Optimizing preoperative portal vein embolization for liver resection

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Publication date
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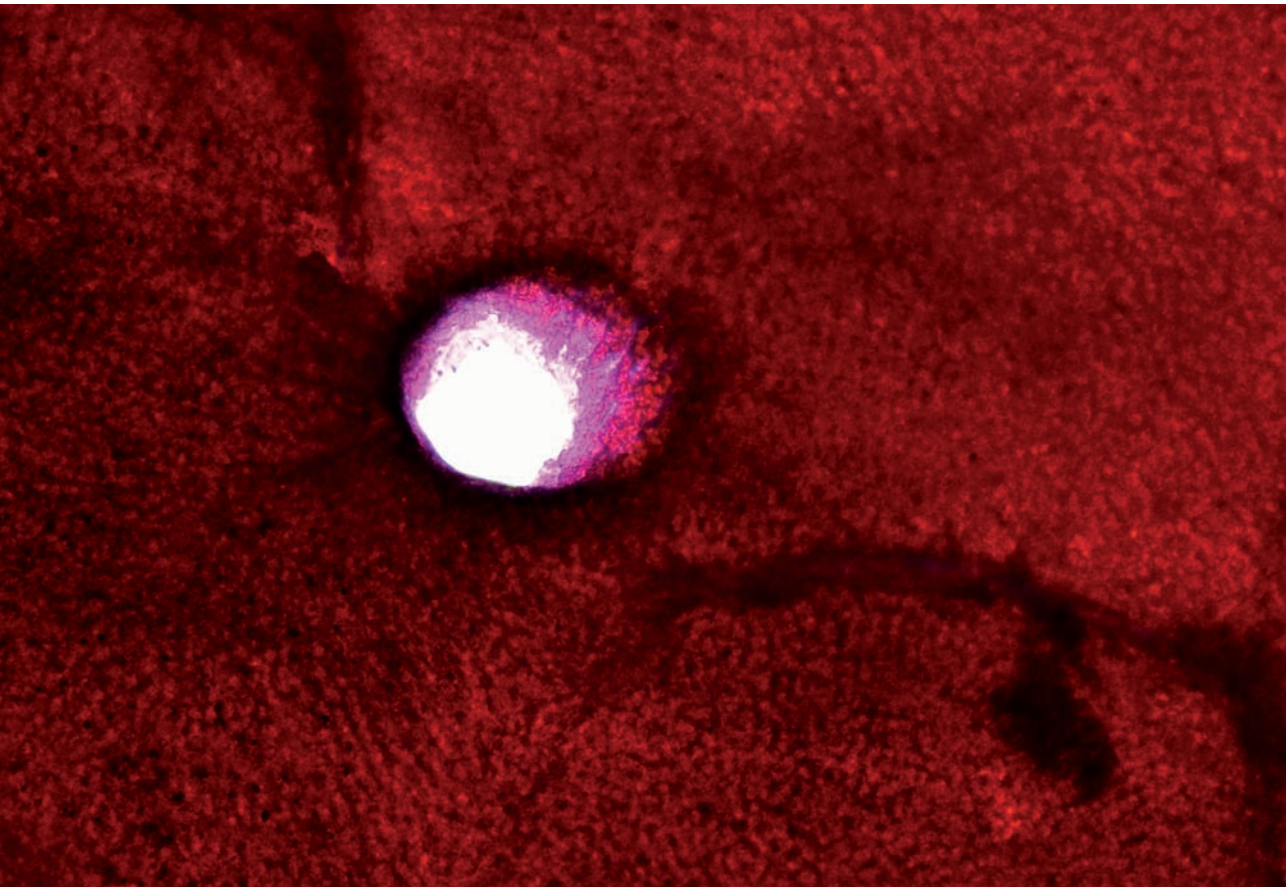

Floor Huisman

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Floor Huisman
PhD thesis, University of Amsterdam, the Netherlands

ISBN: 978-94-6361-140-4
Cover design by: Optima Grafische Communicatie, Rotterdam, The Netherlands
Printing: Optima Grafische Communicatie, Rotterdam, The Netherlands

The research described in this thesis was sponsored by Roba Metals BV, IJsselstein, The Netherlands.

Printing of this thesis was financially supported by: Wetenschappelijk fonds Chirurgie Academisch Medisch Centrum and Chipsoft.

OPTIMIZING PREOPERATIVE PORTAL VEIN EMBOLIZATION FOR LIVER RESECTION

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. ir. K.I.J. Maex
ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
op donderdag 27 september 2018, te 12.00 uur

door Floor Huisman

geboren te Delft

PROMOTIECOMMISSIE:

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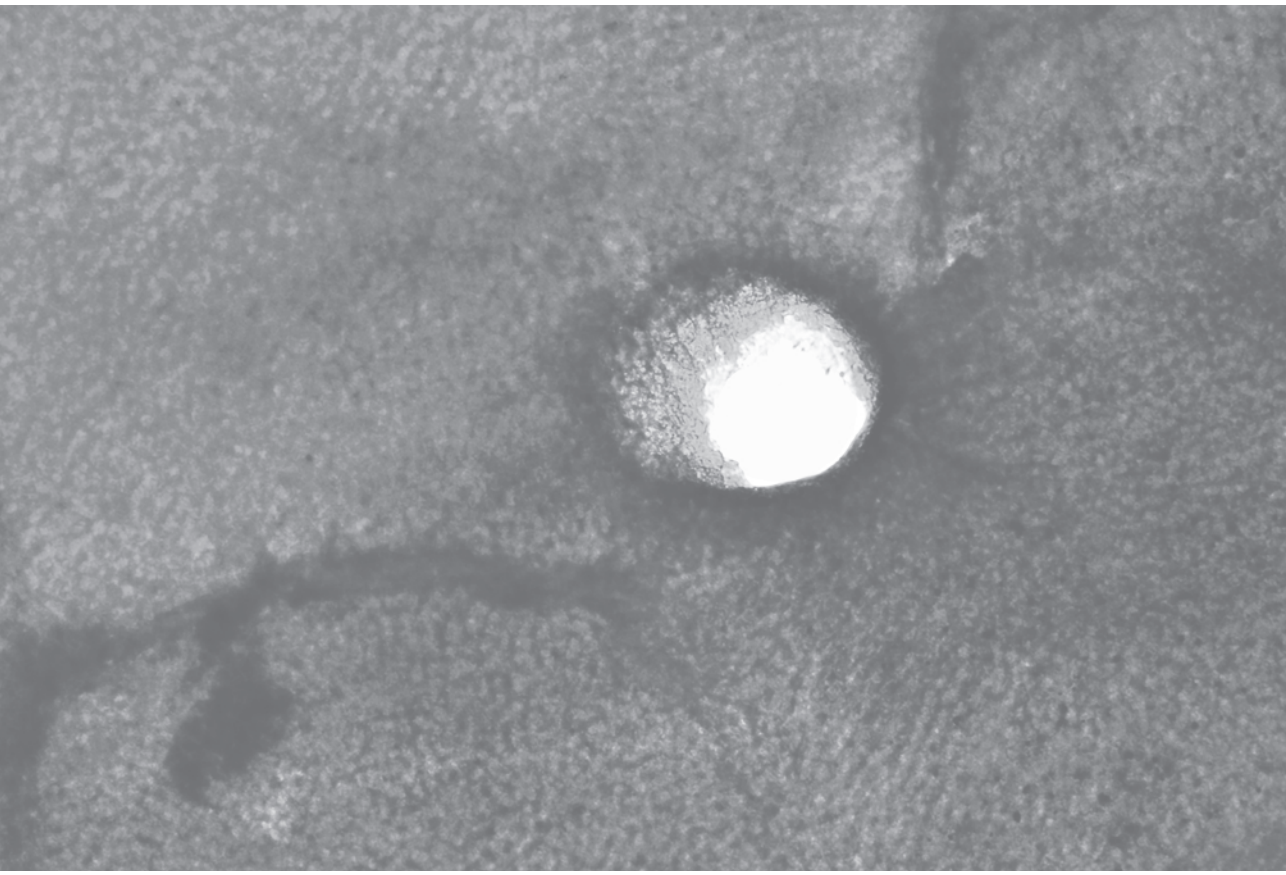
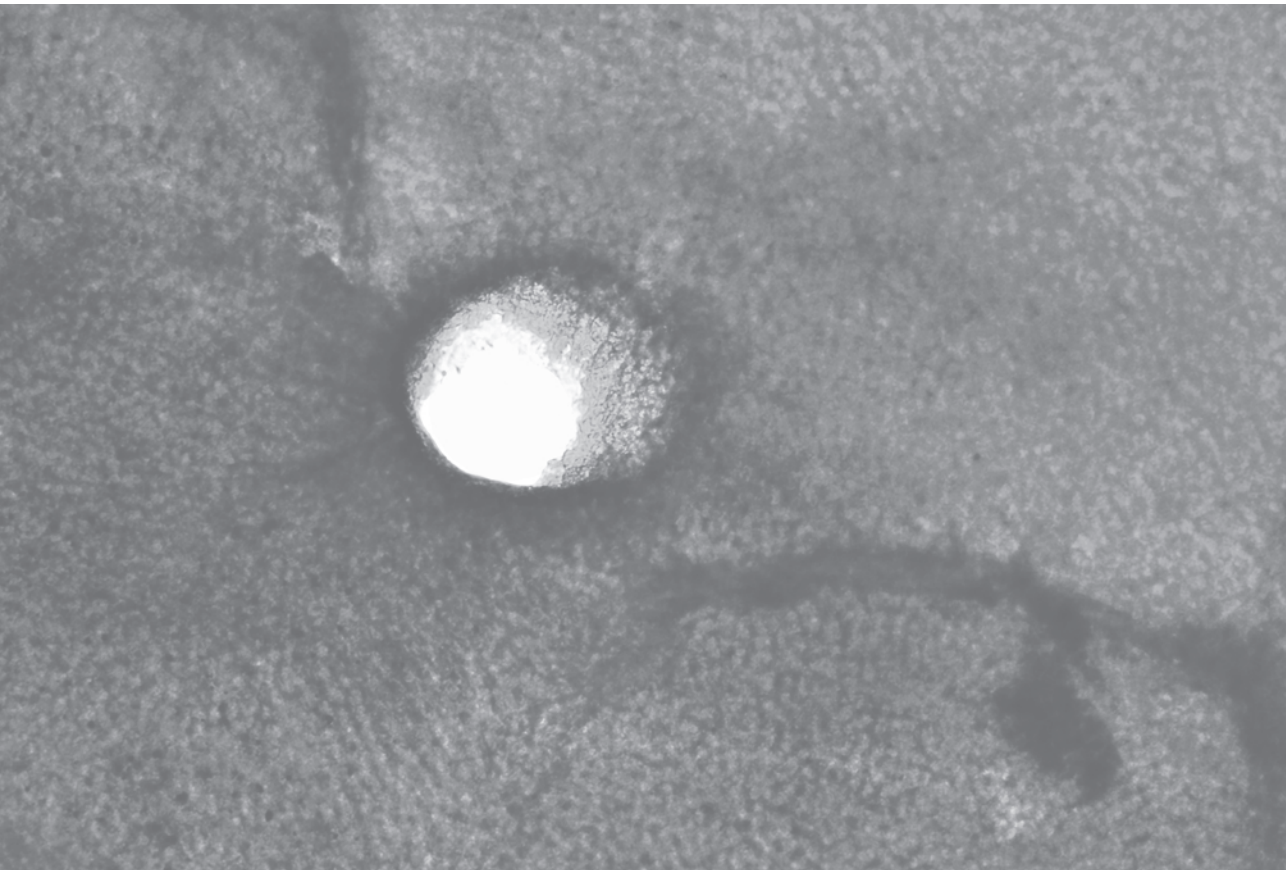
CONTENTS



General introduction and outline of the thesis



GENERAL INTRODUCTION

Surgery is the only curative treatment for primary or secondary hepatic tumors. Through the years, liver resections have become more extensive owing to better surgical techniques, better preoperative workup and new oncological insights. The type of resection depends of the size and the location of the tumor(s). The quality and size of the future remnant liver for a large part dictates the postoperative outcome. Although still a topic of ongoing discussion, it is generally accepted that a liver resection can be performed safely when the future remnant liver (FRL) is larger than 25-30% of the total liver volume (TLV) in patients with normal liver parenchyma. When liver tissue is damaged, e.g. in case of cirrhosis, steatosis, cholestasis, fibrosis, or after extensive chemotherapy, the volumetric cut-off value for a safe liver resection is rather set at 40% of the TLV. [1, 2] When volume and function of the FRL fall short, liver augmenting procedures are necessary to prevent postoperative liver failure. Eighty percent of patients who develop post-operative liver failure will die due to this complication as we still lack effective devices for substitution of liver function. The golden standard for assessment of the FRL is volume measurement using computed tomography (CT volumetry). CT volumetry enables correct measurement of FRL-volume, however it does not provide information on function of the FRL. Our group has extensively studied the functional assessment of the FRL in the preoperative setting. We found that preoperative (99m)Tc-mebrofenin hepatobiliary scintigraphy (HBS) is an accurate technique to estimate the risk of postoperative liver failure, in both patients with healthy or compromised liver parenchyma. [3, 4] An effective method to increase remnant liver volume (RLV) and function preoperatively is portal vein embolization (PVE). Introduced in 1986 in clinical practice, this method preoperatively increases volume of the future remnant liver by completely occluding the right portal venous system.[5-7] PVE now is worldwide, an established percutaneous intervention by which usually the right portal vein branches are occluded preoperatively using permanent embolization agents. Polyvinyl alcohol particles in combination with coils and N-butyl cyano-acrylaat (NBCA) with Lipiodol are the most used agents for this procedure.

This thesis describes several conditions of preoperative augmenting procedures along with aspects of postoperative care in patients undergoing liver resection. Aim of this thesis is to improve the postoperative outcome of major liver resection in terms of postoperative morbidity and mortality.

OUTLINE OF THE THESIS

A vast body of research has been published on indications and outcomes of PVE, both clinical and translational. Our group successfully devised a rabbit model for PVE.[8] An overview of all the animal models which can be used to study PVE is presented in **chapter 1**.

Most of the embolization materials used for PVE are permanent, as it is supposed that permanent occlusion of the portal vein is more effective in inducing an hypertrophy response then temporary occlusion.[9-11] However, there are some relevant shortcomings to the application of permanent embolization materials. Firstly, there is a risk of the material migrating into the contralateral portal vein branches, inadvertently leading to thrombosis of the portal vein branches of the FRL. [12, 13] Secondly, some patients after PVE appear not be resectable at exploratory laparotomy due to tumor progression. In case of unresectability, permanent occlusion of the right portal vein potentially causes infectious complications on the embolized side of the liver. Thirdly, in living donor liver transplantation, the use of an absorbable material can be used to achieve a hypertrophy response in the future donor liver lobes before harvesting, without inducing permanent damage in the embolized lobe of the donor. The hypertrophic graft can be resected after sufficient regeneration has taken place while the embolization material is completely absorbed and function of the residual liver in the donor preserved as much as possible. Using an absorbable embolization material would therefore, have several advantages in clinical application. In **chapter 2**, we tested an absorbable embolization material in the rabbit model of PVE that would give a sufficient hypertrophy response.

In some cases the regeneration capacity after PVE proves to be insufficient to allow safe liver resection. Bile acids seem to play an important role as early mediators of regeneration of the liver, by activating the bile acid Farnesoid X-receptor (FXR). [14] In **chapter 3**, we investigated the effect of a FXR agonist (obeticholic acid, OCA) on liver regeneration in our standardized rabbit model of PVE.

Even more important than volume of the FRL is adequate liver function necessary to prevent post-operative liver failure. In **chapter 4,** we analyzed HBS before PVE as a predictor of a sufficient hypertrophy response after PVE. Little is known about the consequences of a persisting atrophic lobe in patients who are found to be unresectable after PVE. In **chapter 5,** we assessed the complications occurring after PVE in case of unresectability.

Chapter 6 focuses on differences in postoperative outcomes in patients who underwent liver resections in a specialized regional or academic center.

It is widely accepted that laparoscopic liver surgery potentially offers many benefits. The learning curve is difficult to deal with and in **chapter 7**, we evaluated a stepwise introduction of laparoscopic liver resections combined with structured training in our center. Finally, the postoperative management of patients after major liver resection is of paramount importance. Fluid overload is a common problem after major liver resections that may be enhanced due to activation of the renin–angiotensin–aldosterone system (RAAS). In **chapter 8** we conducted a clinical pilot study in which the correlation between the RAAS and fluid overload was investigated.

References

(1) de Graaf, W., et al., *Induction of tumor growth after preoperative portal vein embolization: is it a real problem?* Ann Surg Oncol, 2009. **16**(2): p. 423-30.

(2) van Lienden, K.P., et al., *Portal vein embolization before liver resection: a systematic review.* Cardiovasc Intervent Radiol, 2013. **36**(1): p. 25-34.

(3) de Graaf, W., et al., *Assessment of future remnant liver function using hepatobiliary scintigraphy in patients undergoing major liver resection.* J Gastrointest Surg, 2010. **14**(2): p. 369-78.

(4) de Graaf, W., et al., *(99m)Tc-mebrofenin hepatobiliary scintigraphy with SPECT for the assessment of hepatic function and liver functional volume before partial hepatectomy.* J Nucl Med, 2010. **51**(2): p. 229-36.

(5) de Graaf, W., et al., *Increase in future remnant liver function after preoperative portal vein embolization.* Br J Surg, 2011. **98**(6): p. 825-34.

(6) Kinoshita, H., et al., *Preoperative portal vein embolization for hepatocellular carcinoma.* World J Surg, 1986. **10**(5): p. 803-8.

(7) Makuuchi, M., et al., *Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report.* Surgery, 1990. **107**(5): p. 521-7.

(8) de Graaf, W., et al., *A rabbit model for selective portal vein embolization.* J Surg Res, 2011. **171**(2): p. 486-94.

(9) Guiu, B., et al., *Portal vein embolization before right hepatectomy: improved results using n-butyl-cyanoacrylate compared to microparticles plus coils.* Cardiovasc Intervent Radiol, 2013. **36**(5): p. 1306-12.

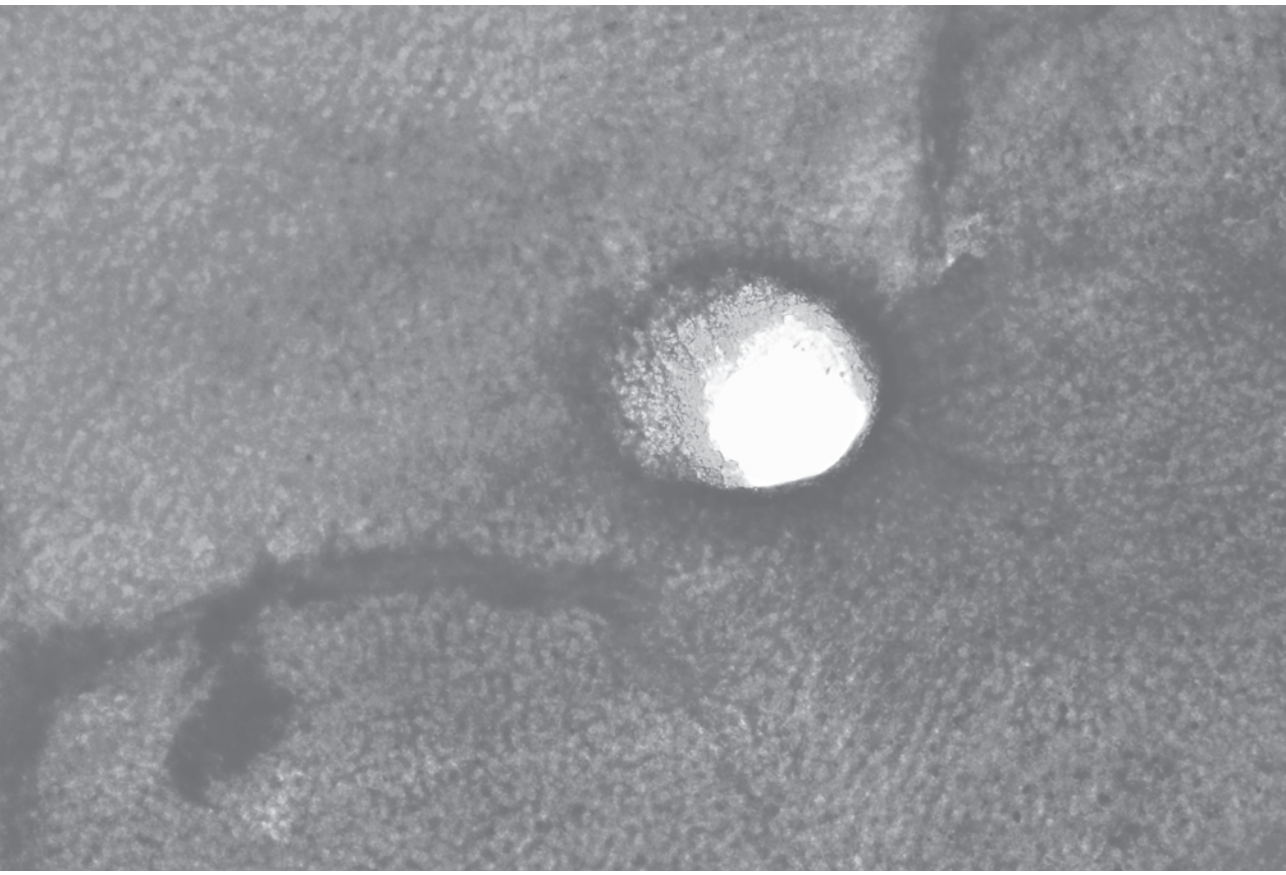
(10) Huang, J.Y., et al., *Portal vein embolization induces compensatory hypertrophy of remnant liver.* World J Gastroenterol, 2006. **12**(3): p. 408-14.

(11) de Baere, T., et al., *Portal vein embolization: utility for inducing left hepatic lobe hypertrophy before surgery.* Radiology, 1993. **188**(1): p. 73-7.

(12) Di Stefano, D.R., et al., *Preoperative percutaneous portal vein embolization: evaluation of adverse events in 188 patients.* Radiology, 2005. **234**(2): p. 625-30.

(13) van Gulik, T.M., et al., *Controversies in the use of portal vein embolization.* Dig Surg, 2008. **25**(6): p. 436-44.

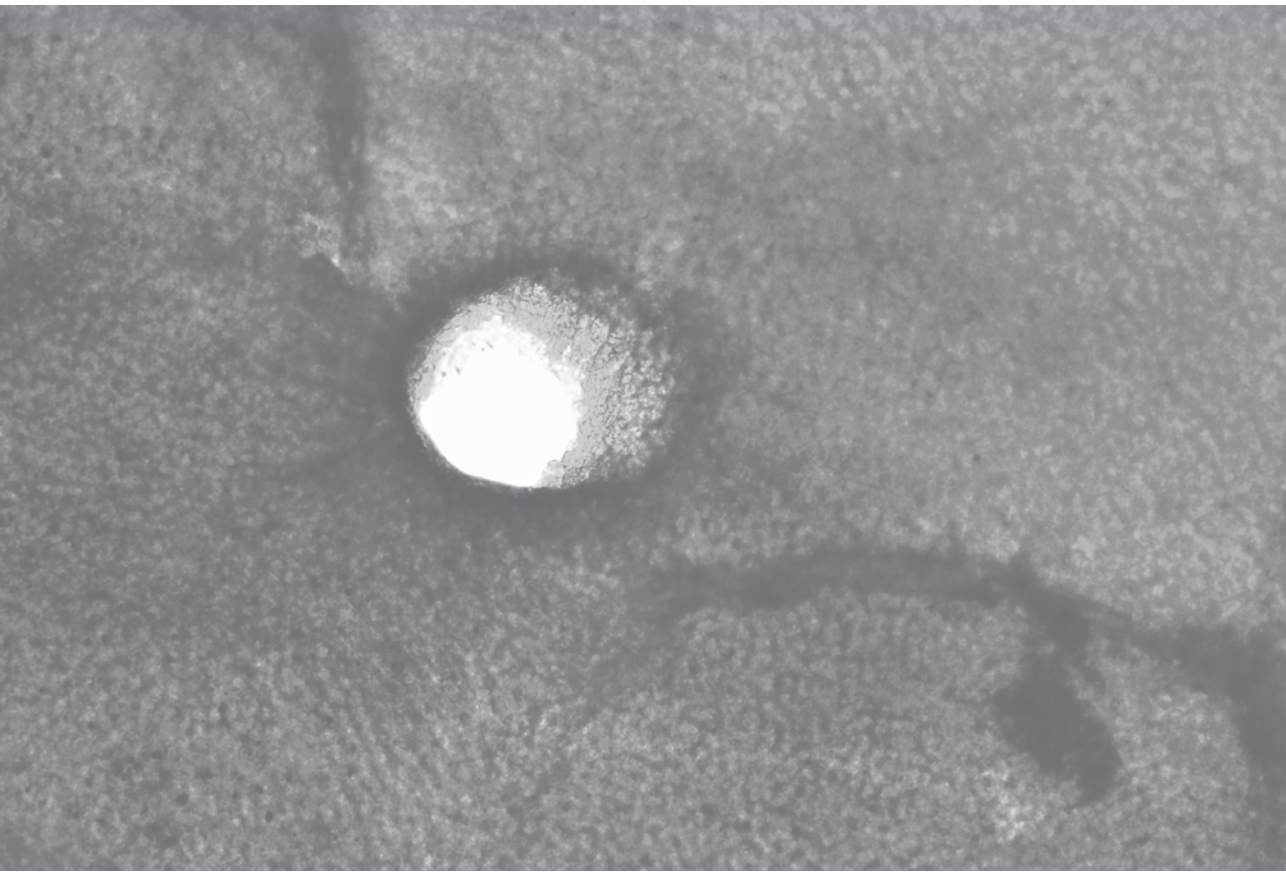
(14) Huang, W., et al., *Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration.* Science, 2006. **312**(5771): p. 233-6.



CHAPTER 1

A review of animal models for portal vein embolization

F. Huisman, K.P. van Lienden, S. Damude, L.T. Hoekstra, T.M. van Gulik



Journal of Surgical Research 2014

ABSTRACT

Background:

Portal vein embolization (PVE) is a preoperative intervention to increase the future remnant liver (FRL) through regeneration of the non-embolized liver lobes. This review assesses all the relevant animal models of PVE available, to guide researchers who intend to study PVE.

Materials and methods:

We performed a systematic literature search in Medline and Pubmed, from 1993-June 2013, using search headings “PVE” and “portal vein ligation”. Articles were included when meeting the selection criteria: experimental animal study on PVE or portal vein ligation and experiments described in 5 animals or more.

Results:

Sixty-one articles were selected, describing six different animal models. Most articles reported experiments with rats, rabbits, and pigs. In rats, the increase in wet-weight ratio of the non-occluded liver or total liver weight is greatest in the first 7 d with values ranging from 75%-80.5% on day 7. The volume increase of FRL in the rabbit model is greatest in the first 7 d with values ranging from 33.6%-80% on day 7. In pigs, the largest gain in volume of the FRL was seen in the first 2 wk.

Conclusions:

The choice of the model depends on the specific aim of the study. Evaluating the increase in liver volume and liver function after PVE, larger animals as the pig, rabbit, or the dog is useful because of the possibility to apply computed tomography volumetry. To evaluate mechanisms of regeneration after PVE, the rat model is useful, because of the variety of antibodies commercially available.

BACKGROUND

Surgical resection of primary or secondary tumors in the liver remains the only curative therapy. The great majority of patients are however, not candidates for surgery because of tumor burden or too small liver remnant leading to increased risk of post hepatectomy liver failure. To undergo a major liver resection, the future remnant liver volume in humans has to be at least 25% based on CT volumetric studies to avoid post resectional liver failure [1]. In livers with compromised parenchyma due to cirrhosis, steatosis or recent chemotherapy, the minimum volume should be at least 30%. Preoperative portal vein occlusion by embolization or ligation is a method to stimulate growth of the non-occluded liver segments, thereby increasing the volume of the future remnant liver [2].

The concept of the atrophy-hypertrophy complex following unilateral portal vein occlusion has initially been demonstrated in a rabbit model by Rous and Larimore in 1920 [3]. They discovered in rabbits that ligation of the portal branches to part of the liver caused atrophy of that part of the liver and concomitant hypertrophy of the non-ligated part of the liver. This phenomenon has already been used in a clinical setting for many years. Although clinical PVE has shown effective, several issues need further investigation. The mechanism of induction of liver regeneration after PVE is still poorly understood, the optimal technique and choice of embolization materials can be improved and (pharmaceutical) interventions to stimulate liver regeneration in addition to PVE must be further investigated. As a downside, PVE not only induces liver regeneration, but it also promotes tumor growth [2, 4, 5]. Strategies to control this potential drawback need to be explored in animal studies. In this review, we describe all relevant animal models, used to study PVE, in relation to the species-specific anatomy techniques and the induced hypertrophy response.

MATERIALS AND METHODS

We performed a systematic literature search in Medline and Pubmed, from 1993 to June 2013. The applied search headings were: “portal vein embolization” and “portal vein ligation”. Limitations were set to English language and animal studies. The abstracts were screened to identify potentially relevant articles and were evaluated by two of the authors (FH, SD, LH), using a predetermined scoring list. Full text articles of potentially relevant papers were screened and were included in this study when meeting the following selection criteria:

- Experimental animal study on PVE or PVL
- Experiments described in at least 5 animals.

RESULTS

Types of animals

Sixty-one articles were selected, describing 6 different animal models; i.e. in monkeys, pigs, dogs, rabbits, rats or mice (**Table 1**). One article discussed both dogs and rats. Two articles described both PVL and PVE in a rat model, rabbit model and pig model.

Table 1. Animal models used for PVE.

Animal	Number of articles PVL	Number articles PVE	Total number of articles
Monkey	0	1	1
Pig	5	9	13
Dog	1	7	8
Rabbit	2	8	9
Rat	20	8	27
Mouse	3	0	3

Mice

Only three articles described the procedure of PVL in a murine model and no reports of PVE in the mouse have been published [6-8]. The diameter of the portal branches of the mouse is small; therefore it is not an ideal animal model to perform PVE.

Monkeys

One article described the use of a monkey model to observe the regeneration response after PVE with an absorbable embolization material [9]. The use of monkeys for research is in many countries restricted or forbidden.

Rats

Twenty-seven articles described PVE or PVL in a rat model.

Anatomy of the rat liver

The liver of the rat consists of four lobes. The left part of the liver part consists of the median lobe and the left lateral lobe. The right part of the liver consists of the superior and inferior right lobe, the anterior and posterior caudate lobe. Each lobe has its own blood supply consisting of branches of the portal vein and hepatic artery (**Figure 1**).

Technical procedure in the rat

In 7 of the 8 articles describing PVE in rats, the left liver lobe was embolized, corresponding to 70% of total liver volume (**Figure 2**; [10-16]). The main portal trunk was dissected and punctured with a needle ranging from 20 – 30-gauge and a catheter was connected through

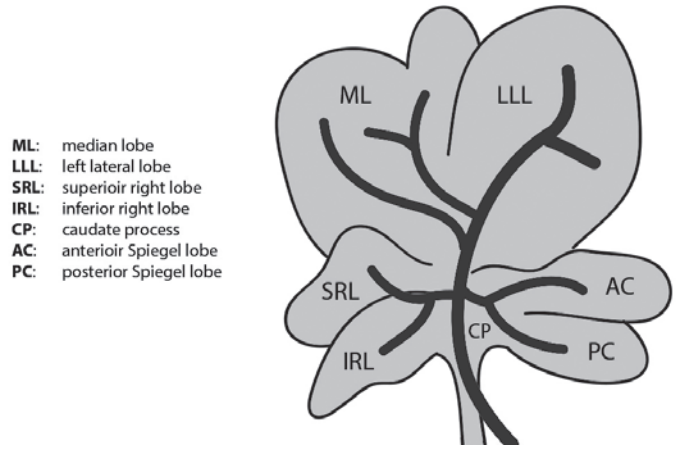


Figure 1 Anatomy of the rat liver.

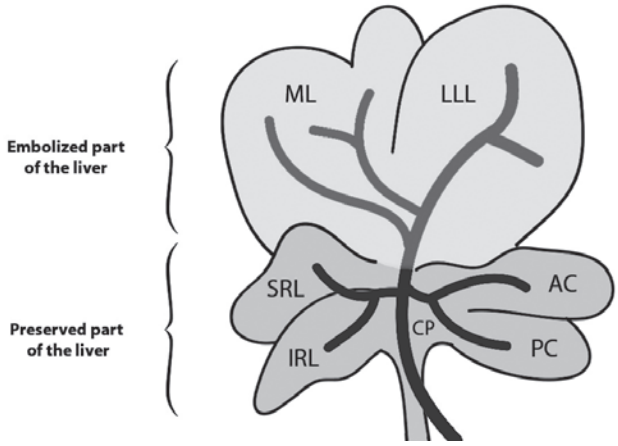


Figure 2 Schematic view of the rat liver lobes showing occlusion of the portal vein branches to the left liver lobe, i.e., the median lobe (ML) and the left lateral lobe (LLL), and preservation of the right liver, i.e., the superior (SRL) and the inferior right lobe (IRL), the caudate process (CP), the anterior (AC) and posterior caudate lobe (PC).

which portography was performed. The tip of the catheter was placed just above the right portal branches and transcatheter embolization was possible. Selective embolization is done by temporary clamping of the portal branches of the liver lobes to be preserved.

Furrer et al. chose to embolize the right liver lobes and the left lateral lobe, leaving only the portal branch to the median lobe open (**Figure 3**; [17]).

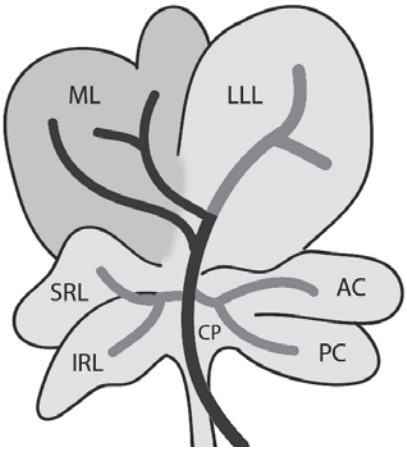


Figure 3 Schematic presentation of the rat liver after PVE (approximately 70%). All lobes are embolized, except the median lobe. The median lobe (ML), the left lateral lobe (LLL), the superior (SRL) and the inferior right lobe (IRL), the caudate process (CP), the anterior (AC) and posterior caudate lobe (PC) are indicated.

Volume increase of future remnant liver and time to maximum hypertrophy

In one study, rats were sacrificed after 4 days. Most studies, however, chose to sacrifice after one week (9 out of 27 studies), or after 2 weeks (8 studies). Accelerated growth of the non-embolized liver was seen in the first 3-4 days after which the hypertrophy response subsequently decreased. In none of the studies, did liver regeneration reach a plateau phase at the time of sacrifice, suggesting that rats should be observed at least 14 days to fully appreciate the regenerative capacity.

To evaluate the hypertrophy response after PVE or PVL in rats, weights of the non-occluded parts of the liver have been measured at sacrifice and volume increase was calculated using the following formulas:

- Weight of the non-embolized liver or non-ligated liver lobes / total liver weight (%) (**Table 2**).
- Weight of the non-embolized liver or non-ligated liver lobes / body weight (%) (**Table 3**).

The increase of wet weight ratio of the non-occluded liver / total liver weight is greatest in the first 7 days with values ranging from 75 to 80.5% on day 7. In two studies, rats were sacrificed on day 14 and 28, with an ultimate increase in volume of 80% and 95%, respectively.

Rabbits

Anatomy

The liver of the rabbit consists of four main lobes: one caudal liver lobe and three cranial liver lobes (**Figure 4**). The caudal liver lobe is separated from the cranial liver lobes and therefore, can be clearly distinguished.

Table 2. Ratio of the wet weight of non-occluded lobes of the liver after PVL/PVE / total liver (%).
(* estimation of the increase found in graphs in the articles)

PVE/PVL	Author	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	Day 10	Day 14	Day 28
PVL	Schweizer et al. [18]	38* %			60*		75*			95*
PVL	Vetelainen et al. [19]		50*	60*					80*	
PVL	Yu et al. [20]		38.39±2.25 (SD)		60.02±4.22 (SD)		77.68±2.34(SD)			
PVL	Mizuno et al. [21]	30±2(SD)					80±1(SD)			
PVE / PVL	Iuchi et al. [11]							63.1±3.5 (SD)/59.9±2.7 (SD)		
PVE	Lu Ming-De et al. [13]	23.0	30.0		31.9		49.8		68.8	
PVL	Lin et al. [22]		48*		60*		80.5±3.9(SD)			
PVL	Sugimoto et al. [23]	9.30±0.97(SD)					23.76±1.67 (SD)		26.66±2.67(SD)	
PVL	Morine et al. [24]					22.49±2.19 (SD)				

Table 3. Ratio of the non-embolized liver or non-ligated liver lobes / body weight. (%)

PVE/PVL	Author	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14
PVL	Kucuktulu et al. [25]		1.4±0.1(SD)	1.8±0.1(SD)		3.0±0.2(SD)		
PVE/PVL	Lee et al. [12]	1.0*/ 1.0*	1.2*/1.7*		1.8*/2.5*		2.8*/2.8*	3.1*/3.0*
PVL	Tanaka et al. [16]	1.39±0.05(SD)		2.0*		2.6*	2.89±0.05(SD)	
PVL	Uemura et al. [26]	1.2*	1.4*	1.8*	1.9*	2.5*	2.4*	
PVL	Kong et al. [27]		1.9*	2.2*	2.8*	3.2*	3.8*	
PVL	Makino et al. [28]	1.4*	1.8*			2.8*	2.9*	
PVL	Sugimoto et al. [23]	0.39±0.04(SD)					0.86±0.07(SD)	1.03±0.05(SD)
PVL	Morine et al. [24]					0.802±0.09(SD)		

(* estimation of the increase found in graphs in the articles)

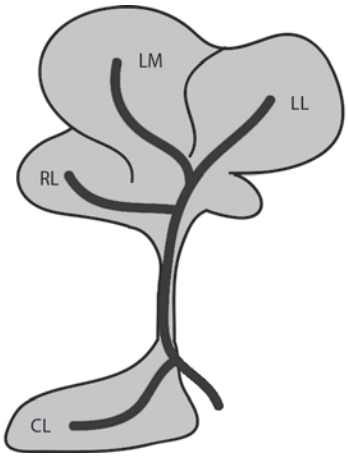


Figure 4 Anatomy of the rabbit liver. Four main lobes: caudal liver lobe and three cranial lobes: left lateral (LL), left medial (LM) and the right liver lobe (RL).

Technical procedure in the rabbit

In 6 of 8 articles, PVE was performed by embolizing the portal branches to the cranial liver lobes. These lobes account for 80% of total liver volume. After a midline laparotomy, the mesenteric vein is cannulated and via a 3 French microcatheter, the portal branches to the cranial liver lobes are embolized [5, 18-22]. In two articles, a different method to embolize a part of the rabbit liver was performed. The authors occluded the external left branch of the portal vein, supplying the left lateral lobe (accounts for 25% of the total liver volume) [23, 24]. A percutaneous transhepatic puncture of the external left branch of the portal vein was undertaken to avoid laparotomy. Besides PVE, the rabbit is an excellent model to perform other procedures including arterial and hepatic venous embolization [22].

Volume increase of future remnant liver and time to maximum hypertrophy

As shown in **table 4,** the regeneration response reaches a plateau-phase already after 7 days, allowing experiments with short observation-time (**Figure 5**).

CT volumetry is a very suitable method to measure the growth of the non-occluded liver non-invasively, similar to the assessment of future remnant liver in patients after PVE. All authors used CT volumetry, which enables repeated measurements within one rabbit. The caudal, non-embolized liver lobe (CLV) can easily be delineated and measured. The increase in CLV is measured with the following formula:

$$\text{Increase CLV} = \frac{CLV_{\text{post-PVE}} - CLV_{\text{pre-PVE}}}{CLV_{\text{pre-embolization}}} \times 100\%$$

The volume increase of the future remnant liver weight is greatest in the first 7 days with values ranging from 33.6% to 80% on day 7. The maximum observation time after PVE reported in rabbits is two weeks.

Table 4. Increase in FRL (%) after PVE in the rabbit model

PVE/PVL	Author	Day 3	Day 7	Day 10	Day 14
PVE	De Graaf et al. [29]	38*	80*	85*	88*
PVE	Van den Esschert et al. [32]	33.6±10	79.8±18.8		
PVE	Van Lienden et al. [33]	33.6±4	79.8±8.4		
HVE		-10.2±5.5	5.6±7.0		
HVE+PVE		43.4±7.5	103.6±10.5		

(* estimation of the increase extracted from graphs in the articles)

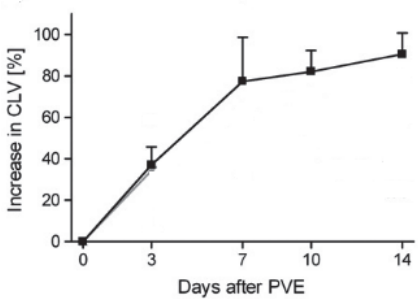


Figure 5 Increase in CLV measured by CT volumetry in the rabbit PVE model.

Dogs

PVE in dogs was described in 7 articles and 1 article described PVL.

Anatomy

The liver of the dog consists of seven lobes; the left portal branch perfuses the left lateral lobe and papillary process, the left medial lobe, the quadrate lobe and the right medial lobe.

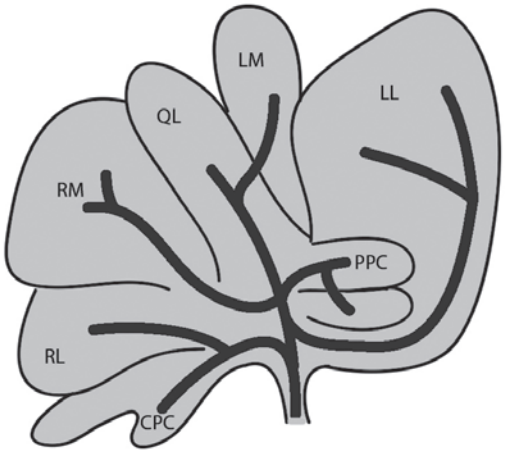


Figure 6 Anatomy of the liver of the dog. LL = left lateral lobe, PPC = papillary process of caudate lobe, LM = left medial lobe, QL = quadrate lobe, RM = right medial lobe, CPC = caudate process of caudate lobe.

The right portal branch perfuses the right lateral lobe and caudate process (**Figure 6**). In dogs, the left liver lobe corresponds to 70% of total liver volume [25].

Technical procedure

The most frequently used procedure to perform PVE in dogs was through laparotomy and direct injection of an embolization material into the left branch of the portal vein under angiographic guidance [26-28]. The materials used were hydrophilic phosphorylcholine, n-butyl cyanoacrylate, hydrophilic gel, polyvinyl alcohol (PVA) particles, polidonacol and gelatine sponge, steel coils, absolute ethanol, gelfoam with and without coils (**Figure 7**).

One of the articles described a procedure of PVL in the dog model in which left portal branch ligation was performed in 23 dogs [27].

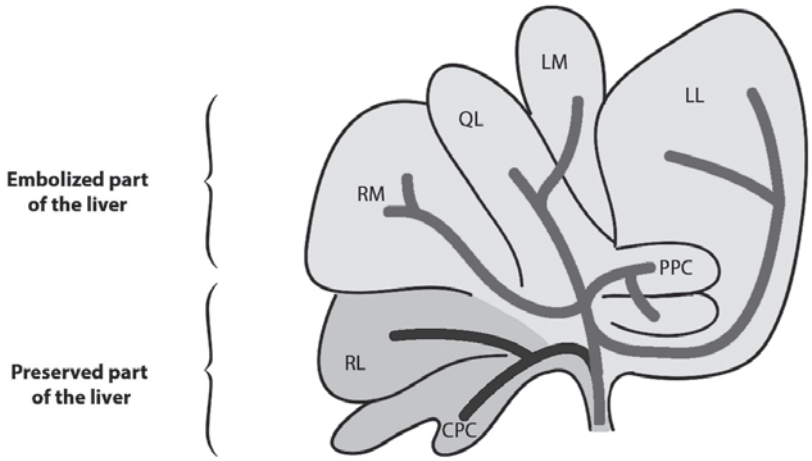


Figure 7 Schematic representation of portal vein embolization in a dog liver. LL = left lateral lobe, PPC = papillary process of caudate lobe, LM = left medial lobe, QL = quadrate lobe, RM = right medial lobe, CPC = caudate process of caudate lobe.

Volume increase of future remnant liver and time to maximum hypertrophy

Various methods to evaluate the hypertrophy response have been used in dogs making it difficult to compare the results described in different articles. Huang et al. measured the hypertrophy response using CT volumetry and showed that regeneration had reached a plateau phase after 6 weeks, suggesting that dogs should be sacrificed after at least 6 weeks following PVE [26]. Kaneko et al. evaluated hepatic regeneration by measuring the relative liver weight ratio (LWR): (Non-embolized liver weight / embolized liver weight) x 100%. They occluded the portal vein branches to the right medial, quadrate, left lateral, and papillary process of caudate lobe. During an 8 weeks waiting period, the LWR increased from 31.8%

in control animals to 220.8% after embolization. They concluded that in dogs the atrophy/hypertrophy response continued for 8 weeks after embolization [29].

Pigs

The pig model has been used in 5 articles concerning PVL and 9 articles concerning PVE.

Anatomy

The pig liver consists of 4 lobes, i.e. the right lobe, right middle lobe, left middle lobe and left lobe. The lobar volume distribution of the porcine liver is similar to human livers (**Figure 8**). The left lateral segments account for 20-25% of total liver volume.

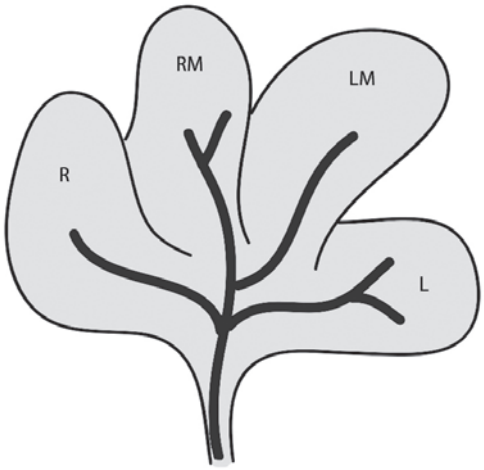


Figure 8 Hepatic lobar anatomy of a pig liver, consisting of the right lobe (R), right middle lobe (RM), left middle lobe (LM) and left lobe (L).

Technical procedure

Nine articles described a pig model for PVE. The most common method to perform embolization of the left portal vein branch perfusing the left middle lobe and left lobe was by percutaneous transhepatic approach [30-36]. Smits et al. tried to find a less invasive method to embolize the right lateral and medial lobe (n=6) together with the left lateral and medial lobe of the porcine liver. They demonstrated that portal vein embolization can be performed by a new technique called retrograde transsinusoidal injection of low-viscosity liquid embolic agent [37]. This technique is based on the phenomenon that contrast fluid is able to pass in retrograde fashion through the sinusoid and reach the portal vein. The authors were the first to demonstrate that PVE can be accomplished by retrograde injection of the embolic agent from a wedged catheter in the hepatic vein. The hypertrophy response was however, not assessed. Madoff et al. have also attempted to find a less invasive approach to perform PVE [32]. They demonstrated another, indirect method to occlude the portal venous system by the transarterial approach. This approach may minimize complications and make the procedure easier. Transarterial PVE

was compared with transhepatic, transportal PVE. For transarterial PVE, the femoral artery was cannulated and a catheter was advanced into the hepatic arterial branches supplying the left and left middle liver lobes. The embolization material was injected across the peribiliary arterioportal plexus into the portal veins resulting in occlusion of the portal venous system. They concluded that transarterial portal vein embolization is safe and effective for inducing liver regeneration in the swine. In most of the articles, the left and median lobes were embolized.

Volume increase of future remnant liver and time to maximum hypertrophy

Due to the lack of reporting standards for PVE in the pig, no conclusions can be drawn from these articles regarding volume increase of the future remnant liver.

The time from PVE to sacrifice was 1-6 weeks. Park et al. found, as a result of embolization with Embol-78, that the volume ratio of the non-embolized liver changed from 55% to 71% at 2 weeks and to 84% at four weeks [33]. This was determined by weighting the liver lobes after sacrificing the pigs. Satake et al. measured a mean future remnant liver / embolized liver volume ratio increase after PVE with absolute ethanol, measured by CT-volumetry after 3 weeks, of 14.2%. The largest gain in volume of the future remnant liver was seen in the first two weeks.

The most frequently used method to evaluate the future remnant liver volume was CT-volumetry [32, 34, 35]. The peak of regeneration in pigs after PVE or hepatectomy is described to occur at 7 days [31].

Embolization materials

Since the first article in 1986 has been published by Kinoshita et al. [38] reporting the use of PVE in patients, various embolization materials have been used to perform PVE. No randomized trials have been performed evaluating the efficacy of the different embolization materials in inducing hypertrophy. Only one retrospective clinical study compared N-butyl cyanoacrylate (NBCA) and Polyvinyl alcohol (PVA) micro particles with coils, concluding that the use of NBCA induces a greater regeneration response of the FRL[39]. **Table 5** shows the various embolization materials that have been used in the animal models described above.

It is not clear which embolization material shows the best results. The embolization materials mentioned are often used in combination and each experiment used its own standard, precluding conclusions on which material is most effective in inducing a hypertrophy response of the FRL.

Tumor models

Besides regeneration of the FRL, several studies describe potential tumor progression after PVE, which is a serious drawback of this technique [4, 40, 41]. Animal tumor models have been used to investigate enhanced tumor growth after PVE.

Table 5. Embolization materials used for PVE in the animal models.

Embolization material	Animal model	References
Polyvinyl alcohol particles + Coils	Pig	[41, 42]
Ethanol	Dog, pig and rat	[13, 15, 37, 38, 45, 51]
Gelfoam	Dog and rat	[11, 36, 37, 52]
Gelfoam + 60% Urografin	Dog	[36]
Gelfoam + coils	Dog	[37]
Gelatin Sponge	Dog, monkey and rabbit	[9, 30, 32, 40]
Gelatin sponge + polidocanol	Dog	[40]
Polyvinyl acetate (Embol-78)	Pig	[44]
Acrylic copolymer trisacryl, cross-linked with gelatin (Embosphere)	Rat	[17]
Ethylene vinyl alcohol copolymer (Onyx)	Pig	[48]
Nbutylcyanoacrylate	Pig and rabbit	[32, 41, 46]
Sodium acrylate-vinyl alcohol copolymer particles	Pig	[46]
Hydrophilic phosphorylcholine & Hydrophilic gel	Pig	[41]
Fibrin glue Lipiodol	Rabbit	[32]
N-butyl-2-cyanoacrylate (Histoacryl) + Lipiodol	Pig, rat	[16, 47]
Coils	Dog	[51]
Cyanoacrylate + metacrylaxysulfolan (Glubran)	Rat	[10, 14]

Qi et al. [23] were the first who used a VX2-tumor model in rabbits in combination with portal vein ligation to investigate the effect on tumor growth. The VX2-tumor used was derived from a virus-induced papilloma tumor in rabbits. Zou et al.[24] were the first using the VX2-tumor model in combination with PVE. The tumor is of non-hepatic origin, but grows rapidly and the blood supply is similar to human hepatocellular carcinoma. Therefore, it is a very well suited tumor model for evaluating the influence of PVE on tumor growth.

To investigate the effect of PVE on tumor growth, the rabbit model was used in two articles [5, 24]. The VX2-tumor cells were implanted in the hind limb of the donor rabbit. After three weeks, the tumor cells were collected and cut into tumor fragments. The fragments were directly injected superficially under the liver capsula in the cranial lobes and PVE was performed two weeks later. In both reports, the authors concluded that PVE promotes tumor growth and that the rabbit model is an ideal model to use because of the isolated, non-embolized caudal liver lobe allowing selective implantation of tumor.

In the rat model, three articles investigated the differences in tumoral responses in the liver after PVL and PVE. Bretagnol et al. found a decrease in liver metastases in the embolized liver after injecting the tumor cells in the left medial liver lobe. Iuchi et al. injected tumor cells in the portal vein to produce liver metastasis on both sides of the liver. They found a significant reduction of tumor growth in the non-embolized lobes after embolization [10, 11, 14]. The other study by Maggiori et al. however, showed an opposite effect of PVL and PVE on tumor growth, in which PVL and PVE both increased tumor growth in both the

embolized and non-embolized liver lobes.[14] The tumor cells used in the latter two rat models were DHD/K12 cells, which were directly injected under the liver capsule. DHD/K12 is an established, transplantable colon carcinoma cell line. This tumor model in rats has the advantage that the DHD/K12 cells provide the biologically most accurate in vivo model of development of early colonic liver metastasis [42].

DISCUSSION

In this article we give an overview of the relevant literature on animal models used to investigate the hypertrophy response after PVE, in order to guide researchers who intend to do research on PVE.

Over the years, several animal models of PVL and PVE have been used describing different techniques, in most instances specific for the animal species used. Every animal model obviously has its own advantages and disadvantages.

The rat model is the most common model for PVE. This is the fastest model with lowest costs. Rabbits are more expensive, but they are easy to handle and to house. Experiments with pigs are labour intensive, expensive and not every research center has facilities to accommodate these pigs. Dogs are the most expensive animals and their use for research purposes is controversial in Europe. Housing facilities are also expensive for dogs.

Concerning the technical approach, in humans, three different techniques to perform PVE have been described, i.e. trans-ileocolic, contralateral and ipsilateral. These techniques have also been described in the animal models. Madoff et al. demonstrated a new, transarterial approach to embolize the portal branches in a pig model [32]. Absolute ethanol was infused through a microcatheter via the hepatic artery braches reaching the portal system via the peribiliary plexus. No adverse events were seen and the hypertrophy response of the FRL was comparable to the transhepatic approach. There are no reports showing applicability of this approach in other animal species or in humans.

Experimental studies and series in humans comparing PVE and PVL showed conflicting results regarding outcomes of the regeneration response. This issue has also been studied in rats, rabbits, dogs and pigs. In the pig model, only one study by Wilms et al. concluded that PVE is more effective than PVL to induce a hypertrophy response [36]. In the rabbit, Van den Esschert et al. found that PVE is superior to PVL in terms of the regeneration response [20]. This may be due to the fact that after PVL, formation of collateral portal vessels leads to portal reperfusion of the parenchyma distal to the ligature. This phenomenon has also been demonstrated in humans as published by Van Lienden et al. The authors visualized on fluoroscopy 3 weeks after PVE, the formation of intrahepatic portoportal, neocollateral vessels reperfusing the ligated lobe, which made them conclude that PVL is less effective in inducing a hypertrophy response of the nonligated lobe [43].

In contrast, Lee et al. found that PVE had the same effect as PVL on DNA synthesis and cell proliferation in rats [12]. They also concluded that the increase in volume of the future remnant liver lobes was higher after PVL compared to the PVE group. Furrer et al., also using rats, concluded that PVL is superior to PVE in inducing a regenerative response of the non-occluded liver [17]. This was thought to be due to a foreign body reaction induced by the embolization material used with PVE leading to less blood flow and less macrophages involved in the regenerating part of the liver.

The rat is much smaller than the human being and experimental results therefore, are less translatable to the clinical situation. The porcine liver is suitable for PVE because of the liver anatomy similar to the liver lobes in humans. For comparative studies, the pig model would be most appropriate because of the large calibre of the portal vein branches.

The mean diameter of the portal vein branches of the rat is too small to apply coils and large embolization particles. PVE in rats and mice is very difficult because of the small size of the veins of the portal system resulting in a high failure rate.

The rabbit provides a unique model, because of the anatomy of the liver. The separated, cranial liver lobe can be readily embolized while hypertrophy of the non-embolized, caudal liver lobe can be accurately monitored by CT-scans.

CT volumetry is used in humans to evaluate the hypertrophy response expressed as a volume increase after PVE. This technique is also often used in the rabbit and the pig model. In rabbits, an accurate measurement of the hypertrophy rate of the non-embolized liver lobes can easily be performed by sequential CT-volumetry on several time points, without sacrificing the animals. This is also possible for the pig, however requires special facilities. The rat liver is small and the resolution of the CT-scan is too low to obtain accurate estimation of the increase in volume.

Another method of assessing the response after selective PVE is to determine functional increase of the non-embolized liver lobes. We found 3 articles in which 99mTc- diisopropyl iminodiacetic acid (DISIDA) dynamic SPECT (single-photon emission computed tomography) was used to measure liver function in a rat model [44-46]. Lin et al. evaluated the functional changes after PVL in cirrhotic and noncirrhotic rats [45]. They concluded that the regenerated, functional liver mass in cirrhotic rats after PVL was less than in non-cirrhotic rats. Tseng et al. used SPECT/CT only to determine liver volume instead of function in a rat model [47].

Rat liver samples can be easily used for screening of gene expression using DNA microarray techniques. This technique allows assessment of the cytokines and growth factors involved in the mechanism of regeneration after PVE. A disadvantage of the rabbit model as compared to the rat model is the lack of available antibodies to determine specific growth factors and cytokines induced by PVE by ELISA or immunohistochemical staining. This also applies to the pig and the dog model.

In conclusion, several animal models are available to study PVE, all with their own advantages and limitations. The choice of the model depends on the purpose of the experiment.

Evaluating increase in liver volume and liver function after PVE, larger animals as the pig, rabbit or the dog are useful because of the possibility to apply CT volumetry. To evaluate underlying mechanisms of regeneration (cytokines or growth factors) after PVE, the rat model is more useful, because of the variety of antibodies commercially available.

Acknowledgements:

We would like to thank E. A. P. Huisman for creating the illustrations.

References

(1) van Gulik TM, van den Esschert JW, de Graaf W, van Lienden KP, Busch OR, Heger M, et al. Controversies in the use of portal vein embolization. Dig Surg 2008;25(6):436-44.

(2) Clavien PA, Petrowsky H, DeOliveira ML, Graf R. Strategies for safer liver surgery and partial liver transplantation. N Engl J Med 2007 Apr 12;356(15):1545-59.

(3) Rous P, Larimore LD. Relation of the portal blood to liver maintenance: a demonstration of liver atrophy conditional on compensation. J Exp Med 1920 Apr 30;31(5):609-32.

(4) Hoekstra LT, van Lienden KP, Doets A, Busch OR, Gouma DJ, van Gulik TM. Tumor progression after preoperative portal vein embolization. Ann Surg 2012 Nov;256(5):812-7.

(5) Hoekstra LT, van Lienden KP, Verheij J, van der Loos CM, Heger M, van Gulik TM. Enhanced tumor growth after portal vein embolization in a rabbit tumor model. J Surg Res 2013 Mar;180(1):89-96.

(6) Heinrich S, Jochum W, Graf R, Clavien PA. Portal vein ligation and partial hepatectomy differentially influence growth of intrahepatic metastasis and liver regeneration in mice. J Hepatol 2006 Jul;45(1): 35-42.

(7) Kollmar O, Corsten M, Scheuer C, Vollmar B, Schilling MK, Menger MD. Tumour growth following portal branch ligation in an experimental model of liver metastases. Br J Surg 2010 Jun;97(6):917-26.

(8) Sakai N, Clarke CN, Schuster R, Blanchard J, Tevar AD, Edwards MJ, et al. Portal vein ligation accelerates tumor growth in ligated, but not contralateral lobes. World J Gastroenterol 2010 Aug 14;16(30):3816-26.

(9) Lainas P, Boudechiche L, Osorio A, Coulomb A, Weber A, Pariente D, et al. Liver regeneration and recanalization time course following reversible portal vein embolization. J Hepatol 2008 Sep;49(3):354-62.

(10) Bretagnol F, Maggiori L, Zappa M, Sibert A, Vilgrain V, Panis Y. Selective portal vein embolization and colorectal liver metastases in rat: a new experimental model for tumor growth study. J Surg Res 2011 Dec;171(2):669-74.

(11) Iuchi M, Ishikawa M, Tashiro S. Growth inhibition of experimental liver metastasis by portal embolization. Hepatogastroenterology 2010 Nov;57(104):1515-21.

(12) Lee KC, Kinoshita H, Hirohashi K, Kubo S, Iwasa R. Extension of surgical indications for hepatocellular carcinoma by portal vein embolization. World J Surg 1993 Jan;17(1):109-15.

(13) Lu MD, YY Y, Ren W. A study of portal vein embolization with absolute ethanol injection in cirrhotic rats. World J Gastroenterol 1998 Oct;4(5):415-7.

(14) Maggiori L, Bretagnol F, Sibert A, Paradis V, Vilgrain V, Panis Y. Selective portal vein ligation and embolization induce different tumoral responses in the rat liver. Surgery 2011 Apr;149(4):496-503.

(15) Ogasawara K, Uchino J, Une Y, Fujioka Y. Selective portal vein embolization with absolute ethanol induces hepatic hypertrophy and makes more extensive hepatectomy possible. Hepatology 1996 Feb; 23(2):338-45.

(16) Tanaka H, Kinoshita H, Hirohashi K, Kubo S, Lee KC. Increased safety by two-stage hepatectomy with preoperative portal vein embolization in rats. J Surg Res 1994 Dec;57(6):687-92.

(17) Furrer K, Tian Y, Pfammatter T, Jochum W, El-Badry AM, Graf R, et al. Selective portal vein embolization and ligation trigger different regenerative responses in the rat liver. Hepatology 2008 May;47(5):1615-23.

(18) de Graaf W, van den Esschert JW, van Lienden KP, Roelofs JJ, van Gulik TM. A rabbit model for selective portal vein embolization. J Surg Res 2011 Dec;171(2):486-94.

(19) Hoekstra LT, Rietkerk M, van Lienden KP, van den Esschert JW, Schaap FG, van Gulik TM. Bile salts predict liver regeneration in rabbit model of portal vein embolization. J Surg Res 2012 Dec;178(2):773-8.

(20) van den Esschert JW, van Lienden KP, de GW, Maas MA, Roelofs JJ, Heger M, et al. Portal vein embolization induces more liver regeneration than portal vein ligation in a standardized rabbit model. Surgery 2011 Mar;149(3):378-85.

(21) van den Esschert JW, van Lienden KP, Alles LK, van Wijk AC, Heger M, Roelofs JJ, et al. Liver regeneration after portal vein embolization using absorbable and permanent embolization materials in a rabbit model. Ann Surg 2012 Feb;255(2):311-8.

(22) van Lienden KP, van den Esschert JW, Rietkerk M, Heger M, Roelofs JJ, Lameris JS, et al. Short-term effects of combined hepatic vein embolization and portal vein embolization for the induction of liver regeneration in a rabbit model. J Vasc Interv Radiol 2012 Jul;23(7):962-7.

(23) Qi YY, Zou LG, Liang P, Zhang D. Establishing models of portal vein occlusion and evaluating value of multi-slice CT in hepatic VX2 tumor in rabbits. World J Gastroenterol 2007 Jun 28;13(24):3333-41.

(24) Zou RH, Li AH, Han F, Hong J, Li BK, Huang W, et al. Liver hypertrophy and accelerated growth of implanted tumors in nonembolized liver of rabbit after left portal vein embolization. J Surg Res 2012 Nov;178(1):255-63.

(25) Ishikawa M, Yogita S, Iuchi M, Yagi K, Chikaishi H, Fukuda Y, et al. Experimental and clinical studies on liver regeneration following transcatheter portal embolization. Hepatogastroenterology 2000 Jan; 47(31):226-33.

(26) Huang JY, Yang WZ, Li JJ, Jiang N, Zheng QB. Portal vein embolization induces compensatory hypertrophy of remnant liver. World J Gastroenterol 2006 Jan 21;12(3):408-14.

(27) Lu MD, Liang LJ, Huang JF, Ye WJ, Yang QS, Peng BG, et al. Portal vein embolization with ethanol injection via a fine needle in dogs. Surg Today 1995;25(5):416-20.

(28) Lu MD, Chen JW, Xie XY, Liang LJ, Huang JF. Portal vein embolization by fine needle ethanol injection: experimental and clinical studies. World J Gastroenterol 1999 Dec;5(6):506-10.

(29) Kaneko T, Nakao A, Takagi H. Experimental studies of new embolizing material for portal vein embolization. Hepatogastroenterology 2000 May;47(33):790-4.

(30) de Baere T, Denys A, Paradis V. Comparison of four embolic materials for portal vein embolization: experimental study in pigs. Eur Radiol 2009 Jun;19(6):1435-42.

(31) Duncan JR, Hicks ME, Cai SR, Brunt EM, Ponder KP. Embolization of portal vein branches induces hepatocyte replication in swine: a potential step in hepatic gene therapy. Radiology 1999 Feb;210(2): 467-77.

(32) Madoff DC, Gupta S, Pillsbury EP, Kan Z, Tinkey PT, Stephens LC, et al. Transarterial versus transhepatic portal vein embolization to induce selective hepatic hypertrophy: a comparative study in swine. J Vasc Interv Radiol 2007 Jan;18(1 Pt 1):79-93.

(33) Park S, Yoon HK, Lee N, Huh SJ, Kang GH, Lee I, et al. Portal vein embolization with use of a new liquid embolic material: an experimental study. J Vasc Interv Radiol 1999 Mar;10(3):339-45.

(34) Satake M, Tateishi U, Kobayashi T, Murata S, Kumazaki T. Percutaneous transhepatic portal vein embolization: effectiveness of absolute ethanol infusion with balloon catheter in a pig model. Acta Radiol 2005 Jul;46(4):344-52.

(35) Tsoumakidou G, Theocharis S, Ptohis N, Alexopoulou E, Mantziaras G, Kelekis NL, et al. Liver hypertrophy after percutaneous portal vein embolization: comparison of N-butyl-2-cyanocrylate versus sodium acrylate-vinyl alcohol copolymer particles in a swine model. Cardiovasc Intervent Radiol 2011 Oct;34(5):1042-9.

(36) Wilms C, Mueller L, Lenk C, Wittkugel O, Helmke K, Krupski-Berdien G, et al. Comparative study of portal vein embolization versus portal vein ligation for induction of hypertrophy of the future liver remnant using a mini-pig model. Ann Surg 2008 May;247(5):825-34.

(37) Smits ML, Vanlangenhove P, Sturm EJ, van den Bosch MA, Hav M, Praet M, et al. Transsinusoidal portal vein embolization with ethylene vinyl alcohol copolymer (Onyx): a feasibility study in pigs. Cardiovasc Intervent Radiol 2012 Oct;35(5):1172-80.

(38) Kinoshita H, Sakai K, Hirohashi K, Igawa S, Yamasaki O, Kubo S. Preoperative portal vein embolization for hepatocellular carcinoma. World J Surg 1986 Oct;10(5):803-8.

(39) Guiu B, Bize P, Gunthern D, Demartines N, Halkic N, Denys A. Portal vein embolization before right hepatectomy: improved results using n-butyl-cyanoacrylate compared to microparticles plus coils. Cardiovasc Intervent Radiol 2013 Oct;36(5):1306-12.

(40) Elias D, de Baere T, Roche A, Mducreux, Leclere J, Lasser P. During liver regeneration following right portal embolization the growth rate of liver metastases is more rapid than that of the liver parenchyma. Br J Surg 1999 Jun;86(6):784-8.

(41) Hayashi S, Baba Y, Ueno K, Nakajo M, Kubo F, Ueno S, et al. Acceleration of primary liver tumor growth rate in embolized hepatic lobe after portal vein embolization. Acta Radiol 2007 Sep;48(7):721-7.

(42) Robertson JH, Yang SY, Iga AM, Seifalian AM, Winslet MC. An in vivo rat model for early development of colorectal cancer metastasis to liver. Int J Exp Pathol 2008 Dec;89(6):447-57.

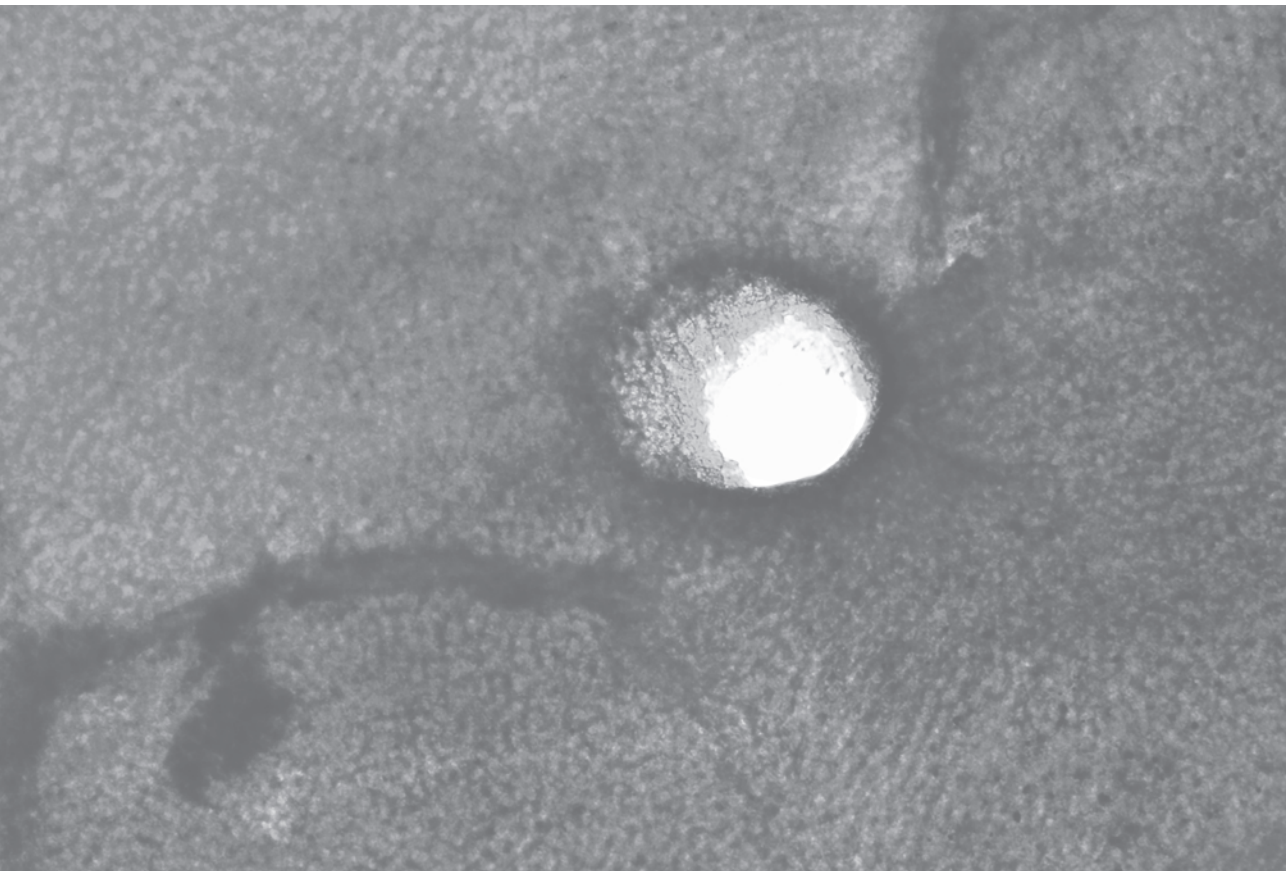
(43) van Lienden KP, Hoekstra LT, Bennink RJ, van Gulik TM. Intrahepatic left to right portoportal venous collateral vascular formation in patients undergoing right portal vein ligation. Cardiovasc Intervent Radiol 2013 Dec;36(6):1572-9.

(44) Hsiao IT, Lin KJ, Chang SI, Yen TC, Chen TC, Yeh TS. Impaired liver regeneration of steatotic rats after portal vein ligation: a particular emphasis on (99m)Tc-DISIDA scintigraphy and adiponectin signaling. J Hepatol 2010 Apr;52(4):540-9.

(45) Lin KJ, Liao CH, Hsiao IT, Yen TC, Chen TC, Jan YY, et al. Improved hepatocyte function of future liver remnant of cirrhotic rats after portal vein ligation: a bonus other than volume shifting. Surgery 2009 Feb;145(2):202-11.

(46) Schweizer W, Duda P, Tanner S, Balsiger D, Hoflin F, Blumgart LH, et al. Experimental atrophy/hypertrophy complex (AHC) of the liver: portal vein, but not bile duct obstruction, is the main driving force for the development of AHC in the rat. J Hepatol 1995 Jul;23(1):71-8.

(47) Tseng JH, Ouyang CH, Lin KJ, Yeh TS. Significance of insulin signaling in liver regeneration triggered by portal vein ligation. J Surg Res 2011 Mar;166(1):77-86.



CHAPTER 2

Use of an absorbable embolization material for reversible portal vein embolization in an experimental model

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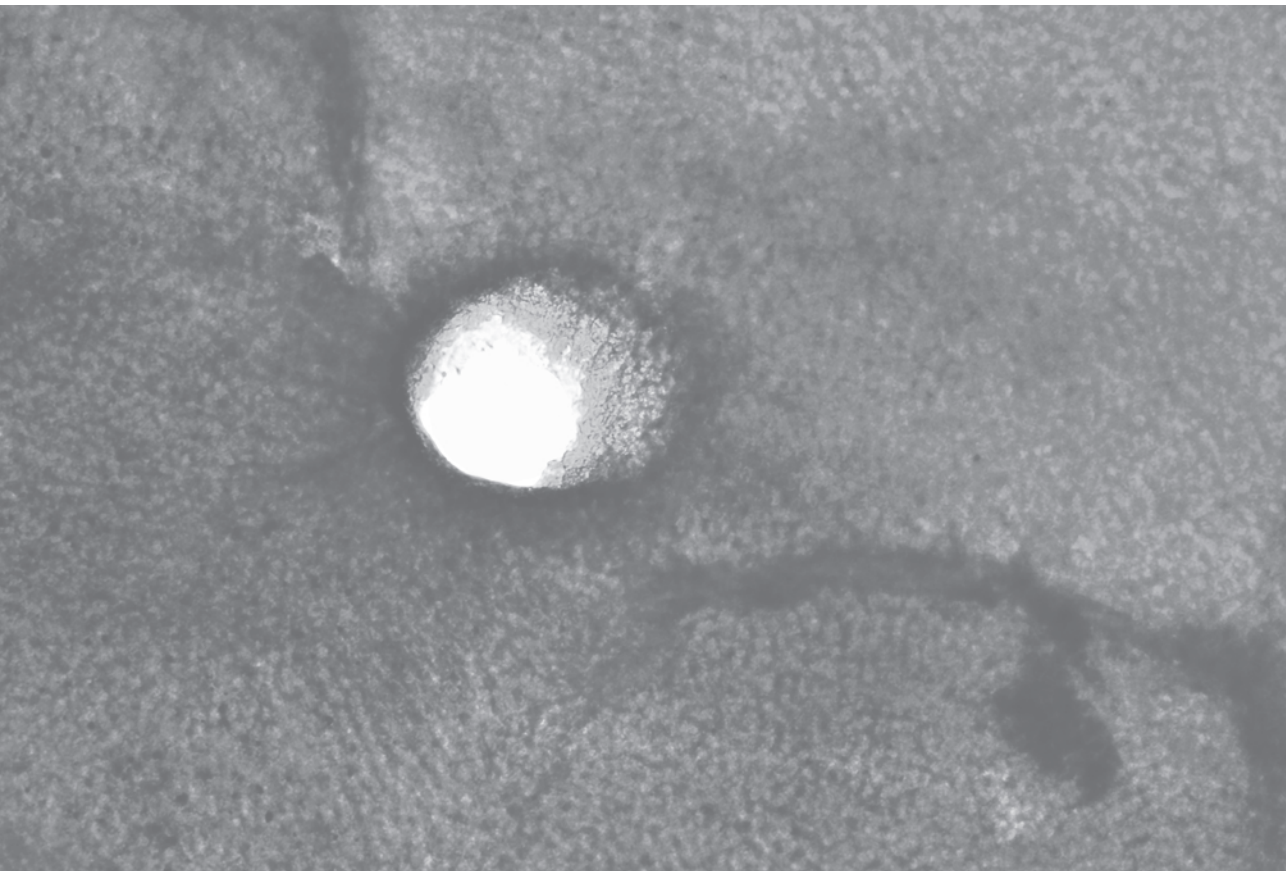


British Journal of Surgery 2016

ABSTRACT

Background:

Portal vein embolization (PVE) is used to increase future remnant liver size in patients requiring major hepatic resection. PVE using permanent embolization, however, predisposes to complications and excludes the use of PVE in living donor liver transplantation. In the present study, an absorbable embolization material containing fibrin glue and different concentrations of the fibrinolysis inhibitor aprotinin was used in an experimental animal model.

Methods:

PVE of the cranial liver lobes was performed in 30 New Zealand White rabbits, which were divided into five groups: fibrin glue + 1000, 700, 500, 300 or 150 kunits/ml aprotinin, and were compared with a previous series of permanent embolization using the same experimental set-up. Caudal liver lobe hypertrophy was determined by CT volumetry and portal recanalization was identified on contrast-enhanced CT images. Animals were killed after 7 or 42 days, and the results were compared with those of permanent embolization.

Results:

PVE using fibrin glue with aprotinin as embolic material was effective, with 500 kunits/ml providing the optimal hypertrophic response. Lower concentrations of aprotinin (150 and 300 kunits/ml) led to reduced hypertrophy owing to early recanalization of the embolized segments. The regeneration rate over the first 3 days was higher in the group with 500 kunits/ml aprotinin than in the groups with 300 or 150 kunits/ml or permanent embolization. In the 500-kunits/ml group, four of five animals showed recanalization 42 days after embolization, with minimal histological changes in the cranial lobes following recanalization.

Conclusion:

Fibrin glue combined with 500 kunits/ml aprotinin resulted in reversible PVE in 80 per cent of animals with a hypertrophy response comparable to that achieved with permanent embolization material.

INTRODUCTION

Liver resection is the only curative treatment for patients with a primary or secondary liver malignancy. Liver resection can be performed safely only when the future remnant liver (FRL) is of sufficient size. To limit the risk of postoperative liver failure, FRL volume is preferably 25 per cent or more of total liver volume in healthy livers and at least 35–50 per cent in compromised livers, as measured before surgery by CT volumetry. [1] When the FRL is too small, portal vein embolization (PVE) is the standard procedure used to increase FRL volume. [2] By occluding the portal vein branch to the tumour-bearing segments, compensatory hypertrophy of the contralateral segments is induced. Makuuchi and colleagues [3] were the first to describe PVE in patients, and since then PVE has made curative liver resection with acceptable postoperative complications possible for many patients.[4]

There is ongoing discussion on the optimal embolization material to be used for PVE. Generally, permanent embolization materials are used because of the higher hypertrophy response compared with that achieved with absorbable embolization materials.[5–7] However, permanent embolization has several disadvantages. First, 20–30 per cent of patients who undergo preoperative PVE are shown to have non-resectable disease at exploration.[8] In these patients, the permanently deportalized liver segments are prone to complications and reversible PVE would hypothetically be safer. The potential risk of permanent embolization of portal segments should thus be avoided in the setting of complex palliative treatment in a patient with unresectable disease after PVE. Second, CT becomes troublesome after permanent embolization owing to stardust formation based on the radio-opaque materials used, which reduces the diagnostic value of these scans. Furthermore, an effective and safe reversible PVE method might have clinical benefit in living donor transplantation, in which the functional volume of the future donor liver can be increased without causing permanent damage to the residual liver segments.

The aim of this study was to evaluate the use of fibrin glue with addition of different concentrations of aprotinin, which inhibits clot lysis, to achieve controllable dissolution of the obstruction and thereby reversible PVE in a standardized rabbit model.

MATERIALS AND METHODS

In vitro clot lysis

The effect of aprotonin on in vitro clot lysis was determined in a purified system consisting of fibrinogen, plasminogen, thrombin and tissue plasminogen activator (tPA). In a volume of 60 µl, fibrinogen (2.5 mg/ml, Haemocomplettan® P; Aventis Behring, Haywards Heath, UK), plasminogen (200 µg/ml), tPA (5 µg/ml, Actilyse®; Boehringer Ingelheim, Ingelheim am Rhein, Germany)[9], thrombin (10 nmol/l; gift from W. Kisiel, University of Albuquerque,

New Mexico, USA) and aprotinin (0–1000 kunits/ml; Roche, Basle, Switzerland) were incubated in buffer containing 10 mmol/l Hepes, 20 mmol/l calcium chloride, 150 mmol/l sodium chloride and 0.1 per cent bovine serum albumin (pH 7.4). Clot formation and subsequent clot lysis were followed by measuring changes in turbidity at 405 nm at 37°C in a microplate reader. Absorbance results were expressed in optical density units.

Animals

Thirty female New Zealand White rabbits (mean(s.d.) weight 3084(241) g) were obtained from Harlan (Gennat, France). Animals were housed individually with a 12-h dark–light cycle and fed standard chow ad libitum. All animals were allowed to acclimatize for 2 weeks before initiation of experiments. The institutional animal ethics and welfare committee approved all animal experimental protocols. The experiments were reported according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Experimental design

The rabbit liver is divided into four lobes: three cranial and one caudal.[10] Because the cranial lobes are isolated from the caudal lobe, the rabbit is ideally suited for selective portal vein occlusion (Figure S1, supporting information). PVE was performed in five groups (n = 5 rabbits per group) using fibrin glue (Beriplast® P Combi-Set; Nycomed, Hoofddorp, The Netherlands) with different concentrations of aprotinin (1000, 700, 500, 300, 150 kunits/ml). Aprotinin was mixed with fibrin glue according to the manufacturer's protocol and was reduced for non-standard aprotinin concentrations (all concentrations except 1000 kunits/ml, which is the standard aprotinin concentration in Beriplast® fibrin glue).

Results were compared with those of a previous PVE series [11] in which permanent embolization material (polyvinyl alcohol (PVA) particles and coils; P and C) was used (n = 5 rabbits); this comprised a combination of PVA (90–180 µm in diameter followed by 300–500 µm in diameter; Cook, Bloomington, Indiana, USA) and three fibred platinum coils (PVAc, 4.0, 5.0 and 6.0 mm; Boston Scientific, Natick, Massachusetts, USA). The methods and technique of PVE were identical to those of the present study. Before death, digital subtraction portography was performed to confirm recanalization of the portal vein.

Rabbit model of portal vein embolization

Anaesthesia was induced by intramuscular injection of 25 mg/kg ketamine (Nimatek; Eurovet, Bladel, The Netherlands) and 0.2 mg/kg medetomidine (Dexdomitor®; Orion, Espoo, Finland). Anaesthesia was maintained using 1–2 per cent isoflurane (Forane®; Abbott Laboratories, Sittingbourne, UK) mixed with oxygen : air (0.5 : 1, 1.5 l/min). All animals received a subcutaneous injection of 0.03 mg/kg buprenorphine (Temgesic®; Reckitt Benckiser Healthcare, Hull, UK) as preoperative analgesia, and 0.2 mg/kg Baytril® (Bayer Healthcare, Berlin Germany) was administered daily as antibiotic prophylaxis until the third postoperative day.

Following a midline laparotomy, a branch of the inferior mesenteric vein was cannulated with an 18-G catheter (Hospira Venisystem, Lake Forest, Illinois, USA). A Renegade 3-Fr microcatheter (Boston Scientific) with a Transend®-ex 0.36-mm × 182-cm guidewire (Boston Scientific) was subsequently introduced into the portal vein. Visualization of the individual portal vein branches by digital subtraction portography was carried out using a mobile C-arm Exposcop 8000 (Ziehm Imaging, Nurnberg, Germany). The microcatheter was placed in the main portal branch to the cranial lobe. The embolization material (fibrin glue with aprotinin) was infused through the catheter to achieve embolization of the portal branches of the cranial liver lobes. Afterwards, the catheter was flushed with 10 ml saline to avoid occlusion. After the embolization procedure, portal occlusion was confirmed by digital subtraction portography. Subsequently, the catheter was extracted and a ligature was used to close the mesenteric vein. The abdomen was closed in two layers using a running Vicryl® 4/0 suture (Ethicon, Johnson & Johnson, Somerville, New Jersey, USA) and interrupted Mersilene 3/0® U-sutures (Ethicon).

Quantification of liver regeneration

Multiphase contrast-enhanced CT was performed using a multislice helical CT scanner (Philips Medical Systems, Eindhoven, The Netherlands) on postoperative days 0, 3 and 7, and weekly thereafter when applicable. Briefly, contrast solution (3 ml Visipaque™; GE Healthcare, Waukesha, Wisconsin, USA) was injected in the lateral ear vein under anaesthesia. Following injection, arterial, portal and venous phase CT images were acquired. Three-dimensional reconstructions of the liver were made by superimposing sequential 2-mm axial slices, and the volumes of the total liver, caudal and cranial lobes were calculated using integrated software (MX-View 3.52; Philips Medical Systems, Best, The Netherlands). Caudal liver volume (CLV) was correlated with caudal liver lobe weight at time of death in order to validate volumetric measurements. The increase in CLV and the CLV regeneration rate were calculated using the following formulas:

$$\text{Increase CLV (\%)} = \frac{CLV_{post\text{-}PVE} - CLV_{pre\text{-}PVE}}{CLV_{pre\text{-}PVE}} \times 100\%$$

$$\text{Regeneration rate (\%/day)} = \frac{\text{Increase } CLV_{post\text{-}PVE}}{\text{Days post-PVE}}$$

On every CT scan, recanalization of the embolized portal branches was evaluated by analyzing ipsilateral portal perfusion. At death, portography was performed to confirm the recanalization status as determined by CT. In all rabbits, portography results corresponded to the recanalization status determined by CT analysis (Figure S2, supporting information).

To exclude congestive hypertrophy as a contributor to the measured increases in liver volume, the wet/dry weight ratio was examined on liver biopsies from all rabbits. Biopsies were weighed, stored at 60°C for 4 weeks, and then weighed again.

Biochemical parameters

Plasma alanine aminotransferase (ALT) was measured by the Department of Clinical Chemistry using a Cobas® 8000 modular analyser (Roche). Plasma total bile acids were measured using a Total Bile Acids Assay Kit (Diazyme, Poway, California, USA) according to the manufacturer's instructions on a Synergy™ HT microplate reader (Biotek, Winooski, Vermont, USA).

Histology

Liver tissue was fixed in formalin and embedded in paraffin. Liver sections (5 µm) were stained using standard haematoxylin and eosin, and van Gieson staining to visualize collagen. Cranial liver lobe histology of the groups treated with 300 and 500 kunits/ml apoprotin was assessed in a descriptive manner by an experienced liver pathologist, who was blinded to the group assignment.

Statistical analysis

Data are expressed as mean (s.d.). Differences in liver hypertrophy between groups were tested by means of the Mann–Whitney U test at individual time points, using values obtained by area under the curve (AUC) analysis. Differences between groups in ALT and total bile acid levels were tested using Kruskal–Wallis tests. Correlations were investigated by Pearson's product-moment correlation. $P < 0.050$ was considered statistically significant. All data analysis was performed using Graphpad Prism® version 5.0 (GraphPad Software, La Jolla, California, USA).

RESULTS

In vitro clot lysis

To select the optimal aprotinin dose for in vivo experiments, the effect of aprotinin on lysis of a preformed clot by tPA was investigated in a purified system in vitro. In the presence of 5 µg/ml tPA, clot lysis was inhibited by aprotinin, whereas at higher tPA concentrations no effect of aprotinin was observed (data not shown). When clot turbidity (increased turbidity reflects increased clot formation) was measured over time, aprotinin had a dose-dependent effect on clot lysis in the presence of 5 µg/mL tPA (Figure 1). In the absence of aprotinin, the clot was immediately degraded. With 250 kunits/ml aprotinin, the clot remained stable for 42 h. Further lowering the concentration of aprotinin resulted in rapid clot lysis, whereas in this set-up increasing concentrations did not provide additional clot stability. Therefore 150, 300, 500, 700 and 1000 kunits/ml aprotinin were selected for the in vivo experiments.

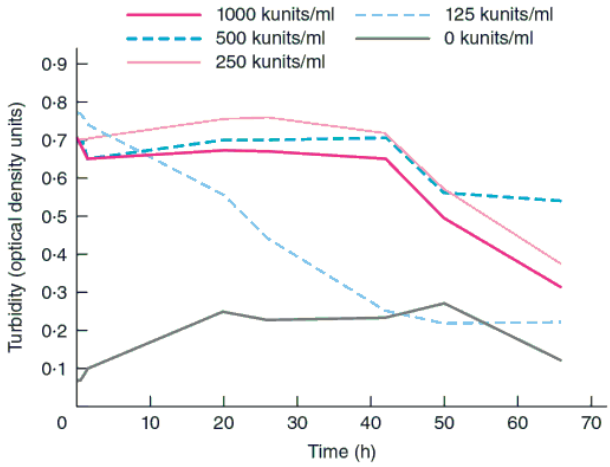


Figure 1. In vitro clot lysis in a purified system in the presence of varying concentrations of aprotinin; 250-1000 kunits/ml of aprotinin resulted in a stable clot for 48 h in the presence of 5 μ/ml tissue plasminogen activator.

Liver regeneration following portal vein embolization

Volumetric measurements of CLV correlated significantly with caudal liver lobe weight at time of death ($r = 0.965$, $P < 0.001$) (Figure S3, supporting information). At baseline, there was no difference between the groups in either total liver volume and CLV (data not shown). PVE was successful in all but one rabbit in the 1000-kunits/ml aprotinin group, determined by direct postprocedural recanalization on the portogram and confirmed by an inadequate hypertrophy response. This rabbit was excluded from further analysis.

All groups showed increases in CLV compared with baseline on days 3 and 7 after PVE (Figure 2a). The effect of aprotinin was optimal in the group that received 500 kunits/ml (Figure 2b); this group showed the greatest increase in CLV on day 7, which did not differ from that of the group treated with P and C, the standard embolization material ($P = 0.962$). Both lower and higher concentrations of aprotinin conferred a smaller degree of hypertrophy. A CLV increase exceeding 50 per cent was observed only in all rabbits in the 300-, 500- and 700-kunits/ml aprotinin groups, and the P and C group. In the 700-kunits/ml group, however, a decrease in regeneration rate was observed on day 7 compared with day 3 (Figure 2c), on the basis of which only the 300- and 500-kunits/ml groups were analyzed further over time until day 42.

The regeneration rate was calculated for all groups on days 3 and 7 after PVE (Figure 2c). On day 3, the regeneration rate in the 500-kunits/ml group was higher than in the groups that received 300 or 150 kunits/ml aprotinin, or P and C. On day 7, the regeneration rate in the 500-kunits/ml group was higher than that in the 700-kunits/ml group. These results demonstrated a rapid and effective hypertrophy response following PVE when fibrin glue was used in combination with 500 kunits/ml aprotinin.

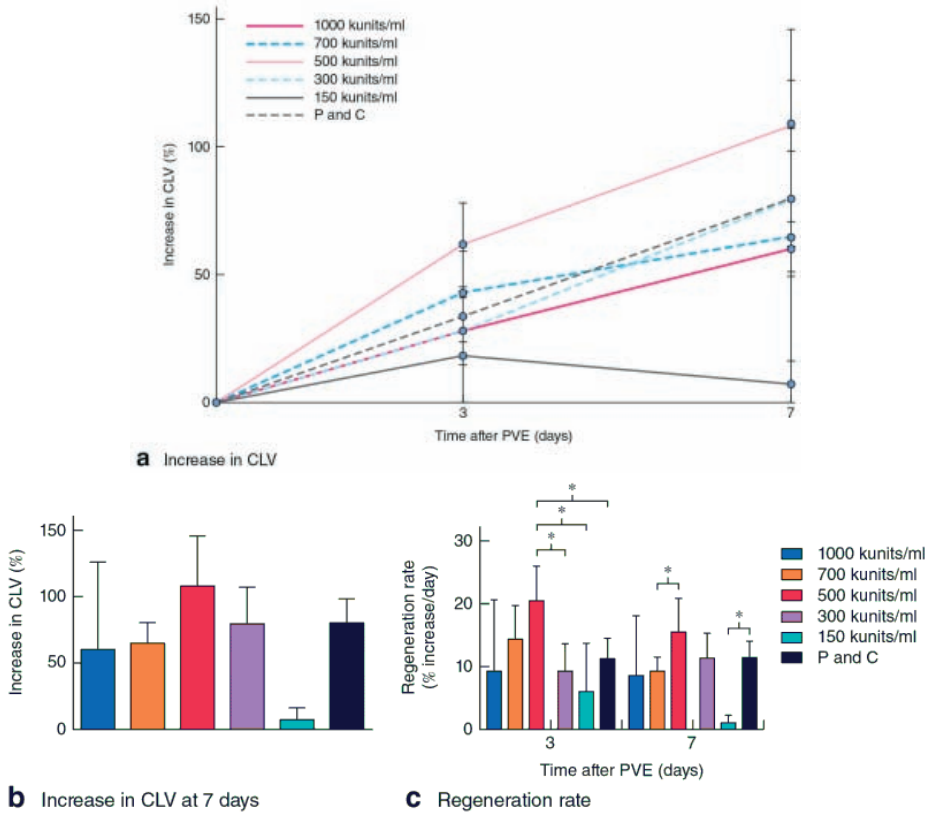


Figure 2a. Increase in caudal liver volume (CLV) on days 3 and 7 after portal vein embolization (PVE) in groups with varying concentrations of aprotinin added to the fibrin glue. Values were normalized to baseline CLV using CT volumetry. **b** Increase in CLV at 7 days after PVE. **c** Differences in regeneration rate between groups on days 3 and 7 after PVE. Values are mean(s.d.) (n = 4–5 per group). *P < 0.050 (Mann–Whitney U test).

Recanalization

After establishing PVE efficacy over an aprotonin concentration range of 300–500 kunits/ml, recanalization of portal branches of the embolized liver lobes was assessed by examining portal flow on CT. On day 7 after PVE, all rabbits in the 150-kunits/ml group showed recanalization, whereas no recanalization was seen in the dose range of 300–1000 kunits/ml. Because it was probable that recanalization was inversely related to the aprotonin concentration used, animals in the 300- and 500-kunits/ml groups were scanned weekly until day 42 to examine recanalization dynamics. On day 42, four of five animals in the 500-kunits/ml group and three of five in the 300-kunits/ml group showed recanalization of the occluded portal system of the cranial lobes (Figure 3a,b).

With respect to recanalization dynamics, two of five animals in the 300-kunits/ml group already had patent portal vein branches to the cranial liver lobes in the first 10 days after

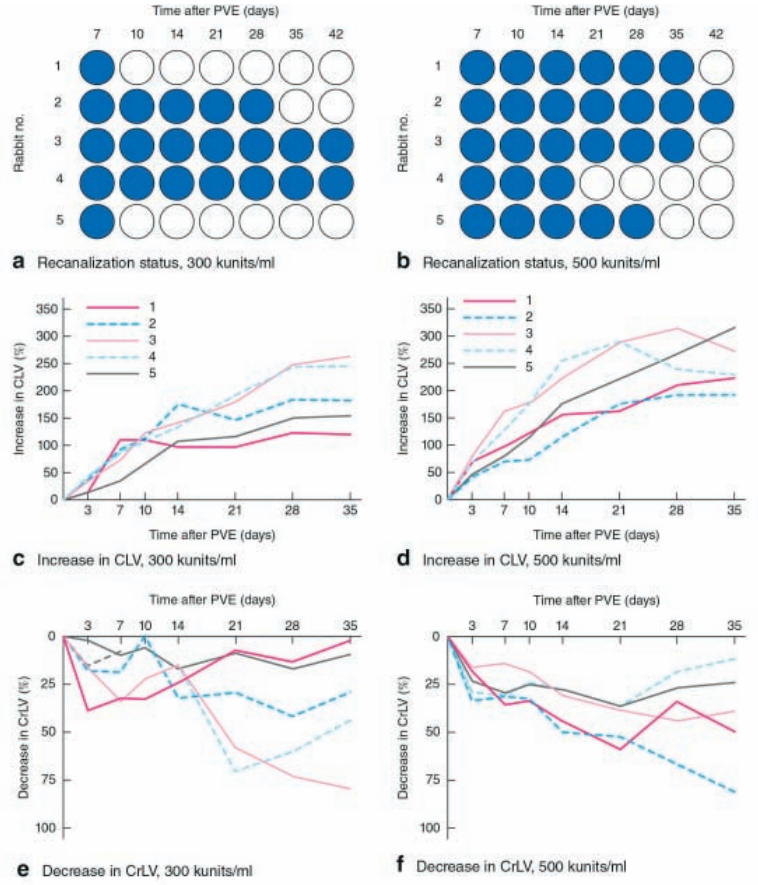


Figure 3 a,b. Recanalization of embolized liver lobes in 300-kunits/ml (a) and 500-kunits/ml (b) aprotinin group determined by examining portal flow on CT images. Numbers 1–5 represent individual animals in each group. Closed circles indicate portal vein obstruction and open circles represent portal vein recanalization. **c,d** Increase in caudal liver volume (CLV) in 300-kunits/ml **(c)** and 500-kunits/ml **(d)** aprotinin group. **e,f** Decrease in cranial liver volume (CrLV) in 300-kunits/ml **(e)** and 500-kunits/ml **(f)** aprotinin group. In **c–f** numbers 1–5 represent the same animals in each aprotinin group as in **a–b.**

PVE (rabbit 1 and 5; Figure 3a), which hampered CLV hypertrophy (Figure 3c). In contrast, earliest recanalization in the 500-kunits/ml group was evident on day 21 after PVE, which did not affect CLV hypertrophy (Figure 3b,d). Therefore, 500 kunits/ml appeared to be the optimal concentration of aprotinin for effective hypertrophy, while allowing recanalization after a sufficiently extended period.

Recanalization had significant effects on the volume of the cranial liver lobes. When recanalization occurred, an increase in cranial liver volume (CrLV) was observed in both groups (Figure 3e,f). In both 300- and 500-kunits/ml groups, there was a significant difference in the

decrease in CrLV on day 35 between animals with non-recanalized portal branches versus those with recanalized portal branches (P = 0.024).

Biochemical parameters

After establishing an adequate hypertrophy response using fibrin glue and aprotinin, with 80 per cent recanalization, serum ALT and total bile acids were measured to ensure the safety of the procedure. Serum ALT levels increased following PVE with a peak on day 1. No significant differences were measured between groups, but there was a trend towards lower peak ALT levels in the aprotinin groups compared with the P and C group (Figure 4a).

Serum total bile acid levels were measured in all rabbits. No significant intergroup differences were seen (Figure 4b). Serum bile acid levels correlated significantly with regeneration on both days 3 and 7 (data not shown).

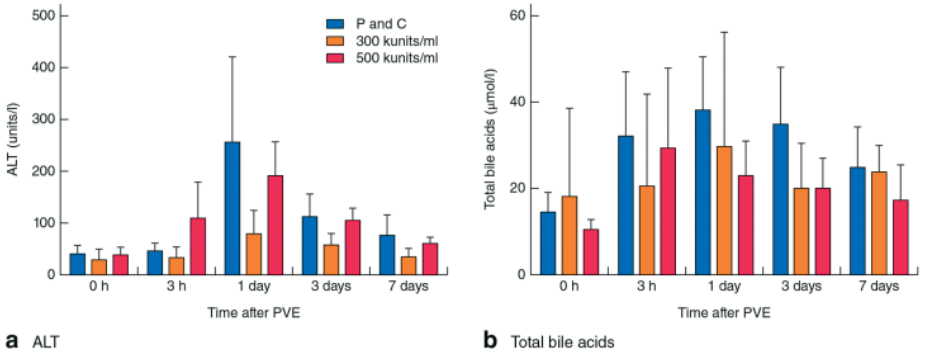
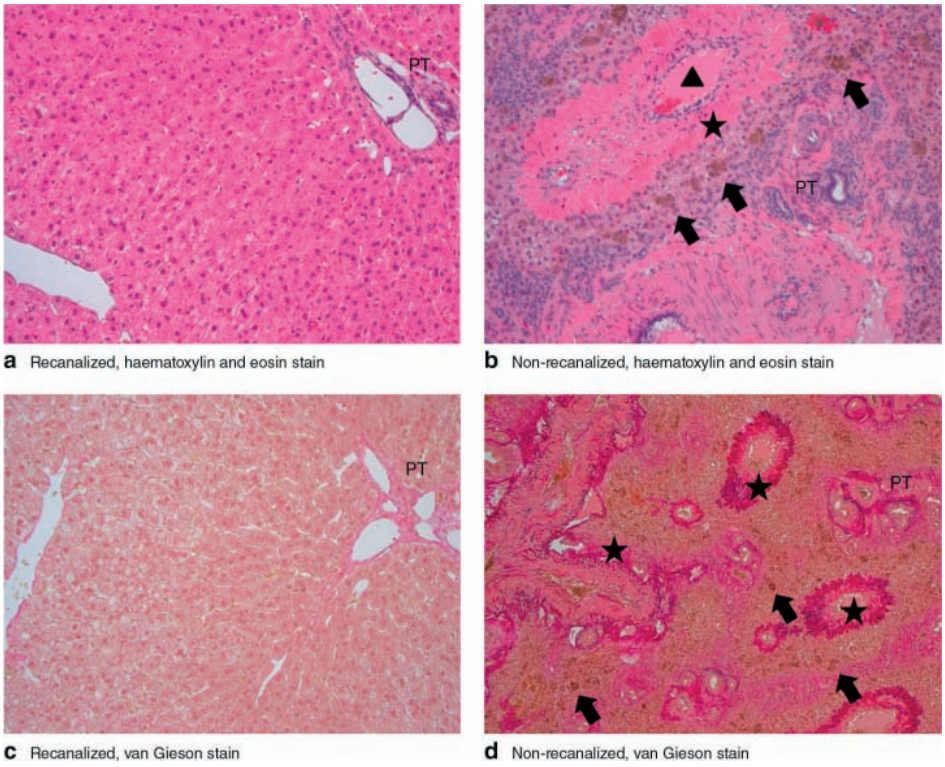


Figure 4 a. Plasma alanine aminotransferase (ALT) levels and **b** total bile acid concentrations in the polyvinyl alcohol particles and coils (P and C), and 300- and 500-kunits/ml aprotinin groups. Values are mean (s.d.). There were no significant differences between groups.

Histology

Cranial liver lobe sections from the 300- and 500 kunits/ml aprotinin groups were examined to determine parenchymal viability following temporary portal occlusion. Marked differences were observed between recanalized (Figure 5a,c) and embolized (Figure 5b,d) liver lobes. In the animals with no recanalization (Figure 5b,d), severe histomorphological changes were evident with severe perivenular and periportal fibrosis and formation of fibrotic portal–portal bridges. Sinusoidal congestion was noted, as well as extensive macrophage influx. Variable venous occlusion was seen in the non-recanalized animals.

Only minor histological changes were found in the cranial liver lobes of recanalized animals (Figure 5a,c). Discrete sinusoidal congestion with minimal inflammation was observed, and in the larger portal tracts mild edema with none to minimal periportal and perivenular fibrosis.



a Recanalized, haematoxylin and eosin stain **b** Non-recanalized, haematoxylin and eosin stain

c Recanalized, van Gieson stain **d** Non-recanalized, van Gieson stain

Figure 5. Representative cranial lobe sections from livers harvested on day 49 after portal vein embolization. **a,b** Haematoxylin and eosin-stained sections from a recanalized animal **(a)** and a nonrecanalized animal **(b)**. **c,d** Van Gieson-stained sections from a recanalized animal **(c)** and a nonrecanalizedanimal **(d)**. Macrophages (arrows), fibrotic changes (asterisks) and occluded vein (triangle) are shown, PT, portal triad (original magnification × 200).

DISCUSSION

This study sought to establish a novel reversible PVE method in a standardized rabbit model by modulating clot lysis with aprotinin. Fibrin glue-based PVE in combination with 500 kunits/ml aprotinin resulted in an adequate hypertrophy response, while providing recanalization in four of five animals after 42 days. These results demonstrate that reversible PVE can be performed safely while preserving procedure efficacy.

Preoperative PVE is part of standard care to prevent postoperative liver failure in patients undergoing major hepatic resections when the FRL volume and/or function is deemed insufficient before surgery. However, using P and C to embolize liver segments severely impairs the diagnostic accuracy of subsequent diagnostic CT. Therefore, absorbable embolization materials hold a potential benefit over the currently used permanent embolization materi-

als. The challenge is to achieve reversible occlusion of the portal branches, while maintaining a regenerative response that is sufficient to allow safe liver resection.

Lainas and colleagues [12] found a sufficient hypertrophy response after PVE with gelfoam in monkeys. Recanalization of the embolized portal branches had taken place after 12–16 days, while adequate volume increase of the non-embolized liver lobes was noted. Van den Esschert and co-workers [11], however, concluded that gelfoam in a rabbit model of PVE was absorbed within 7 days and resulted in clinically insufficient hypertrophy of the non-embolized lobes. In the same study, Beriplast® (fibrin glue with 1000 kunits/ml aprotonin) was evaluated as potential absorbable embolic material. Beriplast®, however, permanently obstructed portal flow, which was attributed to excessive antifibrinolytic activity of aprotonin.

Fibrin glue is a two-component surgical haemostatic agent consisting of the blood coagulation factors fibrinogen, factor XIII, thrombin, an antifibrinolytic agent (aprotinin) and calcium chloride. It mimics the final stages of secondary haemostasis to produce a stable fibrin clot and is commonly used to achieve haemostasis of the resection surface of the liver after parenchymal transection.[13] Fibrin glue has been used routinely for PVE and appears to be safe and effective, yet it is not absorbable and therefore acts as a permanent embolic material. [14,15] The hypothesis of the present study was that lowering the aprotinin concentration would accelerate absorption of the embolic material and hence allow fibrin glue to be used as an absorbable embolic agent.

The in vitro experiments demonstrated that aprotinin effectively inhibits fibrinolysis. Unlike 125 kunits/ml aprotinin, doses of 250–1000 kunits/ml in the presence of 5 µg/ml tPA resulted in stable clots for more than 42 h. Based on these results, the efficacy of the higher aprotonin concentrations were evaluated further in vivo.

In rabbits, PVE using fibrin glue with 500 kunits/ml aprotinin induced the optimal hypertrophy response in the non-embolized liver lobe. During the first 3 days the regeneration rate was significantly higher than in the group embolized with standard P and C. Embolization using less than 500 kunits/ml aprotonin resulted in reduced caudal lobe hypertrophy. This was probably due to early lysis of the embolization material and rapid reperfusion of the embolized portal branches, as demonstrated by recanalization of all portal branches in the 150-kunits/ml aprotinin group by day 3 after PVE. However, the use of aprotonin concentrations exceeding 500 kunits/ml also reduced regeneration, despite stable clot formation. This may be explained by inhibitory effects of aprotinin on liver regeneration pathways. Aprotinin inhibits plasmin, which is required for hepatocyte growth factor expression during liver regeneration [16], as well as for extracellular matrix proteolysis and clearance of cellular debris.[17] In addition, plasminogen mediates liver regeneration by regulating angiogenesis. [18] In high doses, aprotinin also inhibits the kallikrein–kinin system, which in turn reduces plasmin formation.[19] All these effects of increased aprotinin levels probably contributed to the reduced hepatic regenerative capacity observed at higher concentrations.

The clinical potential of reversible PVE is only substantial when the recanalized liver lobes show functional viability. Recanalization of the embolized segments resulted in the regrowth of the atrophied cranial liver lobes, suggesting viability and return of function of the embolized segments upon recanalization. This was supported by lower decreases in CrLV on day 35 in non-recanalized animals compared with those not showing recanalization in the 300- and 500-kunits/ml groups (Figure 3e,f). However, as liver volume does not necessarily reflect liver function [20], functional recovery of recanalized liver segments should be confirmed using quantitative, dynamic liver function tests.[21] Nevertheless, histological analysis showed marked differences between recanalized and embolized cranial liver lobes. Following recanalization, liver histology showed only minor changes, attesting to the hypothesis that the embolized lobes contribute to liver function following recanalization. Considering the minor histological changes in the embolized lobes of rabbits following recanalization, reversible PVE might have potential application in living donor liver donation. However, the technique must be optimized to obtain maximal hypertrophy with an optimal time span to recanalization before clinical introduction.

Associating liver partition with portal vein ligation for staged hepatectomy (ALPPS) combines parenchymal transection and portal vein ligation in patients with a small FRL volume and function, and results in a rapid hypertrophic response.[22] ALPPS resulted in 74 per cent (median) hypertrophy in a median of 9 days, which was faster than similar hypertrophy in a median of 34 days after PVE.[23] Most likely, the parenchymal transection prevents collateral perfusion of the deportalized segments thereby enhancing liver regeneration. Furthermore, with ALPPS, 97 per cent of patients completed liver resection compared with 72 per cent of patients treated with PVE. [23,24] ALPPS might be an alternative technique to reduce the number of patients treated with PVE who do not progress to surgery; however, its effectiveness is counterbalanced by increased morbidity and mortality compared with PVE.[23,25] No ALPPS models have been developed in rabbits. In mice, ALPPS induces more rapid and increased hypertrophy compared with portal vein ligation alone. The regeneration obtained in the present study is comparable to that in the authors' previous reports of PVE in rabbits using permanent embolization materials; however, it is most likely slower than the hypertrophy response after ALPPS.[11] Although ALPPS might result in superior hypertrophy compared with (permanent) PVE, the procedures have distinct indications. In the present study, using a standardized rabbit model of PVE, in which fibrin glue was combined with 500 kunits/ml aprotinin, reversible PVE was obtained in 80 per cent of animals (four of five) while inducing a hypertrophy response comparable with that achieved with standard (permanent) embolization materials. These results pave the way for the clinical evaluation of a fibrin glue based, reversible PVE technique.

References

(1) van Gulik TM, van den Esschert JW, de Graaf W, et al. Controversies in the use of portal vein embolization. Dig Surg 2008;25:436–444.

(2) Abulkhir A, Limongelli P, Healey AJ, et al. Preoperative portal vein embolization for major liver resection: a meta-analysis. Ann Surg 2008;247:49–57.

(3) Makuuchi M, Thai BL, Takayasu K, et al. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. Surgery 1990;107:521–527.

(4) May BJ, Talenfeld AD, Madoff DC. Update on portal vein embolization: evidence-based outcomes, controversies, and novel strategies. J Vasc Interv Radiol 2013;24:241–254.

(5) de Baere T, Roche A, Vavasseur D, et al. Portal vein embolization: utility for inducing left hepatic lobe hypertrophy before surgery. Radiology 1993; 188: 73–77.

(6) Huang JY, Yang WZ, Li JJ, et al. Portal vein embolization induces compensatory hypertrophy of remnant liver. World J Gastroenterol 2006;12:408–414.

(7) Kaneko T, Nakao A, Takagi H. Clinical studies of new material for portal vein embolization: comparison of embolic effect with different agents. Hepatogastroenterology 2002;49:472–477.

(8) van Lienden KP, van den Esschert JW, de Graaf W, et al. Portal vein embolization before liver resection: a systematic review. Cardiovasc Intervent Radiol 2013;36:25–34.

(9) Deutsch DG, Mertz ET. Plasminogen: purification from human plasma by affinity chromatography. Science 1970;170:1095–1096.

(10) Huisman F, van Lienden KP, Damude S, et al. A review of animal models for portal vein embolization. J Surg Res 2014;191:179–188.

(11) van den Esschert JW, van Lienden KP, Alles LK, et al. Liver regeneration after portal vein embolization using absorbable and permanent embolization materials in a rabbit model. Ann Surg 2012;255:311–318.

(12) Lainas P, Boudechiche L, Osorio A, et al. Liver regeneration and recanalization time course following reversible portal vein embolization. J Hepatol 2008;49:354–362.

(13) Dunn CJ, Goa KL. Fibrin sealant: a review of its use in surgery and endoscopy. Drugs 1999;58:863–886.

(14) Ebata T, Yokoyama Y, Igami T, et al. Portal vein embolization before extended hepatectomy for biliary cancer: current technique and review of 494 consecutive embolizations. Dig Surg 2012;29:23 29.

(15) Nagino M, Nimura Y, Kamiya J, et al. Selective percutaneous transhepatic embolization of the portal vein in preparation for extensive liver resection: the ipsilateral approach. Radiology 1996;200: 559–563.

(16) Shanmukhappa K, Matte U, Degen JL, et al. Plasmin-mediated proteolysis is required for hepatocyte growth factor activation during liver repair. J Biol Chem 2009;284:12917–12923.

(17) Okada K, Ueshima S, Imano M, et al. The regulation of liver regeneration by the plasmin/alpha 2-antiplasmin system. J Hepatol 2004;40:110–116.

(18) Drixler TA, Vogten JM, Gebbink MF, et al. Plasminogen mediates liver regeneration and angiogenesis after experimental partial hepatectomy. Br J Surg 2003;90:1384–1390.

(19) Mahdy AM, Webster NR. Perioperative systemic haemostatic agents. Br J Anaesth 2004;93 842–858.

(20) de Graaf W, van Lienden KP, Dinant S, et al. Assessment of future remnant liver function using hepatobiliary scintigraphy in patients undergoing major liver resection. J Gastrointest Surg 2010;14:369–378.

(21) Hoekstra LT, de Graaf W, Nibourg GA, et al. Physiological and biochemical basis of clinical liver function tests: a review. Ann Surg 2013;257:27–36.

(22) Schnitzbauer AA, Lang SA, Goessmann H, et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. Ann Surg 2012;255:405–414.

(23) Shindoh J, Vauthey JN, Zimmitti G, et al. Analysis of the efficacy of portal vein embolization for patients with extensive liver malignancy and very low future liver remnant volume, including a comparison with the associating liver partition with portal vein ligation for staged hepatectomy approach. J Am Coll Surg 2013;217:126–133.

(24) Schadde E, Schnitzbauer AA, Tschuor C, et al. Systematic review and meta-analysis of feasibility, safety, and efficacy of a novel procedure: associating liver partition and portal vein ligation for staged hepatectomy. Ann Surg Oncol 2015;22:3109–3120.

(25) Aloia TA. Associating liver partition and portal vein ligation for staged hepatectomy: portal vein embolization should remain the gold standard. JAMA Surg 2015;150:927–928.

Supportive information

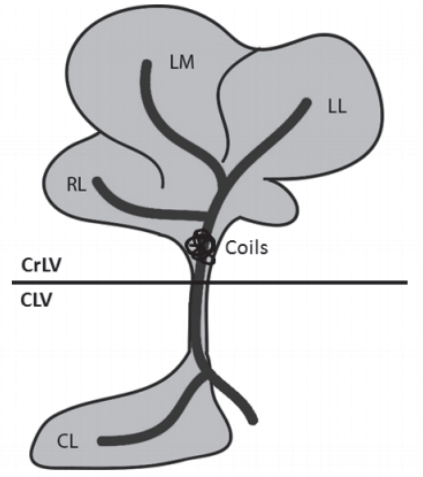


Figure S1. Anatomy of the rabbit liver. Four main lobes: caudal liver lobe and three cranial lobes: left lateral (LL), left medial (LM), and the right liver lobe (RL). CT volumetric assessment is facilitated by the rabbit liver anatomy in which the cranial and caudal lobes are separated. The lobes above the black line represent the cranial liver volume (CrLV), the lobes below the line represent the caudal liver volume (CLV). The coils represent the position of portal catheter placement during PVE and the position of the coils in the Particles & Coils group.

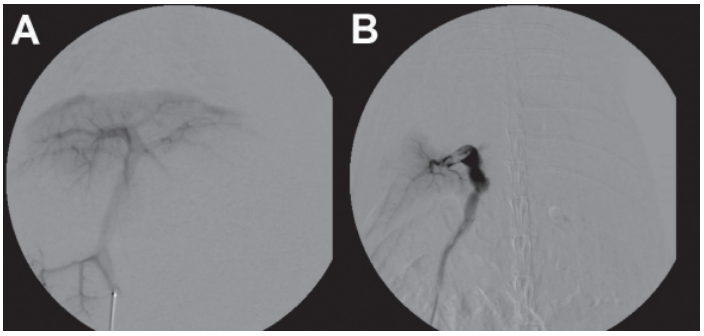


Figure S2. Portography before sacrifice to confirm correctness of recanalization status based on portal perfusion on contrast-enhanced CT-scans. **A**. Portography demonstrating an occluded portal system of the cranial lobes. **B.** Portography demonstrating perfusion of the portal system of the cranial liver lobes in a previously embolized animal.

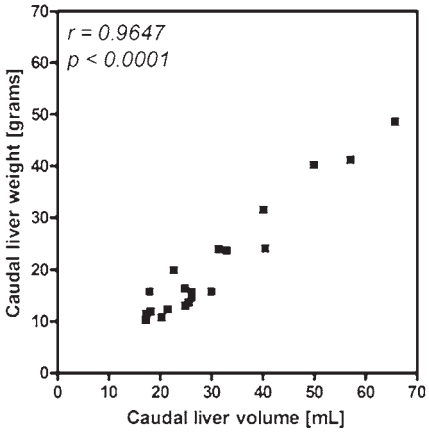
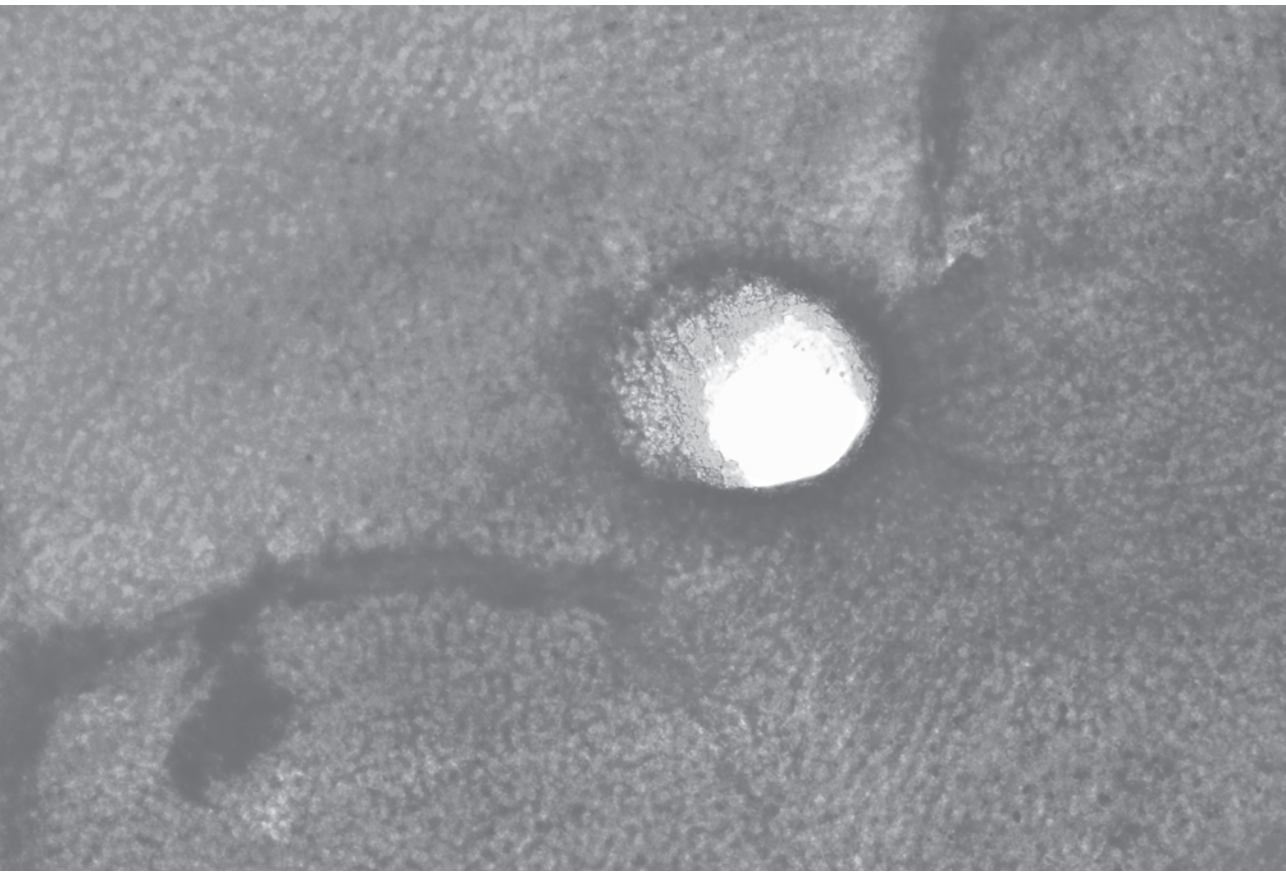


Figure S3. Correlation between CLV determined by CT-volumetric analysis on the CT scan directly before sacrifice and caudal liver weight at sacrifice. Correlation was tested using Pearson's correlation coefficient for 20 animals. r = 0.9647 and p < 0.0001.



CHAPTER 3

Effect of obeticholic acid on liver regeneration following portal vein embolization in an experimental model

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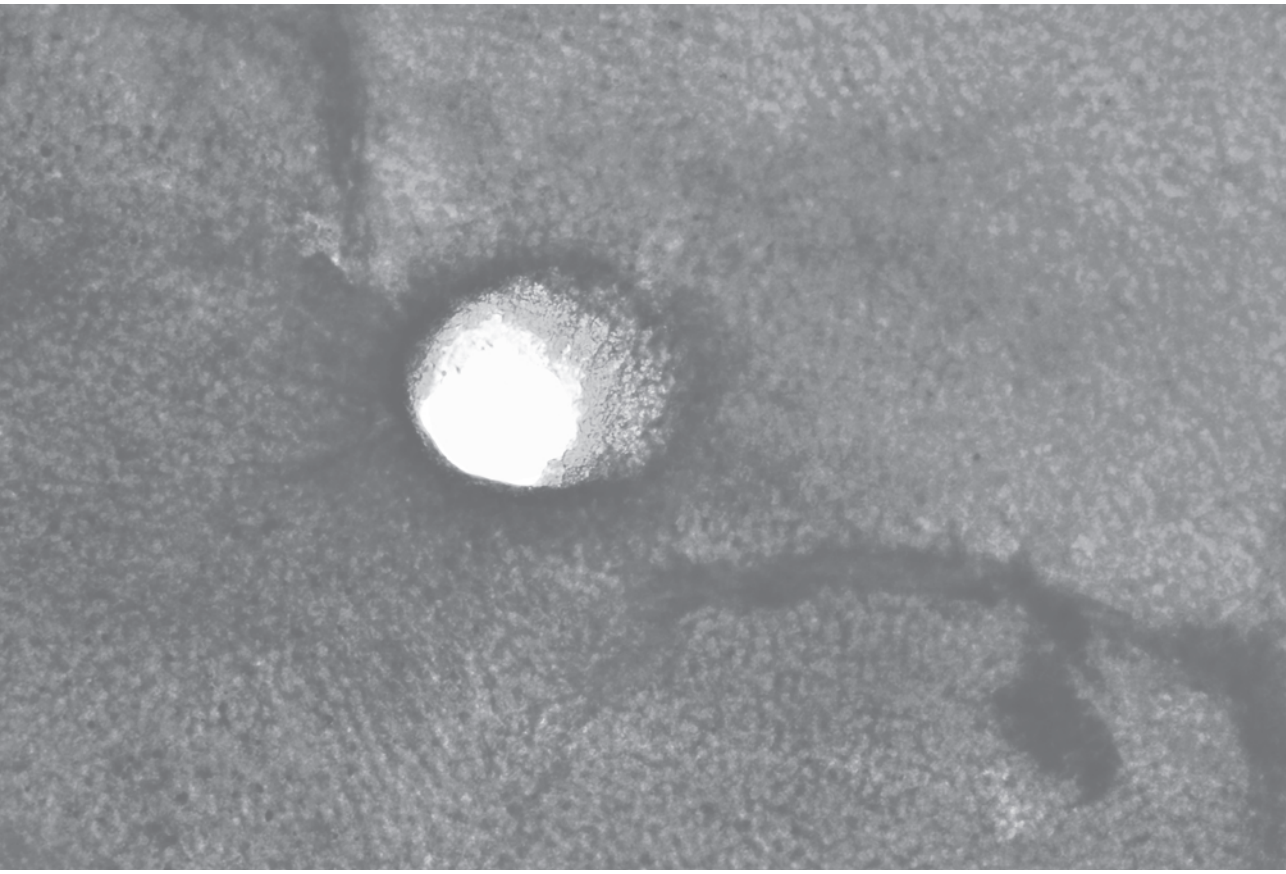


British Journal of Surgery 2017

ABSTRACT

Background:

The bile salt-activated transcription factor farnesoid X receptor (FXR) is a key mediator of proliferative bile salt signalling, which is assumed to play a role in the early phase of compensatory liver growth. The aim of this study was to evaluate the effect of a potent FXR agonist (obeticholic acid, OCA) on liver growth following portal vein embolization (PVE).

Methods:

Rabbits were allocated to receive daily oral gavage with OCA (10 mg/kg) or vehicle (control group) starting 7days before PVE (n=18 per group), and continued until 7days after PVE. PVE of the cranial liver lobes was performed using polyvinyl alcohol particles and coils on day 0. Caudal liver volume (CLV) was analysed by CT volumetry on days –7, –1, +3 and +7. Liver function was determined by measuring mebrofenin uptake using hepatobiliary scintigraphy. Additional parameters analysed were plasma aminotransferase levels, and histological scoring of haematoxylin and eosin- and Ki-67-stained liver sections.

Results:

Three days after PVE of the cranial lobes, the increase in CLV was 2.2-fold greater in the OCA group than in controls (mean(s.d.) 56.1(20.3) versus 26.1(15.4) per cent respectively; $P < 0.001$). This increase remained greater 7 days after PVE (+1.5-fold; $P = 0.020$). The increase in caudal liver function at day +3 was greater in OCA-treated animals (+1.2-fold; $P = 0.017$). The number of Ki-67-positive hepatocytes was 1.6-fold higher in OCA-treated animals 3 days after PVE ($P = 0.045$). Plasma aminotransferase levels and histology did not differ significantly between groups.

Conclusion:

OCA accelerated liver regeneration after PVE in a rabbit model. OCA treatment might increase the efficacy of PVE and, thereby, resectability.

INTRODUCTION

The liver is the only human organ that is able to regenerate following injury or loss of tissue mass. This regenerative capacity is essential in liver surgery, as it allows resection of up to 70 per cent of liver mass with acceptable morbidity and mortality. [1] However, clinical outcomes correlate with the amount and function of the remnant liver and its ability to regenerate. [2,3] Enhancing liver regeneration could be instrumental in overcoming these surgical limitations. The search for pharmacological interventions to stimulate liver regeneration is ongoing and has not yet resulted in a clinical application.

Recently, bile acids have been identified as early mediators of liver regeneration through activation of the nuclear bile acid receptor farnesoid X receptor (FXR). [4] Bile flow is essential for liver regeneration after partial hepatectomy, and disruption of the enterohepatic circulation delays liver regeneration. [4] This is attributed to loss of signalling via FXR, as Fxr-deficient mice display delayed regeneration following partial hepatectomy. [4,5] Intestinal FXR activation might be the primary mediator via the production and portal release of mitogenic and bile salt homeostatic fibroblast growth factor (FGF) 19 (mouse orthologue Fgf15). [5,6] Absence of Fgf15 impaired liver regeneration and increased mortality after partial hepatectomy in mice. [7] Fgf15 acts through the hepatocyte Fgfr4 receptor to regulate bile acid synthesis and stimulate regenerative signaling. [7,8]

Recently, potent FXR agonists have been developed that might stimulate liver regeneration. Obeticholic acid (OCA) is a semisynthetic bile acid analogue with around 100-fold increased potency for activating human FXR compared with the most potent endogenous bile salt agonist, chenodeoxycholic acid. [9] OCA represses hepatic bile acid synthesis, limits hepatocyte bile acid uptake, and stimulates basolateral and canalicular bile acid export. [10] These actions all contribute to the maintenance of low intrahepatic bile acid levels, thereby preventing bile acid hepatotoxicity and promoting normal progression of liver regeneration after partial hepatectomy. Furthermore, OCA might directly stimulate hepatocellular proliferation by inducing expression of cell-cycle regulatory transcription factor Foxm1b. [4,11] Alternatively, OCA-induced expression of FGF-19/Fgf15 in the ileum might lead to similar lowering of bile acid levels in hepatocytes through Fgfr4 and promotion of regeneration inducing pathways. [7,8]

It has been hypothesized that OCA stimulates liver regeneration when applied during portal vein embolization (PVE), a preoperative procedure used to increase future liver remnant (FLR) volume. [12] PVE induces a 37 – 62 per cent increase in FLR volume over 29 – 34 days. [12.13] The relatively long interval between PVE and liver resection in patients might necessitate chemotherapy to limit tumour progression. [14] On the other hand, the regenerative response following PVE is not always sufficient to proceed to safe liver resection. [12] Enhancing PVE-induced liver regeneration has a clear clinical benefit as it renders more patients eligible for surgery.

The aim of this study was to examine the effect of OCA on liver regeneration in a standardized rabbit model of PVE. [15]

METHODS

The animal ethics and welfare committee of the Academic Medical Centre, Amsterdam, approved the experimental protocols (BEX35AC and BEX35AD). Thirty-six New Zealand White rabbits (Charles River, Gennat, France) with a mean(s.d.) weight of 2941(267) g were allowed to acclimatize for 1 week before inclusion in the experiments. Rabbits were housed in groups in a temperature-controlled room with a 12-h light/dark cycle, and free access to water and standard chow. Animal experiments were reported according to the ARRIVE guidelines. [16]

Experimental design

Two groups of 18 rabbits were planned for PVE. Animals were allocated to either OCA (a gift from Intercept Pharmaceuticals, New York, USA) treatment (10mg/kg in 1 per cent methyl cellulose) or vehicle (1 per cent methyl cellulose; Sigma Aldrich, Zwijndrecht, The Netherlands) (control) via oral gavage (1.5 ml for a 3-kg animal). Treatment was started 7 days before PVE, and continued until death of the animal at 3 or 7days after PVE. Seven days of OCA pretreatment was chosen to ensure adequate tissue levels of OCA at the time of PVE. [17] In mice, diet enriched with cholic acid induced spontaneous liver growth in an FXR dependent manner. [4] Therefore, liver volume and function were assessed 7 days and 1 day before PVE (days –7 and –1 respectively; PVE was carried out on day 0).

Portal vein embolization

Animals were anaesthetized by subcutaneous injection of 25 mg/kg ketamine (Nimatek®; Eurovet, Bladel, The Netherlands) and 0.2mg/kg medetomidine (Dexdomitor®; Orion, Espoo, Finland). Isoflurane 2 per cent (Forene®; Abbott Laboratories, Sittingbourne, UK) mixed with oxygen/air (1:1, 3l/min) was used to maintain anaesthesia. Preoperative analgesia consisted of 0.03 mg/kg buprenorphine (Temgesic®; Reckitt Benckiser Healthcare, Hull, UK). Antibiotic prophylaxis consisted of subcutaneous injection of 0.2 mg/kg enrofloxacin (Baytril®; Bayer Healthcare, Berlin, Germany).

PVE was performed as described previously. [15] Following midline laparotomy, a branch of the inferior mesenteric vein was cannulated using an 18-G catheter (Hospira Venisystems, Lake Forest, Illinois, USA). Under digital subtraction portography, a RenegadeTM 3-Fr microcatheter (Boston Scientific, Natick, Massachusetts, USA) with a Transend-ex® 0.36-mm × 182-cm guidewire (Boston Scientific) was positioned in the main portal branch to the cranial liver lobes. Polyvinyl alcohol particles (90 – 180 and 300 – 500 µm in diameter; Cook, Bloomington, Indiana, USA) and two fibred platinum coils (4.0 and 6.0mm; Boston Scientific)

were infused through the catheter to occlude the portal branches to the cranial lobes. PVE was confirmed by portography, and the mesenteric vein was closed using a ligature. The abdomen was closed in two layers. Enrofloxacin (0.2 mg/kg) was administered daily for 3 days following PVE.

CT volumetry

Multiphase CT scans (Brilliance 64TM; Philips, Eindhoven, The Netherlands) were performed on days –7, – 1, +3 and +7. Animals (n = 18 per treatment group) were anaesthetized and a 22-G catheter was placed in the lateral ear vein. A baseline scan was carried out and 3 ml contrast solution (VisipaqueTM; GE Healthcare, Waukesha, Wisconsin, USA) was injected. Arterial-, portal- and venous-phase images were acquired after 15, 30 and 45 s respectively. Volumetric analysis was performed on three-dimensional reconstructions of 5-mm axial slices using manual delineation. Caudal liver volume (CLV) and total liver volume (TLV) were determined, and increase in CLV was calculated as:

$$\% \text{ increase CLV} = \frac{(CLV_{day\ x} - CLV_{baseline})}{CLV_{baseline}} \times 100\%$$

where x is the day on which the image was acquired. The same formula was used to obtain the decrease in cranial liver volume (CrLV), which was calculated as TLV – CLV. Increase in CLV and decrease in CrLV were calculated using day –1 values as the baseline. To validate CT volumetric data, the volumetric measurements at the time of death were correlated with actual liver weight measured using a precision scale (Sartorius, Göttingen, Germany) (**Fig. S1**, supporting information).

Hepatobiliary scintigraphy

Liver function was assessed using hepatobiliary scintigraphy with ^{99m}Tc-labelled (2,4,6 trimethyl-3-bromo) iminodiacetic acid (^{99m}Tc-mebrofenin) (Bridatec®; GE Healthcare, Eindhoven, The Netherlands) on days – 7, – 1, +3 and +7. Rabbits (n = 6 per treatment group) were anaesthetized and placed on an imaging table, with the liver and heart positioned under a large field-of-view single-photon emission CT camera (Siemens Symbia^{TM} T16, The Hague, The Netherlands). Regions of interest were drawn around the left ventricle for blood pool readings, around the entire liver for total liver uptake, and around the caudal liver lobe (**Fig. S2**, supporting information). A dose of 50 MBq ^{99}mTc-mebrofenin per rabbit was administered via a lateral ear vein directly before the start of acquisition.

The geometric mean of data sets from the anterior and posterior cameras was used for analysis. Hepatic ^{99m}Tc-mebrofenin uptake rate was calculated as the increase in ^{99m}Tc-mebrofenin uptake over 2 min, corrected for perfusion by subtraction of activity in the region of interest drawn over the left ventricle. Total liver uptake was represented by the total hepatic ^{99m}Tc-mebrofenin uptake rate, and calculated as a percentage of the injected dose

per minute. The fractional ^{99m}Tc-mebrofenin uptake rate was calculated for the caudal liver lobe, based on the distribution of segmental activity, and was corrected for baseline measurements at –7days. Correction for day –7 was chosen to exclude effects of OCA treatment on mebrofenin uptake before PVE. Scintigraphy measurements were optimized in earlier pilot experiments (data not shown).

Histology

Liver tissue (left lateral and caudal lobes) was fixed in buffered formalin for 48 h, and subsequently dehydrated and embedded in paraffin. Sections of liver tissue (4 µm) were cut, and stained with haematoxylin and eosin. Sections were scored for lobular and portal inflammation, as outlined in **Table S1** (supporting information). In addition, liver sections were stained with Ki-67 antibodies to quantify hepatocyte proliferation, and counterstained with haematoxylin, as described previously. [18,19] Ki-67-positive hepatocytes were counted in a total of five high-power fields per animal, by a hepatopathologist blinded to the group allocation. Liver histology was assessed at days 3 and 7 (n = 6 per treatment group).

Clinical chemistry

Serum alanine aminotransferase (ALT), aspartate amino- transferase (AST), γ-glutamyl transferase (γGT) and alkaline phosphatase (ALP) were determined by the Department of Clinical Chemistry (Academic Medical Centre, Amsterdam, The Netherlands) using a Cobas® 8000 modular analyser (Roche, Basle, Switzerland) (n = 12 per treatment group).

PCR

Total RNA was isolated from terminal ileum and (non)-embolized liver lobes using Tri Reagent® (Ambion, Landsmeer, The Netherlands). Following treatment with DNaseI (Promega, Leiden, The Netherlands), 750 ng total RNA was converted to cDNA using an iSCRIPT™ cDNA synthesis kit (BioRad, Veenendaal, The Netherlands). Quantitative reverse transcriptase–PCR was performed on an IQ™5 Cycler using SYBR Green (SYBR Green Master- Mix; BioRad) and cDNA equivalent to 7.5 ng total RNA as template. Expression levels were calculated using LinReg software [20] and normalized with respect to the geometric mean of Rplp0, Hprt and Gapdh. Primer sequences are provided in **Table S2** (supporting information). Predicted amplicon size was checked by agarose gel electrophoresis. Transcript analysis was performed using tissue obtained on days +3 and +7 (n = 6 per treatment group).

Statistical analysis

Normally distributed data were analysed using Mann-Whitney U test, or two-way ANOVA in the case of serum laboratory values. Differences in data with a non-normal distribution between groups were tested using Mann-Whitney U test or Kruskal-Wallis test. The effects of OCA on CLV increase or CrLV decrease were determined from values obtained by area under

the curve analysis, and assessed by means of the Mann-Whitney U test. Correlations were tested using Spearman's rank correlation coefficient. All statistical analysis was performed using Graphpad Prism® 6.0 (GraphPad, La Jolla, California, USA).

RESULTS

It was first assessed whether OCA induced liver growth by analysing TLV in the pretreatment period (day – 7 until day –1. [4] At both time points, TLV corrected for bodyweight was similar in both groups, and similar between the time points in both groups (**Fig. 1a**). Thus, OCA did not induce spontaneous liver growth.

The effect of OCA on PVE-induced liver growth was examined. The PVE procedure was tolerated well, although two animals died before the end of the experiments because of a

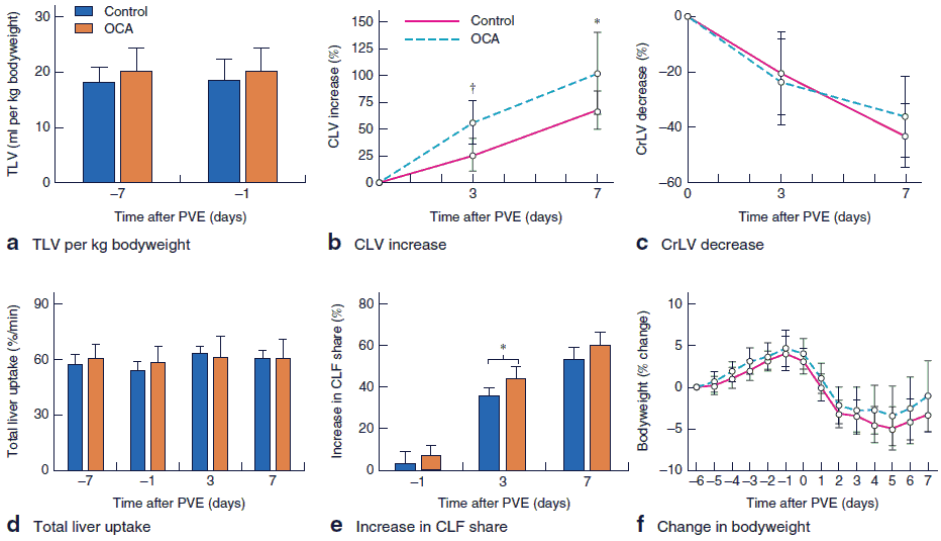


Figure 1. Farsenoid X receptor (FXR) agonism accelerates portal vein embolization (PVE)-induced liver growth. Animals were pretreated with the FXR agonist obeticholic acid (OCA) for 7 days before undergoing embolization of the cranial liver lobes. Volume of the total (TLV), caudal (CLV, regenerating after PVE) and cranial lobes (CrLV, atrophying after PVE) liver volume was assessed during the course of the experiment. Animals were killed at 3 or 7 days after PVE, which was carried out on day 0. **a:** TLV per kg bodyweight in the pretreatment phase (n=17 per group). **b:** Percentage increase in CLV following PVE relative to volume on day –1 (n=17 per group until day +3, n=11 per group on day +7; *P <0.050, †P <0.001 versus control, Mann–Whitney U test on area under the curve values at individual time points). **c:** Percentage decrease in CrLV following PVE relative to volume on day –1 (n=17 per group until day +3, n=11 per group on day +7). **d:** Total liver uptake of 99mTc-labelled mebrofenin determined by hepatobiliary scintigraphy (n=6 per group). **e:** Increase in share of the caudal liver lobe to 99mTc-labelled mebrofenin uptake from baseline measurements on day –7 (CLF, caudate liver function) (n=5–6 per group; *P <0.050, 2-way ANOVA). **f:** Percentage change in bodyweight relative to values on day –7 (n=17 per group). Values are mean(s.d.)

technical complication during induction of anaesthesia and mesenteric vein cannulation. Hence, liver volumetry data on day 3 after PVE were available for 34 animals. At this time, 12 animals were killed, leaving 22 for volumetric assessment on day 7 after PVE.

Liver hypertrophy of the caudal lobe was assessed 3 and 7 days after PVE and expressed as percentage increase from day – 1 values. At 3 days after PVE, the volume of the caudal non-embolized liver had increased 2.2-fold in the OCA group compared with the control group (mean(s.d.) 56.1(20.3) versus 26.1(15.4) per cent; $P < 0.001$) (**Fig. 1b**). On day7 after PVE, the increase in caudal liver volume remained 1.5-fold higher in OCA-treated animals (102.0(38.2) versus 67.6(17.7) per cent; P=0.020). The decrease in volume of the embolized segments was similar in the two groups (**Fig. 1c**).

Total liver uptake of mebrofenin was similar in the two groups on day –1, and remained stable after PVE in both groups (**Fig.1d**). The contribution of the non-embolized caudal liver lobe (caudal liver function share) to total liver mebrofenin uptake increased in both groups 3 and 7 days after PVE. However, the increase was greater in OCA-treated animals 3 days after PVE (44.5(5.4) versus 36.0(3.7) per cent; P=0.017) (**Fig.1e**). To determine whether the

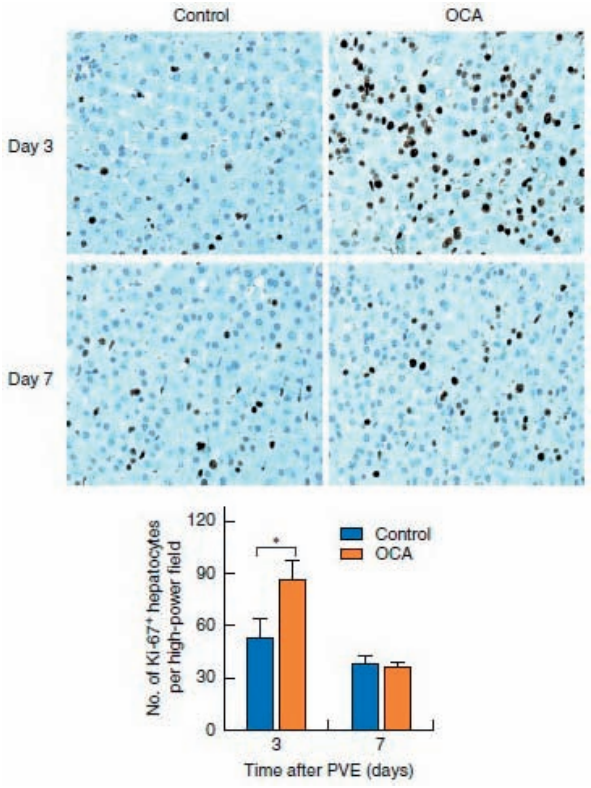
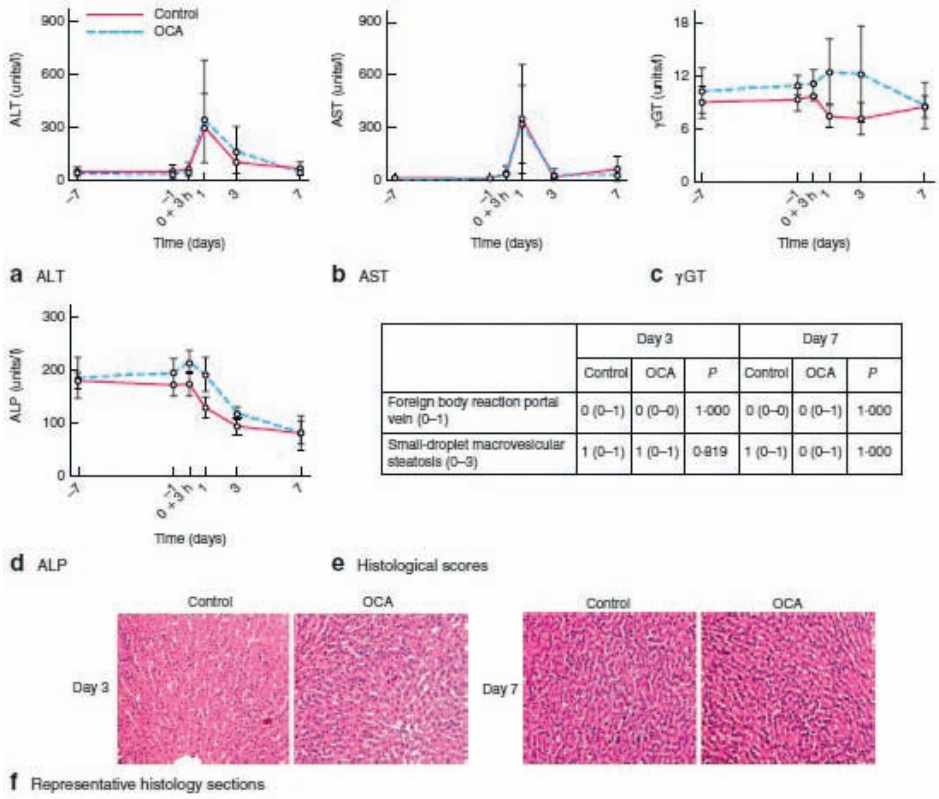


Figure 2. Ki-67-stained liver sections (100 × magnification) and quantification of Ki-67-positive hepatocytes (n=6 per group; *P <0.050, Mann–Whitney U test). OCA, obeticholic acid.

increased volume gain in OCA-treated animals reflected hypertrophy or hyperplasia, liver sections were stained for the proliferation marker Ki-67. Increased numbers of Ki-67-positive hepatocytes were apparent in the OCA group on day +3 (1.6-fold higher in OCA-treated compared with control animals; P = 0.045), with similar numbers in the groups on day +7 (**Fig. 2**).

Animal bodyweight was measured daily to exclude bodyweight changes as a confounding factor in the liver volume calculations. Bodyweight decreased after PVE in both groups to a similar extent (**Fig. 1f**). Bodyweight gain before PVE was similar in both groups.

To examine whether OCA treatment resulted in liver injury, aminotransferase levels were measured during the course of the experiment. PVE induced a transient increase in ALT and AST, with levels peaking at day +1 in both groups and returning to baseline values afterwards (**Fig. 3a,b**). Levels were similar in the two groups throughout the experiment. γGT and ALP



	Day 3			Day 7		
	Control	OCA	P	Control	OCA	P
Foreign body reaction portal vein (0–1)	0 (0–1)	0 (0–0)	1·000	0 (0–0)	0 (0–1)	1·000
Small-droplet macrovesicular steatosis (0–3)	1 (0–1)	1 (0–1)	0·819	1 (0–1)	0 (0–1)	1·000

Figure 3. Plasma **a** alanine aminotransferase (ALT), **b** aspartate aminotransferase (AST), **c** γ-glutamyl transferase (γGT) and **d** alkaline phosphatase (ALP) levels before and after PVE in obeticholic acid (OCA) and control groups. Values are mean (s.d.) (n=5–11 per group). **e** Median (range) scores of haematoxylin and eosin-stained sections of the caudal liver lobe (n=5–6 per group). **f** Representative liver sections of both groups on days +3 and +7 (haematoxylin and eosin stain, 100× magnification).

levels also remained stable in both groups before PVE. After PVE, γGT and ALP levels were slightly higher in OCA-treated animals; however, levels did not increase significantly above baseline after PVE in either group (**Fig. 3c,d**).

To examine the potential effects of OCA on liver histology, haematoxylin and eosin-stained sections of the caudal lobe were scored in a blinded fashion. Mild portal and lobular inflammation and mild sinusoidal dilatation were observed in all animals, with no differences between groups (**Fig. 3e**). A foreign body reaction, caused by back ow of embolic material in the caudal lobe, was observed in four animals (2 in the control group killed on day +3; 1 in the OCA group killed on day +3 and 1 killed on day +7). This was no different between groups and the back ow of embolic material did not appear to affect liver hypertrophy. Small-droplet macrovesicular steatosis was observed in both groups on days +3 and +7, with no differences between groups.

To assess the transcriptional effects of OCA, FXR target gene expression was analysed in terminal ileum and liver harvested at 3 and 7 days after PVE. FXR is expressed in both terminal ileum and liver, and it is conceivable that both contribute to liver regeneration in the rabbit. [5,6]. OCA had no effect on ileal expression of Fxr per se, but resulted in ileal induction of the FXR target gene Shp on day +3 (**Fig. 4a**). Ileal expression of the FXR target gene Ostβ was not, however, affected by OCA. Fgf19 mRNA in intestinal specimens or liver was detectable only in trivial amounts in some tissue samples under optimized PCR conditions (data not shown). Lack of an empirically validated reference sequence for rabbit Fgf19 mRNA may underlie this failure. Hepatic expression of the bile salt synthetic enzyme Cyp7a1 was strongly suppressed in OCA-treated animals on day 3 (**Fig.4b**). This occurred without transcriptional induction of the repressor Shp. Expression of Fgfr4 was not affected by OCA treatment, whereas minor downregulation at the transcript level was observed for βKlotho after 3 days (**Fig. 4b**). OCA treatment resulted in reduced hepatic Fxr expression at day +7. Ostβ expression in the liver was raised in OCA-treated animals on days +3 and +7. Conversely, ileal Shp expression was induced by OCA, but this was not observed in the liver.

DISCUSSION

In this study, the effect of the potent FXR agonist OCA on liver regeneration was examined in a standardized rabbit model of PVE. OCA accelerated liver regeneration early after PVE, producing a 2.2-fold increase in CLV by day +3 and a 1.5-fold increase by day +7 after PVE compared with vehicle-treated controls. In addition, hepatobiliary scintigraphy revealed an enhanced uptake capacity of the caudal liver lobe 3 days after PVE in OCA-treated animals. This was accompanied by an increase in the number of Ki-67-positive hepatocytes, indicating that PVE plus OCA elicited a stronger hyperplastic response than PVE without OCA. The accelerated liver regeneration induced by OCA potentially holds great clinical potential.

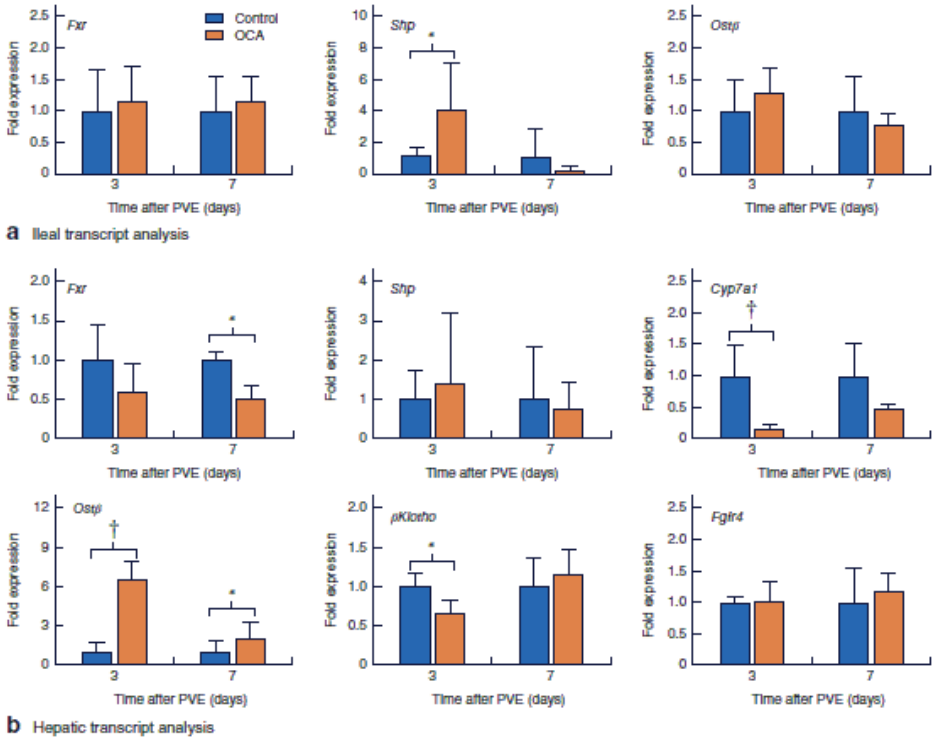


Figure 4. Effect of obeticholic acid (OCA) on ileal and hepatic gene expression. Animals were pretreated with OCA for 7 days and underwent portal vein embolization (PVE) of the cranial liver lobes. **a:** Terminal ileum and **b** caudal liver was harvested 3 and 7 days after PVE. Gene expression was analysed by reverse transcriptase–PCR. Values are mean(s.d.) fold expression relative to that in the control group (n=5–6 per group; *P <0.050, †P <0.010, Mann–Whitney U test). Fxr, farnesoid X receptor; Shp, small heterodimer partner; Ost, organic solute transporter; Cyp7a1, cholesterol 7α-hydroxylase; Fgfr4, fibroblast growth factor receptor 4

The present data show that the increased volume gain of the caudal lobe after PVE in the OCA group is due to hyperplasia, as inferred from enhanced hepatocytic positivity for the proliferative marker Ki-67. These results indicate that OCA might be able to reduce the time from PVE to liver resection. This could have several advantages, such as avoiding the need for chemotherapy after PVE. Moreover, OCA might improve the hepatic growth response to PVE and this could increase the options for liver resection in patients with a very small FLR. Theoretically, when these results are extrapolated to hepatectomy, the increased liver regeneration by OCA may prevent postresectional liver failure. Patients are most prone to liver failure in the first few days after extended liver resection [21] and early initiation of liver regeneration is associated with a lower incidence of liver failure. [22] By enhancing liver regeneration in these first days, OCA could be anticipated to reduce the risk of liver failure, and resulting morbidity and mortality.

The volumetric as well as functional gain, and increased number of Ki-positive hepatocytes in the present study indicate accelerated regeneration early after PVE. It was shown previously that the functional increase in the FLR in patients precedes the volumetric increase after PVE. [23] Thus, OCA-treated rabbits may have already increased uptake capacity over controls in the initial period after PVE, before assessment of liver function on day +3. As the liver volume increase is generally slower than the functional increase [23], the difference in liver volume is still present on day +7. In line with the higher metabolic rate in rabbits, liver regeneration occurs at a higher rate in rabbits than in humans, whereas metabolism in mice and rats is even higher than in rabbits. The median increase in CLV of 67–71 per cent in vehicle-treated rabbits 7days after PVE [15] is comparable to the 62 per cent increase in FLR volume in selected patients a median of 34 days after PVE that included segment IV. [13] In the setting of associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), FLR volume increases by a median of 74 per cent after a median of 9 days following the first stage. [13,24] As ALPPS is associated with substantial morbidity and mortality, enhancement of the hypertrophy response after PVE with OCA might be a safer alternative to increase resectability in patients with a very small FLR.

The present results suggest a direct proliferative effect on the non-embolized liver lobes, without affecting the atrophy of the embolized lobes, and without the toxic effects demonstrated by similar bodyweight and plasma aminotransferase levels in both groups. The exact mechanisms by which OCA stimulates liver growth remain unclear. The results indicate both ileal and hepatic FXR activation, demonstrated by increased ileal Shp expres- sion and decreased hepatic Cyp7a1 expression, which are both FXR target genes. The absence of hepatic Shp upregulation suggests that Shp-independent Fgf19 signalling may be responsible for the observed repression of Cyp7a1, which is further substantiated by hepatic downregulation of Fgf19 receptor Fgfr4. Fgf19 expression was detectable only in trivial amounts under optimized conditions, despite a study reporting Fgf19 expression in rabbits. [25] Future studies should elaborate further on the mechanisms of OCA-induced liver regeneration in animals with established mRNA sequencing data, which are not yet available for rabbits.

OCA is currently being evaluated in phase III clinical trials [26,27], and has been shown to reduce histological features of non-alcoholic steatohepatitis, and to decrease plasma levels of ALP, γGT and ALT in patients with primary biliary cholangitis. Substantial safety data are available, and therefore clinical translation of OCA for stimulation of liver regeneration is within reach. However, there are some uncertainties regarding its use in the setting of PVE and liver resection. Seven days of pretreatment is recommended to ensure adequate tissue levels of OCA at the time of PVE or resection. [17] Tumours in the liver are at the same time exposed to the potent agonistic effects of FXR. Several reports [28-30] have suggested that direct FXR stimulation of tumours poses no oncological concerns, and the increase in circulating FGF-19 levels induced by OCA is not expected to increase the growth of tumours expressing Fgfr4 [31-32]. Pharmacological safety studies in mice have revealed that 2 years'

exposure to a dose (25 mg per kg per day) exceeding that used in the present study and in ongoing clinical trials did not result in OCA-related neoplasms (personal communication; L. Adorini, Intercept Pharmaceuticals, based on the toxicology report by WIL Research, Ashland, Ohio, USA).

The present study was performed in young healthy rabbits, whereas surgical practice is dominated by elderly patients often with hepatic parenchymal disease [33], both of which have been shown to decrease the regenerative potential of the liver. [3] Some reports suggest alleviation of age-related defects in liver regeneration by FXR agonism. [11] The effects of OCA on regeneration of aged and diseased livers remain to be established. In addition, long-term effects as well as OCA dose and timing variations remain to be evaluated.

REFERENCES

(1) Farges O, Goutte N, Bendersky N, et al. Incidence and risks of liver resection: an all-inclusive French nationwide study. *Ann Surg* 2012; 256(5):697-704; discussion 704-5.

(2) de Graaf W, van Lienden KP, Dinant S, et al. Assessment of future remnant liver function using hepatobiliary scintigraphy in patients undergoing major liver resection. *J Gastrointest Surg* 2010; 14(2): 369-78.

(3) van den Broek MA, Olde Damink SW, Dejong CH, et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; 28(6):767-80.

(4) Huang W, Ma K, Zhang J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. *Science* 2006; 312(5771):233-6.

(5) Borude P, Edwards G, Walesky C, et al. Hepatocyte-specific deletion of farnesoid X receptor delays but does not inhibit liver regeneration after partial hepatectomy in mice. *Hepatology* 2012; 56(6):2344-52

(6) Zhang L, Wang YD, Chen WD, et al. Promotion of liver regeneration/repair by farnesoid X receptor in both liver and intestine in mice. *Hepatology* 2012; 56(6):2336-43

(7) Kong B, Huang J, Zhu Y, et al. Fibroblast growth factor 15 deficiency impairs liver regeneration in mice. *Am J Physiol Gastrointest Liver Physiol* 2014; 306(10):G893-902.

(8) Padrissa-Altes S, Bachofner M, Bogorad RL, et al. Control of hepatocyte proliferation and survival by Fgf receptors is essential for liver regeneration in mice. *Gut* 2015; 64(9):1444-53.

(9) Pellicciari R, Fiorucci S, Camaioni E, et al. 6alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J Med Chem* 2002; 45(17):3569-72

(10) Fiorucci S, Clerici C, Antonelli E, et al. Protective effects of 6-ethyl chenodeoxycholic acid, a farnesoid X receptor ligand, in estrogen-induced cholestasis. J Pharmacol Exp Ther. May 2005;313(2):604-612.

(11) Chen WD, Wang YD, Zhang L, et al. Farnesoid X receptor alleviates age-related proliferation defects in regenerating mouse livers by activating forkhead box m1b transcription. Hepatology. Mar 2010;51(3): 953-962.

(12) van Lienden KP, van den Esschert JW, de Graaf W, et al. Portal vein embolization before liver resection: a systematic review. Cardiovasc Intervent Radiol. Feb 2013;36(1):25-34.

(13) Shindoh J, Vauthey JN, Zimmitti G, et al. Analysis of the efficacy of portal vein embolization for patients with extensive liver malignancy and very low future liver remnant volume, including a comparison with the associating liver partition with portal vein ligation for staged hepatectomy approach. J Am Coll Surg. Jul 2013;217(1):126-133; discussion 133-124.

(14) Fischer C, Melstrom LG, Arnaoutakis D, et al. Chemotherapy after portal vein embolization to protect against tumor growth during liver hypertrophy before hepatectomy. JAMA Surg. Dec (2013);148(12): 1103-1108.

(15) van den Esschert JW, van Lienden KP, Alles LK, et al. Liver regeneration after portal vein embolization using absorbable and permanent embolization materials in a rabbit model. Ann Surg. Feb 2012; 255(2):311-318.

(16) Kilkenny C, Browne W J, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 2010; 8: e1000412.

(17) Mookerjee R, Mehta G, Jalan R.Obeticholicacid,a Farnesoid-X receptor agonist, improves portal hypertension by two distinct pathways in cirrhotic rats. Hepatology 2014; 60: 1798–1799.

(18) van der Loos C M, de Boer O J, Mackaaij C, Hoekstra LT, van Gulik TM, Verheij J. Accurate quantitation of Ki67-positive proliferating hepatocytes in rabbit liver by a multicolor immunohistochemical (IHC) approach analyzed with automated tissue and cell segmentation software. J Histochem Cytochem 2013; 61: 11–18.

(19) Marsman HA, de Graaf W, Heger M, van Golen RF, Ten Kate FJ, Bennink R et al. Hepatic regeneration and functional recovery following partial liver resection in an experimental model of hepatic steatosis treated with omega-3 fatty acids. Br J Surg 2013; 100: 674–683.

(20) Ramakers C, Ruijter JM, Deprez RH, Moorman AF. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Lett 2003; 339: 62–66.

(21) Etra JW, Squires MH III, Fisher SB, Rutz DR, Martin BM, Kooby DA et al. Early identi cation of patients at increased risk for hepatic insuf ciency, complications and mortality after major hepatectomy. HPB (Oxford) 2014; 16: 875–883.

(22) Shirabe K, Motomura T, Takeishi K, Morita K, Kayashima H, Taketomi A et al. Human early liver regeneration after hepatectomy in patients with hepatocellular carcinoma: special reference to age. Scand J Surg 2013; 102: 101–105.

(23) de Graaf W, van Lienden KP, van den Esschert JW, Bennink RJ, van Gulik TM. Increase in future remnant liver function after preoperative portal vein embolization. Br J Surg 2011; 98: 825–834.

(24) Schnitzbauer AA, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. Ann Surg 2012; 255: 405 – 414.

(25) Shang Q, Guo GL, Honda A, Shi D, Saumoy M, Salen G et al. Bile acid ux through portal but not peripheral veins inhibits CYP7A1 expression without involvement of ileal FGF19 in rabbits. Am J Physiol Gastrointest Liver Physiol 2014; 307: G479 – G486.

(26) Hirschfield GM, Mason A, Luketic V, Lindor K, Gordon SC, Mayo M et al. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to rsodeoxycholic acid. Gastroenterology. Apr 2015;148(4):751- 761 e758.

(27) Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebocontrolled trial. Lancet. Mar 14 2015;385(9972):956-965.

(28) Dai J, Wang H, Shi Y, Dong Y, Zhang Y, Wang J. Impact of bile acids on the growth of human cholangiocarcinoma via FXR. J Hematol Oncol. 2011;4:41.

(29) Guo F, Xu Z, Zhang Y, et al. FXR induces SOCS3 and suppresses hepatocellular carcinoma. Oncotarget. Oct 27 2015;6(33):34606-34616.

(30) Deuschle U, Schuler J, Schulz A, et al. FXR controls the tumor suppressor NDRG2 and FXR agonists reduce liver tumor growth and metastasis in an orthotopic mouse xenograft model. PLoS One. 2012; 7(10):e43044.

(31) Uriarte I, Latasa MU, Carotti S, et al. Ileal FGF15 contributes to fibrosis-associated hepatocellular carcinoma development. Int J Cancer. May 15 2015;136(10):2469-2475.

(32) Gauglhofer C, Paur J, Schrottmaier WC, et al. Fibroblast growth factor receptor 4: a putative key driver for the aggressive phenotype of hepatocellular carcinoma. Carcinogenesis. Oct 2014;35(10):2331-2338.

(33) Jarnagin WR, Gonen M, Fong Y, et al. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. Ann Surg. Oct 2002;236(4):397-406; discussion 406-397.

Supportive information

Table S1 Histologic scoring

	Scoring			
Lobular inflammation	**0** – None	**1** - Mild	**2** - Moderate	**3** - Severe
Intralobular inflammation	**0** – None	**1** - < 2 foci	**2** - 2 to 5 foci	**3** - > 5 foci
Sinuoidal dilatation	**0** – None	**1** - Mild	**2** - Moderate	**3** - Severe
Foreign body reaction in portal veins	**0** – None	**1** - Yes		
Small droplet microvesicular steatosis	**0** – None	**1** - Mild	**2** - Moderate	**3** - Severe

Table S2 Primer sequences

	Forward	**Reverse**
Rplp0	CCTCGTGAGAGTGACATCGT	CGCCCACGATGAAGCATTTT
Hprt	GACCAGTCAACAGGGGACAT	ATCCAACAAAGTCTGGCCTGT
Gapdh	CCACTACATGGTCTACATGTTCC	TCACCCCACTTGATGTTGGC
Fxr	ACAAGTGACGTCGACAACGA	AGGTCTGAAACCCTGGCAAC
Slc51b	TGGGAACAGGAGCCAGAAAC	CGTCAGGGCAAGGATGGAAT
Shp	GCCCCAAGGAATACGCCTAC	CCGGAATGGACTTGAGGGTG
Fgfr4	GAAAACCAGCAATGGCCGC	GAGCCCCCAAGTGTGAAGAT
Klb	GAGAACGGCTGGTTCACAGA	TCGAAGCCATCCAGGAGAGA
Cyp7a1	ATATGATGAGGAGCTCTGAAGC	GGGACTCCTTGATGATGCTGT

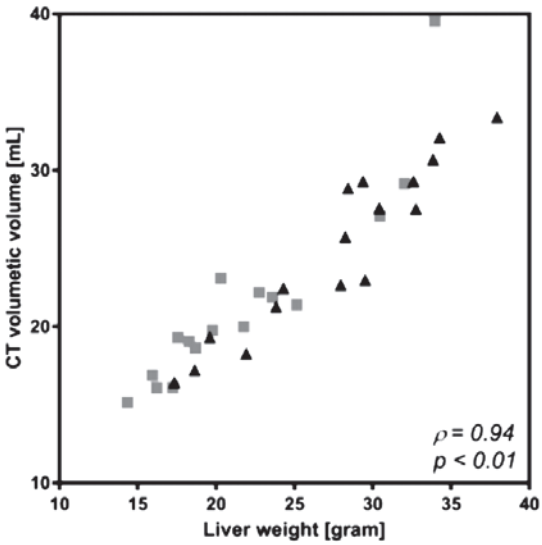


Figure S1 CLV determined by CT volumetry correlated to caudal liver lobe weight at sacrifice. Gray squares represent animals from the control group, black triangles represent animals treated with OCA. Correlation was tested using Spearman's rank correlation coefficient.

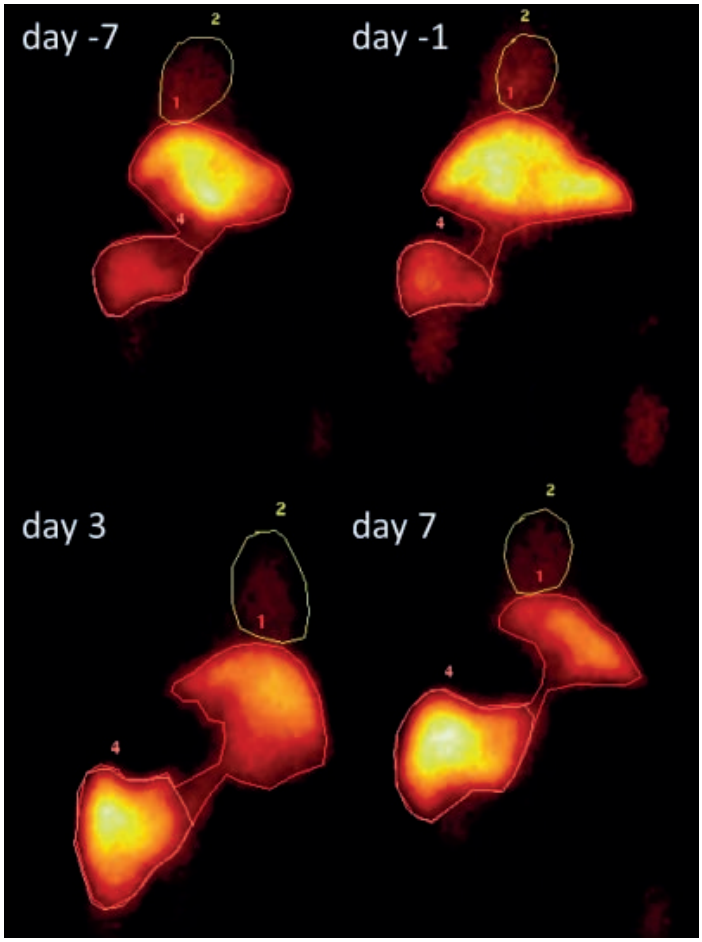
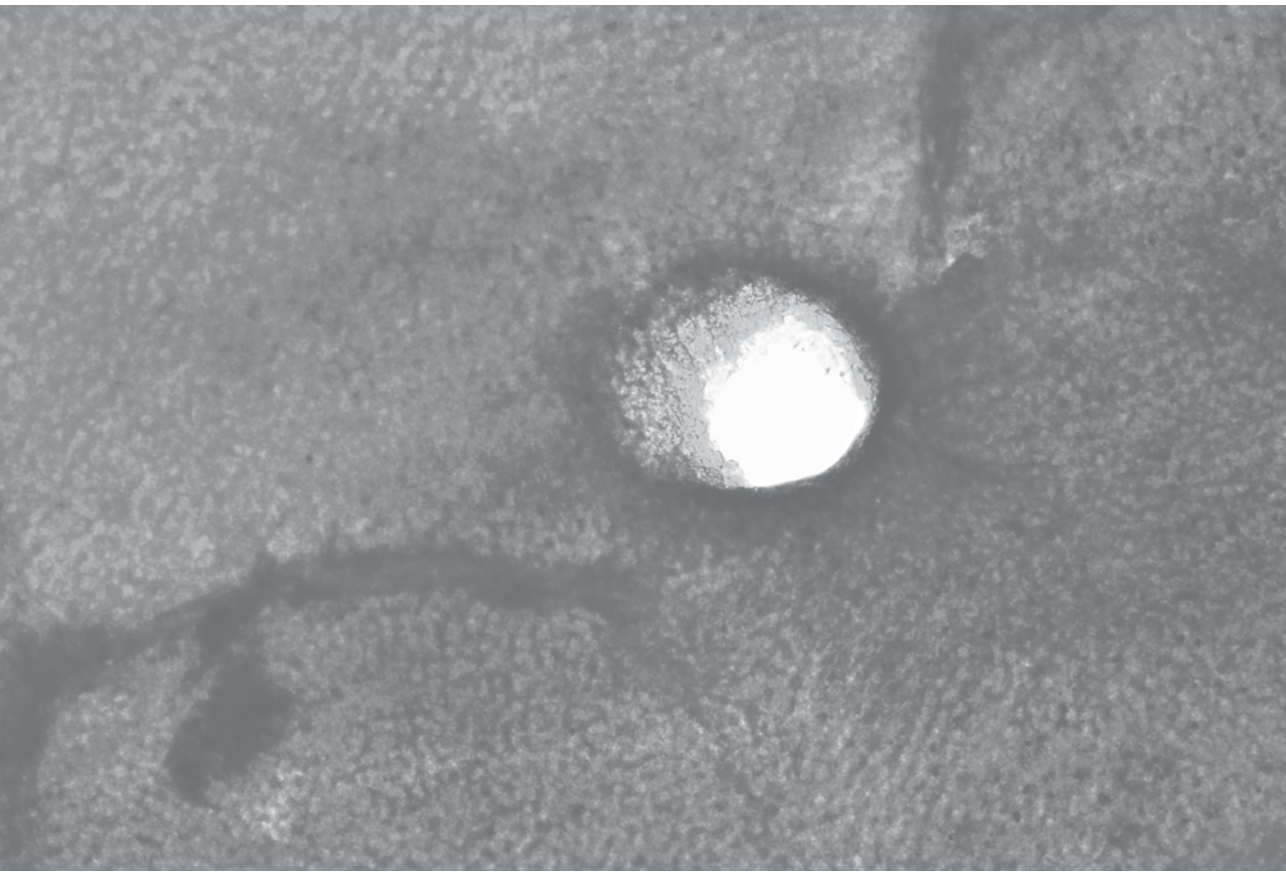


Figure S2 Hepatobiliary scintigraphic images of a single rabbit at all sequential scans. The yellow region of interest (ROI) delineates the left ventricle, the red ROI the total liver and the pink ROI the caudal liver lobe. A marked decrease in cranial liver lobe activity and increase in caudal liver lobe activity was seen following portal vein embolization.



CHAPTER 4

Future remnant liver function as predictive factor for the hypertrophy response after portal vein embolization

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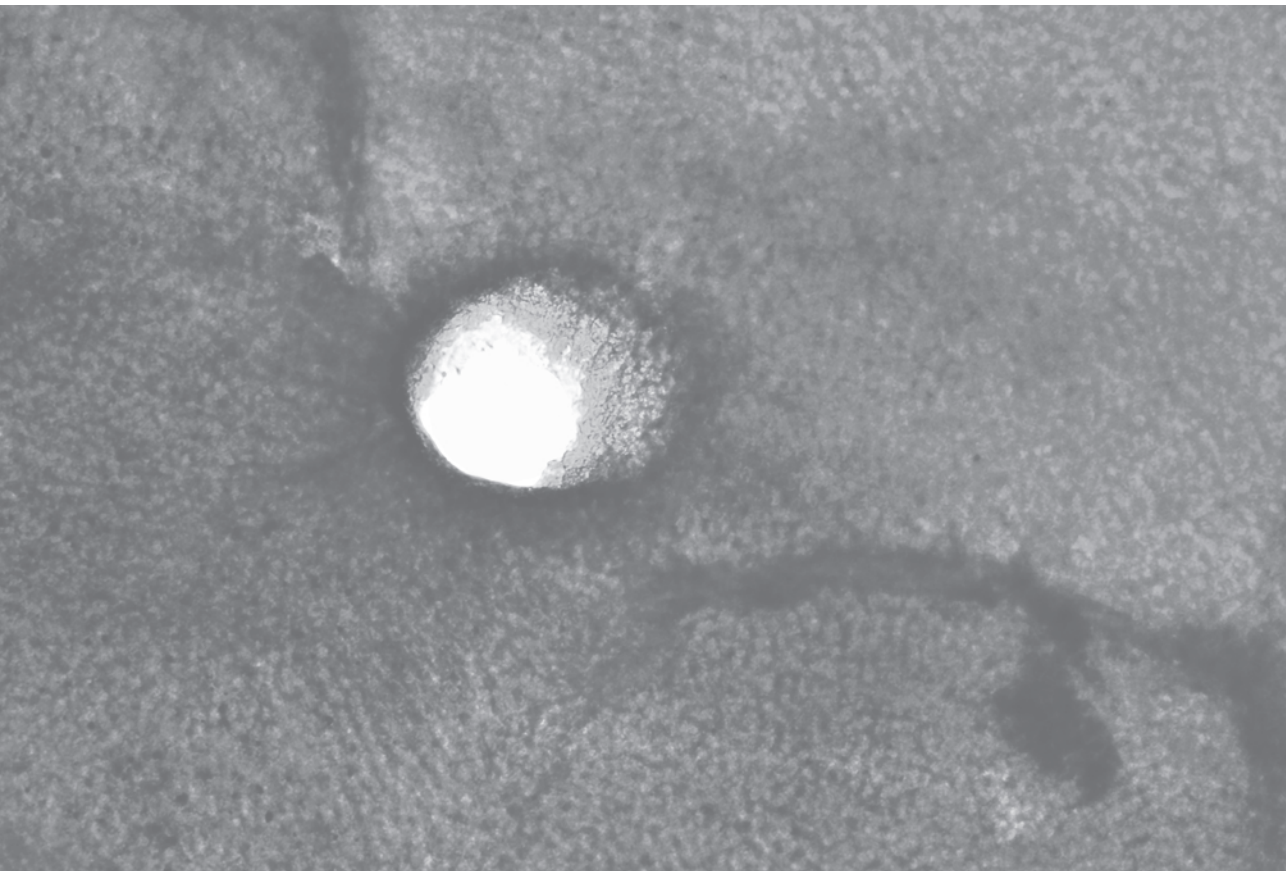


Surgery 2016

ABSTRACT

Background:

Preoperative portal vein embolization (PVE) is widely used to increase the future remnant liver (FRL). Identification of non-responders to PVE is essential because these patients may benefit from associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), which induces a more powerful hypertrophy response. ^{99m}Tc-mebrofenin hepatobiliary scintigraphy (HBS) is a quantitative method for assessment of FRL function with a calculated cut-off value for the prediction of postoperative liver failure. The aim of this study was to analyze FRL function before PVE to predict sufficient functional hypertrophy response after PVE.

Methods:

Sixty-three patients who underwent preoperative PVE and computed tomography imaging were included. HBS was performed to determine pre–PVE and post–PVE FRL function. Receiver operator characteristic (ROC) analysis of pre–PVE FRL function was performed to identify patients who would meet the post–PVE cut-off value for sufficient function (i.e., 2.7%/min/m^2).

Results:

Mean pre–PVE FRL function was 1.80% ± 0.45%/min/m^2 and increased to 2.89% ± 0.97%/min/m^2 post–PVE. ROC analysis in 33 patients who did not receive chemotherapy revealed that a pre–PVE FRL function of ≥ 1.72%/min/m^2 was able to identify patients who would meet the safe FRL function cut-off value 3 weeks after PVE (area under the curve = 0.820). The predictive value was less pronounced in 30 patients treated with neoadjuvant chemotherapy (area under the curve = 0.618). A total of 45 of 63 patients underwent liver resection, of whom 5 of 45 developed postoperative liver failure; 4 of 5 patients had a post–PVE FRL function below the cut-off value for safe resection.

Conclusion:

When selecting patients for PVE, FRL function assessed with HBS can be used as a predictor of insufficient functional hypertrophy after PVE, especially in non-chemotherapy patients. These patients are potential candidates for ALPPS.

INTRODUCTION

The majority of patients with primary or secondary liver tumors are considered unresectable at the time of diagnosis because of (locally) advanced disease, severe comorbidities or due to insufficient future remnant liver (FRL). This concerns approximately 60-80% of patients diagnosed with colorectal liver metastases (CRLM)[1, 2] and 70-80% of patients with hepatocellular carcinoma (HCC).[1]

In case of insufficient FRL, patients are at risk of developing postoperative liver failure, a severe complication for which only supportive treatment options are currently available. Preoperative portal vein embolization (PVE) is a widely accepted technique to increase both FRL volume and function in patients with insufficient FRL.[3] First described by Kinoshita and colleagues in 1986,[4] PVE induces hypertrophy of the non-embolized liver segments and thereby increases resectability rates.

Nonetheless, approximately 20% of planned liver resections in patients who have undergone preoperative PVE will not be carried out, even though PVE was performed successfully.[1] Although unresectability is mainly caused by progression of the disease, some patients are unresectable because of insufficient hypertrophy response. Several methods have been proposed in order to identify patients who will not benefit from PVE, for example, the recently introduced kinetic growth factor, which illustrates the degree of volumetric FRL hypertrophy per week.[5] The available methods, however, only enable identification of the non-responders after the embolization procedure has already been performed.

Avoiding unsuccessful PVE is desirable in view of PVE-related complications and possibly accelerated tumour growth[6-10] all of which can reduce the chance of curative treatment after PVE. Furthermore, predicting the non-responders to PVE is essential because these patients may now benefit from an alternative strategy to increase FRL, that is, ALPPS (Associating Liver Partition and Portal vein Ligation for Staged hepatectomy) which has been shown to induce an accelerated hypertrophy response.[11, 12]

Although computed tomography (CT) is the conventional means of examining the hypertrophy response after PVE in terms of volume increase, several studies have pointed out that monitoring liver function outperforms volumetric measurements because the increase in FRL function proceeds faster than that of its volume.[13-16] Assessment of liver function may therefore shorten interval between PVE and resection. Additionally, functional measurements take into account the quality of the liver parenchyma and consequently generate a more reliable preoperative risk assessment.[17, 18]

Hepatobiliary scintigraphy (HBS) with ^{99m}Tc-labeled iminodiacetic acid analogs (e.g. mebrofenin) is a method for the assessment of liver function that can be used during the preoperative work-up of patients eligible for liver operation, as well as for the monitoring of increase of FRL function after PVE.[14, 17] This technique has the major advantage of allowing measurement of both total and segmental liver function based on patient-specific characteristics

(body surface area). De Graaf *et al.* calculated a FRL-function cut-off value of 2.7%/min/m^2 which demonstrated a negative predictive value of 98% for postoperative liver failure after major liver resection.[17] We recently have updated our experience with preoperative use of HBS in a large series of patients.[19] Several centers around the world have now adopted the use of HBS in the preoperative work-up of patients undergoing major liver resection.[20, 21]

We hypothesize that in patients requiring PVE, the level of pre-PVE FRL function determines whether they will meet the cut-off value for safe resection after PVE. The aim of this study was therefore, to test FRL function measured before PVE as a predictor of sufficient functional hypertrophy response after PVE.

METHODS

Patients

Between November 2001 and December 2014, 105 patients underwent PVE in the Academic Medical Center, Amsterdam, The Netherlands. Of these, 63 (60%) underwent HBS and CT volumetry both prior and 3 to 4 weeks after PVE and could therefore be analyzed. HBS and CT volumetry after PVE were performed on the same day in 47 patients (74.6%). The study was approved by the institutional review board of the Academic Medical Center, and the need for written informed consent was waived.

Computer tomography volumetry

Multiphase contrast-enhanced CT scans were generated by a multislice helical CT scanner (MX-8000 or Brilliance, Philips, Eindhoven, the Netherlands). Three-dimensional reconstructions of the liver were created using reconstructed 5 mm thick axial slices from 2-3 mm original slices. The entire liver, FRL and tumor(s) were manually outlined using portal and hepatic veins as landmarks for segmental division. All delineations were performed by an experienced radiologist (K. P. vL). Integrated software (Mx-View 3.52; Philips Medical Systems) was used to calculate total liver volume, tumor volume and FRL volume. FRL volume was expressed as proportion of total liver volume using the following formula:

$$\%\text{FRL-volume} = \frac{\text{FRL-volume}}{(\text{Total liver volume} - \text{Tumor volume})} \times 100\%$$

The kinetic growth rate (KGR) of the FRL, as proposed by Shindoh and colleagues, was calculated by dividing the % point difference between pre- and post-PVE %FRL-volume by weeks since PVE.[5]

Scintigraphic imaging and data acquisition

HBS was carried out using ^{99m}Tc-labeled (2,4,6 trimethyl-3-bromo) iminodiacetic acid (^{99m}Tc-

mebrofenin, Bridatec; GE Healthcare, Eindhoven, The Netherlands) as described previously.[22, 23] The procedure was performed after a 4-hour fast, because food consumption stimulates hepatic function and bile flow, which might influence test results.

Patients were positioned supine under a large field-of-view (SPECT)/CT camera (Symbia T16; Siemens) located over the liver and heart area. The SPECT/CT camera was equipped with low-energy high-resolution collimators. After intravenous administration of 200 MBq freshly prepared ^{99m}Tc-mebrofenin, dynamic acquisition was obtained (36 frames of 10 s/frame, 128 matrix), which was used to calculate the hepatic mebrofenin uptake rate corrected for body surface area (cMUR).[24-26]

Subsequently, a fast SPECT acquisition was performed (60 projections of 8 s/projection, 128 matrix), centred around the peak of the hepatic time–activity curve to allow for 3-dimensional assessment of liver function and functional liver volume. Directly after SPECT, a low-dose non–contrast-enhanced CT scan was acquired for attenuation correction and anatomic mapping.[27] Data were processed on a Hermes workstation (Hermes Medical Solutions, Stockholm, Sweden).

The HBS parameters related to cMUR in the total liver and cMUR in the FRL (i.e. FRL function) were calculated as described previously.[24, 25] The share of the FRL to the total hepatic uptake (%FRL-F) is expressed as a percentage. A cut-off value of 2.7%/min/m^2 was used to discriminate normal from decreased FRL uptake rates, as was calculated in a previous study.[17]

Portal vein embolization

PVE was indicated when HBS demonstrated an FRL function below the validated cut-off value of 2.7%/min/m^2. The %FRL-volume cut-off value for safe resection was set at ≥ 25% for patients with healthy liver parenchyma and ≥ 35% in case of suspected compromised parenchyma.

After ultrasonography-guided puncture of the anterior branch of the right portal vein, a 5 French sheath was inserted and a reverse catheter was advanced into the main portal trunk. A portogram was performed to identify the individual branches. All branches of the right portal system were subsequently embolized using polyvinyl alcohol particles (300-500 nm; Cook, Bloomington, IN) and coils (Tornado Embolization Microcoil, Cook). At the end of the procedure, the portogram was repeated to confirm deprivation of portal flow in embolized segments and normal flow to the non-embolized liver segments. The puncture tract was closed with a gelfoam plug (Spongostan standard, Ferrosan A/S, Soeborg, Denmark).

Data collection

Demographic, clinical and intraoperative data were extracted from electronic patient records. Data concerning PVE, CT scans and HBS were collected from radiology and nuclear medicine reports. In case of inconsistencies regarding which liver segments were considered as the FRL, FRL function and/or volume were recalculated using reprocessed data, thereby ensuring comparability. Diagnoses were acquired from pathology reports.

Study endpoints

The endpoint of this study was defined as a minimum FRL function before PVE that is required to reach the FRL function cut-off value for safe resection (i.e. 2.7%/min/m^2) after PVE.

Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS 22.0; IBM Inc., Armonk (NY) USA). Normally distributed continuous data are expressed as mean ± standard deviation (SD), while non-normally distributed data are presented as median along with interquartile range (IQR). Univariate analysis was performed with the independent t-test (normally distributed data) or Mann-Whitney U test (non-normally distributed data) for continuous parameters and by the Chi-squared test or Fisher's exact test for categorical data. Receiver operating characteristic (ROC) curve analysis was used to determine a cut-off value for FRL function prior to PVE in predicting the acquisition of 2.7%/min/m^2 at post-PVE HBS. All statistical tests were two-tailed.

RESULTS

Patients

Sixty-three patients were included in this study. The median age was 64.4 years (IQR 56.9-70.1), and the majority of the patients were man (n = 41). CRLM (n = 38) and perihilar cholangiocarcinoma (PHC; n = 13) were the most frequent diagnoses. In 25 of 63 (39.7%) patients, liver parenchyma was compromised as confirmed by microscopic examination of a biopsy or the resection specimen by the pathologist. Thirty out of the 63 (47.6%) patients underwent chemotherapy before PVE.

All patients underwent PVE because of insufficient anticipated FRL function (<2.7%/min/m^2). In 59 of 63 (93.7%) patients, the percutaneous transhepatic ipsilateral approach was used. In the remaining 4 of 63 (6.3%) patients a transhepatic contralateral approach was employed. All patients underwent embolization of the right portal system. Additional embolization of segment IV was performed in 4 of 63 (6.3%) patients. The procedure was uncomplicated in 61 of 63 (96.8%) patients, while in 2 of 63 (3.2%) patients, adverse effects were reported, that is, partial portal vein thrombosis and severe allergic reaction.

In total 18 patients did not undergo resection: 1) In 4 (4/18) patients, insufficient hypertrophy response was the reason not to perform a resection. In 1 of these 4 patients, with a large single metastasis, radiofrequency ablation was performed instead of resection. In these patients, there was no progression of the disease. 2) Progression of the disease was seen in 12 (12/18) patients due to which resection could not be carried out. Of these 12 patients, 4 were diagnosed with PHC. Among these 4 patients, a curative resection was no longer feasible in 3 patients because the tumor appeared to be locally more advanced at

exploration than was expected preoperatively, and in 1 patient, no resection was performed because distant lymph node metastases were found intraoperatively.

The remaining 8 patients with progression of the disease were all diagnosed with CRLM. The resection could not be carried out due to new lesions in the FRL and/or increase of tumor in the embolized lobe, apparent on the follow-up CT after PVE or during laparotomy. It is important to note that in 2 of the latter patients, we already doubted that the resection would be possible during the preoperative work-up. 3) Resection appeared technically not feasible because of local vascular anatomy in one (1/18) patient diagnosed with PHC even though there was a locally resectable tumor without distant metastases. 4) One (1/18) patient suspected of intrahepatic cholangiocarcinoma or hepatocellular carcinoma died before resection could take place.

Three of 45 (6.7%) patients who underwent liver resection developed postoperative liver failure, which eventually led to death, while 2 other patients (4.4%) developed transient hepatic failure. Four of the 5 patients with postoperative liver failure had a post-PVE FRL function below the cut-off value for safe resection. Patient characteristics are shown in **Table 1.**

Table 1 Baseline characteristics of the 63 patients who underwent PVE together with HBS and CT-imaging before and after PVE procedure

Male: female, *n*	41:22
Age in years, median (IQR)	64.4 (56.9-70.1)
BMI in kg/m^2, mean ± SD	25.6 ± 4.1
Diagnosis, *n (%)*	
Colorectal liver metastases	38 (60.3)
Hilar cholangiocarcinoma	13 (20.6)
Hepatocellular carcinoma	4 (6.3)
Hepatocellular adenoma	3 (4.8)
Other	5 (7.9)
Compromised liver parenchyma, *n (%)*	
Steatosis	11 (17.5)
Cholestasis	6 (9.5)
Fibrosis	5 (7.9)
Combined disease	3 (4.8)
None	31 (49.2)
Unknown	7 (11.1)
Neoadjuvant chemotherapy prior to PVE, *n (%)*	30 (47.6)
Type of resection, *n (%)* *	
Right hemihepatectomy	27 (60.0)
Extended right hemihepatectomy	15 (33.3)
Other	3 (6.7)

Table 1 Baseline characteristics of the 63 patients who underwent PVE together with HBS and CT-imaging before and after PVE procedure (*continued*)

Postoperative liver failure*, n (%)*	
Fatal	3 (6.7)
Transient	2 (4.4)

PVE, portal vein embolization; HBS, hepatobiliary scintigraphy; BMI, body mass index; IQR, interquartile range; SD, standard deviation; * presented for the 45 patients who underwent hepatic resection after PVE.

Changes in liver volume

The mean time interval between pre-PVE CT imaging and PVE was 40 ±29 days, while the median interval between PVE and post-PVE CT scanning was 22 (IQR 21-24) days. Total liver volume did not differ significantly between pre- and post-PVE volumetric measurements. FRL volume increased from 409 ml (IQR 306-534) before PVE to 534 ml (IQR 418-708) post-PVE, corresponding to a significant median increase of 137 ml (IQR 94-207, P < 0.001). The %FRL-volume increased from 24.7 ± 7.3% pre-PVE to 34.1 ± 8.7% post-PVE (P < 0.001), corresponding to a mean KGR of 2.86%/wk (IQR 1.78–4.09). Volumetric measurements are presented in **Table 2**.

Table 2 PVE-, CT- and HBS related parameters in all 63 patients who were included in this study, patients who were and were not treated with chemotherapy prior to PVE

	All patients, n=63	**No chemotherapy patients, n=33**	**Chemotherapy patients, n=30**	**P value**
Time intervals in days				
CT - PVE, ± SD	40 ± 29	45 ± 31	35 ± 25	0.149¶
PVE - CT, (IQR)	22 (21-24)	21 (21-25)	22 (21-24)	0.675*
HBS - PVE, (IQR)	6 (2-27)	19 (3-33)	5 (2-16)	0.019*
PVE - HBS, (IQR)	21 (21-23)	22 (21-25)	21 (20-23)	0.387*
PVE approach, n (%)				0.614Ʊ
Transcutaneous ipsilateral	59 (93.7)	30 (90.9)	29 (96.7)	
Transcutaneous contralateral	4 (6.3)	3 (9.1)	1 (3.3)	
PVE complications, n (%)				0.493Ʊ
Partial portal vein thrombosis	1 (1.6)	1 (3.0)	0	
Allergic reaction	1 (1.6)	1 (3.0)	0	
Function before PVE				
Total liver function, %/min/m^2, ±SD	13.94 ± 3.98	13.39 ± 3.99	14.55 ± 3.95	0.250¶
%FRL-function, %, ± SD	24.2 ± 7.6	24.9 ± 8.9	23.5 ± 5.8	0.468¶
FRL function, %/min/m^2, ± SD	1.80 ± 0.45	1.76 ± 0.48	1.84 ± 0.43	0.481¶
Function after PVE				
Total liver function, %/min/m^2, ±SD	13.34 ± 3.14	12.92 ± 3.35	13.79 ± 2.89	0.274¶
%FRL-function, %, (IQR)	36.1 (30.6 - 46.0)	37.0 (30.4 - 46.8)	35.5 (30.6 - 44.3)	0.788*

Table 2 PVE-, CT- and HBS related parameters in all 63 patients who were included in this study, patients who were and were not treated with chemotherapy prior to PVE (*continued*)

	All patients, n=63	No chemotherapy patients, n=33	Chemotherapy patients, n=30	P value
FRL function, %/min/m^2, ± SD	2.89 ± 0.97	2.78 ± 0.97	3.01 ± 0.97	0.338¶
Volume before PVE				
Total liver volume, mL, (IQR)	1845 (1497 - 2346)	2063 (1735 - 2532)	1601 (1388 - 2011)	0.005*
Tumor volume, mL, (IQR)	30 (0 - 161)	7 (0 - 287)	50 (6 - 154)	0.191*
FRL volume, mL, (IQR)	409 (306 - 534)	443 (304 - 583)	374 (306 - 474)	0.122*
%FRL-volume, %, ± SD	24.7 ± 7.3	24.3 ± 7.4	25.0 ± 7.2	0.697¶
Volume after PVE				
Total liver volume, mL, (IQR)	1861(1523 - 2341)	2038 (1694 - 2553)	1656 (1448 - 1936)	0.004*
Tumor volume, mL, (IQR)	38 (0 - 177)	11 (0 - 213)	66 (2 - 193)	0.232*
FRL volume, mL, (IQR)	534 (418 - 708)	622 (472 - 804)	502 (414 - 670)	0.104*
%FRL-volume, %, ± SD	34.1 ± 8.7	33.4 ± 8.1	34.9 ± 9.3	0.505¶
Increase in function and volume after PVE				
Total liver function, %/min/m^2, ± SD	-0.61 ± 2.78	-0.47 ± 2.83	-0.76 ± 2.76	0.683¶
%FRL-function, %, (IQR)	54.5 (30.0 - 85.5)	51.5 (30.5 - 87.2)	64.0 (29.4 - 87.1)	0.650*
FRL function, %/min/m^2, (IQR)	1.00 (0.60 - 1.56)	0.97 (0.51 - 1.33)	1.17 (0.58 - 1.65)	0.397*
FRL function increase / week, %/min/m^2, (IQR)	0.32 (0.16 - 0.48)	0.25 (0.14 - 0.43)	0.38 (0.17 - 0.53)	0.375*
Total liver volume, mL, ± SD	32 ± 302	51 ± 367	11 ± 214	0.602¶
FRL volume, mL, (IQR)	137 (94 - 207)	172 (101 - 215)	130 (93 - 188)	0.177*
%FRL-volume, %, (IQR)	36.1 (25.5 - 53.0)	39.1 (25.5 - 56.5)	33.1 (24.8 - 53.2)	0.660*
KGR, %/week, (IQR)	2.86 (1.78 - 4.09)	2.90 (1.72 - 3.85)	2.78 (1.91 - 4.20)	0.620*
FRL volume increase / week, mL, (IQR)	41 (28 - 64)	43 (27 - 67)	40 (27 - 59)	0.364*

PVE, portal vein embolization; HBS, hepatobiliary scintigraphy; CT, Computed Tomography imaging; FRL, future remnant liver; %FRL-function, FRL function percent of total liver function; %FRL-volume, FRL volume percent of non-tumorous total liver volume; KGR, kinetic growth rate, % point difference between pre- and post-PVE %FRL-volume divided by weeks since PVE; IQR, interquartile range reported in case of median values; SD, standard deviation reported in case of mean values; * Mann-Whitney U test; ¶ independent samples t-test; Ʊ Fisher's exact test.

Changes in liver function

The median interval between pre-PVE HBS and PVE was 6 days (IQR 2-27), and the median interval between PVE and post-PVE HBS was 21 days (IQR 21-23). No significant difference in total liver function was found between pre- and post-PVE measurements (13.94 ± 3.98 and 13.34 ± 3.14%/min/m^2, respectively; P = 0.087). %FRL-F increased from 24.2 ± 7.6% pre-PVE to 36.1% (IQR 30.60-46.00) after the embolization (P < 0.001). Mean FRL function prior to PVE was 1.80 ±0.45%/min/m^2 while post-PVE FRL function was 2.89 ±0.97%/min/m^2. This corresponds to a median increase in the FRL function of 1.00%/min/m^2 (IQR 0.60-1.56, P

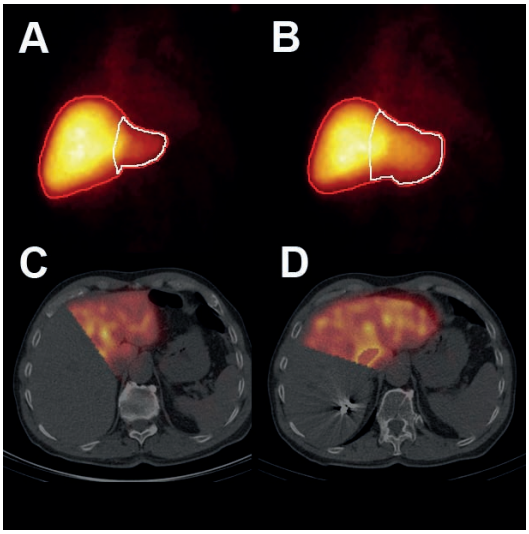


Figure 1. FRL uptake of mebrofenin before (*A, C*) and after (*B, D*) embolization of the right branches of the portal vein in a patient with colorectal liver metastases. *(A) and (B)*, Summed geometric mean dynamic dataset (150–350 sec after injection of ^{99m}Tc-mebrofenin, white line indicates the FRL, red line the total liver); *(C) and (D),* combined ^{99m}Tc-mebrofenin SPECT and low dose CT-images before and after the procedure. Three weeks after PVE, both FRL-volume and FRL-function had increased after which right hemihepatectomy was performed.

< 0.001). **Figure 1** illustrates the differences in the FRL mebrofenin uptake rate before and after PVE. The FRL function increase per week was 0.32%/min/m^2 (IQR 0.16-0.48, **Table 2**).

Post-PVE outcome

Presence of hepatic comorbidity did not substantially influence the increase in FRL function or FRL volume (P = 0.635 and p = 0.336, respectively). The same accounts for the increase in FRL function per week, FRL volume per week, and KGR, as they were not affected by the presence of hepatic comorbidity either (P = 0.721, P = 0.322 and P = 0.216, respectively). Moreover, there was no correlation between the increase in FRL volume and the increase in FRL function in patients with healthy liver parenchyma according to Pearson's correlation coefficient (r = -0.195, P = 0.294). Likewise, we found no correlation between these 2 parameters in patients with compromised liver parenchyma (r = 0.228 with P = 0.499 for steatosis; r = 0.350 with P = 0.496 for cholestasis and r = -0.106 with P = 0.865 for fibrosis).

Similarly, neoadjuvant chemotherapy treatment prior to PVE did not significantly influence the increase in FRL function (P = 0.397) or FRL volume (P = 0.177) after PVE (**Table 2 and Figure 2**). Functional and volumetric FRL increase per week also were not impaired substantially by the administration of chemotherapy (P = 0.375 and P = 0.364, respectively), nor was KGR (P = 0.620).

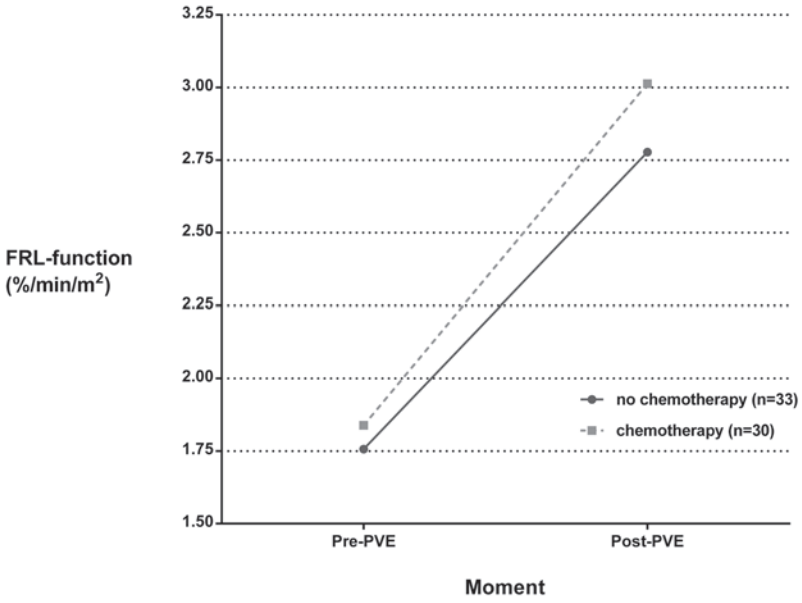


Figure 2. A comparison of the mean increase in FRL-function between patients who were treated with chemotherapy prior to PVE and non-chemotherapy patients during 3 weeks after PVE (p = 0.397).

Prediction of post-PVE outcome

Results of ROC analysis of pre-PVE FRL function for the prediction of whether the cut-off value of 2.7%/min/m^2 for safe resection would be met after PVE are shown in **Table 3**.

Table 3 Results of ROC-curve analysis of FRL function before PVE for the prediction of sufficient hypertrophy response in the entire cohort, patients who were and were not treated with chemotherapy and patients diagnosed with PHC

	AUC	95% CI	FRL, %/min/m^2	Sens, %	Spec, %	PPV, %	NPV, %
Entire cohort, *n=63*	0.721	0.593 - 0.848	1.72	78.1	61.3	66.2	74.3
No chemotherapy, *n=33*	0.820	0.671 - 0.968	1.72	81.3	82.4	83.1	80.6
Chemotherapy, *n=30*	0.618	0.409 - 0.827	1.92	62.5	71.4	65.7	68.5
PHC, *n=13*	0.952	0.839 - 1.000	1.71	85.7	100.0	100.0	89.1

ROC, receiver operator characteristic; PVE, portal vein embolization; AUC, area under the curve; CI, confidence interval; FRL, future remnant liver; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; PHC, perihilar cholangiocarcinoma.

Prediction of post-PVE outcome in non-chemotherapy patients

Of the 33 (52.4%) patients who did not receive chemotherapy prior to PVE, 16 (48.5%) patients reached the cut-off value for sufficient FRL function. Their FRL function before PVE and absolute increase in FRL function after PVE were significantly higher than in patients

who did not reach this value (P = 0.001 and P = 0.014, respectively). The same accounts for the functional increase rate per week which was greater in those patients that reached the cut-off value, as shown in **Table 4**. The %FRL-volume and KGR also were significantly greater in patients who reached the cut-off value (P = 0.023 for both).

In these 33 patients, the ROC analysis revealed that a pre-PVE FRL function of 1.72%/min/m^2 was able to identify patients who did not meet the FRL function cut-off value of 2.7%/min/m^2 after PVE, with a sensitivity of 81.3% and a specificity of 82.4% (**Figure 3**). When the same

Table 4 Comparison of patients who did or did not meet the post-PVE FRL function cut-off value of 2.7 %/min/m^2

	Patients without chemotherapy, *n=33*			**Patients with chemotherapy, *n=30***		
	Post-PVE FRL function			Post-PVE FRL function		
	≥ 2.7 %/min/m^2, *n=16*	≤ 2.7 %/min/m^2, *n=17*	P value	≥ 2.7 %/min/m^2, *n=16*	≤ 2.7 %/min/m^2, *n=14*	P value
Male/female, n	12/4	12/5	0.776¥	9/7	8/6	0.961¥
Age, years (IQR)	65.7 (56.3 - 71.8)	60.2 (48.9 - 67.1)	0.105*	65.1 (60.8 - 70.1)	67.4 (58.1 - 70.7)	0.967*
BMI, kg/m^2 (IQR)	25.7 (22.5 - 28.0)	25.1 (23.6 - 27.7)	0.914*	24.9 (22.2 - 26.8)	26.2 (22.0 - 29.2)	0.418*
Hepatic comorbidity, *n (%)*	6 (37.5)	10 (58.8)	0.119¥	4 (25.0)	5 (35.7)	0.491¥
HBS parameters						
FRL function pre-PVE, %/min/m^2, ± SD	2.02 ± 0.37	1.51 ± 0.44	0.001¶	1.91 ± 0.39	1.76 ± 0.47	0.349¶
FRL function increase, %/min/m^2, (IQR)	1.04 (0.88 - 1.59)	0.70 (0.00 - 1.07)	0.014*	1.61 (1.40 - 1.76)	0.60 (0.19 - 0.70)	<0.001*
%FRL-function increase, %, (IQR)	52.9 (36.8 - 98.7)	48.8 (0.0 - 82.4)	0.249*	82.9 (69.1- 113.9)	31.7 (8.5 - 45.7)	<0.001*
FRL function increase / week, %/min/m^2, (IQR)	0.37 (0.24 - 0.50)	0.18 (0.00 - 0.38)	0.017*	0.51 (0.43 - 0.60)	0.17 (0.07 - 0.23)	<0.001*
Volumetric parameters						
FRL volume pre-PVE, ml, ± SD	525 ± 227	444 ± 219	0.302¶	411 ± 120	371 ± 118	0.370¶
FRL volume increase, ml, (IQR)	157 (80 - 224)	171 (122 - 210)	0.746*	140 (95 - 213)	125 (80 - 141)	0.198*
%FRL-volume increase, %, (IQR)	30.3 (16.3 - 42.5)	53.0 (36.2 - 66.3)	0.002*	39.1 (27.3 - 74.0)	29.4 (22.4 - 44.0)	0.170*
KGR, %/week, (IQR)	2.19 (1.48 - 3.10)	3.48 (2.06 - 4.21)	0.023*	3.10 (2.08 - 4.71)	2.49 (1.67 – 3.83)	0.170*
FRL volume increase / week, ml, (IQR)	41 (26 - 71)	51 (33 - 66)	0.719*	43 (29 - 69)	38 (23 - 48)	0.339*

PVE, portal vein embolization; HBS, hepatobiliary scintigraphy; CT, Computed Tomography imaging; FRL, future remnant liver; %FRL-function, FRL function percent of total liver function; %FRL-volume, FRL volume percent of non-tumorous total liver volume; KGR, kinetic growth rate, % point difference between pre- and post-PVE %FRL-volume divided by weeks since PVE; IQR, interquartile range reported in case of median values; SD, standard deviation reported in case of mean values; * Mann-Whitney U test; ¶ independent samples t-test; ¥ Chi-square test.

analysis was performed for patients with perihilar cholangiocarcinoma (n = 13), a pre-PVE FRL function of 1.71%/min/m^2 was found with a sensitivity of 85.7% and a specificity of 100%.

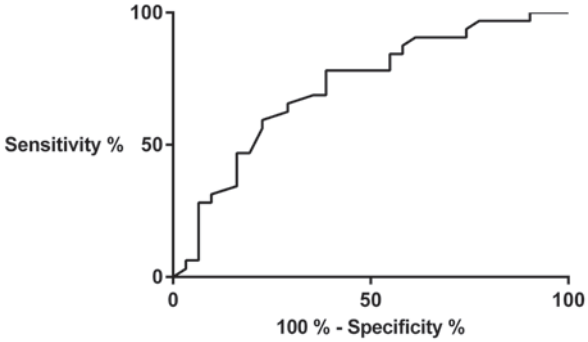


Figure 3. Receiver operator characteristic (ROC) curve analysis of pre-PVE FRL-function for the prediction of post-PVE FRL-function in non-chemotherapy patients (n = 33). Pre-PVE FRL-function of at least 1.72%/min/m^2 was able to identify patients who would meet the validated cut-off value for safe resection (2.7%/min/m^2) after PVE with a sensitivity of 81.3% and specificity of 82.4% (area under the curve = 0.820, 95% confidence interval [CI] 0.671-0.968)

Prediction of post-PVE outcome in chemotherapy patients

Thirty (47.6%) patients had received chemotherapy prior to PVE. ROC analysis showed that for these patients, a pre-PVE FRL function of 1.92%/min/m^2 was able to distinguish responders from non-responders to PVE with a sensitivity and specificity of 62.5% and 71.4%, respectively.

No significant differences in any volumetric or functional hypertrophic parameters were found between patients who received neoadjuvant chemotherapy with platinum agents (n = 22) and those who did not (n = 41). When comparing patients who received neoadjuvant chemotherapy with or without (n = 8) platinum agents, no statistically significant differences in volumetric or functional hypertrophic parameters could be demonstrated either.

DISCUSSION

The aim of this study was to examine if patients who did not reach a ^{99m}Tc-mebrofenin FRL cut-off value of 2.7 %/min/m^2 for sufficient function after PVE, could be identified on the basis of their FRL function prior to PVE. Several factors have been studied in relation to clinical outcomes after PVE, such as preceding chemotherapy, hepatic comorbidity and the influence of different embolization materials. This is the first study to show the possibility of predicting the outcome of PVE using quantitative, scintigraphic FRL function assessment using HBS.

Subgroup analysis in patients who were not treated with chemotherapy revealed a good predictive value of FRL function prior to PVE, showing that an FRL uptake rate of at least 1.72%/min/m^2 is required in order to reach sufficient FRL function of 2.7%/min/m^2 or greater during the first 3 weeks after PVE. The predictive value of pre-PVE FRL function was even more pronounced in patients diagnosed with PHC. However, this subgroup analysis concerned only 13 patients; therefore, conclusions based on such a small group should be interpreted with caution.

The predictive value of pre-PVE FRL function in the entire cohort was not accurate enough for the purpose of patient selection. We suspect that this was caused by the modest ability of pre-PVE FRL function to predict post-PVE outcome in patients who had undergone neoadjuvant chemotherapy. It is important to note is that pre-PVE FRL function, post-PVE FRL function and an increase in FRL function were comparable in patients who did or did not receive chemotherapy. In other words, our data suggests that the administration of chemotherapy prior to embolization does not substantially influence the FRL hypertrophy response after PVE.

This hypothesis was also proposed in a systematic review by van Lienden *et al*.[1] Baere and colleagues suggested that administration of platin agents especially impairs FRL volume regeneration.[28] Our data do not support this contention because no significant differences in FRL volume increase, %FRL-volume increase, KGR, FRL function increase or %FRL-function increase were found between patients who did or did not receive platin agents with their chemotherapy before PVE. Therefore, we presume that the low predictive value of pre-PVE FRL function in the chemotherapy group could be explained by the heterogeneity of the chemotherapy-related patient characteristics among these patients.

Temporary impairment of total liver function after administration of chemotherapy was recently demonstrated by Bednarsch *et al.*[29] The chemotherapy induced liver injury was however, followed by regeneration of liver function in several weeks. Discrepancies in the time intervals between PVE and last administration of chemotherapy and the wide range of applied cytostatic drugs may therefore have led to varying degrees of temporary impairment FRL function in this series.

The relation between hepatic comorbidity and FRL hypertrophy response after PVE has been reported extensively in the literature. We were not able to demonstrate a significant influence of hepatic comorbidity on the regenerative response of the FRL after PVE. This finding is consistent with several other studies that investigated the influence of parenchymal disease on the hypertrophy response after PVE.[1, 30, 31] In the present study, the correlation between increase in FRL volume and increase in FRL function was not significant, regardless of the quality of the liver parenchyma. This finding emphasizes that functional assessment of the FRL provides a more reliable risk analysis for postoperative liver failure than volumetric parameters only, as previously reported.[17, 18]

Curative treatment of patients with extensive hepatic tumors and concurrent insufficient FRL is an increasing challenge in liver operations. On the one hand, avoiding futile PVE is of interest

in the context of procedure related complications and because of accelerated tumor growth due to the hypertrophy response. On the other hand, patients with insufficient FRL are at risk of developing postoperative liver failure with a high postoperative mortality rate (up to 80%).[32]

Recently, an alternative surgical treatment was introduced for patients with insufficient FRL, i.e. ALPPS (Associating Liver Partition and Portal vein Ligation for Staged hepatectomy).[11] ALPPS is a 2-stage procedure in which accelerated hypertrophy of the FRL is induced in the first stage by *in situ* splitting of the liver parenchyma and concomitant portal vein ligation. The resection is completed in 1-2 weeks after the first stage by removal of the deportalized liver.[11] The hypertrophy response seen in ALPPS greatly surpasses the FRL hypertrophy reported in 3 to 4 weeks after PVE. Nearly 100% of patients treated with ALPPS show sufficient hypertrophy response in order to undergo the second stage of the procedure.[12]

Although the rates of postoperative morbidity and mortality until now exceed those seen in patients who underwent PVE followed by resection or a conventional 2-stage resection, ALPPS offers a modality to increase the hypertrophy response in patients who will not show sufficient response after PVE. HBS can be used in order to identify these patients, and additionally, to further monitor functional increase of the FRL after the first stage of ALPPS.[21, 33]

Four of the 5 patients who developed postoperative liver failure had a post-PVE FRL function at or below the cut-off value of 2.7%/min/m^2. The fifth patient in which postoperative liver insufficiency occurred had a post-PVE FRL function well above the cut-off value (3.73%/min/m^2), but died from multi-organ dysfunction in the setting of acute liver ischemia due to partial portal vein thrombosis and ensuing liver failure.

Several authors have studied the relationship between the degree of FRL volume hypertrophy after PVE and the occurrence of postoperative liver failure. Leung *et al.* described that patients who did not develop hepatic failure after surgery had shown significantly higher degrees of FRL hypertrophy and KGR.[34] Shindoh *et al.* found that KGR was a reliable predictor of postoperative liver failure (AUC 0.830), with no postoperative hepatic insufficiency occurring in patients with a KGR of ≥2%/wk.[5] According to our data, the 5 patients who developed postoperative hepatic failure did not show a significantly lower KGR (P = 0.671), with 2 of the 5 patients showing a KGR of >2%/week.

In view of the available literature and findings of this study, we believe functional evaluation of the future remnant liver is superior to volumetric monitoring in the preoperative setting. In this study we presented a new potential application of HBS for patients with insufficient FRL.

CONCLUSION

When selecting patients for PVE, FRL function determined prior to PVE may be used as a predictor of insufficient hypertrophy response after PVE, especially in non-chemotherapy patients. These patients are potential candidates for ALPPS.

References

(1) van Lienden KP, van den Esschert JW, de GW, et al. Portal vein embolization before liver resection: a systematic review. *Cardiovasc. Intervent. Radiol* 2013; 36(1):25-34.

(2) Wicherts DA, de Haas RJ, Andreani P, et al. Impact of portal vein embolization on long-term survival of patients with primarily unresectable colorectal liver metastases. *Br. J. Surg* 2010; 97(2):240-250.

(3) de Graaf W, Bennink RJ, Vetelainen R, et al. Nuclear imaging techniques for the assessment of hepatic function in liver surgery and transplantation. *J. Nucl. Med* 2010; 51(5):742-752.

(4) Kinoshita H, Sakai K, Hirohashi K, et al. Preoperative portal vein embolization for hepatocellular carcinoma. *World J. Surg* 1986; 10(5):803-808.

(5) Shindoh J, Truty MJ, Aloia TA, et al. Kinetic growth rate after portal vein embolization predicts posthepatectomy outcomes: toward zero liver-related mortality in patients with colorectal liver metastases and small future liver remnant. *J. Am. Coll. Surg* 2013; 216(2):201-209.

(6) Huisman F, Cieslak, K.P., van Lienden, K.P., Bennink, R.J., van Gulik, T.M. Liver related complications in unresectable disease after portal vein embolization. HBSN, Vol. Accepted for publication, 2017.

(7) de GW, van den Esschert JW, van Lienden KP, et al. Induction of tumor growth after preoperative portal vein embolization: is it a real problem? *Ann. Surg. Oncol* 2009; 16(2):423-430.

(8) May BJ, Madoff DC. Portal vein embolization: rationale, technique, and current application. *Semin. Intervent. Radiol* 2012; 29(2):81-89.

(9) van Gulik TM, van den Esschert JW, de GW, et al. Controversies in the use of portal vein embolization. *Dig. Surg* 2008; 25(6):436-444.

(10) Hoekstra LT, van Lienden KP, Doets A, et al. Tumor progression after preoperative portal vein embolization. *Ann. Surg* 2012; 256(5):812-817.

(11) Schnitzbauer AA, Lang SA, Goessmann H, et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann. Surg* 2012; 255(3):405-414.

(12) Bertens KA, Hawel J, Lung K, et al. ALPPS: challenging the concept of unresectability--a systematic review. *Int. J. Surg* 2015; 13:280-287.

(13) Beppu T, Hayashi H, Okabe H, et al. Liver functional volumetry for portal vein embolization using a newly developed 99mTc-galactosyl human serum albumin scintigraphy SPECT-computed tomography fusion system. *J. Gastroenterol* 2011; 46(7):938-943.

(14) de Graaf W, van Lienden KP, van den Esschert JW, et al. Increase in future remnant liver function after preoperative portal vein embolization. *Br. J. Surg* 2011; 98(6):825-834.

(15) Hirai I, Kimura W, Fuse A, et al. Evaluation of preoperative portal embolization for safe hepatectomy, with special reference to assessment of nonembolized lobe function with 99mTc-GSA SPECT scintigraphy. *Surgery* 2003; 133(5):495-506.

(16) Nanashima A, Tobinaga S, Abo T, et al. Relationship of hepatic functional parameters with changes of functional liver volume using technetium-99m galactosyl serum albumin scintigraphy in patients undergoing preoperative portal vein embolization: a follow-up report. *J. Surg. Res* 2010; 164(2):e235-e242.

(17) de Graaf W, van Lienden KP, Dinant S, et al. Assessment of future remnant liver function using hepatobiliary scintigraphy in patients undergoing major liver resection. *J. Gastrointest. Surg* 2010; 14(2): 369-378.

(18) Guglielmi A, Ruzzenente A, Conci S, et al. How much remnant is enough in liver resection? *Dig. Surg* 2012; 29(1):6-17.

(19) Cieslak KP, Bennink RJ, de Graaf W, et al. Measurement of liver function using hepatobiliary scintigraphy improves risk assessment in patients undergoing major liver resection. *HPB (Oxford)* 2016; 18(9): 773-80.

(20) Chapelle T, Op De Beeck B, Huyghe I, et al. Future remnant liver function estimated by combining liver volumetry on magnetic resonance imaging with total liver function on (99m)Tc-mebrofenin hepatobiliary scintigraphy: can this tool predict post-hepatectomy liver failure? *HPB (Oxford)* 2016; 18(6):494-503.

(21) Truant S, Baillet C, Deshorgue AC, et al. Drop of Total Liver Function in the Interstages of the New Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy Technique: Analysis of the "Auxiliary Liver" by HIDA Scintigraphy. *Ann Surg* 2016; 263(3):e33-4.

(22) Bennink RJ, Dinant S, Erdogan D, et al. Preoperative assessment of postoperative remnant liver function using hepatobiliary scintigraphy. *J. Nucl. Med* 2004; 45(6):965-971.

(23) Erdogan D, Heijnen BH, Bennink RJ, et al. Preoperative assessment of liver function: a comparison of 99mTc-Mebrofenin scintigraphy with indocyanine green clearance test. *Liver Int* 2004; 24(2):117-123.

(24) Bennink RJ, Tulchinsky M, de GW, et al. Liver function testing with nuclear medicine techniques is coming of age. *Semin. Nucl. Med* 2012; 42(2):124-137.

(25) Ekman M, Fjalling M, Friman S, et al. Liver uptake function measured by IODIDA clearance rate in liver transplant patients and healthy volunteers. *Nucl. Med. Commun* 1996; 17(3):235-242.

(26) Mosteller RD. Simplified calculation of body-surface area. *N. Engl. J. Med* 1987; 317(17):1098.

(27) de Graaf W, van Lienden KP, van Gulik TM, et al. (99m)Tc-mebrofenin hepatobiliary scintigraphy with SPECT for the assessment of hepatic function and liver functional volume before partial hepatectomy. *J. Nucl. Med* 2010; 51(2):229-236.

(28) de BT, Teriitehau C, Deschamps F, et al. Predictive factors for hypertrophy of the future remnant liver after selective portal vein embolization. *Ann. Surg. Oncol* 2010; 17(8):2081-2089.

(29) Bednarsch J, Jara M, Lock JF, et al. Noninvasive diagnosis of chemotherapy induced liver injury by LiMAx test - two case reports and a review of the literature. *BMC. Res. Notes* 2015; 8:99.

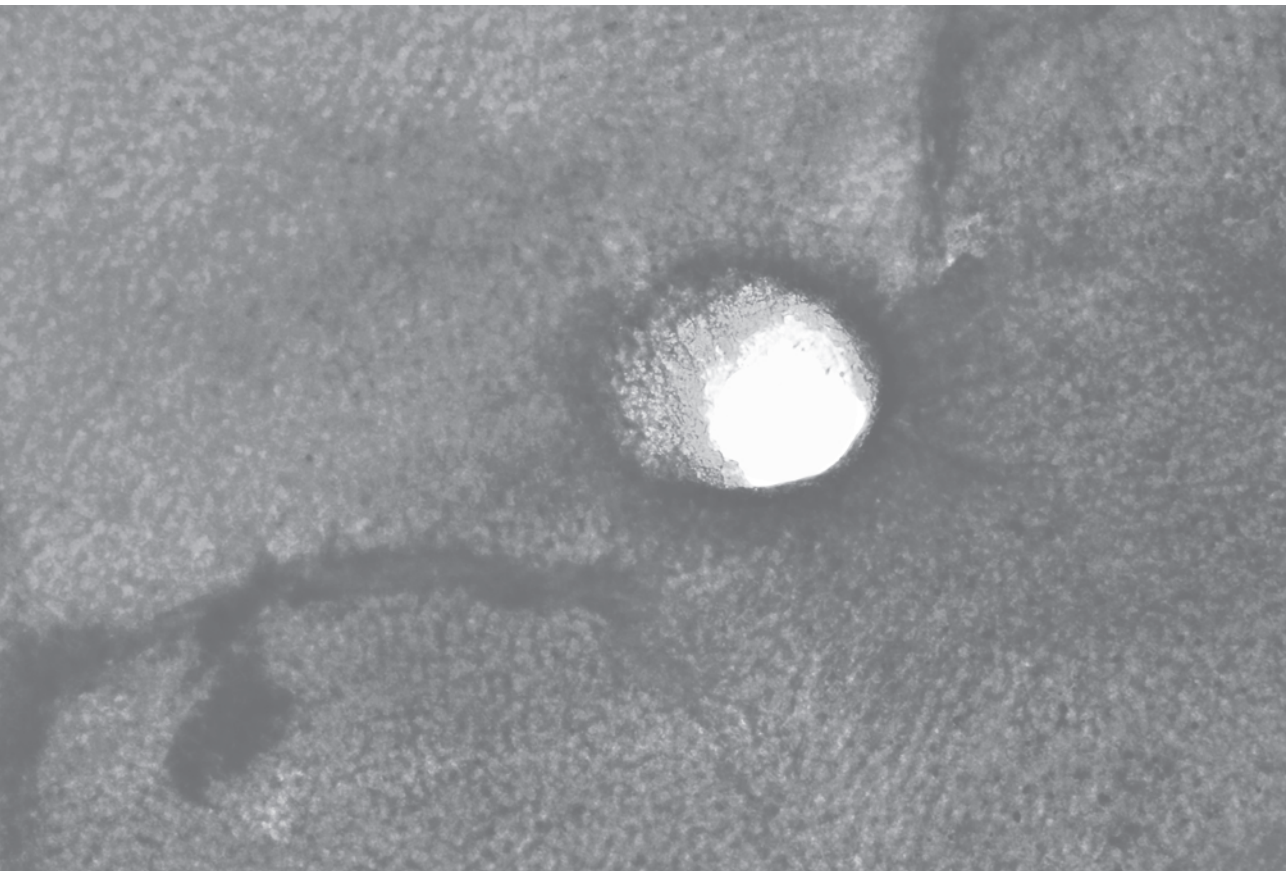
(30) Giraudo G, Greget M, Oussoultzoglou E, et al. Preoperative contralateral portal vein embolization before major hepatic resection is a safe and efficient procedure: a large single institution experience. *Surgery* 2008; 143(4):476-482.

(31) Malinowski M, Stary V, Lock JF, et al. Factors influencing hypertrophy of the left lateral liver lobe after portal vein embolization. *Langenbecks Arch. Surg* 2015; 400(2):237-246.

(32) van den Broek MA, Olde Damink SW, Dejong CH, et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; 28(6):767-780.

(33) Cieslak KP, Olthof PB, van Lienden KP, et al. Assessment of Liver Function Using 99mTc-Mebrofenin Hepatobiliary Scintigraphy in ALPPS (Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy). *Case Reports in Gastroenterology* 2015; 9(3):353-360.

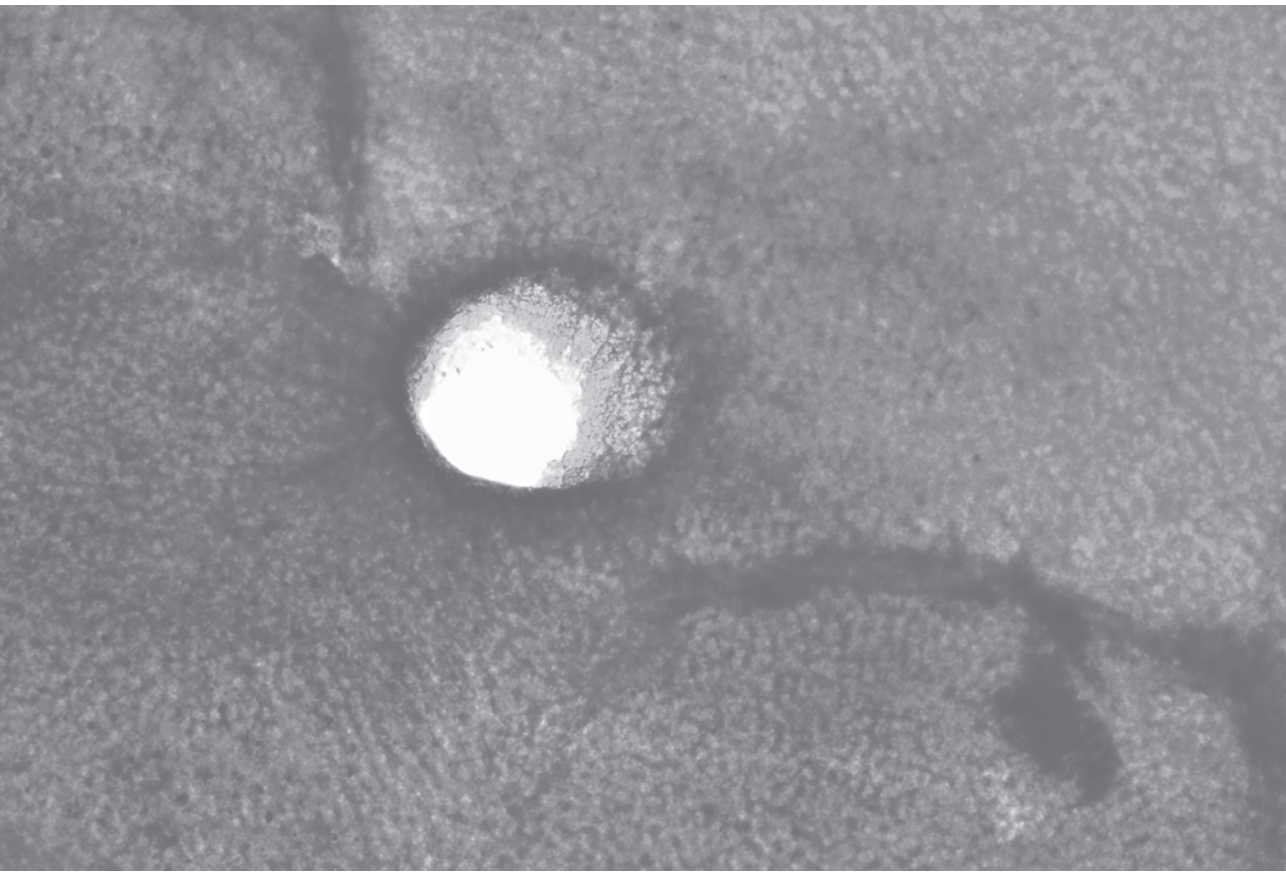
(34) Leung U, Simpson AL, Araujo RL, et al. Remnant growth rate after portal vein embolization is a good early predictor of post-hepatectomy liver failure. *J. Am. Coll. Surg* 2014; 219(4):620-630.



CHAPTER 5

Liver related complications in unresectable disease after portal vein embolization

F. Huisman, K.P. Cieslak, K.P. van Lienden, R.J. Bennink, T.M. van Gulik



Hepato Biliary Surgery and Nutrition 2017

ABSTRACT

Background:

Portal vein embolization (PVE) is used preoperatively in patients to increase future remnant liver (FRL) volume. Unfortunately, some patients are found to be unresectable at exploration due to tumor progression or new lesions. The aim of this study is to evaluate the long-term effects of PVE in the embolized liver lobe when left unresected.

Methods:

Of 85 patients who underwent right PVE, 16 (19%) were unresectable (PVE-group). These patients were compared with 48 randomly matched patients from a pool of 75 unresectable patients who had not undergone PVE. Primary outcome parameter was occurrence of infectious complications (liver abscesses) on follow-up imaging of the liver. The long-term volumetric changes of the hypertrophy/atrophy complex were assessed as secondary outcome parameter.

Results:

Five of 16 (31%) patients in PVE-group developed an abscess *vs.* 4 (8%) patients in non-PVE group (P = 0.022). The volume distribution of left and right liver lobes (hypertrophy-atrophy rate) increased from 26%:74% before embolization to 36%:64% three weeks after PVE and to 51%:49% six months after PVE.

Conclusions:

Persistence of embolized liver lobe in unresectable patients after PVE resulted in abscesses in 31%. This observation calls for developing reversible embolization techniques using absorbable materials in patients with uncertain resectability.

INTRODUCTION

Liver resection is the main curative therapy for primary or metastatic liver tumors. The volume of the liver that remains after resection (future remnant liver, FRL) should be at least 25–30% of the total liver volume (TLV) based on computed tomography (CT) volumetric studies. An insufficient FRL volume is associated with increased rates of postoperative liver failure and liver failure related mortality.[1] In case of compromised liver tissue, e.g., due to steatosis, cirrhosis or recent chemotherapy, FRL volume of at least 40% is preferred.[2] However, in patients with extensive disease who require major liver surgery (resection of 3 or more Couinaud segments), the FRL volume is often lower than the accepted cut-off values for safe resection.

Portal vein embolization (PVE) is an accepted method to preoperatively increase FRL volume. This technique uses the unique capacity of the liver to regenerate. As a result of selective portal vein occlusion, atrophy of the embolized liver lobes occurs with a concomitant hypertrophy response of the non-embolized liver lobes. Permanent occlusion of the portal vein is usually achieved by using non-absorbable embolization materials, such as polyvinyl alcohol particles with coils (PVAc), n-butyl cyanoacrylate (NBCA), fibrin glue, ethanol or combinations of these. Unfortunately, recent studies have shown that approximately 20% of the originally planned liver resections after PVE were cancelled, because of insufficient hypertrophy response of the FRL, intrahepatic tumor progression, new hepatic metastases or extra-hepatic tumor spread.[3] This is consistent with our own experience where 19% of the patients after having undergone PVE (2005–2013) were found to be unresectable. Remarkably, we have observed infectious complications in the embolized liver lobe in a number of patients who had undergone PVE, but eventually did not undergo resection. The long-term effects of permanent occlusion of portal vein on the embolized, atrophied liver lobe have not been described in literature so far. Hence, the aim of this study is to evaluate the long-term effects of PVE in the embolized liver lobe when left unresected.

METHODS

Patients

From March 2005 to August 2013, 85 patients underwent PVE of the right portal system. Sixteen out of the 85 (19%) patients were found to be unresectable (PVE group). Of these patients, 10/16 (62.5%) were diagnosed with colorectal liver metastases (CRLM) and 6/16 (37.5%) with perihilar cholangiocarcinoma (PHC). These patients were compared with 48 randomly matched patients with CRLM and PHC (CRLM-PHC ratio of 3:1) from a pool of 75 patients who did not undergo preoperative PVE and were found unresectable during the preoperative work-up or during laparotomy (non-PVE group) in the same period. We

used IBM SPSS Statistics (SPSS for Windows; SPSS, Chicago, Illinois, USA) to create a random sample. The tumor load (number and size of metastasis) in patients diagnosed with CRLM (n = 30) was equally distributed in the PVE-group and the non-PVE group. Furthermore, patients diagnosed with PHC in both groups (n = 10) were classified as Klatskin type 3 or 4. The baseline characteristics of patients in the PVE and non-PVE group are shown in **Table 1.**

Table 1 Baseline characteristics of patients (March 2005 to August 2013) who had undergone right portal vein embolization (PVE group) and the matched control group who had not undergone PVE (non-PVE group) before being defined as unresectable.

Patients	**PVE group, n=16**	**Non-PVE group, n=48**	p-value
Gender, male (%)	12 (75)	28 (58)	0.233
Age in years, mean ± SD	66 ± 6.9	64 ± 11.3	0.661
BMI, median (IQR25-75)	24 (22-27)	23 (20-26)	0.201
Exploration by laparotomy (%)	11 (68.8)	24 (50.0)	0.244
Diagnosis, (%)			
CRLM	10 (62.5)	30 (62.5)	
PHC	16 (37.5)	18 (37.5)	
Palliative chemotherapy* (%)	9 (56)	26 (54.2)	0.885

SD, standard deviation; **IQR**, interquartile range; **CRLM**, colorectal liver metastases**; PHC**, perihilar cholangiocarcinoma; ***** given after unresectability was defined.

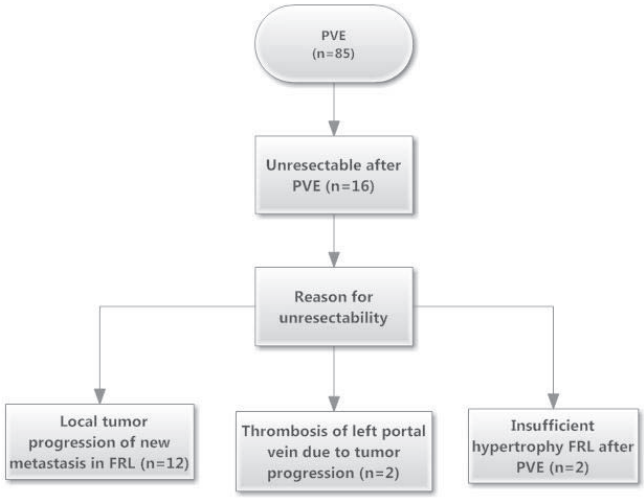
Preoperative work-up in the PVE group

All included patients were evaluated by a multidisciplinary team, where treatment was defined by consensus. PVE was indicated when the FRL was <25–30% of TLV for patients with normal liver parenchyma, and <35–40% in case of underlying liver disease (e.g., steatosis, cirrhosis, recent chemotherapy). In addition to volumetric measurements, the functional capacity of FRL was assessed using technetium-99m (^{99m}Tc)-mebrofenin hepatobiliary scintigraphy (HBS) as described previously.[4] PVE was indicated in case HBS showed FRL function below the validated cut-off value (2.7%/min/m^2).[5]

Volumetric assessment of the liver

Preoperative CT imaging and volumetric assessment of the FRL were performed in all patients. CT volumetry was performed before and three weeks after PVE. The portal-venous phase of the CT scans was used for the volumetry. 3D reconstructions of the liver were made using 5 mm thick axial slices. Portal and hepatic veins were used as landmarks for segmental division. The circumferences of total liver, tumor and FRL were outlined manually. Integrated software (Mx-View 3.52, Philips Medical Systems) was used to calculate TLV, tumor volume (TV) and FRL volume. The percentage FRL volume was calculated using the following formula: [FRL volume/ (TLV – TV)] ×100. Tumor progression was documented when detected after PVE.

When unresectability was concluded after PVE (**Figure 1A**), follow-up imaging (CT scan or ultrasound) of the liver was performed in all 16 patients in the PVE group. In 11 (68.8%) of these patients, a follow-up CT scan was available after a mean interval of 6 ±2 months following PVE. Possible recanalization of the occluded portal venous system was evaluated using the portal phase images. Volumetric studies were performed to measure the long-term hypertrophy-atrophy rates of embolized and non-embolized lobes, respectively. Three



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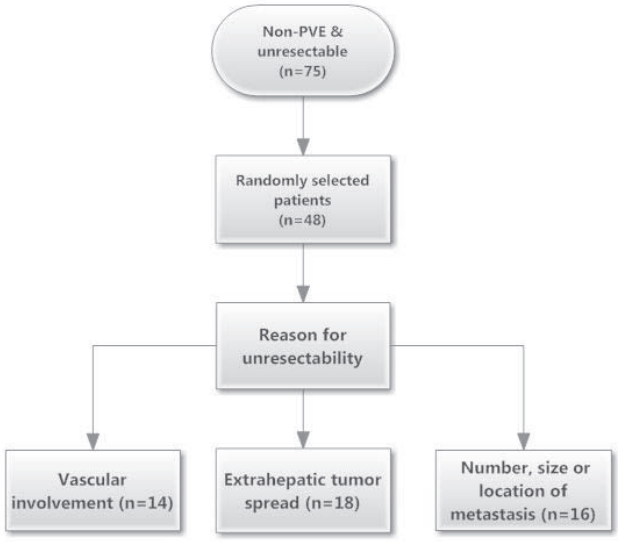


B

Figure 1. Reasons for unresectability in patients who underwent portal vein embolization (PVE) (A) and in patients of the non-PVE group (B)% CI 0.671-0.968).

patients had died without follow-up scans and two patients had only follow-up ultrasound examinations; therefore, no volumetric data were available of these patients. One patient diagnosed with PHC underwent follow-up HBS at 20 months after PVE to evaluate long-term changes in function of the embolized and non-embolized liver lobes.

HBS evaluation of liver function

Liver function was evaluated quantitatively using HBS before and 3 weeks after PVE as published before.[4, 5] A cut-off value of at least 2.7%/min/m^2 was considered compatible with sufficient residual liver function. Data were processed on a Hermes workstation (Hermes Medical Solutions, Sweden).

Unresectable disease and follow-up in non-PVE patients

Patients in the control group were deemed unresectable by a multidisciplinary team because of number and location of lesions as evidenced by radiological findings, or were found to be unresectable during laparotomy, see **Figure 1B**. Follow-up imaging was performed to evaluate the response to chemotherapy after unresectability was defined.

Chemotherapy

Palliative chemotherapy was offered to all patients who were considered unresectable. Chemotherapeutic regimens were scheduled according to the local protocols, but if needed, adapted to the individual patient. The combination of oxaliplatin and capecitabine with or without bevacizumab was given in most cases (CRLM). Some patients received capecitabine, irinotecan, panitumumab, or oxaliplatin with 5-fluorouracil/leucovorin. The chemotherapy regime for cholangiocarcinoma consisted of a combination of gemcitabine and cisplatin. [6] Due to the large variation of the received chemotherapy regimens, we only recorded whether patients received palliative chemotherapy or not.

Study outcomes

The primary outcome parameter of this study was the occurrence of infectious complications in unresected patients, defined as the finding of liver abscesses on follow-up imaging of the liver. All patients received follow-up CT scans. When a liver abscess was clinically suspected, an ultrasound and subsequent CT were made. Most of the patients presented with the clinical picture of an abscess, i.e., fever and elevated CRP.

The secondary outcome parameter was volumetric changes of the embolized and non-embolized liver lobes reflecting long-term hypertrophy/atrophy rates after PVE. The volume distribution was defined as the rate between the percentage FRL volume of the TLV and the percentage right liver volume of the TLV, i.e., %FRLV : %RLV. In one patient, we were able to assess long-term changes in the hepatic uptake function of the embolized and non-embolized lobes using HBS.

Statistical analysis

The data were analysed by statistical software (SPSS for Windows; SPSS, Chicago, Illinois, USA) and GraphPad Prism (Graph-pad, San Diego, CA, USA). The non-parametric Mann Whitney U test was used for comparing data that was not normally distributed between the PVE-group and the non-PVE group. Survival curves were generated by the Kaplan-Meier method. All statistical tests were two-tailed, and differences were considered significant at a P value of ≤0.05.

RESULTS

PVE

PVE of the right portal venous system was successfully performed in all 16 unresectable patients in the PVE-group. No procedure related complications were noted. The median time interval between PVE and surgical exploration was 36 days (IQR 32–58 days). The mean %FRLV measured by CT volumetry before PVE was 28% ±7%. Three to 4 weeks (mean 21 ±3 days) after PVE, the %FRLV had increased to 37% ±6% (P < 0.001), **Table 2**. HBS showed a mean ^{99}Tc-mebrofenin uptake rate in the FRL of 2.04% ±0.95%/min/m^2 before PVE. Three to 4 weeks (mean 22 ±2 days) after PVE, the mean ^{99}Tc-mebrofenin uptake rate in the FRL had increased to 3.37% ±1.13%/min/m^2, P = 0.001. The mean hepatic mebrofenin uptake rate of the atrophic, right liver lobes was 3.90% ±1.75%/min/m^2 before embolization and 3.03% ±1.17%/min/m^2 after PVE, P = 0.010. There were no differences in the mean uptake of the right liver lobes after embolization between patients who developed liver abscesses and patients in whom no abscesses were seen after the procedure, P = 0.699.

Table 2 Volumetric measurements of the total liver, FRLV and RLV before and 3 weeks after PVE in all 16 patients in the PVE group.

CT-volumetry	Before PVE	After PVE	p-value
Total liver volume in ml, mean ±SD	2003 ± 390	2083 ± 595	p=0.40
FRLV in ml, mean ±SD	519 ± 167	699 ± 209	p<0.001
RLV in ml, mean ±SD	1405 ± 324	1198 ± 543	p=0.005
%-FRL, mean ±SD	28 ± 7	37 ± 6	p<0.001
%-RLV, mean ±SD	72 ± 7	63 ± 7	p<0.001

PVE, portal vein embolization; **FRLV**, future remnant liver volume; **RLV**, right liver volume; **SD**, standard deviation; **%-FRL**, future remnant volume as percentage of the total liver volume; **%-RLV**, right liver volume as percentage of the total liver volume.

Reasons for unresectability

In the PVE-group, five patients out of the 16 (31%) did not undergo exploration due to evident tumor progression on the follow-up CT scans 3 weeks post PVE. The remaining 11 patients were found unresectable during exploration. In the non-PVE group, 24 out of the 48 (50.0%) patients were found to be unresectable at exploration while the remaining 24 patients were considered unresectable based on preoperative imaging. Baseline characteristics of the PVE and the non-PVE patients are shown in **Table 1**. The causes of unresectability in both groups are shown in **Figure 1**.

Follow-up imaging and occurrence of PVE related long- term complications

Recanalization of the occluded portal venous system did not occur in any of the 16 patients in the PVE group. Five out of the 16 (31%) patients in the PVE group developed one or more abscess diagnosed on CT scan or ultrasound *vs.* 4 (8%) patients in the non-PVE group, P = 0.022. Of the 5 patients who had developed an abscess in the PVE group, 3/5 (60%) were diagnosed with PHC and 2/5 (40%) with CRLM. In the non-PVE group, 1/4 (25%) patient with abscesses was diagnosed with CRLM and the remaining 3 (75%) patients with PHC.

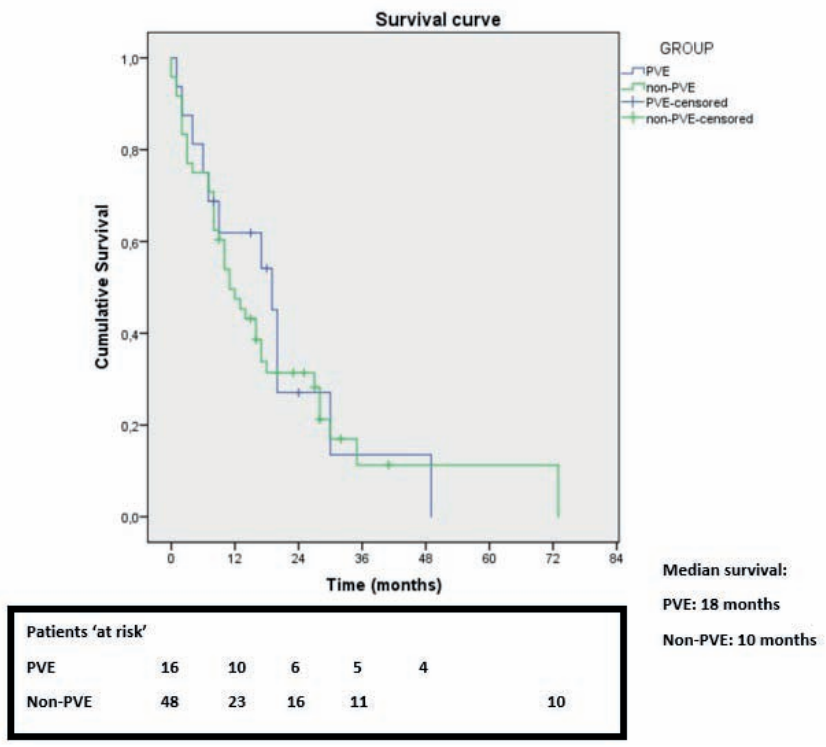


Figure 2. Cumulative survival of patients who underwent portal vein embolization (PVE) and were unresectable and patients who did not undergo PVE.

In the PVE-group, the median survival of unresectable patients was 18 months (IQR 6–20 months), including the 4 patients with liver abscesses. Median survival in the control group was 10 months (IQR 7–17 months), which was not significantly different from the PVE-group (P = 0.66, **Figure 2**).

The long-term hypertrophy/atrophy rates

After a mean time of 6 ±2 months, volumetric measurements were performed using follow-up CT scans in 11/16 (68.8%) patients who had undergone PVE. In this subgroup, the median %FRLV before PVE was 26% ±7% and after PVE had increased to 36% ±6%, P < 0.001. Ongoing hypertrophy was seen 6 months after PVE as the %FRLV had increased to 51% ±13% (P < 0.001, **Table 3**).

Table 3 Volumetric measurements of the 11 patients in the PVE group in whom follow-up CT imaging was performed.

CT-volumetry	Before PVE	3 weeks after PVE	p-value[#]	6 ± 2months after PVE*	p-value[$]
Total liver volume in ml, mean ±SD	1992 ± 398	2034 ± 605	p=0.73	1877 ± 459	p=0.22
FRLV in ml, mean ±SD	517 ± 187	721 ± 245	p<0.001	945 ± 368	p=0.001
RLV in ml, mean ±SD	1461 ± 305	1045 ± 357	p=0.12	932 ± 321	p=0.001
%-FRLV, mean ±SD	26 ± 7	36 ± 6	p<0.001	51 ± 13	p<0.001
%-RLV, mean ±SD	74 ± 7	64 ± 6	p<0.001	49 ± 13	p<0.001
RLL uptake (HBS), %/min/m^2, mean ± SD	3.90 ± 1.75	3.03 ± 1.17	p=0.01		

PVE, portal vein embolization; **FRLV**, future remnant liver volume; **RLV**, right liver volume; **RLL**, right liver lobes; **SD**, standard deviation.

[#] comparison of values before vs. 3 weeks after PVE * after a median time of 6±2 months; [$] comparison of values 3 weeks after PVE vs. 6 months.

The volume distribution of the left and right liver lobes (hypertrophy-atrophy rate) increased from 26%:74% before embolization to 36%:64% three weeks after PVE and to 51%:49% six months after PVE, **Figure 3**.

As mentioned in the methods section, one patient (65 years, female) with biopsy proven PHC underwent functional assessment of the FRL during follow-up with HBS. She underwent HBS scans before PVE and 3 weeks, 17 and 20 months after PVE, respectively, see **Table 4**. The measured uptake function of the embolized, atrophied liver lobe had decreased considerably after 20 months compared to the initial function, i.e., from 5.8%/min/m^2 to 1.1%/min/m^2. **Figure 4** illustrates the changes and distribution of the hepatic uptake function in the embolized and non-embolized liver lobes.

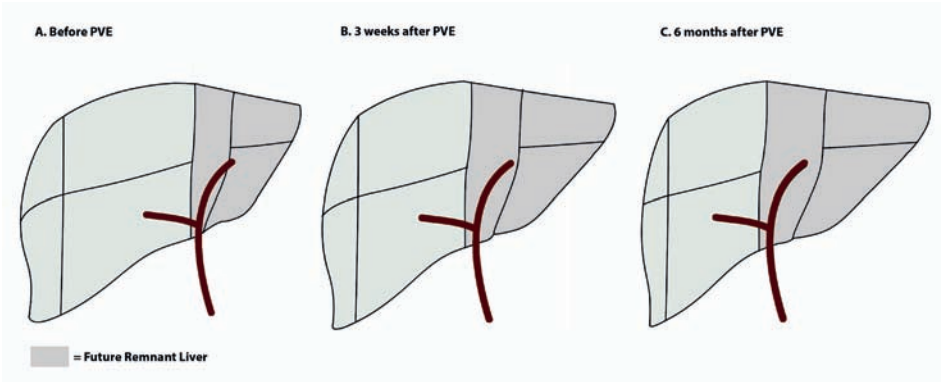


Figure 3. Volume distribution of the future remnant liver (FRL) and right (embolized) liver. Median %FRL increased from 26% ±7% pre portal vein embolization (PVE) to 36% ±6% (P < 0.001) at 3 weeks after PVE and to 51% ±13% (P < 0.001) 6 months after PVE.

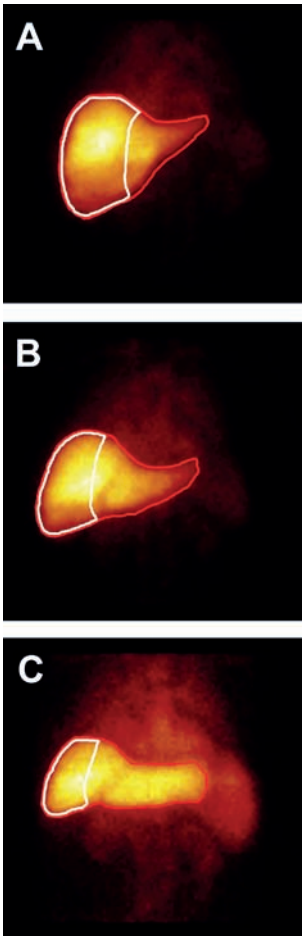


Figure 4. Hepatobiliary scintigraphy (HBS) imaging performed before (**A**), 3 weeks (**B**) and 20 months (**C**) after right portal vein embolization in a patient diagnosed with perihilar cholangiocarcinoma (female; 64 years). The red line delineates the total liver and the white line delineates the right (embolized) liver segments.

Table 4 Follow-up HBS in one unresectable patient who underwent PVE.

CT-volumetry	Before PVE	3 weeks after PVE	17 months after PVE*	20 months after PVE
Total liver uptake, %/min	15.5	15.1	4.9	7.3
%FRL of total liver uptake	24	46	42	68
%embolized lobes of total liver uptake	76	54	58	32
FRL uptake, %/min/m^2	1.8	3.4	0.9	2.3
Embolized liver lobes uptake, %/min/m^2	5.8	4.1	1.3	1.1

PVE, portal vein embolization; **FRL**, future remnant liver; **HBS**, Hepatobiliary-scintigraphy (HBS) using ^{99m}Tc-mebrofenin.

*** Cholangitis due to insufficient biliary drainage during follow-up HBS at 17 months after PVE showing a relevant decrease in FRL uptake rate.**

DISCUSSION

PVE is a safe and frequently used method to induce hypertrophy of the FRL. Owing to PVE, more patients have become eligible for curative liver resection with less postoperative complications.[6] Unfortunately, in approximately 20% of the cases, patients are found to be unresectable in the time between PVE and operation due to tumor progression or the occurrence of extra-hepatic metastases.[7]

Tumor growth of microscopic and macroscopic liver lesions after PVE has been increasingly reported in literature, and can be considered a short-term complication of the procedure.[8] The long-term effects of the persisting embolized lobe after PVE in unresectable patients are largely unknown. The atrophy-hypertrophy complex stabilizes in time while providing sufficient liver function and complications of the atrophied liver lobe have not been reported so far.[9] We assumed that, especially in patients with PHC, troublesome septic complications may occur due to infected and incompletely drained bile ducts while the hilar tumor progresses and continues to occlude (sub)segmental bile ducts. We have shown in this study that unresected patients with not only PHC but also with CRLM who had undergone PVE are more prone to develop abscesses compared to unresected patients with the same diagnoses and a patent portal vein of the right liver. In case of CRLM, the etiology of abscess formation is less clear. Possibly parenchymal damage following extensive chemotherapy and/or local ischemia due to tumor progression plays a role. Although the occurrence of abscesses differed significantly between unresectable PVE and non-PVE patients, this did not influence long-term survival as the survival between the groups was comparable. However, the consequences of intrahepatic abscesses impose considerable burden in patients who are undergoing palliative treatment and already have a limited quality of life. To avoid the abovementioned complications, patients who require PVE probably could benefit from the use of absorbable embolization materials, provided that these materials induce an adequate hypertrophy response of the FRL after a predictable absorption period.

Lainas *et al.*[10] were the first to describe PVE with an absorbable embolization material. Using gelatin sponge powder, the authors embolized the right liver lobes of 9 monkeys and reported a 43% volume increase of the FRL after 1 month. Recanalization of the embolized portal system was seen 12–16 days after PVE in all animals indicating that the gelatin sponge had been absorbed. Van den Esschert *et al.*[11] compared the hypertrophy response after PVE in rabbits using two absorbable embolization materials. A recent study published by Olthof *et al.*[12] found an adequate hypertrophy response using a combination of fibrin glue and aprotinin as embolization material showing 80% recanalization after 42 days in a rabbit model of PVE. The use of absorbable embolization materials therefore seems a promising approach in preventing the long-term complications of permanent embolization. Reversible embolization techniques and their clinical application await validation in further studies.

We evaluated the role of hepatic uptake-function of the embolized liver lobes, as measured with HBS, and its possible contribution to the development of liver abscesses after PVE. The mean uptake function of the embolized liver lobes 3 weeks after PVE decreased significantly in this series. One of the patients with a remarkable long-term survival despite unresectable disease, received several HBS scans during the follow-up period after PVE, showing a decrease of total liver function and relative function of the embolized liver segments in 20 months. This observation corroborates the notion that in case of unresectability after PVE, an absorbable embolization material potentially prevents functional deterioration of the embolized liver lobes and additionally prevents abscess formation in these lobes.

This study has a few limitations. Firstly, we compared initially resectable patients after PVE with unresectable patients without PVE, possibly introducing a selection bias. However, this bias is difficult to avoid since in order to evaluate undesirable long-term complications after PVE we need to compare with patients who did not undergo the procedure. Secondly, we disposed of a relatively small sample size for the PVE group. Notwithstanding these limitations, this is the first report on long-term complications after PVE in unresectable patients.

In conclusion, persistence of the embolized liver lobe in unresectable patients after PVE resulted in abscesses in 31%. This observation calls for developing reversible embolization techniques of the portal vein using absorbable materials in patients with uncertain resectability.

References

(1) Kishi Y, Abdalla EK, Chun YS, et al. Three hundred and one consecutive extended right hepatectomies: evaluation of outcome based on systematic liver volumetry. *Ann Surg* 2009; 250(4):540-8.

(2) van Gulik TM, van den Esschert JW, de Graaf W, et al. Controversies in the use of portal vein embolization. *Dig Surg* 2008; 25(6):436-44.

(3) van Lienden KP, van den Esschert JW, de Graaf W, et al. Portal vein embolization before liver resection: a systematic review. *Cardiovasc Intervent Radiol* 2013; 36(1):25-34.

(4) de Graaf W, van Lienden KP, Dinant S, et al. Assessment of future remnant liver function using hepatobiliary scintigraphy in patients undergoing major liver resection. *J Gastrointest Surg* 2010; 14(2): 369-78.

(5) de Graaf W, van Lienden KP, van den Esschert JW, et al. Increase in future remnant liver function after preoperative portal vein embolization. *Br J Surg* 2011; 98(6):825-34.

(6) May BJ, Talenfeld AD, Madoff DC. Update on portal vein embolization: evidence-based outcomes, controversies, and novel strategies. *J Vasc Interv Radiol* 2013; 24(2):241-54.

(7) Pommier R, Ronot M, Cauchy F, et al. Colorectal liver metastases growth in the embolized and non-embolized liver after portal vein embolization: influence of initial response to induction chemotherapy. *Ann Surg Oncol* 2014; 21(9):3077-83.

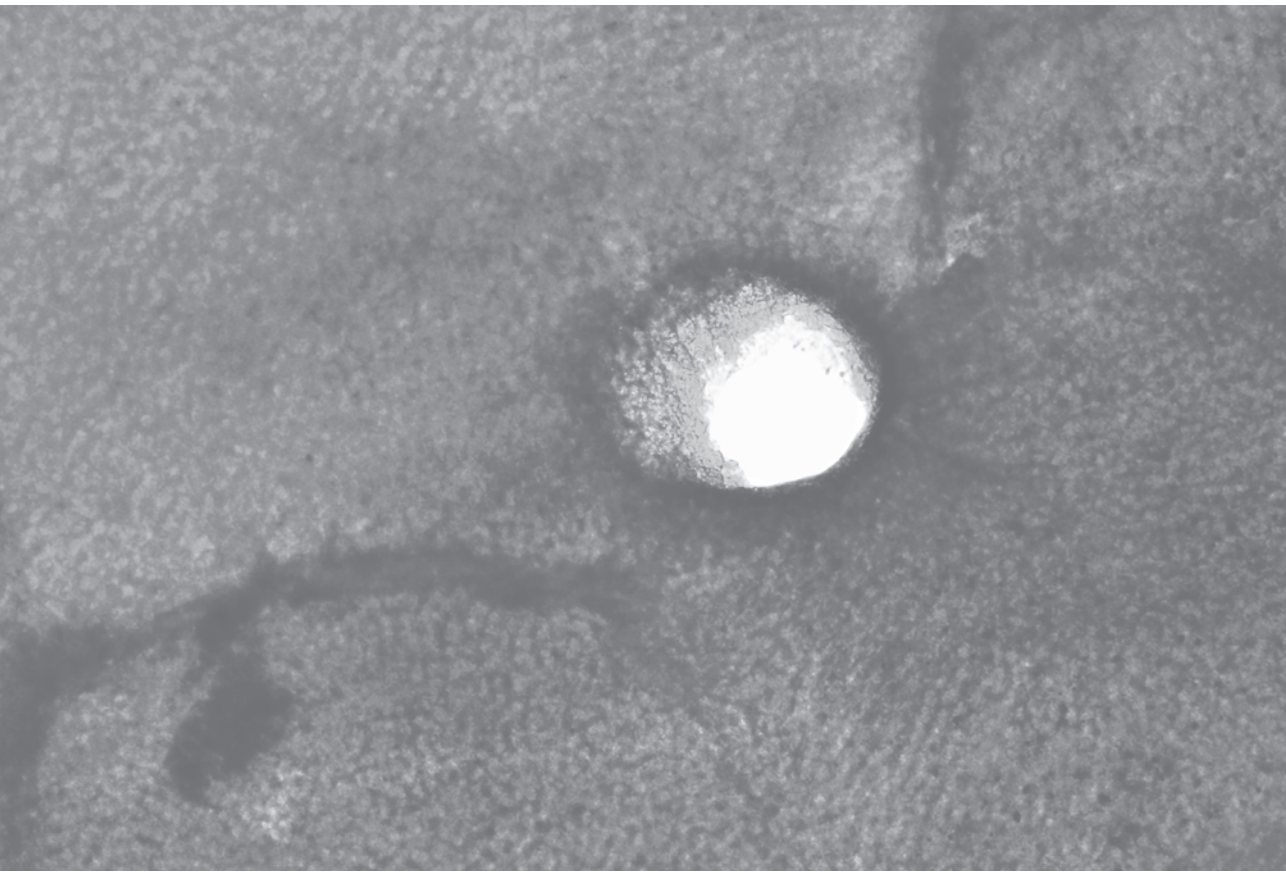
(8) Hoekstra LT, van Lienden KP, Doets A, et al. Tumor progression after preoperative portal vein embolization. *Ann Surg* 2012; 256(5):812-7; discussion 817-8.

(9) Mueller L, Grotelueschen R, Meyer J, et al. Sustained function in atrophying liver tissue after portal branch ligation in the rat. *J Surg Res* 2003; 114(2):146-55.

(10) Lainas P, Boudechiche L, Osorio A, et al. Liver regeneration and recanalization time course following reversible portal vein embolization. *J Hepatol* 2008; 49(3):354-62.

(11) van den Esschert JW, van Lienden KP, Alles LK, et al. Liver regeneration after portal vein embolization using absorbable and permanent embolization materials in a rabbit model. *Ann Surg* 2012; 255(2): 311-8.

(12) Olthof PB, Huisman F, van Golen RF, et al. Use of an absorbable embolization material for reversible portal vein embolization in an experimental model. *Br J Surg* 2016; 103(10):1306-15.



CHAPTER 6

Treatment of colorectal liver metastasis at a regional hospital versus a university medical centre

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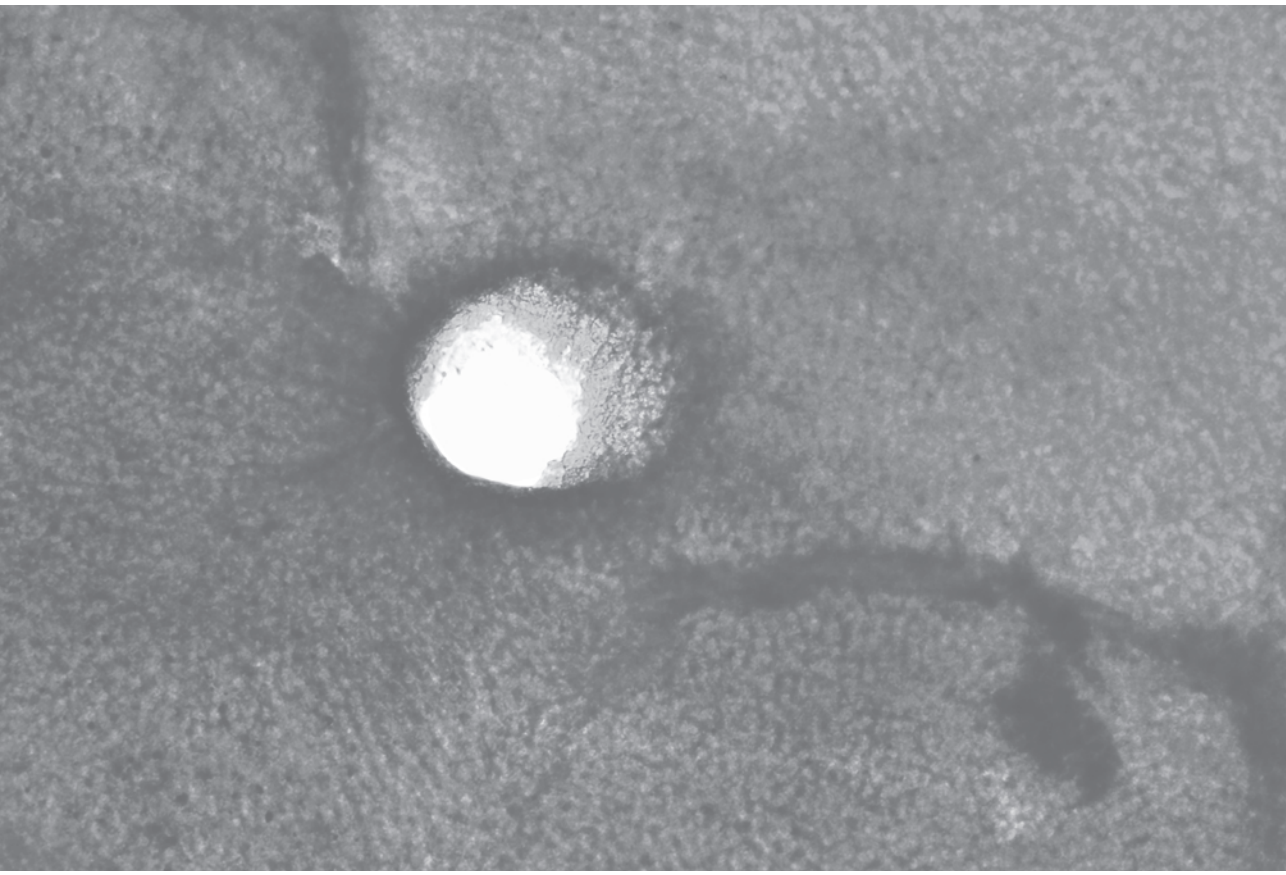


NTvG, 2013

ABSTRACT

Objective:

To evaluate the results of liver surgery for colorectal liver metastasis performed at a specialized regional hospital in comparison with the operation performed at a university medical center (UMC).

Design:

Retrospective study at 2 hospitals.

Methods:

All patients with colorectal liver metastasis who had undergone a partial liver resection and/or radiofrequency ablation (RFA) at Amphia Hospital or at the Academic Medical Centre - University of Amsterdam (AMC) from January 2005-June 2011 were included. Data on patient characteristics, type of resection, pathology results and (disease-free) survival were collected. The primary outcome measures were surgical complications and survival.

Results:

A total of 232 patients were included. No differences in patient characteristics between centres were identified. At the AMC, 121 patients (98.4%) had undergone a resection; 6 in combination with RFA. Two patients (1.6%) had undergone RFA only. At Amphia Hospital, 85 patients (78%) had undergone a resection; 30 in combination with RFA. In 24 patients (22%), only RFA was performed. There was a significant difference in the type of treatment ($p < 0.01$). Not significantly different between the two centres were the average lengths of hospital stay, surgical complications and recurrence rates. After resection, no significant differences in the 1- and 3-year (disease-free) survival rates were found between the two centres. At Amphia Hospital, the overall survival at 1, 3 and 5 years was 86, 47 and 29%, respectively. These rates were significantly better at AMC with 91, 78 and 53%, respectively ($p < 0.05$). The difference in (disease-free) survival for the entire group of patients can be explained by the more frequent performance of RFA at Amphia Hospital.

Conclusion:

Postoperative morbidity, mortality and survival rates after liver surgery obtained from a specialized regional hospital were similar to results obtained from a UMC.

INTRODUCTION

Currently more patients with colorectal liver metastasis qualify for curative treatment and the prognosis of these patients has improved. [1-4] The golden standard for surgical treatment of colorectal liver metastases is a partial liver resection or for limited tumors, radio frequentcy ablation (RFA) and in some patients a combination of these two methods. Various other treatments have become available.[5] For this type of liver surgery, expert teams of surgeons, internist, oncologists and radiologists are required. The intervention is therefore performed in specialized hospitals, typically in university medical centers (UMC's) and more recently, in larger peripheral hospitals. Whether this type of care should be performed only in the UMC's, is the subject of an ongoing discussion. The quality of care in the peripheral centers must be equal to the quality of an UMC. The aim of this study is to evaluate the results of liver resections and RFA treatments for metastases of colorectal carcinoma performed in a specialized regional hospital in comparison to an UMC.

Techniques

Despite radical resection of the primary tumor, approximately 50-60% of patients will develop hepatogenic metastases. Fifteen to 20% of the patients with colorectal cancer will present with synchronous metastatic liver disease, which means that the primary tumor is detected at the same time as the liver metastases. [2,3,6] A partial liver resection is the only curative option with a 5-years survival of 25-50% and a 10-years survival of 20% after microscopically radical resection (R0). [2,7-9]

According to current insights, a patient is eligible for resection if the liver metastases can be radically removed with enough remaining functional liver volume (approximately 30% of the total liver volume).

However, more than 80% of the tumors are not resectable at presentation due to localization of the tumor in multiple organs, ingrowth of the tumor in the central vessels or insufficient estimated liver remnant function. In approximately 13% of the patients for whom resection is not possible in the first instance, a resection can be carried out after response to chemotherapy, resulting in a decrease in size and/or number of metastases ('downsizing').[4,10,11] Another treatment option for resectabel and irresectabel liver metastases is RFA, in which a needle electrode is placed in the center of a metastasis. [3,12-14] The tissue is heated to above 60°C, which leads to irreversible cell damage and cell death.[14-17] This method can be done percutaneously, laparoscopically or during laparotomy. It is also possible to apply RFA in combination with a resection, when one or more metastases are outside the planed field of resection.

PATIENTS AND METHODS

Data of two groups of patients with colorectal liver metastases who underwent a liver resection or RFA, were collected. One group consisted of patients from a specialized, peripheral hospital, the Amphia Hospital in Breda, while the second group of patients originated from the Academic Medical Center (AMC) in Amsterdam, both in the Netherlands. All patients who underwent partial liver resection or RFA for colorectal liver metastases in the period January 2005-June 2011 were included in this study. Patients who were found to be unresectable intraoperatively due to peritonitis carcinomatosa or metastases elsewhere were excluded. Data such as patient characteristics, surgical data, outcomes of pathological examination, survival and disease-free survival were collected prospectively. The Charlson-index was used to compare the comorbidity between the different patient populations.[19] A resection consisted of a metastasectomy, resection of 1 or multiple segments or ('extended') left or right hemihepatectomy. Laparoscopic resection was performed in some patients with a lesion in the left lateral segments of the liver. Patients with multiple or large liver metastases that were irresectable due to localization, were treated with neo-adjuvant chemotherapy to reduce metastases and to achieve resectability. RFA was done in collaboration with the radiologist and was performed percutaneously, laparoscopically or via laparotomy. A combination of resection and RFA was performed in patients who had one or more metastases outside the area to be resected. Postoperative complications were scored based on the clinical patient records. The Clavien-Dindo classification was used to classify the complications, with 7 different scales representing the severity of a complication. Complications within scale 1 and 2 are relatively innocent and only need simple therapy. The serious complications range from 3 to 7 and represent complications that require an intervention or lead to death of the patient.[20] The date of the operation is counted as the start of follow-up. The follow-up consisted of a visit at the outpatient clinic every 3 months during the first 2 years after the liver resection or RFA and then once every 6 months up to 5 years after the resection or RFA. The follow-up consisted of plasma CEA-determination, chest x-ray and an ultrasound or a 4-phase CT scan of the liver. By telephone verification with the GP and by consulting the population registry, it was checked whether patients were still alive. Total survival was defined as the time between the operation and the eventual death or the end of follow-up. Total disease-free survival was defined as the time between the operation and the occurrence of a possible recurrence.

Statistical analysis

SPSS 19.0 (SPSS inc, Illinois, USA) for Microsoft Windows was used for statistical analyses. Patient characteristics were compared using the $\chi 2$ test. Survival was determined with Kaplan-Meier curves and the log rank test was used to test for significance.

RESULTS

A total of 232 patients were eligible for surgical treatment of colorectal liver metastases: 123 patients in the AMC and 109 patients in Amphia Hospital. Patients treated in the AMC were on average younger and had a higher number of liver metastases at the time of presentation (**Table 1**).

At the AMC, a resection was performed in 121 patients (98.4%). Six of the 123 patient underwent liver resection in combination with RFA. At the Amphia Hospital, 85 patients (78.0%) underwent a resection; 30 of them were in combination with RFA. Furthermore, 24 patients (22.0%) at Amphia Hospital were treated with only RFA, in the AMC only 2 patients (1.6%). There was a significant difference in type of treatment (p <0.01) (**Table 2**). Resection

Table 1 Patient characteristics in 232 patients submitted to liver resection for colorectal liver metastases in AMC and Amphia hospital

	Amphia (n = 109)	**AMC (n = 123)**	**p-value***
Gender (%)			0,53
male	62 (56,9)	75 (61,0)	
woman	47 (43,1)	48 (39,0)	
Age (years) ± SD	66,5 ± 9,1	62,0 ± 12,9	<0,01
Charlson index score (%)			0.08
5	77 (70,6)	93 (75,6)	
6	16 (14,7)	25 (20,3)	
≥ 7	16 (14,7)	5 (4,1)	
Primary tumor localisation (%)			0,84
Colon (right)	25 (22,9)	29 (23,6)	
Colon (left)	5 (4,6)	7 (5,7)	
Sigmoid	40 (36,7)	42 (34,1)	
Rectum	39 (35,8)	45 (36,6)	
Dukes classification			0,27
A	0 (0)	1 (0,8)	
B	22 (20,2)	36 (29,3)	
C	32 (29,4)	28 (22,8)	
D	55 (50,4)	58 (47,1)	
Metastasis			0,36
Synchronous	58 (53,2)	58 (47,2)	
Metachronous	51 (46,8)	65 (52,8)	
Number of metastases (n) ± SD	1,85 ± 2,0	2,47 ± 1,5	<0.01

*Chi-Square test and One-way ANOVA
AMC= Academic Medical Center
SD = Standard deviation

of 3 or more segments was performed in 32 patients (37.6%) in Amphia Hospital compared to 52 patients (43.0%) in the AMC, p = 0.37.

In 19 patients in the AMC group, liver resection was combined with additional surgical procedures, e.g ileostomy closure surgery, colectomy or repair of abdominal wall herniation.

In 16 patients, a selective portal vein embolization was performed and 3 patients underwent a two-stage hepatectomy. In the Amphia hospital, three patients underwent a partial liver resection in combination with a colectomy and one patient a combination of RFA of a liver metastasis and a low anterior resection. A R0-resection was achieved in 103 patients (85.1%) in the AMC and in 80 patients (94.1%) in the Amphia hospital.

Recurrences

Recurrences occurred in 52 patients (47.7%) during follow up in de Amphia hospital. In the group of patients who underwent resection only (n=55), 22 patients (40%) developed recurrent disease. In the AMC, 48 patients who underwent a liver resection only developed recurrent disease (39.0%), which was not significantly different compared to the Amphia hospital (p=0.18). The recurrence rate in the Amphia hospital in the patient group treated with RFA (n=24) was 62.5% and 50% in the group of patients who underwent a combination of RFA and resection (n=30) (**Table 2**). In the Amphia hospital, twelve patients (22.2%) of the

Table 2 Type of resection and surgical outcome in both centers

	Amphia (n = 109)	**AMC (n = 123)**	**p-value***
Treatment (%)			<0,01
resection	55 (50,5)	115 (93,5)	
RFA	24 (22,0)	2 (1,6)	
resection + RFA	30 (27,5)	6 (4,9)	
Resection (%)			0,37
< 3 segments	53 (62,4)	69 (57,0)	
> 3 segments	32 (37,6)	52 (43,0)	
R0-resection (%)	80 (94,1)	103 (85,1)	<0,01
Recurrence total (%)	52 (47,7)	48 (39,0)	0,18
Local recurrence after RFA (%)	12 (22,2)		
Re-resection recurrence (%)	5 (9,6)	20 (41,7)	0,04
RFA treatment recurrence (%)	13 (25,0)	8 (16,7)	0,14
Extra-hepatic metastases (%)	39 (35,8)	44 (35,8)	0,64
Mean hospital stay in days ± SD	11 ± 14,1	12 ± 9,7	0,60
Postoperative mortality (within 30 days)	0 (0)	3 (2,4)	0,10

AMC = Academic Medical Center
SD = standard deviation
RFA = radiofrequent ablation

54 patients who were treated with RFA, developed a local recurrence. The number of RFA procedures done in the AMC was too low for comparison. In case of a recurrence in the liver, 5 of the 52 patients underwent a repeat hepatic resection in the Amphia hospital (9.6%), compared to 20 of the 48 patients (41.7%) in the AMC (p=0.04). In addition, 25 and 16.7 % of the patients with a recurrence, respectively, were treated with RFA (p=0.14). The remaining patients with a recurrence were no longer eligible for surgical treatment (**Table 2**).

Hospital stay and follow-up

The mean hospital stay in the Amphia hospital was 11 days, which didn't differ significantly from the AMC (12 days). The postoperative period was uneventful in 87 patients in the Amphia Hospital (79.8%) compared to 96 patients in the AMC (78%) (p=0.16)(**Table 3**). The postoperative mortality was 0 and 2.4 %, respectively. No significant difference was seen in severity of complications scored by Clavien-Dindo classification (p=0.65)(**Table 3**). The median time of follow-up was 22 months (range 0-81 months) in the Amphia hospital and 31 months (range 0-78 months) in the AMC. There were no significant differences in 1- and 3-years survival in the group of patients who underwent resection only. The numbers of patients were too low to compare the 5-years survival (p=0.24) (**figure 1**). In the Amphia hospital, the 1-, 3- and 5-years survival of all the patients included was 86, 47 and 29%, respectively. The 1-, 3-, and 5- years survival of the total group patients in the AMC was significantly better with 91, 78 and 53% (p<0.05), respectively. The disease-free survival in the Amphia hospital after 1, 3 and 5 years was 55, 32 and 28%, respectively, compared

Table 3 Postoperative complications in both centers

	Amphia (n = 109)	AMC (n = 123)	p-value*
Biliary leakage	4	2	
Subphrenic abscess	1	8	
Liver failure	0	1	
Sepsis	2	0	
Bleeding	1	1	
Infection	2	4	
Ileus	1	3	
Pneumonia	1	1	
Other	10	7	
Total	22 (20,2)	27 (22,0)	0,16
Clavien-Dindo classification;			0.65
n (%)			
1 or 2	6 (27.3)	9 (33.3)	
>2	16 (72.7)	18 (66.7)	

*Chi-Square test; AMC = Academic Medical Center

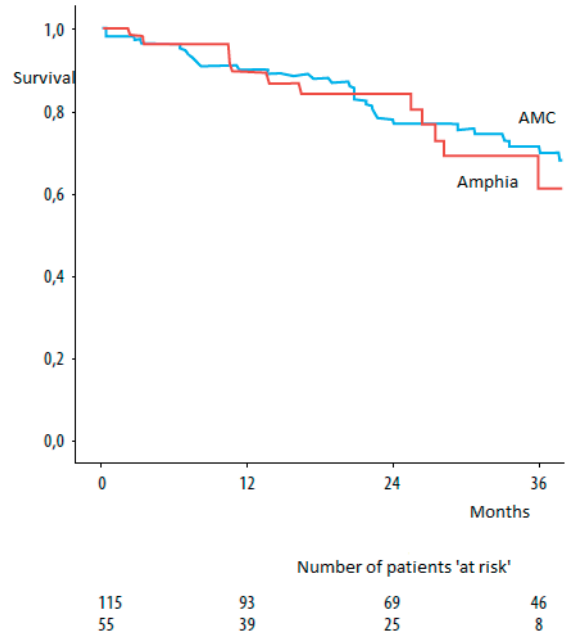


Figure 1. Survival curve after colorectal liver resection. Blue indicates AMC and red indicates Amphia hospital.

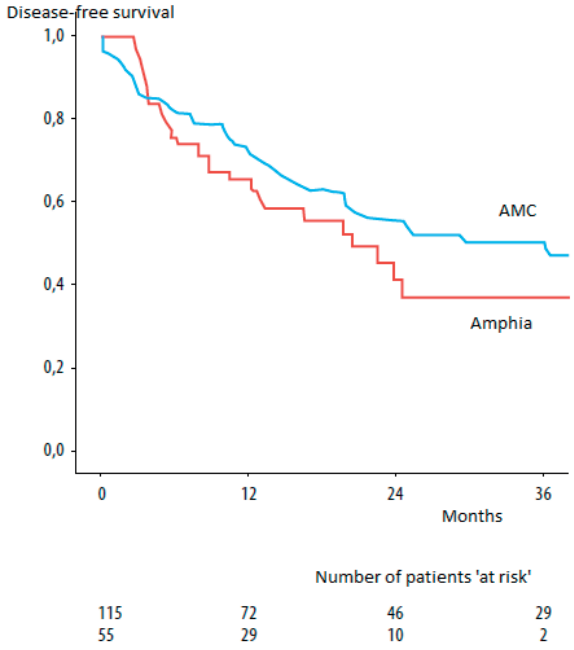


Figure 2. Kaplan-Meier survival plot of disease free survival after colorectal liver surgery in AMC (blue) and Amphia (red).

to 70, 47 and 40% in the AMC, which was significantly different ($p<0.05$). There was no significant difference in disease-free survival after 1 and 3 years in the group of patients who underwent resection only between both hospitals (65 and 37% in the Amphia hospital and 73 and 49% in the AMC, respectively; p=0.26)(**Figure 2**).

DISCUSSION

Liver surgery is a relatively safe procedure, as shown in previous studies. [2-4,6-10] In this study, the morbidity of liver surgery in the Amphia hospital was 20.2 % with no mortality. The morbidity in the AMC was 20.0% and the mortality 2.4%. This is in line with the literature, which describes a morbidity of 20-60% and a mortality less than 5%.[12,21] The recurrence rate in this study (47.7% in Amphia hospital and 39.0% in AMC) is also in line with current literature (25-64%). [4] In the AMC, there was a better survival and disease-free survival for the total patient population. There was however, no difference when comparing patients who underwent only resection at both centers.

A limitation of this study is the difference in type of offered treatment between the two centers. Combined surgery and portal vein embolization was performed more often in the AMC, which may result in a bias in comparable patient populations between the two centers. The difference in (disease-free) survival between the Amphia Hospital and the AMC for the entire group of patients can be explained by the more frequent use of RFA in Amphia Hospital. No randomized studies have been done comparing resection with RFA. However, a recent meta-analysis does show that RFA should be reserved for patients in whom a resection is not or no longer possible because of a higher recurrence rate after RFA. [22] No significant difference in total and disease-free survival after 1 and 3 years was found in patients who underwent resection only. A few years ago, RFA was still seen as a potentially curative option for the treatment of liver metastases. The outcomes of the initial results of RFA treatment in the Netherlands have changed the policy at Amphia Hospital and as result, the percentage of RFAs has declined strongly in recent years.[12] Partial liver resection can be seen as gold standard for curative treatment of patients with liver metastases. [4,21,23] According to current insights, a patient is eligible for resection if liver metastases can be radically removed with enough functional residual liver volume (about 30%). Extrahepatic metastases which can be removed radically, such as synchronic lung metastases, are no contraindication for surgical treatment.[3,4] In case of synchronic metastases, a ‘ liver first’ approach can be considered in which after neo-adjuvant chemotherapy, the liver is operated first before the primary tumor is removed. [24] The patient is no longer eligible for resection in case of progression of the tumor under neo-adjuvant chemotherapy.

CONCLUSION

Postoperative morbidity, mortality and survival rates after liver surgery in a specialized regional hospital were comparable to results of a University Medical Centre. Liver surgery can be adequately applied in a regional hospital with a center function in this field.

References

(1) Hardy KJ. Liver surgery: the past 2000 years. Aust. N.Z. J. Surg. 1990;60;811-17

(2) Dols LF, Verhoef C, Eskens FA, Schouten O, Nonner J, Hop WC, et al. Verbetering in 5-jaarsoverleving na leverresectie voor colorectale metastasen tussen 1984-2006. Ned Tijdschr Geneeskd. 2009 Mar 14; 153(11):490-5.

(3) Wicherts DA, de Haas RJ, Borel Rinkes IH, Voest EE, van Hillegersberg R.Betere behandelingsmogelijkheden voor patiënten met colorectale levermetastasen. Ned Tijdschr Geneeskd. 2006 Feb 18; 150(7):345-51.

(4) De Jong KP, Slooff MJ. Resectie van levermetastasen: hogere kans op overleving. Ned Tijdschr Geneeskd. 2002 Feb 2;146(5):196-9.

(5) Grünhagen DJ, IJzermans JNM, Verhoef C. Focale behandeling van colorectale levermetastasen en andere leverafwijkingen. Ned Tijdschr Oncol. 2012;9:350-7.

(6) Vlot J, Kazemier G, Heijsterkamp J, Tilanus HW, van der Sijp JR, Ijzermans JN. De behandeling van colorectale levermetastasen: goede 5-jaarsoverlevingskans na partiële leverresectie in het Academisch Ziekenhuis Rotterdam, 1984/'99. Ned Tijdschr Geneeskd. 2002 Feb 2;146(5):214-8.

(7) Abdalla EK, Vauthey JN, Ellis LM, Ellis V, Pollock R, Broglio KR, et al. Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. Ann Surg. 2004 Jun;239(6):818-25

(8) Rees M, Tekkis PP, Welsh FK, O'Rourke T, John TG. Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patiënts. Ann Surg. 2008 Jan; 247(1):125-35.

(9) Choti MA, Sitzmann JV, Tiburi MF, Sumetchotimetha W, Rangsin R, Schulick RD, et al. Trends in long-term survival following liver resection for hepatic colorectal metastases. Ann Surg. 2002 Jun;235(6): 759-66.

(10) Fioole B, Liem MS, Hennipman A, Borel Rinkes IH. partiële leverresecties: sterfte, morbiditeit en risicofactoren voor postoperatieve complicaties bij 133 patiënten/137 operaties; Universitair Medisch Centrum Utrecht, 1991/'00. Ned Tijdschr Geneeskd. 2002 Feb 2;146(5):210-3.

(11) Adam R, Delvart V, Pascal G, Valeanu A, Castaing D, Azoulay D, et al. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. Ann Surg. 2004 Oct;240(4):644-57

(12) Blussé van Oud-Alblas M, Fioole B, Jansen MC, van Duijnhoven FH, van Hillegersberg R, Rijken AM, et al. Radiofrequente ablatie van colorectale levermetastasen. Initiële resultaten in Nederland. Ned Tijdschr Geneeskd 2008; 152: 880-886.

(13) Jansen MC, van Duijnhoven FH, van Hillegersberg R, Rijken AM, van Coevorden F, van der Sijp J, et al. Adverse effects of radiofrequency ablation of liver tumours in the Netherlands. Br J Surg. 2005 Oct; 92(10):1248-54.

(14) van Duijnhoven FH, Jansen MC, Junggeburt JM, van Hillegersberg R, Rijken AM, van Coevorden F, et al. Factors influencing the local failure rate of radiofrequency ablation of colorectal liver metastases. Ann Surg Oncol. 2006 May;13(5):651-8.

(15) Adam R, Avisar E, Ariche A, Giachetti S, Azoulay D, Castaing D, et al.Five-year survival following hepatic resection after neoadjuvant therapy for nonresectable colorectal. Ann Surg Oncol. 2001 May;8(4): 347-53.

(16) Curley SA. Radiofrequency ablation of malignant liver tumours. Oncologist. 2001;6;14-23.

(17) Kroft LJM, Linden E van der, Obermann WR. Radiologische percutane thermocoagulatiebehandeling van lever-, nier- en longtumoren. Ned Tijdschr Geneeskd. 2005;149;1448-53.

(18) Fioole B, Jansen MC, Dijnhoven FH van, Hillergersberg R van, Gulik TM van, Borel Rinkes IH. Combining partial liver resection and local ablation of liver tumours: a preliminary Dutch experience. World J Surg Oncol. 2006;4:46.

(19) Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373-83.

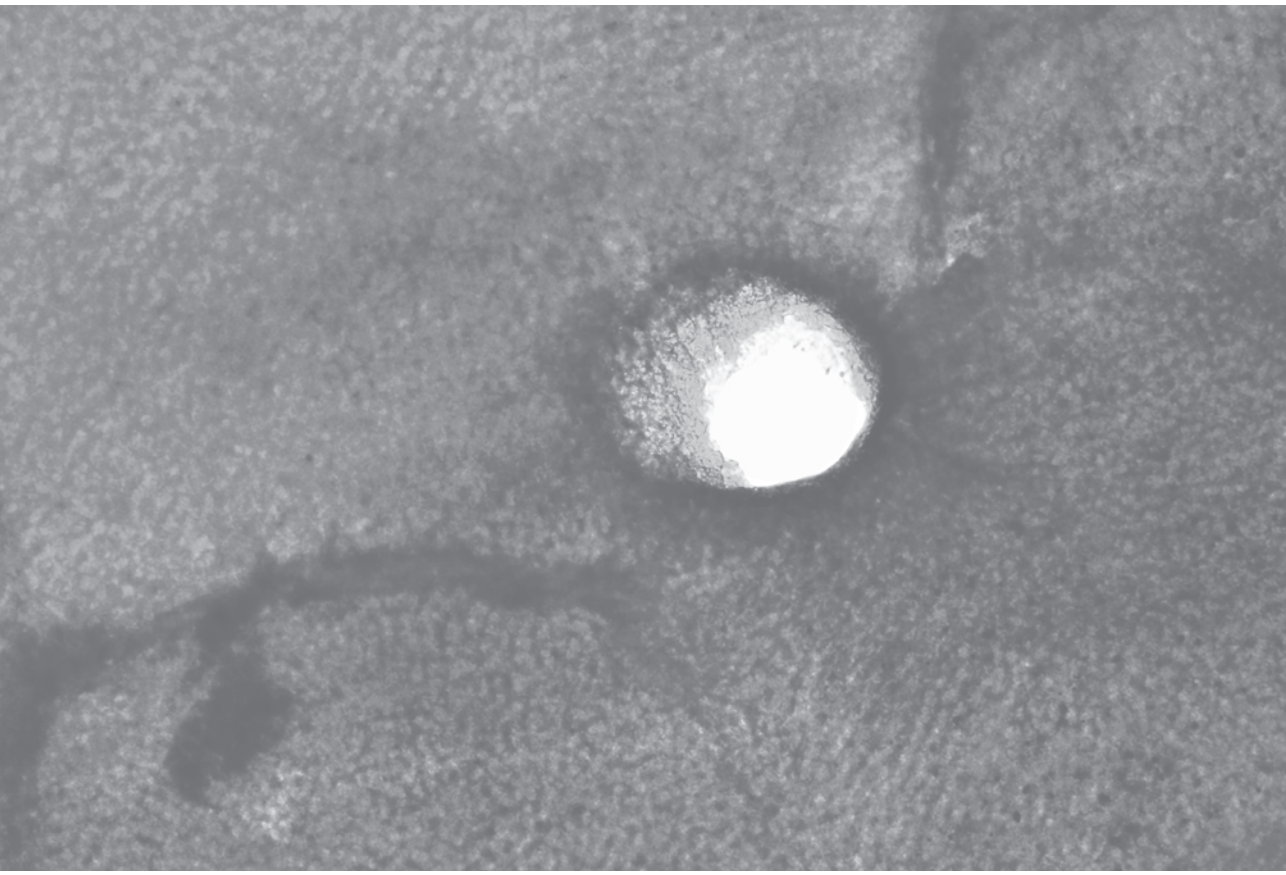
(20) Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004;240:205-13.

(21) Yong TL, Bohmer R, Pande GK, Birks SE, Loh DC, Hewitt PM. Liver resection: a regional hospital experience. ANZ J Surg. 2010 Oct;80(10):710-3. doi: 10.1111/j.1445-2197.2010.05267.x. Erratum in: ANZ J Surg. 2011 Jan;81(1-2):107.

(22) Weng M, Zhang Y,Zhou D, Yang Y, Tang Z, Zhao M, et all.Radiofrequency ablation versus resection for colorectal cancer liver metastases: a meta-analysis. PLoS One. 2012;7(9).

(23) Chua TC, Saxena A, Chu F, Zhao J, Morris DL. Predictors of cure after hepatic resection of colorectal liver metastases: an analysis of actual 5- and 10-year survivors. J Surg Oncol. 2011 Jun;103(8):796-800. doi: 10.1002/jso.21864. Epub 2011 Jan 18.

(24) De Jong MC, van Dam RM, Maas M, Bemelmans MH, Olde Damink SW, Beets GL, et al. The liver-first approach for synchronous colorectal liver metastasis: a 5-year single-centre experience. HPB (Oxford). 2011 Oct;13(10):745-52.



CHAPTER 7

Stepwise introduction of laparoscopic liver surgery: validation of guideline recommendations

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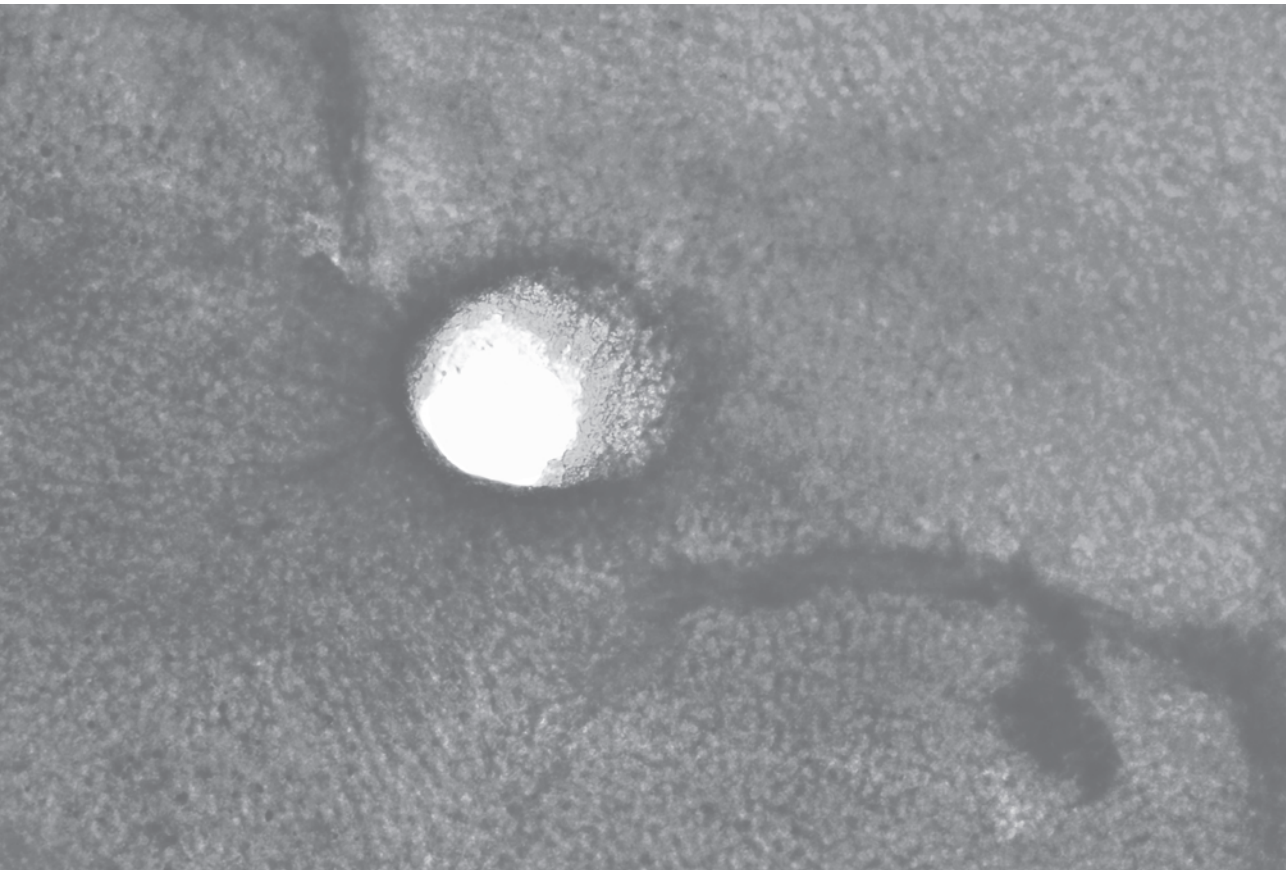


HPB (Oxford) 2017

ABSTRACT

Background:

Uncontrolled introduction of laparoscopic liver surgery (LLS) could compromise postoperative outcomes. A stepwise introduction of LLS combined with structured training is advised. This study aimed to evaluate the impact of such a stepwise introduction.

Methods:

A retrospective, single-center case series assessing short term outcomes of all consecutive LLS in the period November 2006–January 2017. The technique was implemented in a stepwise fashion. To evaluate the impact of this stepwise approach combined with structured training, outcomes of LLS before and after a laparoscopic HPB fellowship were compared.

Results:

A total of 135 laparoscopic resections were performed. Overall conversion rate was 4% (n = 5), clinically relevant complication rate 13% (n = 18) and mortality 0.7% (n = 1). A significant increase in patients with major LLS, multiple liver resections, previous abdominal surgery, malignancies and lesions located in posterior segments was observed after the fellowship as well as a decrease in the use of hand-assistance. Increasing complexity in the post fellowship period was reflected by an increase in operating times, but without comprising other surgical outcomes.

Conclusion:

A stepwise introduction of LLS combined with structured training reduced the clinical impact of the learning curve, thereby confirming guideline recommendations.

INTRODUCTION

Laparoscopic liver surgery (LLS) had a relatively slow start due to initial concerns about bleeding, gas embolism, increased complications during the early phases of the learning curve and the ability to perform adequate radical oncological resections. Through the pioneering work of high-volume, expert centers, an increasing body of evidence has emerged in recent years confirming the possible advantages of LLS.[1-9] Benefits of LLS include less intraoperative blood loss, less postoperative complications, decreased need for analgesics, faster functional recovery, shorter postoperative stay, and a cosmetic benefit.[1-9] In addition, some studies have demonstrated the cost-effectiveness of LLS [10-12], thus resulting in benefits for both individual patients and healthcare institutions. These promising results have promptly increased the interest in LLS worldwide [1,2] and the first randomized controlled trials of laparoscopic vs. open liver surgery have been performed.[13,14]

Despite these promising results, LLS remains challenging and should not be started without appropriate training and acquired surgical skills. During the 2015 Morioka consensus meeting[15] and more recently during the 2017 European guideline meeting on LLS in Southampton (EGMLLS) the importance of structured implementation plans, providing education and a stepwise introduction of LLS, was stressed. Starting with minor resections and gaining experience along the way, surgeons can eventually begin to take on more difficult procedures such as hemihepactectomies. The results of such an approach and its effect on the learning curve have not been specifically addressed before and could further encourage surgeons to implement LLS into their center.

The aim of this study was to present the results of a single center that followed a stepwise approach in setting up a LLS practice, including structured training, with assessment of a potential learning curve effect on short-term postoperative outcomes.

METHODS

Patients

In a retrospective case series, all consecutive patients undergoing LLS for any indication between November 2006 and January 2017 in the Academic Medical Center (AMC) in Amsterdam were evaluated. No LLS was performed prior to November 2006. All primary LLS or combined laparoscopic colorectal and liver resections were included.

Prior to surgery, all patients were discussed in a multidisciplinary team (MDT) meeting with HPB surgeons, radiologists, gastroenterologists, hepatologists, medical oncologists and pathologists. The surgical indication was established independently of the decision regarding the surgical approach, which was made later considering a number of factors including the available experience and skill. Initially, only minor resections, defined according to the

Louisville consensus meeting in 2008 [16], were considered candidates for the laparoscopic approach whilst major LLS procedures were only considered after experience and skills were obtained by performing minor LLS and one surgeon (MB) had completed an eight month fellowship in laparoscopic HPB surgery in 2013.

In addition, complex resections such as those of large lesions or lesions in close proximity to major vascular structures were not considered during the early stages. Attention was paid during the MDT meetings to patient- and tumor characteristics (e.g. tumor location, obesity) that could increase the difficulty of the operation, in order to select the patients most suitable for LLS, especially during the early stages.

Outcomes

Baseline patient- and procedure characteristics included patient demographics, body mass index (BMI, kg/m^2), American Society of Anaesthesiology (ASA) classification, liver cirrhosis, previous abdominal surgery, previous liver resection, simultaneous colorectal resection, tumor pathology (benign/malignant), extent of resection (minor/major/technically major [17]), type of resection, hand–assistance, multiple simultaneous liver resections and approach to liver resection (one-stage only, one-stage + radio frequency ablation (RFA), two-stage without portal vein embolization (PVE) and two-stage with PVE. Intra- and post-operative outcomes included operative time (mins), intraoperative blood loss (ml), blood transfusion, conversion, resection margins (margin negative (R0) or margin involved (R1)), length of postoperative hospital stay (days), clinically relevant complication rate (defined as Clavien-Dindo score 3 or higher) [18] and mortality (defined as death related to liver and/or colorectal complications within 90 days after surgery or within hospital stay).

Surgical experience

All resections were performed or supervised by one or two out of three liver surgeons (OB, PT and MB), all of whom had completed a fellowship in open liver surgery, had experience in advanced laparoscopic gastrointestinal surgery (defined here as anything beyond laparoscopic cholecystectomy, appendectomy or hernia repair surgery) and had taken at least two hands-on courses on minor LLR. OB had ten years of experience in open liver surgery and advanced laparoscopic gastrointestinal procedures after his fellowship. PT and MB each had two years of experience after their fellowship including advanced laparoscopic gastrointestinal procedures. OB started with LLS in 2006, PT in 2010 and MB in 2012. MB completed a fellowship in laparoscopic HPB surgery (Jan-Aug 2013; University Hospital Southampton NHS Foundation Trust).

Surgical technique

A standardized approach was used. Patients were placed in a supine position with legs apart and if required on a beanbag. After placement of 3-5 trocars, parenchymal dissection was

performed with ultrasonic shears (Harmonic Ace®; Ethicon Endo-Surgery, Cincinnati, OH, USA) and, for larger/posterior lesions or resections, laparoscopic cavitron ultrasonic surgical aspirator (CUSA) (Valleylab, Boulder, CO, USA). For left lateral sectionectomy, only ultrasonic shears and endostaplers were used. Rarely, for posterior lesions, a handport was used (n=4). Specimens were extracted in a plastic endoscopic bag (Endocatch; Ethicon Endo-Surgery, Cincinnati, OH, USA) via a Pfannenstiel incision or, in case of lesions <3cm, through a widened trocar incision. Pringle manoeuvre was applied for laparoscopic major procedures, including posterior metastasectomies and larger, atypical metastasectomies. For metastasectomies the 'diamond technique' was preferred.[19] All laparoscopic hemihepatectomies and laparoscopic resections involving segment 7 were performed by a team of two surgeons (MB, PT).

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows version 23.0 (IBM corp., Armonk, NY, USA). To evaluate the stepwise approach and its impact on the learning curve, the cohort was divided into two groups: before (group A) and after (group B) a dedicated fellowship in major laparoscopic HPB surgery. Continuous non-parametric variables were reported as median with interquartile range (IQR). A Mann Whitney *U* test was used to compare continuous variables between the groups. Categorical variables were reported as proportions and compared between groups using chi-square test or Fisher's exact test as appropriate. A two-tailed *p* value of <0.05 was considered statistically significant.

RESULTS

Between November 2006 and January 2017, 135 LLS were performed in 132 patients (one patient underwent two procedures and one underwent three). During this period, the percentage of liver resections performed laparoscopically increased from 2% in 2006 to 37% in 2016 (p <0.001) (**Fig.1**).

Baseline patient and procedure characteristics are shown in **table 1** and **2** respectively. Of all resections, 100 (74%) were for malignant disease, mostly CRLM (n=58) and HCC (n=27). Operations were performed by combinations of two surgeons in 33% (n=45) of procedures.

Perioperative outcomes are shown in **table 3**. Conversion to an open procedure occurred in 5 patients (4%) for the following reasons: bleeding (n=2), inadequate access to the lesions (n=2) and concern about oncological efficiency (n=1). Clinically relevant postoperative complications occurred in 18 patients (13%), including biloma/abscess requiring drainage (5%, n=7) and anastomotic leak in combined laparoscopic colorectal procedures (15%, n=4) as the most frequently observed complications. One patient died of decompensating liver cirrhosis with hepatorenal syndrome after hand-assisted resection of HCC from segment 8, resulting in a 0.7% mortality rate.

Sensitivity analysis

Outcomes did not change when major LLS was excluded from the analysis (data not shown).

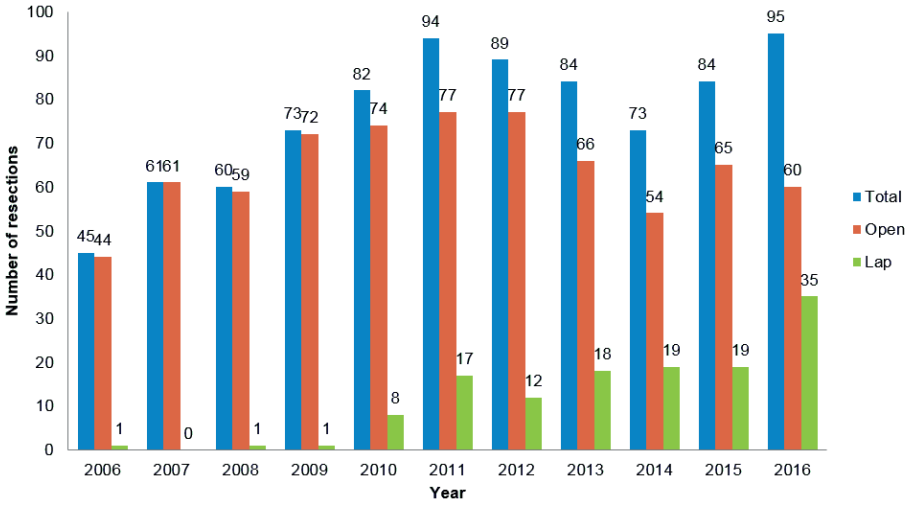


Figure 1. Number of open and laparoscopic liver resections through the years.

Table 1 Patient characteristics

	Overall (n=135)	Group A (n=52)	Group B (n=83)	*P*
Age, years (IQR)	59 (46-67)	56 (40-66)	61 (47-71)	0.276[a]
Gender, males, n (%)	75 (56%)	28 (54%)	47 (57%)	0.859[b]
BMI, kg/m^2 (IQR)	26 (23-29)	26 (24-30)	26 (23-29)	0.947[a]
ASA score (%)				0.887[c]
- ASA I	27 (20%)	12 (23%)	15 (18%)	
- ASA II	84 (62%)	31 (60%)	53 (64%)	
- ASA III	22 (16%)	8 (15%)	14 (17%)	
- ASA IV	2 (2%)	1 (2%)	1 (1%)	
Liver cirrhosis, n (%)	21 (16%)	9 (17%)	12 (15%)	0.808[b]
Previous abdominal surgery, n (%)	63 (47%)	12 (23%)	51 (61%)	<0.001[b]
Simultaneous colorectal surgery, n (%)	26 (19%)	8 (15%)	18 (22%)	0.263[b]
Previous liver resection, n (%)	6 (4%)	3 (6%)	3 (4%)	0.676[c]
Malignancy, n (%)	100 (74%)	32 (62%)	68 (82%)	0.010[b]
Lesion size, cm (IQR)	3 (1.8-5.5)	4 (2-5.9)	2.5 (1.9-5)	0.055[a]
Tumor location, n (%)				0.008[b]
- Anterior/left lateral segments (2,3,4b,5,6)	96 (71%)	45 (87%)	51 (61%)	
- Posterior/superior segments (4a,7,8,1)	34 (25%)	7 (14%)	27 (33%)	

IQR = interquartile range, [a] Independent Samples Test, [b] Chi-square test, [c] Fisher's exact test
Group A = before fellowship in laparoscopic HPB surgery, group B = after fellowship

Table 2 Procedure characteristics

	Overall (n = 135)	Group A (n=52)	Group B (n=83)	*P*
Extent of resection, n (%)				0.032[a]
- Minor	118 (87%)	49 (94%)	69 (83%)	
- Major	9 (13%)	0	9 (11%)	
- Technically major	8	3 (6%)	5 (6%)	
				0.158[b]
Type of resection, n (%)				
- Non-anatomic/metastasectomy	64 (47%)	23 (44%)	41 (49%)	
- Left lateral sectionectomy	27 (20%)	12 (23%)	15 (18%)	
- Segmentectomy	27 (20%)	14 (27%)	13 (16%)	
- Bisegmentectomy	8 (6%)	3 (6%)	5 (6%)	
- Right hepatectomy	5 (4%)	0	5 (6%)	
- Left hepatectomy	4 (3%)	0	4 (5%)	
Hand assistance, n (%)	4 (3%)	4 (8%)	0	0.020[b]
Additional wedge resection, n (%)	21 (16%)	2 (4%)	19 (23%)	0.003[a]
Approach, n (%)				0.402[b]
- One stage resection	121 (90%)	47 (90%)	74 (89%)	
- One stage resection + RFA	5 (4%)	3 (6%)	2 (2%)	
- Two stage resection without PVE	5 (4%)	2 (4%)	3 (4%)	
- Two stage resection with PVE	4 (3%)	0	4 (5%)	

RFA = radiofrequency ablation, PVE = portal vein embolization, [a] Chi-square test, [b] Fisher's exact test
Group A = before fellowship in laparoscopic HPB surgery, group B = after fellowship

Table 3 Perioperative outcomes

	Overall (n = 135)	Group A (n=52)	Group B (n=83)	*P*
Operation time, minutes (IQR)	154 (101-267)	12 (94-188)	215 (130-370)	0.001[a]
Intraoperative blood loss, ml (IQR)	250 (100-700)	375 (200-775)	200 (50-700)	0.048[a]
Blood transfusion, n (%)	7 (5%)	2 (4%)	5 (6%)	0.707[b]
Conversion, n (%)	5 (4%)	2 (4%)	3 (4%)	1[b]
Resection margins for malignancies, R0 resection (%)	93 /100 (93%)	28/32 (88%)	63/67 (94%)	0.131[b]
Postoperative stay, days (IQR)	4 (3-5)	5 (3-5)	4 (2-5)	0.058[a]
Postoperative complications, Clavien-Dindo ≥III, n (%)	18 (13%)	4 (8%)	14 (17%)	0.193[c]
Mortality, n (%)	1 (0.7%)	1 (2%)	0	0.385[b]

DISCUSSION

This single-center, retrospective study confirms the guideline recommendations that a stepwise introduction of LLS combined with specific surgical training and mentoring is a

valuable and safe strategy for centers starting with LLS. In this series, the stepwise introduction was evidenced by a significant increase of more complicated procedures and less favorable patient characteristics over time and was combined with structured education before implementing major LLS. Despite increasing complexity of the procedures, intra- and postoperative outcomes were not compromised.

During the 2015 Morioka [15] and 2017 Southampton EGMLLS guideline meetings on LLS, a stepwise approach combined with formal training in LLS was advised in order to decrease the impact of the learning curve in the early stages. Very few studies, however, report on the results of such an approach to setting up a LLS practice in starting centers. More frequently, authors report on the surgical learning curve in LLS, often displayed as the number of resections needed before optimal outcomes are reached.[20-22] The variables to assess the presence of a learning curve vary between studies. In some, the learning curve is clinically obvious with improving perioperative results such as operative time [21,23-29], intraoperative blood loss [21,24-28], conversion [21], postoperative stay [21,23,24] and morbidity [28] over time. In a study by Robinson et al. [30] in 37 patients, increasing complexity of LLS with stable perioperative results was defined as a learning curve, similar to the current study. Both improving outcomes and increasing complexity with stable outcomes are used to define the learning curve, even though the developments of improving results and increasing difficulty of procedures are distinctly different. Obviously, both are a result of growing experience, but they are not the same. This distinction when addressing the learning curve is relevant, since implementing a new technique in clinical practice should always be done in a safe way and without compromising patient outcomes. The concept and clinical relevance of this “proficiency curve”, defined by patient outcomes such as complications, hospital stay and mortality, as opposed to the “feasibility curve”, defined by intra-operative outcomes such as operative time, conversion rate and blood loss, have previously been described in laparoscopic distal pancreatectomy.[31]

In standardized resections like distal pancreatectomy, improving results over time can be expected. One might argue that our improving results are masked by the heterogeneity of LLS. Improving results in minor LLS could have been counterbalanced by the introduction of major LLS. A sensitivity analysis, however, showed that excluding major resections had no detectable impact on outcomes, although numbers were small.

Previous reports have described learning curves for LLS varying from 24 to 295 patients, clearly demonstrating the heterogeneity of these studies as to when the learning curve is completed. Despite the findings reported in the current series (93% R0 resection, 3.7% conversion, 13.3% complications and 0.7% mortality), the learning curve for major LLS (e.g. for laparoscopic hemi-hepatectomy) still might not have been overcome. This requires constant monitoring. The institutional experience of hemihepactectomies (n=9) is rather small. This was partly overcome by performing all major resections with two senior surgeons. Major LLS was only performed after a dedicated laparoscopic HPB fellowship, during which over

20 major LLS procedures had been performed. Previous studies have demonstrated that there are no differences in the rates of R1 margins between open and laparoscopic liver surgery.[5,32-34] The 93% R0 resection rate in this study is within the 82-100% range as previously described for LLS.[1] As stressed in the 2015 and 2017 guideline meetings, adequate training is crucial. In the AMC, major LLS was only introduced after one surgeon had completed a laparoscopic HPB fellowship and experience was obtained through minor LLS (n=48). Furthermore, one third of all procedures were performed by combinations of two surgeons to enhance the learning process. Besides the steps of implementation, all surgeons had significant experience in open and advanced laparoscopic gastrointestinal surgery and had followed at least two hands-on courses in minor LLS prior to starting with LLR. This level of experience and surgical training was considered an essential pillar before the stepwise introduction of LLS was even considered and has very likely contributed to the low conversion and complication rates from the beginning.

Consensus on how to design structured training and formal education programs is lacking and should be a focus for further research. In the current era of highly specialized and complex (laparoscopic) HPB surgery, a plea has been made to move away from 'see one, do one, teach one' [35], and progress is made, with several expert HPB units starting hands-on courses in LLS and specialized laparoscopic HPB fellowships.[36,37]

This series clearly has its shortcomings. The retrospective study design introduces significant risk of selection bias and that the series is relatively small. However, since this study reports on consecutive (selected) patients and the decision when to start with major resections was made prospectively, this series confirms the benefits of the stepwise introduction of LLS combined with structured training.

In conclusion, the current retrospective, single-center study supports the guideline recommendations of a stepwise introduction of LLS combined with structured surgical training. This approach can help to decrease the clinical impact of the learning curve and can be an appropriate method for technique implementation in starting centers and on a larger, nationwide scale. Future studies should focus on an effective design and structure for education and training programs for LLS.

References

(1) Nguyen KT, Gamblin TC, Geller DA. (2009) World review of laparoscopic liver resection - 2,804 patients. *Ann Surg* 250:831-41.

(2) Ciria R, Cherqui D, Geller DA, Briceno J, Wakabayashi G. (2016) Comparative Short-term Benefits of Laparoscopic Liver Resection: 9000 Cases and Climbing. *Ann Surg* 263:761-77.

(3) Abu Hilal M, McPhail MJ, Zeidan B, Zeidan S, Hallam MJ, Armstrong T *et al*. (2008) Laparoscopic versus open left lateral hepatic sectionectomy: A comparative study. *Eur J Surg Oncol* 34:1285-8.

(4) Wakabayashi G, Cherqui D, Geller DA, Han HS, Kaneko H, Buell JF. (2014) Laparoscopic hepatectomy is theoretically better than open hepatectomy: preparing for the 2nd International Consensus Conference on Laparoscopic Liver Resection. *J Hepatobiliary Pancreat Sci* 21:723-31.

(5) Abu Hilal M, Di Fabio F, Teng MJ, Lykoudis P, Primrose JN, Pearce NW. (2011) Single-centre comparative study of laparoscopic versus open right hepatectomy. *J Gastrointest Surg* 15:818-23.

(6) McPhail MJ, Scibelli T, Abdelaziz M, Titi A, Pearce NW, Abu Hilal M. (2009) Laparoscopic versus open left lateral hepatectomy. *Expert Rev Gastroenterol Hepatol* 3:345-51.

(7) Vibert E, Perniceni T, Levard H, Denet C, Shahri NK, Gayet B. (2006) Laparoscopic liver resection. *Br J Surg* 93:67-72.

(8) Aldrighetti L, Pulitanò C, Catena M, Arru M, Guzzetti E, Casati M *et al*. (2008) A prospective evaluation of laparoscopic versus open left lateral hepatic sectionectomy. *J Gastrointest Surg* 12:457-62.

(9) Kazaryan AM, Pavlik Marangos I, Rosseland AR, Røsok BI, Mala T, Villanger O *et al*. (2010) Laparoscopic liver resection for malignant and benign lesions: ten-year Norwegian single-center experience. *Arch Surg* 145:34-40.

(10) Cannon RM, Scoggins CR, Callender GG, Quillo A, McMasters KM, Martin RC 2nd. (2013) Financial comparison of laparoscopic versus open hepatic resection using deviation-based cost modeling. *Ann Surg Oncol* 20:2887-92.

(11) Abu Hilal M, Di Fabio F, Syed S, Wiltshire R, Dimovska E, Turner D *et al*. (2013) Assessment of the financial implications for LLS: a single-centre UK cost analysis for minor and major hepatectomy. *Surg Endosc* 27:2542-50.

(12) Polignano FM, Quyn AJ, de Figueiredo RS, Henderson NA, Kulli C, Tait IS. (2008) Laparoscopic versus open liver segmentectomy: prospective, case-matched, intention-to-treat analysis of clinical outcomes and cost effectiveness. *Surg Endosc* 22:2564-70.

(13) Fretland ÅA, Kazaryan AM, Bjørnbeth BA, Flatmark K, Andersen MH, Tønnessen TI *et al*. (2015) Open versus laparoscopic liver resection for colorectal liver metastases (the Oslo-CoMet Study): study protocol for a randomized controlled trial. *Trials* 16:73.

(14) Wong-Lun-Hing EM, van Dam RM, van Breukelen GJ, Tanis PJ, Ratti F, van Hillegersberg R *et al*.; ORANGE II Collaborative Group. (2017) Randomized clinical trial of open versus laparoscopic left lateral hepatic sectionectomy within an enhanced recovery after surgery programme (ORANGE II study). *Br J Surg* 104;525-535.

(15) Wakabayashi G, Cherqui D, Geller DA, Buell JF, Kaneko H, Han HS *et al*. (2015) Recommendations for laparoscopic liver resection: a report from the second international consensus conference held in Morioka. *Ann Surg* 261:619-29.

(16) Buell JF, Cherqui D, Geller DA, O'Rourke N, Iannitti D, Dagher I *et al*. (2009) The international position on LLS: The Louisville Statement, 2008. *Ann Surg* 250:825-30.

(17) Di Fabio F, Samim M, Di Gioia P, Godeseth R, Pearce NW, Abu Hilal M. (2014) Laparoscopic major hepatectomies: clinical outcomes and classification. *World J Surg* 38:3169-74.

(18) Dindo D, Demartines N, Clavien PA. (2004) Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 240:205-13.

(19) Cipriani F, Shelat VG, Rawashdeh M, Francone E, Aldrighetti L, Takhar A *et al.* (2015) Laparoscopic Parenchymal-Sparing Resections for Nonperipheral Liver Lesions, the Diamond Technique: Technical Aspects, Clinical Outcomes, and Oncologic Efficiency. *J Am Coll Surg* 221:265-72.

(20) Vigano L, Laurent A, Tayar C, Tomatis M, Ponti A, Cherqui D. (2009) The learning curve in laparoscopic liver resection: improved feasibility and reproducibility. *Ann Surg* 250:772-82.

(21) Nomi T, Fuks D, Kawaguchi Y, Mal F, Nakajima Y, Gayet B. (2015) Learning curve for laparoscopic major hepatectomy. *Br J Surg* 102:796-804.

(22) van der Poel MJ, Besselink MG, Cipriani F, Armstrong T, Takhar AS, van Dieren *S et al*. (2016) Outcome and Learning Curve in 159 Consecutive Patients Undergoing Total Laparoscopic Hemihepatectomy. *JAMA Surg* 151:923-928.

(23) AbuHilal M, Pearce NW. (2008) Laparoscopic left lateral sectionectomy: a safe, efficient, reproducible technique. *Dig Surg* 25:305-8.

(24) Cai X, Li Z, Zhang Y, Yu H, Liang X, Jin R *et al.* (2014) Laparoscopic liver resection and the learning curve: a 14-year, single-center experience. *Surg Endosc* 28:1334-41.

(25) Choi SH, Choi GH, Han DH, Choi JS. (2015) Laparoscopic liver resection using a rubber band retraction technique: usefulness and perioperative outcomes in 100 consecutive cases. *Surg Endosc* 29:387-97.

(26) Spampinato MG, Arvanitakis M, Puleo F, Mandala L, Quarta G, Baldazzi G. (2015) Assessing the learning curve for totally laparoscopic major-complex liver resections: A single hepatobiliary surgeon experience. *Surg Laparosc Endosc Percutan Tech* 25:45-50.

(27) Wang X, Li J, Wang H, Luo Y, Ji W, Duan W *et al*. (2013) Validation of the laparoscopically stapled approach as a standard technique for left lateral segment liver resection. *World J Surg* 37:806-11.

(29) Ratti F, Barkhatov L, Tomassini F, Cipriani F, Kazaryan AM, Edwin B *et al*. (2016) Learning curve of self-taught laparoscopic liver surgeons in left lateral sectionectomy: results from an international multi-institutional analysis on 245 cases. *Surg Endosc* 30:3618-29.

(30) Robinson SM, Hui KY, Amer A, Manas DM, White SA. (2012) Laparoscopic liver resection: is there a learning curve? *Dig Surg* 29:62-9.

(31) de Rooij T, Cipriani F, Rawashdeh M, van Dieren S, Barbaro S, Abuawwad M et al. (2017) Single-surgeon learning curve in 111 laparoscopic distal pancreatectomies: does operative time tell the whole story? J Am Coll Surg 224:826-832.

(32) Medbery RL, Chadid TS, Sweeney JF, Knechtle SJ, Kooby DA, Maithel SK *et al.* (2014) Laparoscopic vs open right hepatectomy: a value-based analysis. *J Am Coll Surg* 218:929-39.

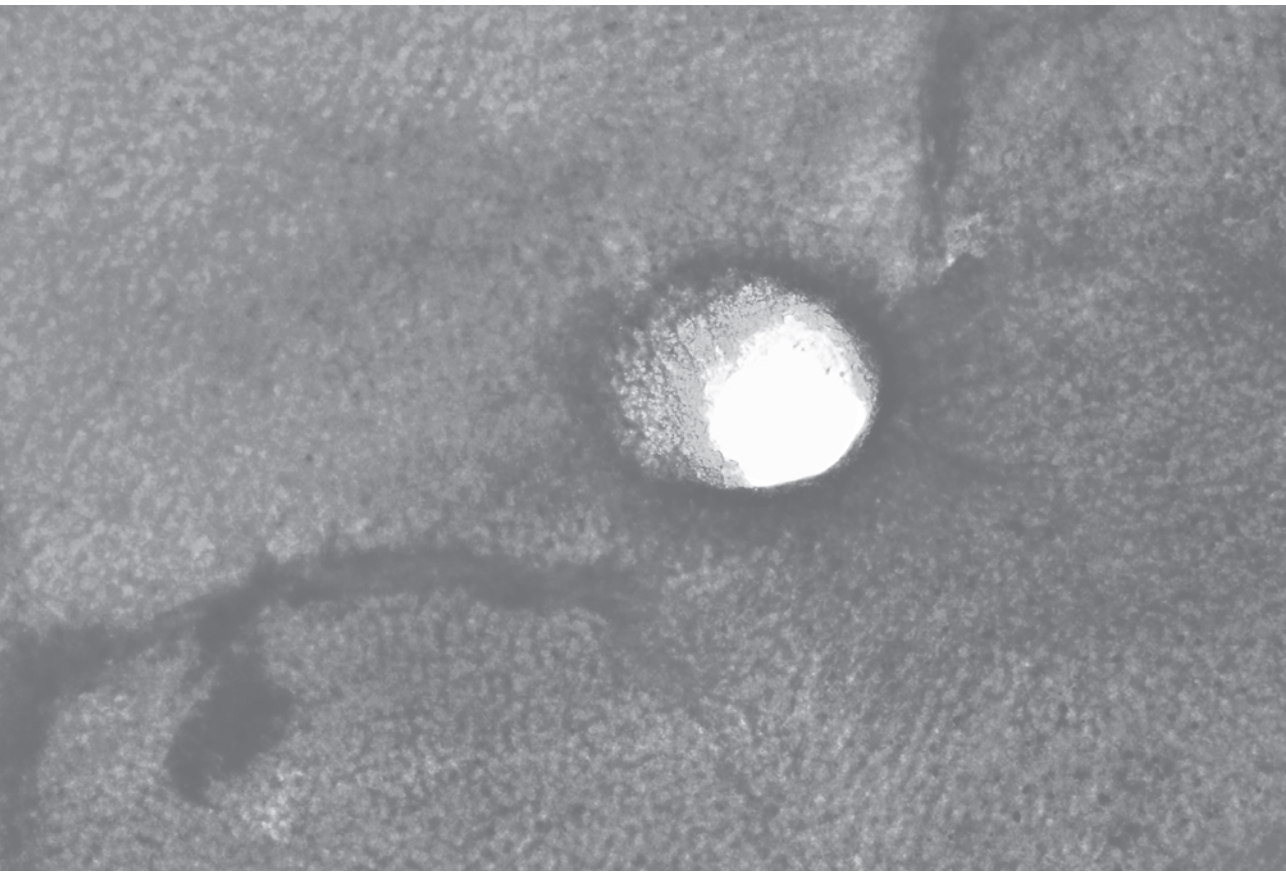
(33) Topal H, Tiek J, Aerts R, Topal B. (2012) Outcome of laparoscopic major liver resection for colorectal metastases. *Surg Endosc* 26:2451-5.

(34) Martin RC, Scoggins CR, McMasters KM. (2010) Laparoscopic hepatic lobectomy: advantages of a minimally invasive approach. *J Am Coll Surg* 210:627-36.

(35) Hogg ME, Besselink MG, Clavien PA, Fingerhut A, Jeyarajah DR, Kooby DA et al. (2017) Training in minimally invasive pancreatic resections: a paradigm shift away from “See one, Do one, Teach one”. HPB 19:234-245.

(36) Gumbs AA, Gayet B. (2007) The Medical and Surgical Department of Digestive Diseases and Minimally Invasive HPB Fellowship at IMM. *HPB (Oxford)* 9:470-1.

(37) Subhas G, Mittal VK. (2011) Training minimal invasive approaches in hepatopancreatobilliary fellowship: the current status. *HPB (Oxford)* 13:149-52.



CHAPTER 8

Evaluation of cardiac index, fluid retention, and the RAAS system during and after major hepatic resection

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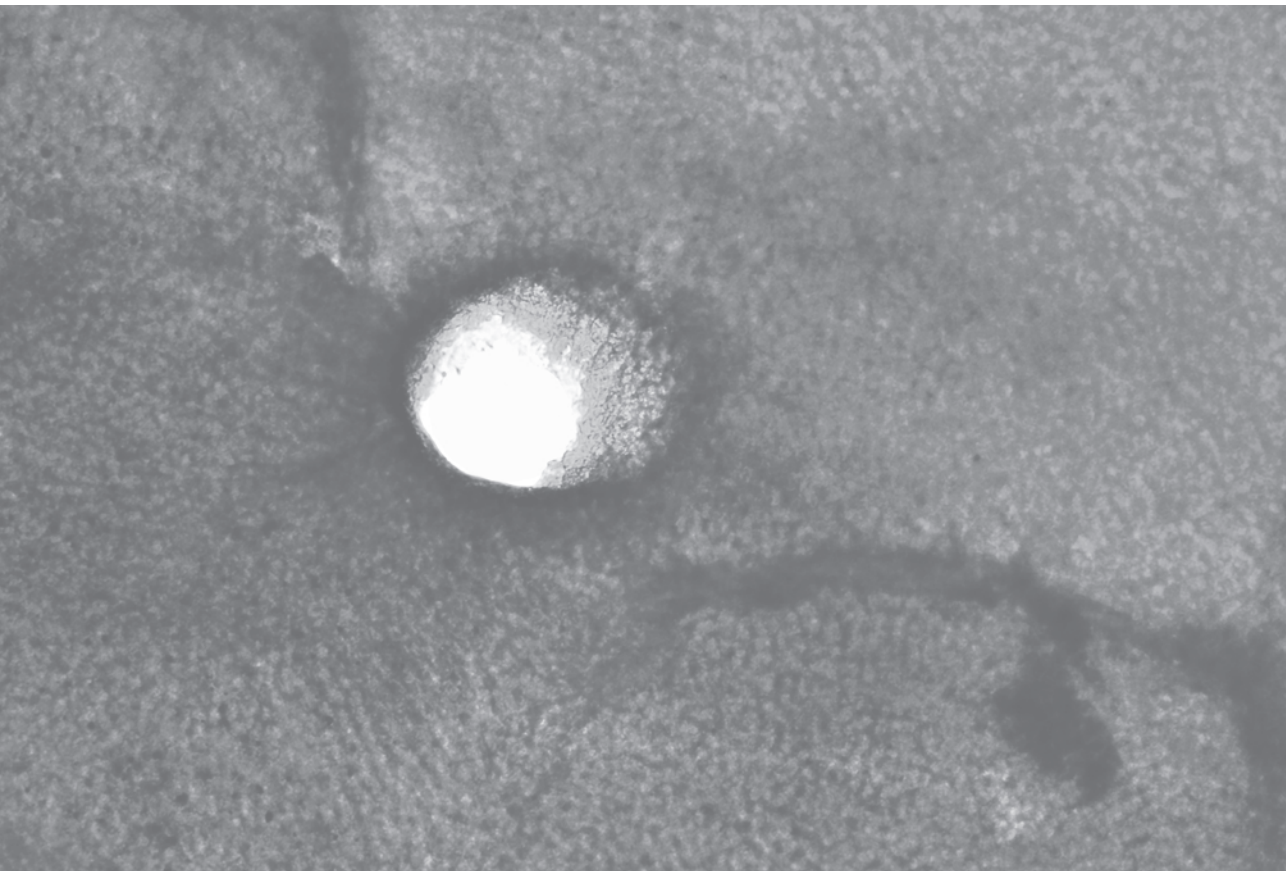


Submitted

ABSTRACT

Background:

Major liver resection is regarded as one of the highest risk surgeries. It is known that fluid overload leads to complications. We assessed renin–angiotensin–aldosterone system (RAAS) activity after liver surgery, and the correlation between RAAS activity and fluid hemostasis postoperatively.

Methods:

Between October 2013 and April 2016 seventeen patients underwent a major liver resection. Included patients were classified as ASA I or II and had no renal and/or cardiac failure. Inclusion took place one day preoperatively and follow-up was continued until five days postoperatively. Fluid overload was represented as. weight gain. Preoperatively we measured baseline weight and ADH, aldosterone and renin activity levels. These measurements were continued postoperatively, i.e. daily weight and hormone levels.

Results:

Increases of aldosterone, ADH and renin were seen in the first 2 postoperative days. Aldosterone was positively correlated with weight gain ($p = 0.007$ with a 95% confidence interval of 0.597-3.543).

Conclusion

The RAAS system is stimulated during liver surgery, which leads to elevated concentrations of these hormones in especially the first 2 postoperative days. Elevated levels of aldosterone are associated with weight gain due to fluid overload during the first five postoperative days.

INTRODUCTION

The only curative treatment for patients with primary or secondary liver malignancies is surgery. Although postoperative outcomes after major liver surgery have improved over the past years owing to better surgical techniques and postoperative care, liver surgery is still regarded as high risk surgery. Postoperative complications often occur. [1-4] It is known that fluid overload during and after the operation can play a significant role in the etiology of complications like cardiopulmonary and renal events, decreased wound healing, decreased bowel motility and an increase in length of hospital stay. [5, 6]

Fluid administration seems to be a logical step in the management of decreased diuresis, hypotension and/or tachycardia and also in the maintenance of sufficient oxygen delivery. [7, 8] However, the attempts to control haemodynamic instability often lead to fluid overload. The exact etiology of haemodynamic instability in these patients is often unclear. Proposed mechanisms are absolute or relative hypovolemia, vascular leakage and activation of the renal regulatory hormones for reabsorption, or cardiac dysfunction. However, the levels of physiological, compensatory mechanisms may differ between patients while a direct relation between RAAS activation and postoperative weight gain may exist. [9, 10] We hypothesized that the renin–angiotensin–aldosterone system (RAAS) is activated during liver resection leading to higher fluid responsiveness and retention of fluids. [11, 12] The aim of this study was to evaluate if 1) the RAAS is activated after liver surgery, and if 2) a correlation exists between activation of RAAS, intraoperative fluid responsiveness (change in cardiac index ≥ 10%) and fluid overload (weight gain) postoperatively.

METHODS

Study design and patients inclusion

This study was designed as a prospective, single-centre pilot study including patients undergoing major liver resection (≥ 3 liver segments). Data was collected during the perioperative period of patients between July 2013 and April 2016. The study was approved by the Institutional Review Board of the Academic Medical Center in Amsterdam (METC 2013_028; NL43476.018.13; Dutch trial register ID NTR4020). Informed consent was obtained from all patients prior to the start of the study.

Study population

A total of 29 patients were included in the study. Inclusion criteria were patients between the age of 18 and 86, who underwent a major liver resection with ASA (American Society of Anaesthesiologists classification) I or II. Exclusion criteria were renal failure (Estimated GFR < 30 ml/min using the Modification of Diet in Renal Disease formula MDRD), cardiac

failure (LVEF <30%), cardiac arrhythmias, liver cirrhosis, admission on intensive care unit postoperatively and severe sepsis.

Seventeen of 29 patients were eventually included in the analysis. Eight patients did not meet the inclusion criteria because of unresectability due to progression of the disease or vascular infiltration into the portal vein. One patient died due to sepsis after the operation. Three patients did not want to continue with the study after the operation.

Hemodynamic monitoring, standard care

Standard assessment of the hemodynamic parameters of all patients who are scheduled for major liver surgery includes measurement of blood pressure, heart rate and arterial oxygen saturation. The day before the operation, a baseline measurement of these parameters was performed. During the operation additional monitoring of central venous pressure (CVP) and continuous invasive arterial blood pressure was carried out.

Hemodynamic monitoring, non-invasive measurements using Nexfin Additional Indicators of the hemodynamic state were stroke volume, cardiac output and cardiac index. In this study Nexfin® (Edwards Lifesciences, USA (CA)) was used for measurements of the cardiac index during the intra- and postoperative period. The Nexfin is a device which forms a continuous, non-invasive finger blood pressure. Blood pressure and cardiac output measurements using Nexfin have been validated, and the device allows continuous monitoring of parameters that reflect the cardiovascular state of the patient. [13-15]

Study endpoints

Primary outcome measures of the study were weight in kilograms, intraoperative measurement of change in cardiac index after bolus of fluid and the serum levels of RAAS hormones pre- and postoperatively.

Secondary outcome parameters were complications after surgery, fluid balance during surgery and Mean Arterial Pressure (MAP), Heart Rate (HR), saturation and production of urine during the first 5 days after surgery.

Pre-operative management

Plasma samples for aldosterone, antidiuretic hormone (ADH) and renin were taken at the admission day as a baseline measurement. Aldosterone was collected via venous puncture in a serum tube. Analysis took place via Semi-Automated Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) using a 2D Xevo LC-MSMS (Waters) device. Plasma renin activity was collected in a chilled EDTA (EthyleneDiamineTetra-Acetic acid) tube and measured by hand using a radio-immunoassay. A special EDTA tube with 140 microliter aprotinin was used for the examination of serum ADH concentrations. The tube was placed directly in ice after the blood drawing and analyzed by hand using radio-immunoassay after extraction.

In addition, baseline measurements of cardiac index using Nexfin were performed one day before the operation.

Perioperative management

During surgery, patients received standard anaesthesiological treatment according to the protocol used in our center. After induction patients routinely received a central venous catheter to measure the central venous pressure (CVP), radial arterial cannulation and a urethral catheter. CVP, mean arterial pressure (MAP), end-tidal carbon dioxide, heart rate, oxygen saturation and urine output were monitored throughout the operation.

Nexfin was connected to the finger of the patient to monitor the cardiac index. Every hour, the patients received a fluid challenge of 250 ml colloids (tetraspan®), after which the change in cardiac index was monitored. A CI change of ≥ 10% was defined as a positive response to the fluid challenge. Patients were defined as fluid responsive in case of a response with a CI change of ≥ 10% in ≥ 50% of the fluid challenges. Additional fluids and vasopressors were given according to the attending anesthesiologists' insight. In general, MAP was kept above 65 mmHg, blood loss was compensated with colloids in a 1:1 ratio. Other fluid losses were compensated with cristalloids (Sterofundin®). Fluid balance was recorded during the operation.

Postoperative management

According to our standard postoperative protocol, all patients were kept at the recovery unit for overnight observation. Fluid and haemodynamic management were carried out according to the attending anaesthesiologist's judgement.

During the first 5 postoperative days (starting from the recovery unit) patients' haemodynamics were measured using the conventional method as well as with Nexfin. The conventional method included measurement of the Mean Arterial Pressure (MAP), Heart Rate (HR), saturation and production of urine. Measurements took place at two different time points during the day. The first measurement took place in the morning (11 a.m.). The second measurement was performed in the afternoon (15.00 p.m.).

On the first five days postoperatively, we collected plasma samples every morning. We assessed aldosterone, antidiuretic hormone and renin.

Finally, we measured daily the body weight. The gain of weight was used to estimate the degree of fluid overload.

Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS 22.0, IBM Inc., Armonk (NY) USA). Distribution was assed using Shapiro-Wilk test. Normally distributed continuous data are expressed as mean ± standard deviation (SD), while non-normally distributed data are presented as median along with interquartile range (IQR). The levels

of RAAS hormones were compared with one sample T tests. In order to explore if RAAS hormones are correlated with weight gain after the operation, mixed model analysis was performed. In case of missing data analysis was performed with discrete missing data as imputation of data is not advisable because of the low number of patients included in the study. A chi-square tests was used to explore the relationship between fluid responsiveness and posteropative weight gain.

RESULTS

Most patients were men (59%) and the median age was 67 years (IQR 61.5-73). Other baseline characteristics are shown in **Table 1**. Preoperatively, median CI was 2.5 L/min^{-1}/m^2 (IQR 2.2-3.0). The mean arterial pressure (MAP) was 92.3 mmHg (SD 10.3). The baseline

Table 1 Baseline characteristics of the 17 patients included in the analysis

Gender, male, *n (%)*	10 (59)
Age in years, median [IQR]	67 [61.5-73]
Height (cm), median [IQR]	170 [165-181]
Weight (kg), median [IQR]	73 [62-80]
ASA classification, *n (%)*	
ASA 1	4 (24)
ASA 2	13 (76)
Diagnosis, *n (%)*	
CRLM	9 (53)
HCC	1 (6)
PHC	5 (29)
Benign lesion	2 (12)
Type of resection, *n (%)*	
Right hemihepatectomy	9 (52.9)
Extended right hemihepatectomy	3 (17.6)
Left hemihepatectomy	4 (23.5)
Extended left hemihepatectomy	1 (5.9)
Duration of hospitalization, days, median (IQR)	8 (7-20.5)
Blood	
ADH, pmol/L, median (IQR)	1 (1-1)
Aldosterone, nmol/L, median (IQR)	0.14 (0.09-0.19)
Renin, microgA1/L/U, median (IQR)	1.2 (0.3-3)

ASA, ASA-classification, physical status classification according to American Society of Anaesthesiologists; CRLM, colorectal liver metastases; HCC, Hepatocellular carcinoma; PHC, Perihilar cholangiocarcinoma; IQR, interquartile range; ADH, antidiuretic hormone.

mean ADH concentration was 1.313 (SD 1.283) picomol/liter. Aldosterone concentration was 0.626 (SD 1.808) nanomol/liter. The plasma renin activity at baseline was 7.01 (SD 1.283) microgA1/L/U.

Intraoperative phase

The haemodynamic changes due to the hourly fluid challenges are reported in Table 2. The amount of challenges differs among patients in line with the duration of the procedure. The mean operation time was 416 min (SD 130) while the median number of fluid challenges was 5 (IQR 5-7). Changes that were observed in cardiac index as an effect of the fluid challenges are also illustrated in **Table 2**. The mean pecentage changes in CI after each challenge were 8.3% (SD 3.3), 2.1% (SD 11.4), 6.3% (SD 9.2), 7.7% (SD 8.3), 21.0% (SD 21.6), 26.7% (SD 17.9), 1.43% (SD 13.3) and 4,1% (SD 1,7), respectively. Five of the 17 patients responded with a CI change of ≥ 10% in ≥ 50%. There was no significant difference in weight gain postoperatively between fluid responders and non-responders (p=0.41).

Appendix Table 1 shows the individual cardiac index changes after the perioperative fluid challenges and fluid balance intra-operatively.

Postoperative phase

Postoperatively, the mean weight was significantly increased (8.9 % (SD 3.3)) in comparison to the baseline measurement (p=0.000) and still remained above the baseline measurement at day 5. (**Table 1, appendix**). An increase in aldosterone, ADH and renine was seen in the postoperative days (**Table 4 and figure 1**). ADH concentration was siginificantly increased during the 5 postoperative days compared to baseline measurements except for day 3: p=0.000, p=0.004, p=0.0099, p=0.004 and p=0.007, respectively. Plasma renine concentration was significantly different compared to the baseline measurement during day 1-4 postoperatively; p=0.005, p=0.001, p=0.000, p=0.0042 and p= 0.142, respectively. The concentration of aldosterone was also significantly different during the first 4 postoperative days compared to baseline measurements; p=0.001, p=0.003, p=0.001, p=0.011 and p=0.053, respectively.

Aldosterone was positively correlated with weight gain (p = 0.007 with a 95% confidence interval of 0.597-3.543). There was no significant correlation between ADH and renin and increase in weight; p=0.551 and p=0.188, respectively.

DISCUSSION

This study shows that postoperatively elevated levels of aldosterone are associated with weight gain due to fluid overload during the first five postoperative days. Fluid overload after liver resection is frequently seen despite the recent developments and experiences in peri-operative management. In general, intra-operative fluid management is advocated to

Table 2 Effect of the hourly fluid challenges of 250mL during the operation on hemodynamic parameters

	Fluid challenge administered during the operation																
Parameter	BI	BFC1 *n=13*	AFC1 *n=13*	BFC2 *n=13*	AFC2 *n=13*	BFC3 *n=13*	AFC3 *n=13*	BFC4 *n=14*	AFC4 *n=14*	BFC5 *n=11*	AFC5 *n=11*	BFC6 *n=5*	AFC6 *n=5*	BFC7 *n=4*	AFC7 *n=4*	BFC8 *n=2*	AFC8 *n=2*
CI $L/min^{-1}/m^2$	2.6 (0.6)	2.0 (0.4)	2.1 (0.5)	2.6 (0.4)	2.6 (0.4)	2.4 (0.4)	2.5 (0.4)	2.5 (0.5)	2.7 (0.5)	2.2 (0.6)	2.6 (0.5)	2.5 (0.5)	3.1 (0.5)	2.6 (0.5)	2.6 (0.3)	2.7 (1.1)	2.8 (1.1)
MAP mmHg	92.3 (10.3)	-	74 (9.4)	74.9 (8.7)	83.7 (11.6)	68.4 (12.0)	79.6 (10.8)	70.9 (11.2)	86.5 (29.7)	62.9 (13.1)	78.5 (14.6)	67.0 (9.3)	79.7 (13.4)	64.0 (8.9)	70.6 (13.3)	63.5 (3.5)	64.5 (7.8)

BI, before induction; BFC, before fluid challenge; AFC, after fluid challenge; CI, cardiac index; MAP, mean arterial pressure; CVP, central venous pressure;
All values concern mean values with SD (Standard deviation).
Green cells indicate a CI change of < 10% after fluid challenge, which is defined as a stable state. Red coloured cells indicate a change in CI of ≥ 10%, which means that the patients was in an hypovoluemtic state.

Table 3 Hemodynamic parameters observed during the first 5 postoperative days

	Baseline	High care	Day 1	Day 2	Day 3	Day 4	Day 5
Weight, kg, mean (SD)	75.0 (15.1), n=17		-	80.8 (12.9), n=11	87.7 (11.2), n=9	83.2 (11.4), n=7	80.4 (16.2), n=11
Nexfin parameters							
MAP, mm Hg	92.3 (10.3), n=17	85.1 (13.5) n=11	72.3 (17.3), n=15	71.3 (11.6), n=10	82.5 (12.8), n=15	83.0 (13.8), n=6	86.2 (19.5), n=7
CI L/min^{-1}/m^2	2.6 (0.6), n=17	3.0 (0.8), n=9	3.1 (0.8), n=13	3.5 (1.0), n=9	3.1 (0.7), n=13	3.3 (0.8), n=11	3.1 (1.0), n=13

MAP, mean arterial pressure; CI, cardiac index;.

Table 4 Blood parameters at baseline and during the first 5 postoperative days and urine parameters at baseline and after the operation

	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5
Serum						
Aldosterone, nmol/L, mean (SD)	0.63 (1.81)	0.55 (0.53)	0.29 (0.31)	0.31 (0.20)	0.17 (0.21)	0.31 (0.40)
Anti duretic hormone, pmol/L, mean (SD)	1.31 (1.28)	13.41 (9.32)	6.28 (6.75)	6.14 (3.67)	8.36 (6.81)	2.48 (1.24)
Renin, microgA1/L/U, mean (SD)	7.01 (19.88)	8.36 (10.3)	6.12 (5.13)	3.30 (2.28)	2.34 (3.48)	5.05 (6.90)

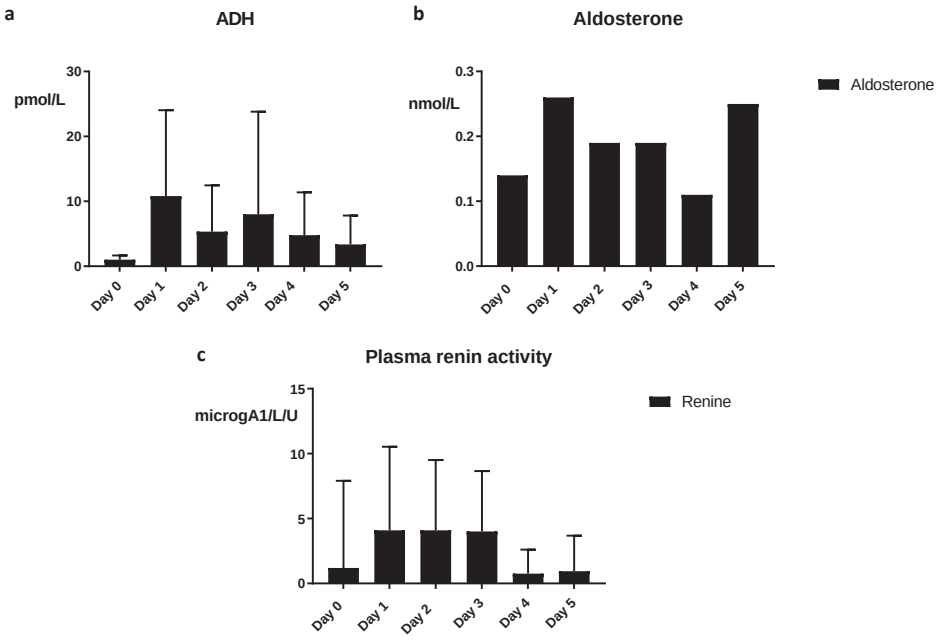


Figure 1. Plasma concentrations of ADH, aldosterone and renin activity at baseline and during the 5 postoperative days.

be restrictive which is associated with a lower incidence of complications. [5] Less evidence is available for fluid management during the early postoperative phase. Differentiation of the underlying cause of postoperative symptoms, such as tachycardia, hypotension and decreased diuresis, is of great importance as it determines the choice of treatment. While on one hand fluid administration should be the treatment of choice in case of hypovolemia, it might lead to worsening of the symptoms in case of other mechanisms behind these symptoms, such as cardiac failure.

RAAS is a complex system that regulates fluid homeostasis. The RAAS plays a role in the homeostatic control of the arterial pressure, tissue perfusion, and extracellular volume.[16] This study shows a significant increase of aldosterone, ADH and renin after major liver resection. This effect was most pronounced on the first two postoperative days in which all three hormones were elevated in comparison to baseline. Furthermore, a significant correlation was seen between the increase in aldosterone and weight gain after liver resection. This observation suggests that RAAS is activated during the operative phase, probably contributing to the fluid overload. During the operation, systemic hemodynamics change. Liver resections are specifically associated with fluid overload postoperatively. One mechanism for fluid overload may be related to changes in the portal venous pressure during the operation. Increased portal vein pressure is associated with in an increase in the mesenteric blood flow and subsequent activation of the systemic vasoconstrictor system including RAAS and vasopressin.[17] Possibly this sequence of events is also activated during liver resections. Interventions with angiotensin-converting enzyme (ACE) or potassium sparing drugs might be helpful to prevent weight gain postoperatively and could lead to a shorter hospital stay with less complications.

A major limitation of this study is the small number of patients included in this cohort. However, the study was performed in a prospective manner allowing measurements of the included parameters at the same moments during the postoperative course which is a great advantage of this study in comparison to the available retrospective literature. Another limitation of this study is the missing clinical parameters. The condition of the patients during the early postoperative phase did not always allow measurement of the abdominal and the ankle girth. For this reason, we did not include this data in our analysis. Although, we cannot be sure that these missing parameters were of no importance for the results, this loss has been compensated by the remaining clinical parameters.

Because of the low number of participants and the lack of a control group, we cannot draw conclusions about predictors of fluid overload. Cardiovascular diseases or kidney diseases might play a role in this pathophysiology. In the future, a randomized controlled trial should be performed in which two groups of patients are compared; one group with major liver resections and one group with major abdominal resections without manipulation of the portal vein. We hypothesize that RAAS will not be stimulated in the absence of manipulation of the portal vein.

In conclusion, the RAAS system is stimulated during major liver surgery, which leads to elevated concentrations of these hormones in especially the first 2 postoperative days. Elevated levels of aldosterone are associated with weight gain due to fluid overload during the first five postoperative days.

References

(1) Cummings LC, Payes JD, Cooper GS. Survival after hepatic resection in metastatic colorectal cancer: a population-based study. Cancer. 2007;109(4):718-26.

(2) Jarnagin WR, Gonen M, Fong Y, DeMatteo RP, Ben-Porat L, Little S, et al. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. Ann Surg. 2002;236(4):397-406; discussion -7.

(3) Melendez JA, Arslan V, Fischer ME, Wuest D, Jarnagin WR, Fong Y, et al. Perioperative outcomes of major hepatic resections under low central venous pressure anesthesia: blood loss, blood transfusion, and the risk of postoperative renal dysfunction. J Am Coll Surg. 1998;187(6):620-5.

(4) Thasler WE, Bein T, Jauch KW. Perioperative effects of hepatic resection surgery on hemodynamics, pulmonary fluid balance, and indocyanine green clearance. Langenbecks Arch Surg. 2002;387(7-8): 271-5.

(5) Chappell D, Jacob M, Hofmann-Kiefer K, Conzen P, Rehm M. A rational approach to perioperative fluid management. Anesthesiology. 2008;109(4):723-40.

(6) Slinger PD. Perioperative fluid management for thoracic surgery: the puzzle of postpneumonectomy pulmonary edema. J Cardiothorac Vasc Anesth. 1995;9(4):442-51.

(7) Perel A. Automated assessment of fluid responsiveness in mechanically ventilated patients. Anesth Analg. 2008;106(4):1031-3.

(8) Holte K, Sharrock NE, Kehlet H. Pathophysiology and clinical implications of perioperative fluid excess. Br J Anaesth. 2002;89(4):622-32.

(9) Gupta R, Gan TJ. Peri-operative fluid management to enhance recovery. Anaesthesia. 2016;71 Suppl 1:40-5.

(10) Brandstrup B, Tonnesen H, Beier-Holgersen R, Hjortso E, Ording H, Lindorff-Larsen K, et al. Effects of intravenous fluid restriction on postoperative complications: comparison of two perioperative fluid regimens: a randomized assessor-blinded multicenter trial. Ann Surg. 2003;238(5):641-8.

(11) Atlas SA. The renin-angiotensin aldosterone system: pathophysiological role and pharmacologic inhibition. J Manag Care Pharm. 2007;13(8 Suppl B):9-20.

(12) Braith RW, Mills RM, Wilcox CS, Davis GL, Hill JA, Wood CE. High-dose angiotensin-converting enzyme inhibition restores body fluid homeostasis in heart-transplant recipients. J Am Coll Cardiol. 2003; 41(3):426-32.

(13) Hofhuizen C, Lansdorp B, van der Hoeven JG, Scheffer GJ, Lemson J. Validation of noninvasive pulse contour cardiac output using finger arterial pressure in cardiac surgery patients requiring fluid therapy. J Crit Care. 2014;29(1):161-5.

(14) Chen G, Meng L, Alexander B, Tran NP, Kain ZN, Cannesson M. Comparison of noninvasive cardiac output measurements using the Nexfin monitoring device and the esophageal Doppler. J Clin Anesth. 2012;24(4):275-83.

(15) Ameloot K, Van De Vijver K, Broch O, Van Regenmortel N, De Laet I, Schoonheydt K, et al. Nexfin noninvasive continuous hemodynamic monitoring: validation against continuous pulse contour and intermittent transpulmonary thermodilution derived cardiac output in critically ill patients. Scientific-WorldJournal. 2013;2013:519080.

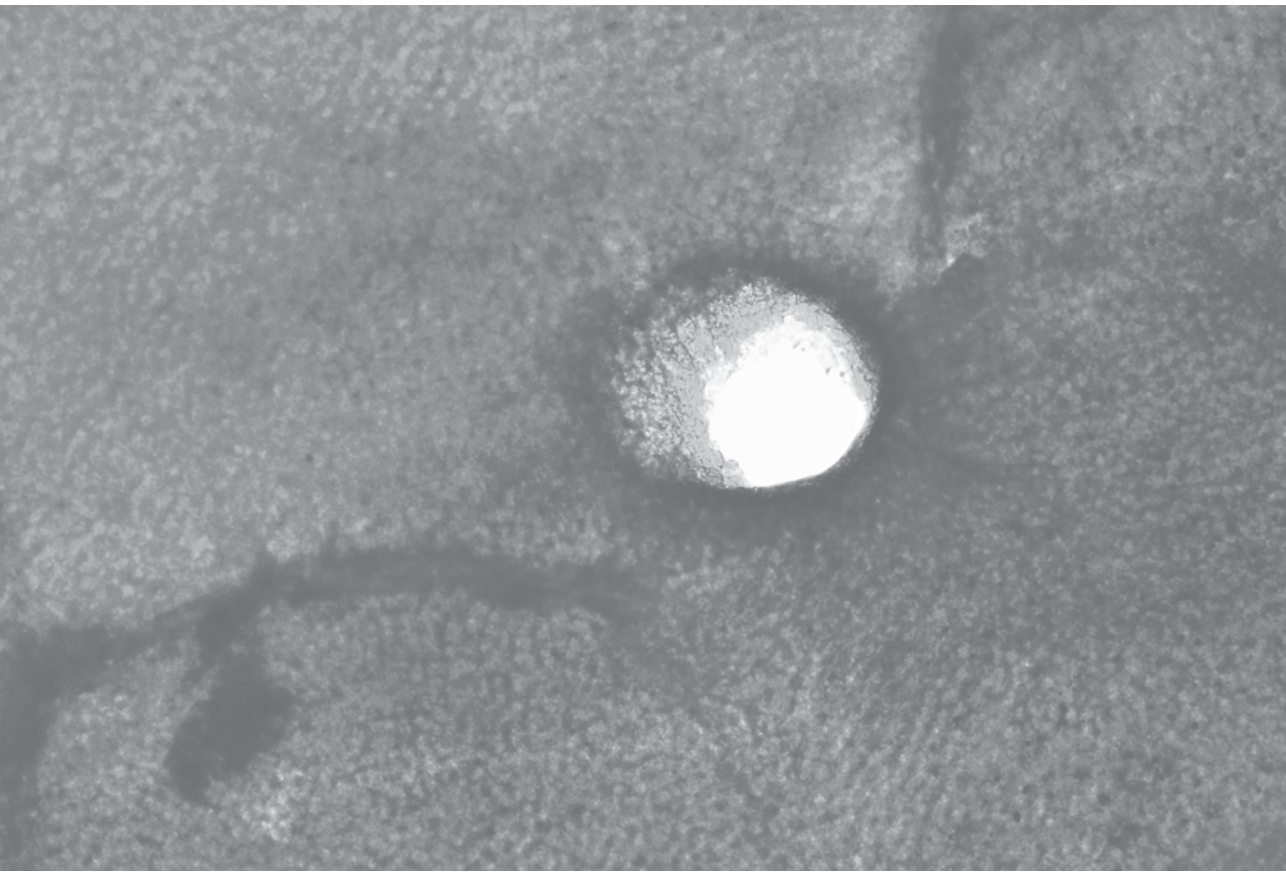
(16) Tox U, Steffen HM. Impact of inhibitors of the Renin-Angiotensin-aldosterone system on liver fibrosis and portal hypertension. Curr Med Chem. 2006;13(30):3649-61.

(17) Sola E, Gines P. Challenges and Management of Liver Cirrhosis: Pathophysiology of Renal Dysfunction in Cirrhosis. Dig Dis. 2015;33(4):534-8.

Appendix table 1 Increase in percent body weight postoperative, bloodloss during surgery and percentual increase in cardiac index during fluid challenges peroperatively

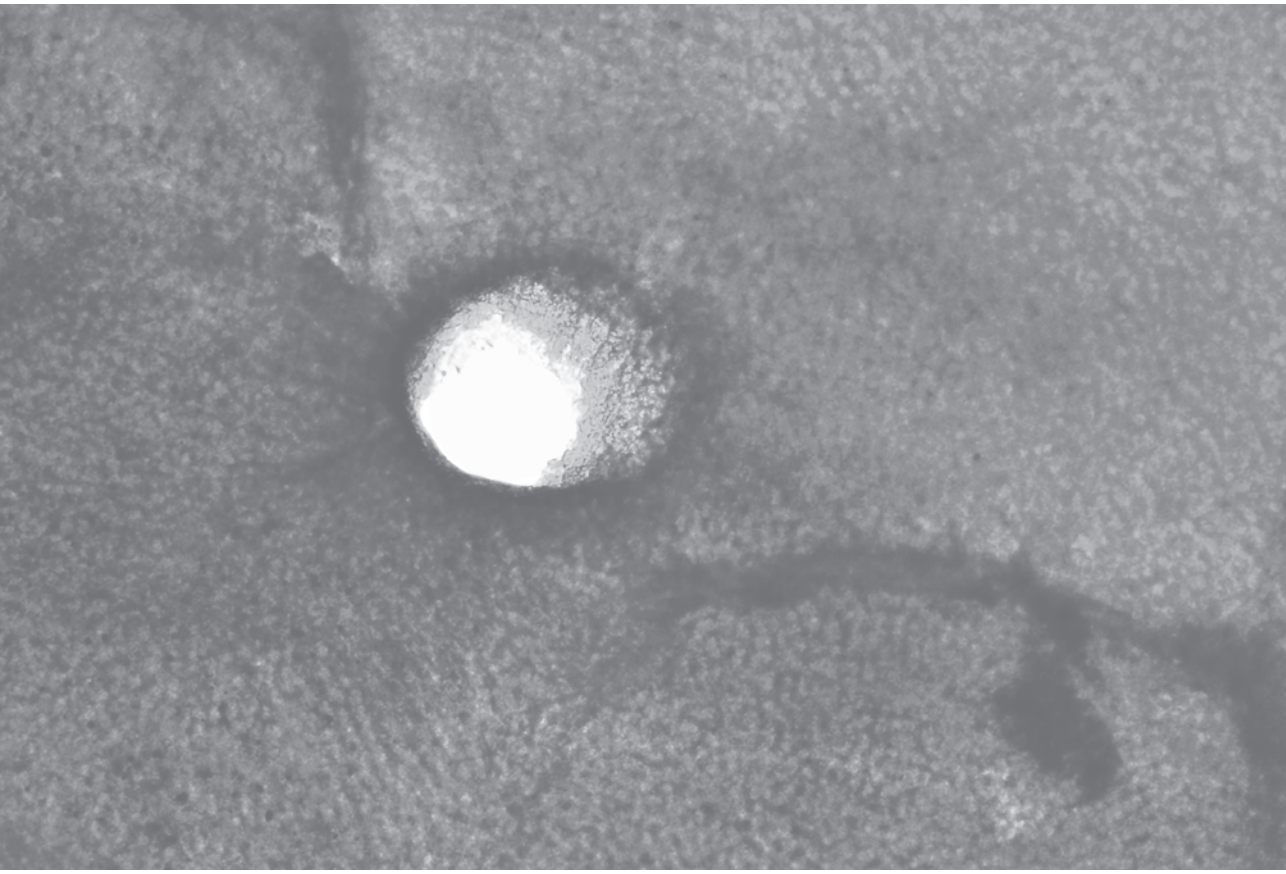
Patient	Percent weight gain	Fluid balance peri-operative (ml)	Percent increase CI FC 1	Percent increase CI FC2	Percent increase CI FC3	Percent increase CI FC4	Percent increase CI FC5	Percent increase CI FC6	Percent increase CI FC7
1	6.7	1461	6.25	12.00	-3.85	18.18	12.50		
2	12.9	-136	18.75	10.00	5.00	5.26	35.71		
3	8.6	13	-5.26	13.64	10.00	4.55			
4	4.5	Missing	13.00	-8.10	20.00	0.00	-5.60	17.60	5.00
5	11.6	93	10.00	-14.00	-17.00	0.00	9.00	8.00	
6	6.2	1343	25.00	18.18	3.23	16.67	63.64		
7	9.1	-725	-5.88	-4.55	15.00	8.70	50.00		
8	11.1	1688	0.00	0.00	0.00	19.35	7.70		
9	10.3	167	19.05	-13.33	0.00	3.70	16.00		
10	6.0	-900	3.57	7.41	0.00	0.00	3.57		
11	4.9	687	-4.55	Missing	3.70	0.00	12.50		
12	8.6	2327	Missing	-14.71	22.22	-4.00	-6.45		
13	4.9	284	14.30	13.64	23.81	8.00	31.25		
14	14.0	2273	0.00	-7.41	Missing	22.22			
15	8.5	560	15.00	-11.54	8.00	0.00			
16	10.7	1142	Missing	Missing	Missing	Missing			
17	14.1	1240	5.26	4.76	-4.76	4.55	4.35		

Red means an increase of the cardiac index of 10% or more
CI = cardiac index, FC = hourly fluid challenge during surgery



SUMMARY

Summary and future perspectives
Nederlandse samenvatting



SUMMARY

A great deal of research has been done to enhance the outcome of liver surgery, both clinical as translational. The unique capacity of the liver to regenerate has been a topic of interest for many of researchers. The exact mechanisms still remain unclear. In the field of liver surgery, a future remnant liver volume of at least 25% of the total liver volume is necessary to prevent postoperative liver failure. Besides enough liver volume, the functional capacity of the future remnant liver is also of great importance. 99mTc-mebrofenin hepatobiliary scintigraphy (HBS) is a quantitative liver function test, which measures the uptake function of the hepatocytes using a radioactive agent. A well-established method to enhance both liver volume and liver function preoperatively, is portal vein embolization (PVE). With PVE, a compensatory regeneration response of the non-embolized liver lobe is induced, resulting in increased volume and function of the future remnant liver, which contributes to performing safer resection. This thesis explores several conditions of preoperative liver augmenting procedures along with aspects of perioperative care in patients undergoing major liver resection in order to improve postoperative outcomes in terms of postoperative morbidity and mortality.

The mechanisms behind hepatic regeneration after PVE are still not fully understood. Animal models are used to mimic the clinical setting. In **chapter 1**, an overview is given of all available animal models to investigate PVE in relation to the species-specific anatomy and the induced hypertrophy response. All experimental studies on PVE or portal vein ligation (PVL) that used at least 5 animals were reviewed. The choice of the model depends on the specific aim of the study. Larger animal models such as the pig or rabbit are useful for the evaluation of the increase of liver volume and function after PVE, because of the possibility of applying clinical imaging techniques such as computed tomography (CT) volumetry in these animals. The rat model is useful to evaluate mechanisms of regeneration after PVE, because of the variety of antibodies commercially available for use in rats.

In **chapter 2**, a PVE rabbit model was used to investigate an absorbable embolization material, to prevent complications that can occur due to the use of permanent embolization materials. Absorbable embolization materials are also of interest in living liver donation, in case the intended graft is too small for size. In this way the hypertrophy response is induced without endangering the donor liver. In this study we have found that in the PVE rabbit model, fibrin glue with aprotinin resulted in an adequate hypertrophy response and in reversible occlusion of the embolized liver lobe in 80 per cent of the animals. Further clinical evaluation of fibrin glue-based embolization material is recommended, especially in the field of living liver donation.

The search for pharmacological interventions to promote liver regeneration is an area of great interest. Bile acids have been identified to play an important role in the early phase of liver regeneration by activating bile salt-activated transcription factor farnesoid X-receptor

(FXR). FXR-agonist obeticholic acid (OCA) is a potent bile salt agonist. In **chapter 3**, we used the PVE rabbit model to demonstrate that OCA stimulates liver regeneration after PVE with a sufficient increase of future remnant liver volume and function. Histology showed no signs of hepatocellular injury. This suggests that OCA treatment could increase the effectiveness of PVE and increase the rates of resectability while decreasing the risk of post-operative liver failure.

In approximately 20% of patients undergoing preoperative PVE, resection cannot be performed because of disease progression or insufficient hypertrophy response. In **chapter 4**, we aimed to identify predictors for successful PVE during the preoperative work-up of patients scheduled for major liver resection. In this retrospective study, HBS was used in 63 patients who underwent preoperative PVE to determine pre- and post-PVE future remnant liver function. Among the 33/63 patients who didn't receive chemotherapy, a pre-PVE future remnant liver uptake function of at least 1.72%/min/m2 was necessary to reach the cut-off value for safe resection (2.7%/min/m2) during the first 3 weeks after PVE. The results of this study aid in identifying patients who will develop a sufficient hypertrophy response of the future remnant liver after PVE. In case of a pre-PVE future remnant function < 1.72%/min/m2 in chemo-naïve patients, the ALPPS procedure (Associating Liver Partition and Portal vein ligation for Staged hepatectomy), which induces a stronger hypertrophy response in a shorter period of time, may be advised.

PVE is an effective procedure to enhance the future remnant liver volume and function pre-operatively. As already mentioned, it provides permanent occlusion of the embolized portal venous system. In case of unresectability after PVE, the embolized liver lobes with permanent embolization material remain in situ. There is a paucity of literature on the long-term consequences of this permanent occlusion. In **chapter 5**, we evaluated the long-term effects in unresected embolized liver lobes after PVE. The primary outcome of the study was the occurrence of liver abscesses on follow-up CT imaging. In this study 85 patients underwent right PVE, and of these patients 16 (19%) were found to be unresectable for different reasons after the procedure was already performed (PVE group). These unresectable patients were randomly matched with 75 patients who were unresectable but did not undergo PVE (non-PVE group). In the PVE group, 5 patients (31%), developed an abscess versus 4 (8%) in the non-PVE group. Although the incidence of this complication is rather low, the consequences for this group of patients who are already in a palliative setting with a limited quality of life, pose a considerable burden. The use of absorbable embolization materials seems to offer a hopeful approach to prevent the long-term complications of permanent occlusion.

The surgical techniques and perioperative management of patients undergoing liver surgery have improved in recent years. An expert team of surgeons, internists, oncologists and radiologist are necessary to sustain a favorable postoperative outcome. In **chapter 6**, the results after liver resection and radio frequency ablation (RFA) are compared for colorectal

carcinoma liver metastases performed in a specialized regional hospital vs. an university medical center over the last 5 years. A total number of 232 patients were included in a retrospective cohort study: 123 patients in the Academic Medical Center in Amsterdam (AMC) and 109 patients in the Amphia hospital, Breda, which is a specialized regional hospital in the Netherlands. There was a significant difference in type of treatment (P<0,01) with more patients treated with RFA in the Amphia hospital, which led to a significant difference in disease-free survival in favor of patients treated at the AMC. There were no differences in outcomes between the centers among patients who underwent resection only. This suggests that liver surgery can be adequately applied in a high-volume regional hospital with a center function in this field.

The interest in laparoscopic liver surgery (LLS) has grown in recent years. In literature, several advantages have been described which include less postoperative complications, shorter hospital stay, decreased need for analgesics and less intraoperative blood loss. In **chapter 7**, the need for stepwise introduction of LLS combined with a structured training is presented. The AMC started to perform LLS since November 2006 and this technique was implemented along with specific surgical training and mentoring. All consecutive patients undergoing LLS in the AMC for any indication between November 2006 and January 2017 were evaluated retrospectively. An increase of more complicated operations and more complex patient characteristics over time was seen without compromising perioperative outcomes. Structured training, hands-on courses in LLS, mentoring and specialized laparoscopic HPB fellowships help to overcome the clinical impact of the learning curve.

Postoperative fluid overload is frequently seen after major liver resections and comes with complications like cardiopulmonary and renal events, decreased wound healing, decreased bowel motility and an increase in length of hospital stay. The exact physiological basis of this fluid overload is poorly understood. In **chapter 8** we hypothesized that the renin–angiotensin–aldosterone system (RAAS) is activated during liver resection which leads to higher fluid responsiveness and retention of fluids. A pilot study was performed to evaluate the activation of the RAAS hormones during liver surgery. Furthermore, we investigated the correlation between the activation of RAAS and intraoperative 'fluid responsiveness'. We included 17 patients and found a significant increase of the studied hormones in the first two postoperative days. Aldosterone was positively correlated with weight gain due to fluid overload during the first five postoperative days. There was no significant difference in postoperative weight gain between fluid responders and non-responders during the operation. This is the first study describing the activation of RAAS during the operative phase, which probably contributes to fluid overload.

FUTURE PERSPECTIVES

This thesis focuses on preoperative augmenting procedures along with aspects of postoperative care in patients undergoing liver resection with the aim to improve the postoperative outcome of major liver surgery in terms of postoperative morbidity and mortality.

In a PVE rabbit model, we defined an absorbable embolization material based on fibrin-glue which provides an adequate hypertrophy response with recanalization of the portal vein in 42 days, without causing histological injury to the liver. The influence of temporary embolization on the function of the embolized liver lobes is a topic of interest that needs to be further investigated in this animal model, preferably using a quantitative liver function test such as hepato-biliary scintigraphy (HBS). The fibrin-glue based embolization material is a safe and commonly used product. [1-3] Clinical evaluation of this absorbable embolization material in a randomized controlled trial would be a logical next step after the encouraging results presented in this study.

The exact mechanism of the atrophy-hypertrophy complex after PVE is still unknown. Further clinical and translational research is needed to unravel the features of this regeneration response. We found that the potent FXR agonist obeticholic acid (OCA) accelerates liver regeneration in our standardized rabbit model of PVE. OCA stimulates liver regeneration, which potentially leads to an increase in the resectability rate in patients requiring PVE to undergo liver resection in the clinical setting. Some liver tumors (e.g. hepatocellular carcinoma and perihilar cholangiocarcinoma) express FXR, which means care should be taken to use FXR agonists in patients with these tumor types in order not to stimulate tumor growth along with the regenerative boost induced by OCA. Further translational research in an animal tumor model is a advisable to examine the effect of OCA on tumor growth. [4-6]

Fluid overload in the postoperative period after major liver resection is an often encountered problem. In the pilot study described in **chapter 8**, an increase in RAAS hormones after major liver resections was found. We noted that postoperative weight gain was positively correlated with the hormone aldosterone. Future studies are needed to assess the effectiveness of an ACE inhibitor in the postoperative period to prevent fluid overload. The mechanism of activation of the RAAS system after liver resection might relate to changes in the portal venous pressure during the operation. Increased portal vein pressure is associated with an increase in the mesenteric blood flow and subsequent activation of the systemic vasoconstrictor system including RAAS and vasopressin.[7] A prospective study comparing patients with and without portal vein manipulation should be able to answer this question.

References

(1) Dunn, C.J. and K.L. Goa, *Fibrin sealant: a review of its use in surgery and endoscopy.* Drugs, 1999. **58**(5): p. 863-86.

(2) Ebata, T., et al., *Portal vein embolization before extended hepatectomy for biliary cancer: current technique and review of 494 consecutive embolizations.* Dig Surg, 2012. **29**(1): p. 23-9.

(3) Nagino, M., et al., *Selective percutaneous transhepatic embolization of the portal vein in preparation for extensive liver resection: the ipsilateral approach.* Radiology, 1996. **200**(2): p. 559-63.

(4) Guo, F., et al., *FXR induces SOCS3 and suppresses hepatocellular carcinoma.* Oncotarget, 2015. **6**(33): p. 34606-16.

(5) Hirschfield, G.M., et al., *Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid.* Gastroenterology, 2015. **148**(4): p. 751-61 e8.

(6) Dai, J., et al., *Impact of bile acids on the growth of human cholangiocarcinoma via FXR.* J Hematol Oncol, 2011. **4**: p. 41.

(7) Sola E, Gines P. *Challenges and Management of Liver Cirrhosis: Pathophysiology of Renal Dysfunction in Cirrhosis*. Dig Dis. 2015;33(4):534-8.

NEDERLANDSE SAMENVATTING

De afgelopen jaren is veel translationeel en klinisch onderzoek gedaan om de uitkomsten van leverchirurgie te verbeteren. Met name de unieke mogelijkheid van de lever om te kunnen regenereren, is een belangrijk onderwerp dat veel onderzoekers heeft geboeid. De exacte mechanismen voor deze regeneratie is echter nog grotendeels onbekend. Bij chirurgische ingrepen die de lever betreffen, is een toekomstig levervolume van 25 % van het totale levervolume nodig om postoperatief leverfalen te voorkomen. De functionele capaciteit van de toekomstige restlever is, naast voldoende levervolume, ook van groot belang. ^{99m}Tc-mebrofenine hepatobiliaire scintigrafie (HBS) is een kwantitatieve leverfunctietest, die de opnamefunctie van de hepatocyten meet middels een radioactieve stof. Vena porta embolisatie (VPE) is een veel toegepaste methode om preoperatief zowel levervolume als functie van de toekomstige restlever te vergroten. Met embolisatie van de vena portae wordt een compensatoire regeneratierespons van de niet geëmboliseerde leversegmenten geïnduceerd, wat resulteert in toename van de functie en van het volume van de toekomstige restlever. Hierdoor komen meer patiënten in aanmerking voor een veilige resectie. Dit proefschrift onderzoekt verschillende perioperatieve procedures bij patiënten die grote leverresecties ondergaan met als doel de postoperatieve morbiditeit en mortaliteit te verbeteren.

Het mechanisme achter het ontstaan van leverregeneratie na VPE is nog steeds een onderwerp van onderzoek. Vaak worden diermodellen gebruikt om de klinische situatie na te bootsen. **Hoofdstuk 1** geeft een overzicht van alle beschikbare diermodellen om VPE te onderzoeken, waarbij specifiek gekeken wordt naar specifieke anatomische voordelen van de dieren en naar de mogelijkheden voor het meten van de hypertrofierespons. Alle experimentele dierenstudies met tenminste 5 dieren, waarin VPE of vena porta ligatie (VPL) was onderzocht, zijn geïncludeerd in deze review. De keuze van het diermodel hangt af van het doel van de studie. Grote diermodellen, zoals het varken of konijn zijn met name goed bruikbaar voor evaluatie van de toename van leverfunctie en levervolume na VPE, vanwege de mogelijkheid tot het toepassen van klinische beeldvormende technieken zoals CT-volumetrie. Het rattenmodel is, vanwege het breed beschikbaar zijn van antilichamen, goed bruikbaar voor het onderzoeken van de mechanismen van VPE.

In **hoofdstuk 2** wordt een VPE-konijnenmodel gebruikt voor het ontwikkelen van een absorbeerbaar embolizatiemateriaal, zodat complicaties die kunnen ontstaan bij het gebruik van permanente embolizatiematerialen voorkomen kunnen worden. Absorbeerbare embolisatiematerialen zijn met name interessant in het geval van leverdonatie bij levende donoren. De hypertrofierespons kan dan worden geïnduceerd, zonder de leverrest in de donor te beschadigen. In deze studie hebben we gevonden dat fibrinelijm met een bepaalde concentratie aprotinine in het VPE-konijnenmodel een adequate hypertrofierespons induceerde met rekanalisatie van de vena portae in 80% van de dieren. Verdere klinische evaluatie van

fibrinelijm als absorbeerbaar embolisatiemateriaal is nodig, met name voor toepassing in het gebied van leverdonatie met levende donoren.

De laatste jaren wordt veel onderzoek gedaan naar farmacologische interventies om leverregeneratie te vergroten. Galzouten blijken een belangrijke rol te spelen in de beginfase van leverregeneratie, doordat zij de farnesoid x-receptor (FXR) activeren. De FXR-agonist obeticholzuur (OCA) is een krachtige galzoutagonist. In **hoofdstuk 3** laten we zien dat OCA leverregeneratie stimuleert in een VPE konijnenmodel met een adequate toename van het toekomstig restlevervolume en restleverfunctie. Histologisch onderzoek liet geen hepatocellulaire schade zien. Deze studie toont aan dat behandeling met OCA de effectiviteit van VPE kan verhogen en daarmee, het aantal patiënten dat voor leverresectie in aanmerking zou kunnen komen.

In ongeveer 20% van de patiënten die preoperatief VPE ondergaat, kan resectie niet uitgevoerd worden wegens tumorprogressie of een insufficiënte hypertrofierespons. In **hoofdstuk 4** wordt een vroege voorspeller van de hypertrofierespons na VPE gepresenteerd die gebruikt kan worden bij de preoperatieve voorbereiding van patiënten die VPE zullen ondergaan voor een grote leverresectie. In deze retrospectieve studie werd in 63 patiënten HBS gebruikt om de toekomstige leverfunctie voor en na VPE te bepalen. In 33 van de 63 patiënten, die niet met chemotherapie behandeld werden, werd gevonden dat een pre-VPE opnamefunctie van de toekomstige restlever van tenminste 1.72%/min/m^2 nodig is om de veilige afkapwaarde van 2.7%/min/m^2 te bereiken in de eerste drie weken na de VPE procedure. Voor chemotherapie-naïeve patiënten, die deze pre-VPE afkapwaarde van 1.72%/min/m^2 niet halen, zou een ALPPS-procedure (Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy) uitgevoerd kunnen worden vanwege het feit dat deze interventie zorgt voor een sterkere hypertrofierespons in een relatief korte periode.

VPE is een effectieve methode om het volume en de functie van de toekomstige restlever preoperatief te vergroten. Zoals al eerder genoemd zorgt VPE voor een permanente occlusie van de vena portae tak naar de geëmboliseerde leverlob. In het geval van irresectabiliteit blijven de geëmboliseerde leversegmenten in situ. In de literatuur is er weinig geschreven over de consequenties van deze permanente occlusie in het geval van irresectabiliteit. In **hoofdstuk 5** worden de langetermijneffecten van niet-gereseceerde, geëmboliseerde leversegmenten onderzocht. De primaire uitkomstmaat van de studie is het ontwikkelen van abcessen, die gediagnosticeerd werden tijdens vervolg beeldvorming van de lever. In deze studie werden uit een groep van 85 patiënten die VPE ondergingen, 16 patiënten geselecteerd die niet-resectabel waren om verschillende redenen. Deze 16 patiënten (VPE-groep) werden vergeleken met een groep van 48 willekeurig patiënten, die geen VPE hadden ondergaan en ook niet resectabel waren (niet-VPE groep). In de VPE-groep ontwikkelden 5 patiënten (31%) een abces versus 4 (8%) patiënten in de niet-VPE groep. Alhoewel de incidentie van deze complicatie relatief laag is, kunnen de consequenties op de kwaliteit van leven door deze intrahepatische abcessen groot zijn voor deze fragiele patiënten, die vaak

toch al palliatief behandeld worden. Een oplosbaar embolisatiemateriaal kan mogelijk een oplossing bieden om de lange-termijn gevolgen van permanente occlusie te voorkomen.

Chirurgische technieken en de perioperatieve zorg rondom patiënten die leverchirurgie ondergaan zijn de laatste jaren sterk verbeterd. Om de postoperatieve uitkomsten na leverchirurgie verder te optimaliseren is een expertteam nodig van chirurgen, internisten, oncologen en radiologen. **Hoofdstuk 6** vergelijkt de resultaten na leverresectie en na radiofrequente thermo-ablatie (RFA) van colorectale levermetastasen uitgevoerd in een perifeer ziekenhuis (met een centrumfunctie) met die van een universitair medisch centrum. In totaal werden 232 patiënten geïncludeerd in een retrospectief cohort: 123 patiënten in het Academisch Medisch Centrum te Amsterdam (AMC) en 109 patiënten in het Amphia ziekenhuis in Breda. Een significant verschil in type behandeling ($p<0.01$) tussen beide centra werd gevonden, waarbij in het Amphia ziekenhuis meer patiënten werden behandeld met RFA met als gevolg een significant betere ziektevrije overleving in het AMC. Er werden geen significante verschillen gevonden tussen patiënten die alleen resectie ondergingen. Deze resultaten laten zien dat leverchirurgie goed kan worden toegepast in een perifeer ziekenhuis met een centrumfunctie op dit gebied.

Laparoscopische lever resecties (LLR) worden steeds vaker toegepast. In de literatuur worden verschillende voordelen beschreven, zoals minder postoperatieve complicaties, kortere opnameduur, verminderde behoefte aan pijnstilling en minder operatief bloedverlies. In **hoofdstuk 7** wordt het belang van een stapsgewijze introductie van LLR in combinatie met een gestructureerde training beschreven. In november 2006 is in het AMC gestart met het uitvoeren van LLR. Deze start ging gepaard met een specifieke chirurgische training en begeleiding. Alle patiënten die LLR ondergingen in het AMC tussen november 2006 en januari 2017 werden in een retrospectieve studie geëvalueerd. De resultaten laten zien dat gedurende deze periode complexere operaties werden verricht bij complexere patiënten, zonder dat dit de intra- en postoperatieve uitkomsten beïnvloedde. Gestructureerde training, hands-on cursussen, begeleiding en gespecialiseerde laparoscopische HPB fellowships kunnen de klinische gevolgen van de leercurve voor de chirurg verminderen.

Postoperatieve overvulling wordt vaak gezien na grote leverresecties en zorgt voor cardiopulmonale of renale complicaties. Daarnaast veroorzaakt het vertraagde wondgenezing, verminderde darmmotiliteit en verlengde opnameduur. Het exacte mechanisme achter postoperatieve overvulling na leverresecties is niet bekend. In **hoofdstuk 8** wordt de hypothese besproken dat activatie van het renine-angiotensine-aldosteron systeem (RAAS) tijdens leverresecties zorgt voor postoperatieve vochtretentie. Een pilotstudie werd opgezet om de activatie van RAAS-hormonen te evalueren tijdens leverchirurgie. Daarnaast hebben we gekeken naar de correlatie tussen de activatie van RAAS en intra-operatieve 'fluid responsiveness'. In de geïncludeerde 17 patiënten vonden we een significante stijging van de RAAS-hormonen gedurende de eerste twee postoperatieve dagen. Aldosteron was positief gecorreleerd met gewichtstoename gedurende de eerste vijf postoperatieve dagen. Geen

verschil werd gevonden tussen postoperatieve gewichtstoename en 'fluid responsiveness' gedurende de operatie. Dit is de eerste studie die activatie van RAAS correleert aan postoperatieve gewichtstoename bij leveroperaties.

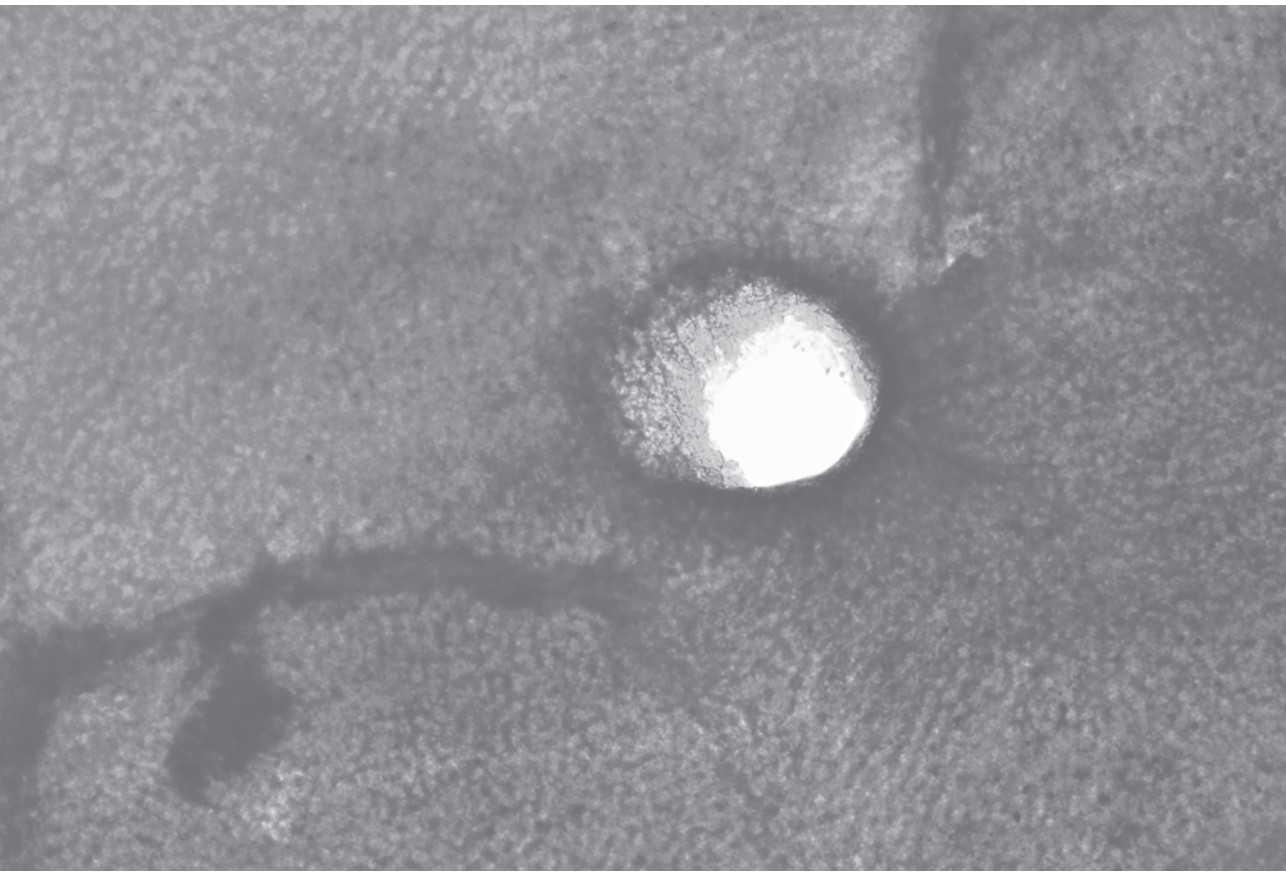
TOEKOMSTPERSPECTIEVEN

Dit proefschrift concentreert zich op preoperatieve procedures die hypertrofie van de lever induceren en op aspecten van postoperatieve zorg bij patiënten die een leverresectie ondergaan met als doel de postoperatieve uitkomst van uitgebreide leverchirurgie in termen van postoperatieve morbiditeit en mortaliteit te verbeteren. We hebben, in een vena porta embolisatie (VPE) konijnenmodel, een oplosbaar embolizatiemateriaal gebaseerd op fibrine lijm ontwikkeld dat een adequate hypertrofie respons laat zien waarbij de vena portae weer doorgankelijk wordt zonder dat het leverweefsel schade ondervindt. Het effect van deze tijdelijke embolisatie op de functie van de geëmboliseerde lever lobben is een onderwerp van discussie hetgeen nog verder uitgezocht dient te worden in dit konijnenmodel, bij voorkeur door gebruik te maken van kwantitatieve leverfunctietesten, zoals hepato-biliaire scintigrafie (HBS). Het absorbeerbare embolisatiemateriaal, bestaande uit fibrine lijm, is een veilig en veel toegepast product. Klinische evaluatie van dit absorbeerbare embolisatiemateriaal in de vorm van een gerandomiseerd onderzoek zou een logische volgende stap zijn op grond van de bemoedigende resultaten van deze studie.

Het exacte mechanisme van het atrophy-hypertrophy complex bij VPE is nog steeds niet opgehelderd. Verder klinisch en translationeel onderzoek is nodig om het mechanisme van de regeneratie respons te ontrafelen. We hebben in het gestandaardiseerde VPE-konijnenmodel gevonden dat de krachtige FXR agonist obeticholic acid (OCA) de leverregeneratie versnelt. OCA stimuleert leverregeneratie, hetgeen uiteindelijk kan leiden tot meer patiënten die na VPE een uitgebreide leverresectie kunnen ondergaan. Sommige levertumoren (bijvoorbeeld hepatocellulair carcinoom en perihilair cholangiocarcinoom) hebben FXR-receptoren, zodat voorzichtigheid geboden moet worden bij het gebruik van FXR agonisten in patiënten met deze typen tumoren, teneinde naast de regeneratie respons ook niet versnelde tumorgroei door OCA te veroorzaken. Verder onderzoek naar de relatie tussen OCA en tumorgroei dient in een diermodel onderzocht te worden.

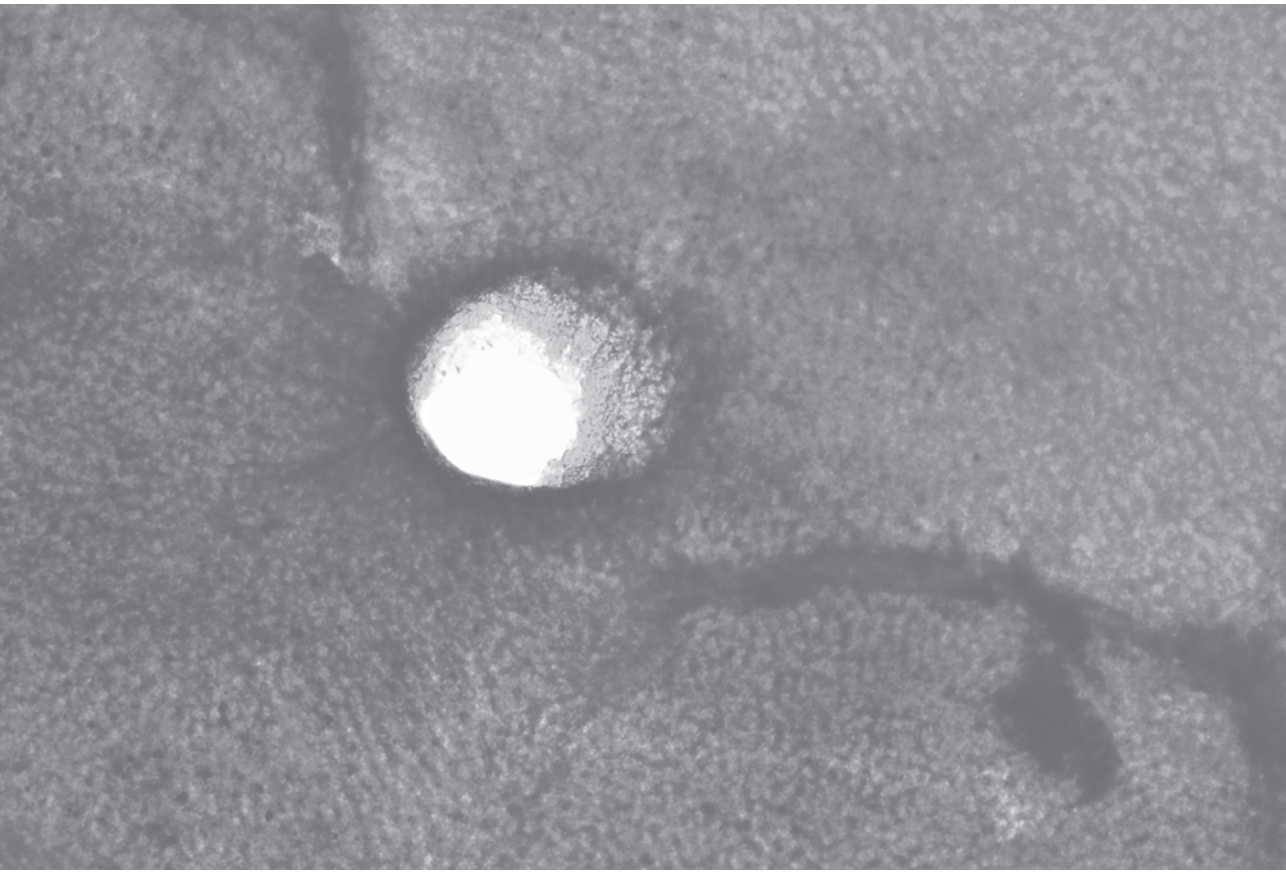
Overvulling in de postoperatieve periode na uitgebreide leverresecties komt veel voor. In de pilotstudie beschreven in **hoofdstuk 8**, is een verhoging van de renine-angiotensine-aldosteronsysteem (RAAS) gevonden. Een positieve correlatie werd gevonden tussen postoperatieve gewichtstoename en het hormoon aldosteron. Verder onderzoek is nodig om te bepalen of een ACE-remmer mogelijk zou kunnen bijdragen aan het voorkomen van postoperatieve overvulling. Het mechanisme achter de activatie van RAAS tijdens lever resecties zou veroorzaakt kunnen worden door veranderingen in de vena portae druk tijdens

de operatie. Een verhoogde druk in de vena portae is geassocieerd met een verhoogde bloedstroom door de mesenteriale vaten en daaruit volgend een activatie van systemen die vasoconstrictie bewerkstelligen, zoals RAAS en vasopressine. Dit zou verder onderzocht kunnen worden in een prospectieve studie, waarin patiënten die manipulatie met als gevolg drukverhoging van de vena portae tijdens operatie hebben ondergaan worden vergeleken met patiënten bij wie dat niet verricht is.



APPENDICES

List of publications
List of contributing authors
PhD portfolio
Dankwoord
Curriculum Vitae



LIST OF PUBLICATIONS

* authors contributed equally

1. F. Huisman, K.P. van Lienden, S. Damude, L.T. Hoekstra, T.M. van Gulik. A review of animal models for portal vein embolization'. *J Surg Research* 2014 Sep; 191(1): 179-88
2. F. Huisman*, P.B. Olthof*, R.F. van Golen, K.P. Cieslak, K.P. van Lienden, T. Plug, J.C. Meijers, M. Heger, J. Verheij, T.M. van Gulik. Use of an absorbable embolization material for reversible portal vein embolization in an experimental model. *British Journal of Surgery* 2016 Sep; 103(10): 1306-15
3. F. Huisman*, PB Olfthof, FG Schaap, KP van Lienden, RJ Bennink, RF van Golen, M Heger, J Verheij, PL Jansen, SW Olde Damink, TM van Gulik. Effect of obeticholic acid on liver regeneration following portal vein embolization in an experimental model. *British Journal of Surgery* 2017 Apr; 104(5): 590-599
4. F. Huisman*, K.P. Cieslak*, T. Bais, R.J. Bennink, K.P. van Lienden, J. Verheij, M.G. Besselink, O.R.C. Busch, T.M. van Gulik. Future remnant liver function as predictive factor for the hypertrophy response after portal vein embolization. *Surgery* 2017 Jul; 162(1):37-47
5. F. Huisman, K.P. Cieslak, K.P. van Lienden, R.J. Bennink, T.M. van Gulik. Liver related complications in unresectable disease after portal vein embolization. *HBSN* 2017 Dec; 6(6): 379-386
6. F. Huisman*, K. de Leur*, P. D. Gobardhan, P. A. M. Kint, J. H. Wijsman, A. J. ten Tije, R. Krause, T.M. van Gulik, A. M. Rijken. Treatment of colorectal liver metastases at a regional hospital versus a university medical centre. *NTvG* 2013; 157(34): A6417
7. M. J. van der Poel, F. Huisman, O.R.C. Busch, M. A. Hilal, T. M. van Gulik, P. J. Tanis, M. G. Besselink. Stepwise introduction of laparoscopic liver surgery: validation of guideline recommendations. *HPB* 2017 Oct; 19(10):894-900
8. F. Huisman, S.K. Ouwehand, D.P. Veelo, K.P. van Lienden, B.F. Geerts, M.W. Hollmann, T. M. van Gulik, E.J.M Nieveen van Dijkum. Evaluation of cardiac index, fluid retention, and the RAAS system during and after major hepatic resection. *Submitted*

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PhD PORTFOLIO

Name PhD student: Floor Huisman
PhD period: January 2012 – April 2015
Name PhD supervisor: Prof. dr. T.M. van Gulik

PhD training		
	Year	**Workload (Hours/ECTS)**
General courses		
BROK (Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers)	2012	0.9
Crash course (bio)chemistry and biology	2012	0.4
Computing in R	2012	0.4
Practical Biostatistics	2012	1.1
Specific courses		
Laboratory animals (art. 9)	2013	3.9
Advanced Topics in Biostatistics	2014	2.1
Seminars, workshops and master classes		
Weekly department seminars	2012-2015	3
One day on liver surgery symposia AMC	2012, 2013, 2014	0.75
Oral presentations		
Plasma bile salt, triglycerides and apoA-V levels in the prediction of liver volume and function after portal vein embolization *IHPBA, Paris*	2012	0.5
Efficacy of absorbable embolization materials for portal vein embolization to induce liver regeneration in a rabbit model		
NVGE, Veldhoven	2013	0.5
ESSR, Budapest	2014	0.5
Introduction of laparoscopic minor liver surgery in a HPB unit: not necessarily associated with a learning curve		
NVGE, Veldhoven	2014	0.5
Poster presentation		
Plasma bile salts predict liver regeneration in a rabbit model of portal vein embolization		
IHPBA, Paris	2012	0.5
Tumor progression after preoperative portal vein embolization		
IHPBA, Paris	2012	0.5
Efficacy of absorbable embolization materials for portal vein embolization to induce liver regeneration in a rabbit model		
E-AHPBA Congress Belgrade	2013	0.5
NVGE Voorjaarsvergadering	2014	0.5

Introduction of laparoscopic minor liver surgery in a HPB unit: not necessarily associated with a learning curve		
Chirurgendagen, Veldhoven	2014	0.5
(Inter)national conferences		
SEOHS, Amsterdam, the Netherlands	2012	0.25
SEOHS, Maastricht, the Netherlands	2013	0.25
Chirurgendagen, Veldhoven, the Netherlands	2012	0.5
Chirurgendagen, Veldhoven, the Netherlands	2013	0.5
NVGE voorjaarscongres, Veldhoven, the Netherlands	2013	0.5
NVGE voorjaarscongres, Veldhoven, the Netherlands	2014	0.5
EAHPBA, Belgrade, Serbia	2013	0.75
ESSR, Budapest, Hungary	2014	0.75
EAHPBA, Manchester, United Kingdom	2015	0.75
Other		
GUT club	2013-2015	1
Local researcher Orange Trial	2013-2015	2
Reviewer for journals	2012-2014	1

Teaching		
	Year	**Workload (Hours/ECTS)**
Mentoring/supervising of students		
- S Damude, bachelor student, review animal models	2012-2013	2
- T. Bais, bachelorthesis, prediction of hypertrophy response after PVE	2014-2015	1.5

DANKWOORD

Wat ben ik blij dat het uiteindelijk gelukt is om dit proefschrift af te ronden. Veel mensen hebben mij hierbij geholpen.

Allereerst wil ik mijn promotor Thomas van Gulik bedanken. In 2012 kon ik bij u beginnen en het is niet altijd makkelijk gegaan. Na de eerste twee jaar onderzoek doen, kwam ik er achter toch niet verder te willen in het vak chirurgie. Ik wil u bedanken voor het vertrouwen dat u altijd in mij heeft blijven houden. Daarnaast bedankt voor alle veelzijdige ervaringen die ik heb mogen beleven tijdens de 3,5 mooie jaren op het lab!

Krijn, copromotor, heel erg bedankt voor jouw bijdrage aan het proefschrift. De minuscule bloedvaten van de konijnen emboliseren is iets wat bijna niemand jou kan nadoen. Jouw peptalks en positieve insteek hebben bijgedragen aan het uiteindelijk afronden van het geheel.

Dr. E.J.M. Nieveen van Dijkum, prof. dr. M.W. Hollmann, prof. dr. O.M. van Delden, prof. dr. O.R.C. Busch, prof. dr. C.H.C. Dejong en dr. I.Q. Molenaar, dank dat u deel wilt nemen aan de promotiecommissie.

De collega's van het lab. Waar moet ik beginnen. Het was een fantastische tijd, waarin we veel met elkaar hebben meegemaakt. Liefdes die opbloeiden en ook weer uitdoofden. Met elkaar naar congressen, theatervoorstellingen, borrels, feesten en natuurlijk de MOM. Jim, Robert-Jan, Rowan, Megan, Pim, Michal, Ruud, Martien, Vincent, Mans, Joost, Pim, Sebas, Ivo, Matthanja en Marcel, bedankt voor de fijne samenwerking.

Dan de vaste medewerkers:

Andrea, je weet de antwoorden op alle vragen. Bedankt voor je hulp de afgelopen jaren. Ook bedankt voor alle toneelvoorstellingen, die we dankzij jou hebben mogen bijwonen. Ik hoop er nog veel te kunnen zien in de toekomst. Adrie, alle experimenten hadden niet kunnen plaatsvinden zonder jouw chirurgische skills. Van 's ochtends vroeg tot 's avonds in de weer met de konijnen. Je bent een topper.

Albert, dank voor je hulp bij de experimenten en voor jouw bulderende lach. Lindy, jouw bijdrage bij de konijnenproeven was onmisbaar. Ik heb veel van je geleerd en ik hoop dat we snel samen een bessen met ijs gaan drinken. Goos, jouw kennis over dieren is fenomenaal. Bedankt voor de eindeloze gesprekken die we hebben gehad. Ruurdtje, dankzij jou weet ik dat het dankwoord een belangrijk onderdeel is van het boekje. Bedankt voor de fijne tijd en de gezellige lunches op het lab. Esther, bedankt voor het ordenen van het onderzoeksmateriaal en veel succes met de nieuwe stappen die je aan het ondernemen bent.

Oma Ann en opa Leen. Wat prachtig dat u dit nog mag meemaken! Opa, u bleef maar vragen wanneer ik nou ging promoveren. Dit heeft mij gemotiveerd om het af te ronden en ik ben dankbaar dat u dit nog mag meemaken. Oma, ik hoop dat ik uw optimisme geërfd heb. Ik kan niet wachten om met u het glas te heffen na de plechtigheid, cheerio.

Lieve vrienden, het was een bewogen periode. Ik wil jullie bedanken voor alle mooie momenten, die vooral niks te maken hadden met promoveren. Mede hierdoor is het uiteindelijk gelukt om de ruimte te vinden dit proefschrift af te ronden. Enkele wil ik graag toelichten. Elisabeth en Sari, wat geniet ik van de mega fijne uren samen kletsen en samen drankjes drinken. Jullie hebben mij er echt doorheen gesleept en altijd een luisterend oor geboden. Ik hou van jullie! Cordial Bizar! Sonia, Rianne, Flora, Dagmar, Annemarije, Laura, Helen, Vera, Kelly en Marjolein. In 2003 zijn we elkaar tegengekomen op de Augiebar in Leiden en sindsdien zijn we vriendinnen. Samen reizen, feesten, lachen en huilen. Ik ben blij dat we elkaar nog steeds zien en ik hoop dat we nog veel mooie dingen met elkaar mee mogen maken. Niki en Haroen, partners in crime! Tante Marja, wij kennen altijd al 34 jaar. Dat is een lange periode, waarin ik het altijd heel leuk vind om je te zien. Ik hoop dat we er nog vele jaren aan mogen vastplakken!

Lieve paranimfen:

Linda, we kennen elkaar al een lange tijd en zijn altijd in elkaars leven gebleven. Ik bewonder je om je creativiteit en je geduld. Fijn dat je mijn vriendinnetje bent!

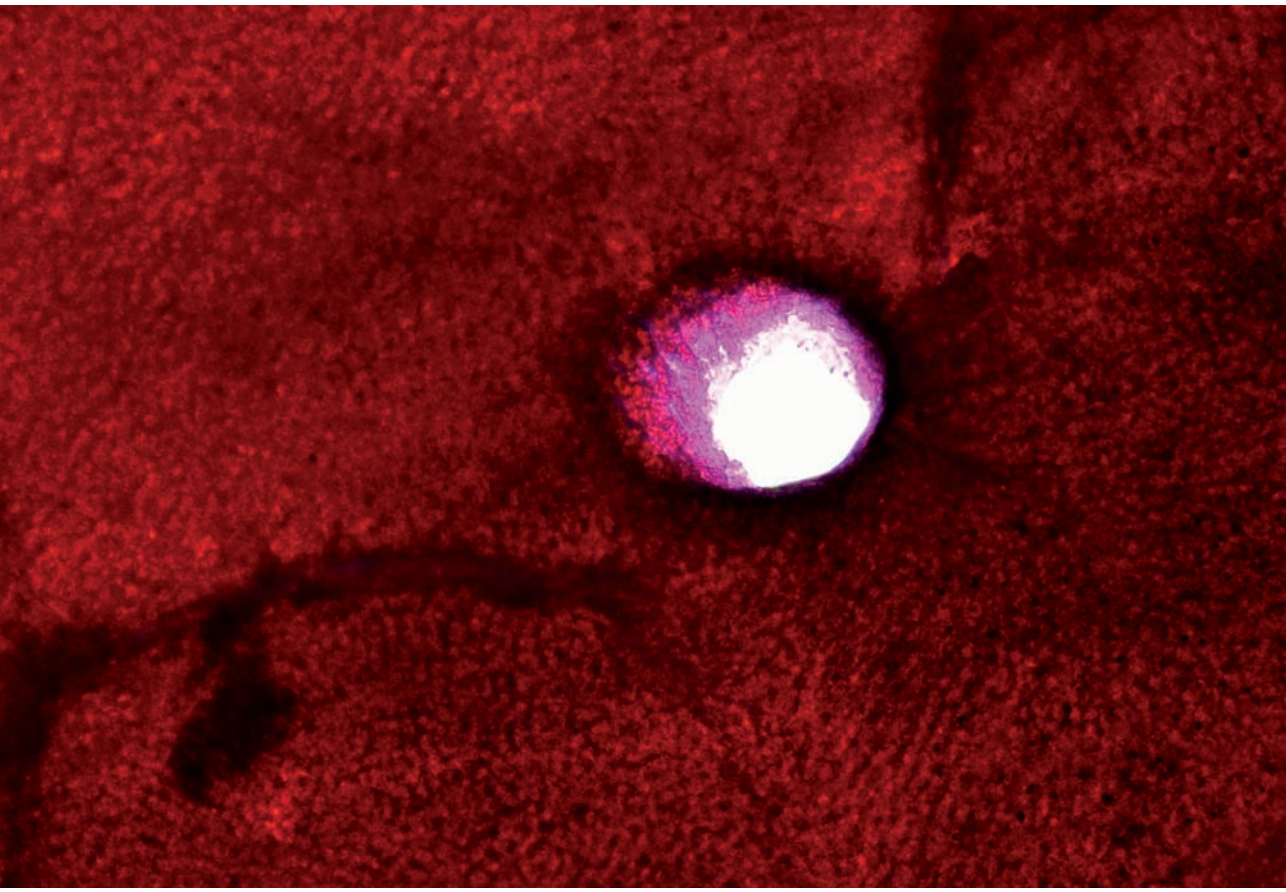
Werrie beertje, wat ben ik blij dat je naast mij mag staan tijdens dit officiële moment. Fijn dat je er altijd voor mij bent. Ik heb veel van jou geleerd en hoop nog veel van je te kunnen leren. Ik hou van jou.

Mijn dierbaren, mama, papa Co, Anto, Timo, Evelien & Wouter: fijn dat jullie altijd naast mij staan! Mama, bedankt voor je eindeloze vertrouwen. Fijn dat je er altijd voor mij bent. Pap, toch wel fijn dat het nu af is he? Ik prijs mezelf gelukkig met zulke ouders als jullie. Daarnaast vind ik het fijn dat Nynke en jij het zo gezellig hebben. Anto, ik ben blij met zo'n hippe tante als jij en ik hoop dat we nog vaak samen leuke dingen zullen doen. Timo, bedankt voor je luisterende oor en voor alles wat je voor mij hebt gedaan de afgelopen jaren. Ik heb veel bewondering voor jouw doorzettingskracht. Je bent lekker bezig! Lieve Evelien, het was het afgelopen jaar niet altijd makkelijk voor jou. Ik ben trots op de manier waarop jij je erdoor heen geslagen hebt. Mooi huis, nieuwe baan, nieuw oog: top. Lieve Wouter, het is fijn om te weten dat er zo'n lieve jongen naast mijn zusje staat. Ik hou van jullie. Laten we nog veel mooie momenten met elkaar meemaken!

Lieve Kasia, van vriendinnen naar lovers en mede mogelijk gemaakt door de Experimentele chirurgie. Wie had dat gedacht…Hoe jij je door de afgelopen 2 jaar heen hebt geslagen, vind ik heel knap. Je bent begin dit jaar gepromoveerd en ik heb alles bij jou af kunnen kijken! Als dit achter de rug is, gaan we lekker samen op reis. Ik hou van jou.

CURRICULUM VITAE

Floor Huisman werd geboren op 18 augustus 1984 in Delft. Zij groeide op in de omgeving van Delft, waar zij in 2003 haar gymnasium diploma haalde aan het Christelijk Lyceum Delft. Ze studeerde geneeskunde aan de Universiteit van Leiden en werd lid van studentenvereniging Augustinus. Samen met haar cordial Bizar beleefden zij een enerverende studententijd met als hoogtepunten een wetenschappelijke stage in Bali en een keuze coschap in Tanzania. Na het behalen van haar artsexamen in 2010 werkte zij 1,5 jaar als ANIOS IC en ANIOS chirurgie in het Reinier de Graaf Gasthuis in Delft, alwaar haar interesse voor het vak chirurgie ontstond. In 2012 werd zij aangenomen voor een promotietraject onder leiding van prof. T.M. van Gulik op de afdeling Experimentele Chirurgie van het AMC en verhuisde zij van Leiden naar Amsterdam. Haar onderzoek naar de effecten van vena porta embolisatie en leverregeneratie hebben geresulteerd in de totstandkoming van dit proefschrift. Tijdens het promotietraject werd haar interesse voor de psyche van de mens aangewakkerd en koos zij er voor om in mei 2015 te starten als ANIOS psychiatrie in de opnamekliniek Rivierduinen in Leiden. Zij is sinds 2016 in opleiding tot psychiater bij Arkin in Amsterdam.



ISBN 978-94-6361-140-4