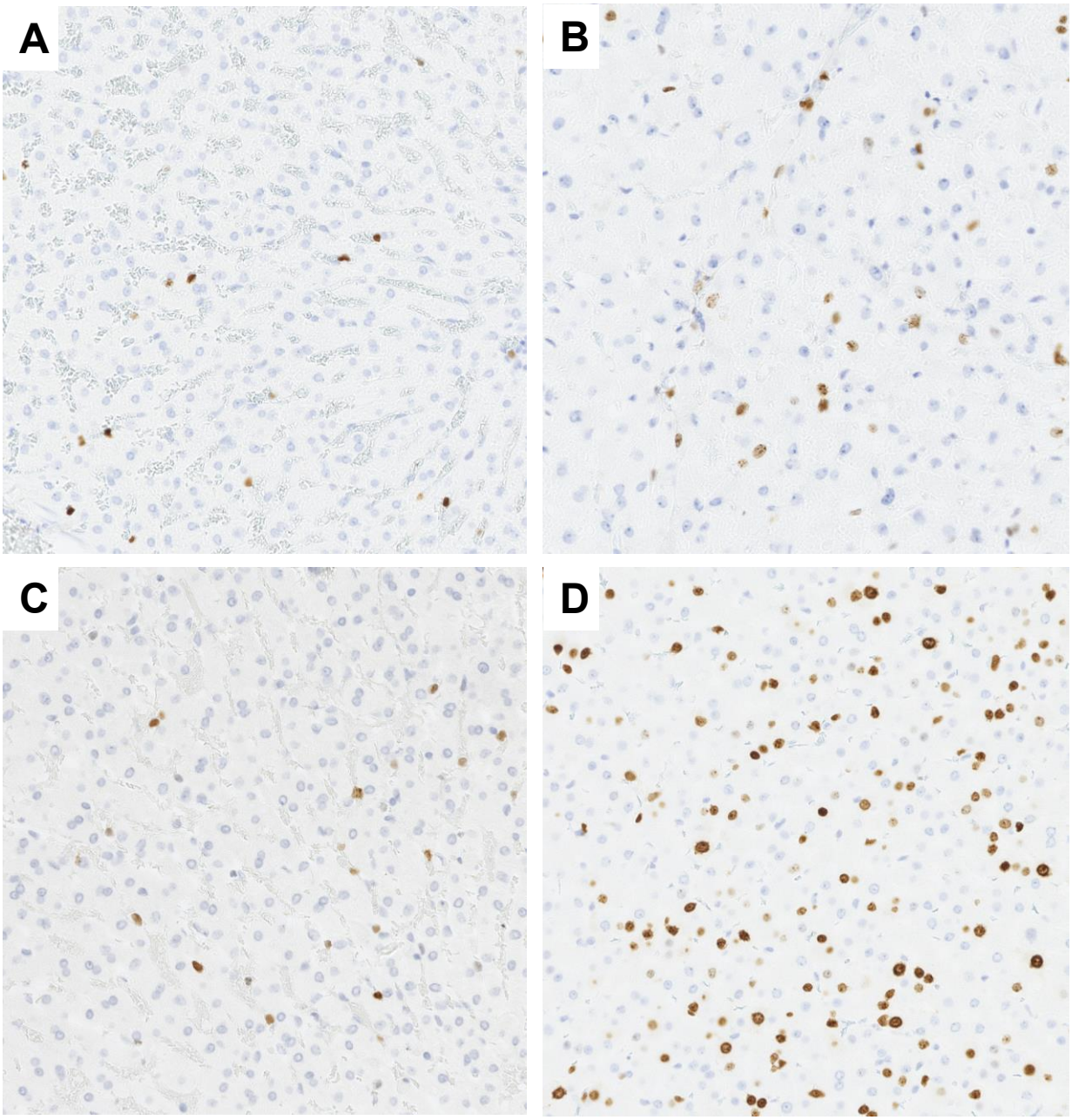

Figure 8. Ki-67 Staining. Cell nuclei that are actively replicating and expressing Ki-67 stain brown. (A) PVE cranial lobe. (B) PVE caudal lobe. (C) ALPPVE Cranial lobe. (D) ALPPVE Caudal lobe. The number of Ki-67 positive cells is greatest in the ALPPVE Caudal lobe (D)

V. DISCUSSION

A. Interpretation of Results

The results of this study suggest that ALP-PVE results in greater growth of the FLR compared to PVE alone over the same time period when comparing, absolute caudal masses, both wet and dry, and caudal percentage of total liver mass between cohorts. The greater number of Ki-67 positive staining cells in the ALP-PVE caudal group compared to the PVE caudal group supports this and provides evidence of increased proliferation. The lack of a significant difference in absolute masses may be attributable to the small sample size of the study. The difference in growth when comparing caudal percentage may however be exaggerated as there is also a significant decrease in the size of the cranial lobes in ALP-PVE compared to PVE, most likely due to necrosis. This results in an increase in the numerator and decrease in the denominator resulting in an exaggerated increase. The difference in mass between the two cohorts is also not due to edema post-intervention because there was no significant difference in percentage of water between groups.

The greater decrease in the size of the cranial lobes due to increased necrosis, supports the claim that microwave ablation can effectively partition the caudal and cranial lobes thus preventing the formation of collaterals from the vascularized caudal lobe to the devascularized cranial liver lobes. This provides evidence that the procedure performs technically as described.

B. Implications for Clinical Practice

ALP-PVE results in greater FLR growth compared to PVE alone suggesting that microwave ablation can create a similar liver parenchymal partition as ALPPS (22) without the associated physical separation of the components. It could possibly be utilized instead of PVE alone as optimal therapy for FLR hypertrophy prior to hepatectomy. However, this study is only the first step, and even with promising results, further randomized animal studies and histopathological studies are required to fully determine the feasibility, growth rate of the FLR, complications, and long term effects of the proposed procedure before transitioning from a pre-clinical to a clinical model.

Another procedure that has also seen promise in FLR hypertrophy is radiation lobectomy (32). Radiation lobectomy involves directed radioembolization of the lobes to be resected resulting atrophy of the lobes to be resected secondary to radiation injury and hypertrophy of the FLR. Radiation lobectomy synchronously provides treatment to the metastatic or primary tumors while providing FLR growth however the rate of growth is slower when compared to PVE (32). Future research aimed at comparing this procedure to radiation lobectomy to assess for superiority is also needed.

C. Limitations

This study has several limitations. First, the sample size of each cohort was small. Second, there was a learning curve to performing the procedures, which may have impacted the early data points in each cohort and thus the changes observed.

Third, there is only data on a single time point post-intervention preventing evaluation of changes over time. Fourthly, there is no baseline liver mass data for each animal so changes in liver mass per animal cannot be determined, which would provide more robust information. The addition of CT volumetric data would help alleviate this shortcoming. Finally, the procedure was performed open and not minimally invasively as intended. While this allowed for better proof of concept testing, the results shown do not account for challenges that may be encountered when performing the ALPPVE procedure minimally invasively.

In conclusion, the novel proposed procedure, ALPPVE, appears to result in increased hypertrophy of the FLR compared to PVE alone providing evidence that it may serve as a superior intervention. The clinical utility however requires further evaluation including proving that the procedure can be performed minimally invasively as intended, as well as performing the procedure in an animal model more closely related to humans both anatomically and physiologically such as a porcine model.

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APPENDIX - Animal Care and Use Approval

UIC UNIVERSITY OF ILLINOIS AT CHICAGO

June 2, 2015

Ron Gaba
Radiology
M/C 931

Office of Animal Care and Institutional
Biosafety Committee (M/C 672)
Office of the Vice Chancellor for Research
206 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612

Dear Dr. Gaba:

The modifications requested in modification indicated below pertaining to your approved protocol indicated below have been reviewed and approved in accordance with the Animal Care Policies of the University of Illinois at Chicago on ***06/02/2015.***

Title of Application: Ablative Liver Partition and Portal Vein Embolization (ALP-PVE): Proof of Concept Testing in a Rabbit Model

ACC Number: 15-022

Modification Number: ***01***

Nature of Modification: ***Addition of Departmental funding.***

Protocol Approved: ***3/27/2015***

Current Approval Period: ***3/27/2015 to 3/17/2016. Protocol is eligible for 2 additional years of renewal prior to expiration and resubmission.***

Current Funding: ***Portions of this protocol are supported by the funding sources indicated in the table below.***
Number of funding sources: 1

Funding Agency	**Funding Title**			**Portion of Funding Matched**
Departmental	*Ablative Liver Partition and Portal Vein Embolization (ALP-PVE): Proof of Concept Testing in a Rabbit Model*			*Departmental funding*
Funding Number	**Current Status**	**UIC PAF NO.**	**Performance Site**	**Funding PI**
713018	*Funded*	*N/A*	*UIC*	*Ron Gaba*

This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare, NIH. **This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the grant are matched to this ACC protocol.**

Thank you for complying with the Animal Care Policies and Procedures of UIC.

Sincerely yours,

Bradley Merrill, PhD
Chair, Animal Care Committee
BM/*ss*
cc: BRL, ACC File, James Bui, Janesh Lakhoo

Phone (312) 996-1972 • Fax (312) 996-9088

VITA

NAME: Janesh Lakhoo

EDUCATION: B.S. in Biomedical Engineering, Northwestern University, Evanston, Illinois 2011

M.D., College of Medicine, University of Illinois at Chicago, Chicago, Illinois 2016

M.S., School of Public Health, University of Illinois at Chicago, Chicago, Illinois 2016

PROFESSIONAL MEMBERSHIP: Society of Interventional Radiology
Radiological Society of North America
American Medical Student Association

AWARDS/GANTS: University of Illinois at Chicago Department of Radiology Faculty Development Grant 2015
Project Title: Ablative Liver Partition and Portal Vein Embolization (ALP-PVE): Proof of Concept Testing in a Rabbit Model

RSNA Research Medical Student Grant 2015
Project Title: Ablative Liver Partition and Portal Vein Embolization (ALP-PVE): Proof of Concept Testing in a Rabbit Model

SIR In-Training Conference Travel Scholarship Award 2014,2015

PUBLICATIONS: **Lakhoo J,** Bui JT, Zivin SP, Lokken RP, Minocha J, Ray CE Jr, Gaba RC. Root Cause Analysis of Rebleeding Events Following Transjugular Intrahepatic Portosystemic Shunt Creation for Variceal Hemorrhage. Journal of Vascular and Interventional Radiology. 2015 Oct;26(10):1444-53.

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