Clinical and experimental studies on portal vein embolization / Diagnosis of hepatocellular adenoma and focal nodular hyperplasia

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Clinical and experimental studies on portal vein embolization/
Diagnosis of hepatocellular adenoma and focal nodular hyperplasia
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Clinical and experimental studies on portal vein embolization/
Diagnosis of hepatocellular adenoma and focal nodular hyperplasia

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aan de Universiteit van Amsterdam
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ten overstaan van een door het college voor promoties ingestelde
commissie, in het openbaar te verdedigen in de Agnietenkapel
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door

Jacomina Willemke van den Esschert
geboren te Breda
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Faculteit der Geneeskunde
aan mijn ouders
## Contents part I

**Clinical and experimental studies on portal vein embolization**

- **Introduction and outline of part I of the thesis**
- **Chapter 1**: James Cantlie's early messages for hepatic surgeons: how the concept of pre-operative portal vein occlusion was defined
- **Chapter 2**: Portal vein embolization prior to liver resection: a systematic review
  - Submitted
- **Chapter 3**: Volumetric and functional recovery of the remnant liver after major liver resection with prior portal vein embolization
- **Chapter 4**: Controversies in the use of portal vein embolization
- **Chapter 5**: Induction of tumor growth after preoperative portal vein embolization: is it a real problem?
- **Chapter 6**: A rabbit model for selective portal vein embolization
  - *Journal of Surgical Research* 2010; May 21 [Epub ahead of print]
- **Chapter 7**: Portal vein embolization induces more liver regeneration than portal vein ligation in a standardized rabbit model
- **Chapter 8**: Liver regeneration after portal vein embolization using absorbable and permanent embolization materials in a rabbit model
  - *Annals of Surgery* 2011; [In press]
- **Chapter 9**: Short-term effects of combined hepatic vein embolization and portal vein embolization on the induction of liver regeneration
  - Submitted
- **Summary, discussion and future perspectives of part I of the thesis**
- **Nederlandse samenvatting van deel I van het proefschrift**
- **Dankwoord**
Contents part 2

Diagnosis of hepatocellular adenoma and focal nodular hyperplasia

Introduction and outline of the thesis 5

Chapter 10. Imaging modalities for focal nodular hyperplasia and hepatocellular adenoma
  *Dig Surg* 2010; 27:46-55. 11

Chapter 11. Systematic review of hepatocellular adenoma in China and other regions
  *J Gastroenterol Hepatol* 2011; 26:28-35. 29

Chapter 12. Gadolinium-EOB-DTPA enhanced MR in differentiating focal nodular hyperplasia from hepatocellular adenoma
  Submitted 47

Chapter 13. Differentiation of hepatocellular adenoma and focal nodular hyperplasia using 18F-fluorocholine PET/CT

Summary, discussion and future perspectives 75

Nederlandse samenvatting 78

Curriculum Vitae 80
Part I

Introduction and outline of the thesis

Clinical and experimental studies on preoperative portal vein embolization
Introduction

Liver resection remains the main curative treatment for patients with liver malignancies. Although for single tumors, non-anatomical resections can be performed, for larger or multiple tumors anatomical liver resections are usually undertaken following the so called Couinaud segments (Figure 1). Unfortunately not all patients are suitable for liver surgery, because of the extent of the resection necessary to completely remove all tumor or the quality of the liver parenchyma which may be affected by pre-existing disease or induction chemotherapy. The part of the liver that remains after surgery must be sufficient in terms of volume and function to meet the metabolic needs of the body, otherwise postoperative liver failure occurs. The current method for determining the size of the liver that remains after liver resection is preoperative volumetry using computed tomography (CT). Liver volume is used as an indirect measure of liver function. The minimally required amount of remnant liver volume is under debate, but in general, 25-30% of the initial total liver volume is thought to be sufficient in patients with otherwise normal liver parenchyma. In patients with a compromised liver function (e.g. cirrhosis, cholestasis, steatosis) however, a remnant liver volume at least 40% is considered to be safe.

Figure 1. Liver segments according to Couinaud.
Fortunately, the hepatocellular mass of the liver has the unique possibility to regenerate. This phenomenon can be used to preoperatively treat patients who are at risk of developing postoperative liver failure because of a small for size liver remnant. Since the first publication of the clinical use of portal vein embolization (PVE) in 1990, the procedure has become a widely used method to preoperatively increase the future remnant liver. After occlusion of the portal vein to the part of the liver that has to be resected, atrophy of this part occurs while inducing a compensatory hyperplasia and hypertrophy of the contralateral liver segments. Although PVE is largely applied worldwide, many issues concerning PVE and its consequences have emerged. Many questions still need to be elucidated, such as: What is the trigger for liver regeneration following portal vein occlusion? What is the underlying mechanism of liver regeneration after PVE? Which technique of portal vein occlusion results in the greatest hypertrophy response? What is the effect of PVE on tumor growth? These issues form the basis of the studies discussed in the first part of this thesis.

Outline of the thesis

The observation that portal vein occlusion leads to hypertrophy of the contralateral liver segments goes back much earlier than the 1980s. Chapter 1 gives an overview of how the concept of preoperative portal vein occlusion was defined.

Since its first clinical application, PVE has developed into a widely accepted, preoperative intervention to increase the future remnant liver. In Chapter 2, a systematic review is performed of all studies on PVE published in the last 20 years (1990-2011). The review discusses in particular, the effect of different embolization materials, prior treatment with chemotherapy, and the consequence of preexisting liver cirrhosis for the extent of hypertrophy response of the future remnant liver.

Preoperative PVE might hamper postresectional liver regeneration because a supposed trigger of liver regeneration, i.e. the instant increase of portal blood flow to the remnant liver, is largely unchanged after hemihepatectomy and previous PVE. A retrospective case-control study is described in Chapter 3 assessing the effect of preoperative PVE on liver volume and function, 3 months after major liver resection.

Unfortunately, PVE also has its drawbacks. The aim of Chapter 4 is to point out and discuss current controversies in the application of PVE. Chapter 5 reviews the clinical and experimental evidence regarding the effect of PVE on tumor growth in both the embolized and non-embolized liver lobes, as well as potential strategies to control tumor progression after PVE.
In an attempt to unravel some underlying mechanisms of liver regeneration after PVE and in order to optimize the technique for preoperative induction of liver regeneration, a standardized animal model is desired. In Chapter 6, a standardized rabbit model is described for PVE in experimental studies.

Clinical and experimental studies show opposite results regarding the issue if ligation or embolization of the portal vein leads to a greater regenerative response. The aim of Chapter 7 is to compare the hypertrophy response of the liver after ligation or embolization of the portal vein using this standardized rabbit model.

Many embolization materials have been used for PVE in the clinical setting. In Chapter 8, the hypertrophy response after the use of absorbable or permanent embolization materials is compared in the same rabbit model.

In order to further optimize the hypertrophy response, the effect of hepatic vein embolization on liver regeneration in addition to PVE is assessed in Chapter 9.
References

James Cantlie’s early messages for hepatic surgeons: how the concept of pre-operative portal vein occlusion was defined

T.M. van Gulik
J.W. van den Esschert
Abstract

In 1897, James Cantlie from Scotland published his findings of an autopsy on a patient in which the right side of the liver was atrophied whereas the left side of the liver showed a marked hypertrophy. He noted the hepatic vessels to the atrophied side to be obliterated. From this observation, he drew two important conclusions. First, that the transition of the atrophied part to the hypertrophied part defined the anatomical mid-line of the liver, according to the portal division of blood supply to the liver. This line we now know as Cantlie's line which he described connecting the fundus of the gallbladder with the centre of the inferior vena cava. Second, he foresaw that the potential of one half of the liver to hypertrophy when the other half is deprived of its blood supply, could be used to the advantage of hepatic resection. It would take another 85 years, however, before the first clinical, pre-operative portal vein embolization was carried out in Japan in 1982.
As early as 1897, Sir James Cantlie published a series of observations of extraordinary significance in the face of how we now look upon portal blood supply and the pre-resectional use of portal vein ligation or embolization to induce hypertrophy of the part of the liver we intend to preserve. In the Proceedings of the Anatomical Society of Great Britain and Ireland, he describes performing an autopsy on a patient in which the right side of the liver was reduced to a mass of fibrotic tissue whereas the left side of the liver showed a marked hypertrophy. He noted that the hypertrophy of the left side joined with the atrophied right side, at a line drawn through the fundus of the gallbladder to the center of the inferior vena cava at the back of the liver. He assumed that an abscess had destructed the right lobe of the liver, and that this resulted in a compensatory hypertrophy of the contralateral part of the liver. Hence, he concluded that the line connecting the fundus of the gallbladder with the centre of the inferior vena cava indicated the mid-line of the liver, unlike common opinion at that time which considered the umbilical fissure as the division of the right and left liver lobes.

He corroborated his observations by performing experiments in which he injected the right and left divisions of the portal vein with coloured dyes showing that the injected areas met along a line connecting the fundus of the gallbladder with the centre of the inferior vena cava grooves the back of the liver. This line we still refer to as Cantlie’s line (Figure 1) indicating the anatomical midline of the liver and defining the border between the right and left liver segments in the plane of the middle hepatic vein.

As he realized that the right and left liver were perfused by two separate streams of the portal vein, he recognized the potential this phenomenon could have for hepatic surgery.

![Figure 1. Cantlie’s line represents the anatomical mid-line of the liver connecting the fundus of the gallbladder with the centre of the inferior vena cava (Reproduced from Cantlie 1897).](image)

*: All phrases shown in italics are quoted from reference 1.
He perceived the consequences the watershed between the right and left liver lobes could have for trauma of the liver by writing 'The liver, when split or fissured by a blow, as between the buffers of railway-carriages, splits along the mid-line of the liver in preference to any other'. He also foresaw that this would not necessarily result in major bleeding as 'haemorrhage has less to be dreaded as the liver is incised or torn in the neighborhood of that line (i.e. the mid-line)'. Indeed in blunt abdominal trauma, the liver may be completely fractured along Cantlie's line without any major bleeding from that plane. We were able to confirm this message recently in a patient admitted after blunt abdominal trauma who had fractured his liver along Cantlie’s line (Figure 2) and who had been successfully managed by conservative treatment without the need for any blood transfusion.²

Figure 2. Contrast enhanced abdominal computed tomography (CT) scan of a patient admitted after blunt abdominal trauma showing a fracture of the liver along Cantlie’s line, running between the medial borders of segment IV and segments V/VIII. The patient sustained a contained intraparenchymal liver bleed without massive haemorrhage.

Coming back to his initial observation at the autopsy, he noted the 'almost elephantine' hypertrophy of the left side of the liver at the expense of a greatly atrophied right side 'which looked like, and practically was, a mere appendage to the left side of the organ'. On dissection of the liver he found the veins, artery and duct of the right side of the liver to be obliterated whereas those to the left side were proportionally increased in diameter. From this observation he conceived that by eliminating blood supply to one side of the liver, a functional advantage for the spared half of the liver could be created resulting in hypertrophy of that part of the liver. He then wrote 'It is theoretically possible to tie the vessels of one side at the gate of the liver, supplying an abnormal growth in one or other of the liver lobes, leaving the other side to do the work'. Realizing the importance this phenomenon could have for resecting the liver, he continued
‘I commend this subject to all those who are working at the surgery of the liver; and I believe that if, in the hands of future observers, the statements I have made receive closer investigation, the surgery of the liver will be advanced a step’. The foresight he had was amazing, with the first formal right hemihepatectomy being performed 55 years later in Beaujon Hospital in Paris and the first clinical portal vein embolization being performed in Japan 85 years after his report.

Sir James Cantlie (Figure 3) was born in 1851 in Banffshire, Scotland. After finishing his medical studies at Aberdeen University, he trained as a surgeon at Charing Cross Hospital in London. He became a fellow of the Royal College of Surgeons in 1877 and went on to work as a surgeon at Charing Cross. Interestingly, in 1887 he moved to Hong Kong where he became a co-founder of a new medical school, the Hong Kong College of Medicine for Chinese, the forerunner of the Faculty of Medicine of the University of Hong Kong. In this institution, of which he led the surgical department, Cantlie carried out the autopsy described above. One of his students was SunYat Sen who would later become the first provisional president of the Republic of China. When this Chinese leader was detained at the Chinese Legation in London in 1896, Cantlie played a key role in his release. In 1897 Cantlie returned to practice in London. The division of the portal vein into a right and left branch at the liver hilum was
already reported by the anatomists of the 17th century. Francis Glisson (1598–1677) in his textbook Anatomia Hepatis described cannulating the portal vein and making casts of the portal venous system. Cantlie, however, showed that by the separate portal vasculature, the liver could be functionally divided into an anatomically distinct right and left half. The potential of one half of the liver to hypertrophy when the other half is deprived of its blood supply was further confirmed in experimental studies by Rous and Larimore in 1920 and Schalm and colleagues in 1956. The latter authors from Arnhem, the Netherlands, made reference to Cantlie’s work and ideas on unilateral occlusion of the portal vein. Surprisingly, portal vein occlusion found its way to clinical application only in 1982, when Makuuchi and later, Kinoshita, published their first experiences with portal vein embolization in patients. Hence, although the credit for the first clinical portal vein occlusions goes to these colleagues in Japan, it should be remembered that in 1897, James Cantlie from Scotland had already laid down the concept of pre-operative portal vein occlusion.
References

2

Portal vein embolization prior to liver resection: a systematic review

K.P. van Lienden
J.W. van den Esschert
W. de Graaf
S. Bipat
J.S. Laméris
T.M. van Gulik
O.M. van Delden
Abstract

Background: To review the literature on the use and outcome of preoperative portal vein embolization (PVE) in the last 20 years.

Methods: A systematic literature search on the use and outcome of PVE from January 1st 1990 to May 1st 2011 was performed in Medline, Cochrane, and Embase. Clinical articles, written in English, containing information on patient characteristics, indication for PVE, pre- and post-PVE liver volumes or percentages of the future remnant liver, the technique, results, and complications of PVE, as well as results of liver surgery were selected.

Results: Finally, 44 articles were selected for this review, including 1791 patients (1139 men (63.6%) and 617 women (34.4%)). The mean age was 61 ± 4.1 years. Overall technical success rate was 99.1% (range 86%-100%). The mean hypertrophy rate of the FRL after PVE was 37.9 ± 0.1% (20.5 - 69.4%). In 52 patients (2.9%), surgery was not performed because of failure of PVE. In 35 patients (1.9%) the hypertrophy response was insufficient to perform liver resection, although the embolization procedure was successful. In the other 17 cases, 10 did not technically succeed (0.6%) and 7 caused a complication leading to unresectability (0.4%). Major complications were seen in 2.5% and the mortality rate was 0.1%. A meta-analysis on the influence of chemotherapy, pre-existing liver cirrhosis, cholestasis, and the use of different embolization materials on the hypertrophy response could not be performed because of the small number of articles and the inhomogeneity of the subgroups. However, a head-to-head comparison of the articles shows no significant differences, except for the use of embolization materials in favor of n-butyl cyanoacrylate.

Conclusion: Preoperative PVE is an effective method to increase the FRL volume with a high technical and clinical success rate. Pre-existing liver damage due to cirrhosis, cholestasis, or chemotherapy seems to have no influence on the hypertrophy response. However, the use of n-butyl cyanoacrylate seems to result in a greater hypertrophy response compared to the other materials used.
Introduction

Liver resection is still the only option for long-term survival for patients with primary or secondary liver malignancies. Unfortunately, only 10-20% of patients with colorectal liver metastases are candidates for liver resection. The resectability rate for hepatocellular carcinoma is about 20-30% in normal livers, but reduced in patients with cirrhotic livers.\(^1\) In literature, the postoperative liver failure rate ranges from 0 - 30% and is still the major cause of death following major liver resection.

When patients prove unresectable because of insufficient remnant liver volume, portal vein embolization (PVE) is one of the methods to stimulate growth of the future remnant liver (FRL), thereby sustaining the possibility of extensive liver resection. The first to demonstrate the regenerative capacity of the liver following portal vein occlusion were Rous and Larimore in the 1920s. In a rabbit model, they showed atrophy of the hepatic lobe ipsilateral to the ligated portal branches, while compensatory hypertrophy was observed in the contralateral lobe.\(^2\) In 1961, portal vein ligation was reported in humans as part of a two-stage extended hepatectomy.\(^3\) Kinoshita et al. reported in 1986 the first preoperative PVE in a human being.\(^4\) Since then, numerous reports have shown the efficacy of inducing compensatory hypertrophy of the FRL after PVE in preparation for surgery to resect primary or metastatic cancers in the liver.\(^5-7\)

Several techniques of portal vein occlusion have been reported, including intra-operative portal branch ligation,\(^8-10\) transileocolic PVE,\(^11-13\) and the percutaneous transhepatic ipsilateral\(^14,15\) or contralateral\(^16,17\) PVE technique. The underlying principle is to block the portal venous blood flow to the liver segments containing the tumor which are planned to be resected. This induces atrophy of the ipsilateral liver segments and compensatory hypertrophy of the contralateral liver segments, resulting in increase of the size of the FRL. In this end, different embolization materials are used clinically, e.g. polyvinyl alcohol particles (PVA), coils, gelatin sponge, n-butyl cyanoacrylate and lipiodol, or fibrin glue.

Many studies have been published on the effects of PVE on hypertrophy of the FRL in small and larger patient cohorts. However, only few data have been published on the difference between the use of different embolization materials and the effect of chemotherapy or pre-existing liver cirrhosis on the growth of the FRL after PVE. In 2008, a meta-analysis was published by Abulkhir et al., reviewing all publications on PVE between 1990 and 2005.\(^8\) They focused especially on the differences in accession technique (transhepatic vs. transileocolic) regarding the ensuing hypertrophy response and surgical outcome. However, with the growing availability of radiological intervention suites, the percutaneous transhepatic technique has become the standard technique for PVE. In addition, many new articles on PVE have been published since.

In this review, we systematically evaluated the publications on PVE in the last 20 years, with special interest in the influence of chemotherapy, pre-existing liver cirrhosis, cholestasis, and the use of different embolization materials on the hypertrophy response.
Methods

Search strategy
A systematic literature search was performed in Medline, Cochrane, and Embase, from January 1st 1990 to May 1st 2011. The applied search heading was: “portal vein embolization”, limited to clinical studies including at least 10 cases, published in the English language. Titles and abstracts were screened to identify potentially relevant articles. Referred and related articles were also checked. Articles were selected following the selection criteria and were independently evaluated by two of the authors (vL, vdE), using a scoring list. The final selection was made in consensus.

Selection criteria
All clinical studies on PVE were included for further analysis. Full text articles were retrieved and were included if they contained information on patient characteristics, indication for PVE, pre- and post-PVE liver volumes or percentages of the FRL, the technique, results, and complications of PVE, as well as results of liver surgery. After the initial search, many articles were excluded, because they were written in a non-English language, were reports about portal vein ligation, were animal studies, were articles concerning chemo-embolization, or were review articles. Furthermore, articles were excluded when patient characteristics, indications, methods, and results were not adequately described or when the FRL data were not sufficient and could not be extracted from the published data. Articles which overlapped with previously published data, which were published by the same author or when overlap with patient cohorts from the same study group or combined study groups was suspected, were excluded.

Study quality
All included studies were checked on study quality characteristics by 2 reviewers (vL, vdE) independently. Study quality was assessed using an adapted version of a checklist of the Dutch Cochrane Centre. The main points of interest included (1) patient characteristics, (2) indication for PVE, (3) embolization technique, (4) data on CT volumetry, and (5) follow-up. Articles were valid and used for data extraction if most of the above mentioned points were described clearly.

Data extraction
Data extraction included patient characteristics (number of patients, age, sex, type of liver tumor, liver fibrosis, chemotherapy), PVE indication (minimal percentage FRL based on CT volumetry data or indocyanin green (ICG) clearance), PVE technical approach (transileocolic, transhepatic ipsilateral, transhepatic contralateral), embolization materials used (PVA, gelatin sponge, n-butyl cyanoacrylate, fibrin glue, ethanol, coils, vascular plug, or a combination), PVE success rate (successful occlusion of the portal vein), post-PVE complications and morbidity, and postoperative complications, morbidity and mortality.
We had special interest in the effect of used embolization materials, cirrhotic/fibrotic liver parenchyma (HCC vs. non-HCC), chemotherapy, cholestasis (colorectal metastases (CRM) vs. cholangiocarcinoma), and additional embolization of segment IV branches on the hypertrophy response.

**Table 1. Underlying Pathology**

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Nr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal metastasis</td>
<td>709 (39.6%)</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>516 (28.8%)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>365 (20.4%)</td>
</tr>
<tr>
<td>Gallbladder carcinoma</td>
<td>164 (9.2%)</td>
</tr>
<tr>
<td>Other (NET, angiosarcoma, cystadenocarcinoma)</td>
<td>32 (1.8%)</td>
</tr>
<tr>
<td>Benign (adenoma, hemangioma)</td>
<td>5 (0.3%)</td>
</tr>
</tbody>
</table>

**Results**

The broad initial search using the search heading “portal vein embolization” resulted in 961 publications. Primary survey of the abstracts and articles excluded 684 articles dealing with subjects other than PVE, experimental animal studies or articles in a non-English language.

After critical evaluation of the remaining full text articles, 84 articles remained for the final scoring, using an item-list with the minimum requirements for final inclusion. Finally, 44 publications were included for meta-analysis (Figure 1), consisting of 1791 patients including 1139 men (63.6%) and 617 women (34.5%). The sex of the remaining 35 patients was not extractable for the articles. The mean age was 61 ± 4.1 years. The underlying pathology is summarized in Table 1.

**Indications for PVE**

The indication for PVE, varied in literature, but the percentage of the FRL was mainly used as the criterion for PVE. A resection of more than 70-75% of the total liver volume in normal livers, and more than 60-65% in compromised livers (i.e. cirrhosis, fibrosis) was mainly the threshold for performing preoperative PVE. Three studies used the ICG plasma disappearance rate or retention rate at 15 minutes. A 15 minutes retention rate of more than 15-20% in combination with a large liver resection constituted an indication for PVE.
In the pre-procedural work-up computed tomography (CT)-scans are performed to measure the volumes of the total liver, the part that is planned to be resected, total tumor volume and the FRL. In most studies (30/44, 68.2%) the absolute volumes are used to calculate the percentage FRL\(^5,9,11,12,14-17,24-47\):

\[
\% \text{FRL} = \frac{\text{FRL-V}}{\text{TLV} - \text{TV}} \times 100\%
\]

In the other studies, (14/44, 31.8%) the total estimated volume (TELV) is calculated using CT-volumetry in combination with the body surface area, in the equation:

\[
\text{calTL-V} = -794.41 + 1267.28 \times \text{BSA},
\]

as previously described\(^4,8,3,11,12,14-17,24-47\), or using the standardized FRL (sFRL) with was calculated by dividing FRL-V (measured by CT volumetry) by total liver volume (\(^\text{TL-V}\)) which was calculated using a formula described by Vauthey et al.\(^49\):

\[\text{TL-V} = -794.41 + 1267.28 \times \text{BSA}, \text{ with } \text{BSA} = \sqrt{\text{height (cm)} \times \text{weight (kg)}} / 3600\]
Table 2. Technique of PVE

<table>
<thead>
<tr>
<th>Procedural approach:</th>
<th>No. of procedures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transileocolic</td>
<td>223 (12.5%)</td>
</tr>
<tr>
<td>Transhepatic</td>
<td></td>
</tr>
<tr>
<td>ipsilateral</td>
<td>963 (53.8%)</td>
</tr>
<tr>
<td>contralateral</td>
<td>605 (33.8%)</td>
</tr>
<tr>
<td>Embolized branches:</td>
<td></td>
</tr>
<tr>
<td>Segment 5-8</td>
<td>1430 (79.9%)</td>
</tr>
<tr>
<td>Segment 4-8</td>
<td>209 (11.7%)</td>
</tr>
<tr>
<td>Segment 1-4</td>
<td>41 (2.3%)</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>111 (6.2%)</td>
</tr>
</tbody>
</table>

PVE technique

PVE is performed using a transileocolic or transhepatic approach (Table 2). The transileocolic approach needs a mini-laparotomy or can be performed as part of a two-stage resection. Using the transhepatic approach, the procedure can be performed by ipsilateral or contralateral puncture.

The embolization materials mainly used for PVE were PVA, gelatin sponge, fibrin glue, n-butyl cyanoacrylate with lipiodol, polidocanol-foam, or combinations of these materials with coils or Amplatzer vascular plugs (Table 3). Gelatin sponge and n-butyl cyanoacrylate were used the most in the evaluated studies (59.5%), often in combination with other materials.

Success rate of PVE and its effect on the hypertrophy response

The mean technical success rate of the PVE procedures was 99.1% (range 86%-100%). Reasons for failure were the impossibility of canulating the portal system,17,35,43,50 because of difficult altered portal anatomy caused by the tumor mass or unexpected thrombosis of the portal system due to tumor progression/invasion.17,26,50,51

Hypertrophy response

The growth of the FRL as a result of PVE can be calculated /expressed in two ways:
1. The difference in FRL volume before and after embolization in relation to the FRL volume before embolization (percentage volume increase):

   \[
   \% \text{FRL volume increase} = \frac{\% \text{FRL post-PVE} - \% \text{FRL pred-PVE}}{\% \text{FRL pred-PVE}} \times 100\%
   \]
2. The difference between the percentage FRL, before and after embolization (in literature referred to degree of hypertrophy (DH)):

\[
DH = \frac{\%FRL_{pre-PVE} - \%FRL_{post-PVE}}{\%FRL_{post-PVE}}
\]

When available, the percentage FRL volume increase was extracted from the article, otherwise it was calculated from the available data. The mean time-interval between PVE and the follow-up CT-scan was 25.9 ± 10.1 days (range 14-42 days).
The mean increase of the FRL volume was 37.9 ± 0.1% (20.5-69.4%).

Table 3. Embolization materials used

<table>
<thead>
<tr>
<th>Embolization materials</th>
<th>No. of patients</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA particles + coils</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>PVA + alcohol</td>
<td>3</td>
<td>14.7</td>
</tr>
<tr>
<td>PVA + Amplatzer vascular plug</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Gelatin sponge + lipiodol</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Gelatin sponge + coils</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Gelatin sponge + thrombin + urografin</td>
<td>102</td>
<td>26.3</td>
</tr>
<tr>
<td>Gelatin sponge + urografin</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Gelatin sponge + polidocanol</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Gelatin sponge + Amplatzer vascular plug</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Fibrin glue/Beriplast + lipiodol</td>
<td>177</td>
<td>9.9</td>
</tr>
<tr>
<td>N-butyl cyanoacrylate + lipiodol</td>
<td>554</td>
<td></td>
</tr>
<tr>
<td>N-butyl cyanoacrylate + gelatin sponge</td>
<td>11</td>
<td>32.5</td>
</tr>
<tr>
<td>N-butyl cyanoacrylate + Amplatzer vascular plug</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Embol-78</td>
<td>51</td>
<td>2.8</td>
</tr>
<tr>
<td>Ethanol + lipiodol</td>
<td>159</td>
<td>10.2</td>
</tr>
<tr>
<td>Ethanol + gelfoam + lipiodol</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Ethoxyacrol/air-foam</td>
<td>30</td>
<td>1.8</td>
</tr>
<tr>
<td>Ethiblock + lipiodol</td>
<td>33</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Influence of several variables on the hypertrophy response

A meta-analysis on the variables influencing the hypertrophy response was not possible because of inhomogeneity of the studies, and a limited number of articles within the subgroups (Table 4 and 5). Insufficient data were available to make a strong comparison between the effect of right PVE and additional embolization of segment 4 branches on the hypertrophy response. The same applies to the effect of cholestasis. For evaluation the effect of chemotherapy and cirrhosis/fibrosis on hypertrophy enough studies are available for a head-to-head comparison. Both variables seem to have no influence on the hypertrophy response. Table 6 shows studies which used only a single embolization material. There seems to be a trend that the use of n-butyl cyanoacrylate results in a greater %FRL volume increase compared to gelatin sponge, fibrin glue, and PVA.

Table 4. Influence of chemotherapy on the hypertrophy response

<table>
<thead>
<tr>
<th>Article</th>
<th>No. of patients</th>
<th>Chemo/non-chemo</th>
<th>%FRL chemo</th>
<th>%FRL non-chemo</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covey</td>
<td>100</td>
<td>43 / 57</td>
<td>22</td>
<td>26</td>
<td>Not known</td>
</tr>
<tr>
<td>Nafidi</td>
<td>20</td>
<td>13 / 7</td>
<td>45.8</td>
<td>41.2</td>
<td>NS</td>
</tr>
<tr>
<td>Ribero</td>
<td>112</td>
<td>28 / 80</td>
<td>9.0 (DH)</td>
<td>8.5 (DH)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=not significant in the studies. DH=degree of hypertrophy.

Table 5. Influence of cirrhosis/fibrosis on the hypertrophy response

<table>
<thead>
<tr>
<th>Article</th>
<th>No. of patients</th>
<th>Cirrhosis/non-cirrhosis</th>
<th>%FRL cirrhosis</th>
<th>%FRL non-cirrhosis</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotroneo</td>
<td>31</td>
<td>7 / 24</td>
<td>32.1</td>
<td>44.2</td>
<td>Not known</td>
</tr>
<tr>
<td>Farges</td>
<td>27</td>
<td>14 / 13</td>
<td>24.4</td>
<td>41.6</td>
<td>Not known</td>
</tr>
<tr>
<td>Ko</td>
<td>51</td>
<td>22 / 29</td>
<td>38.4</td>
<td>46.0</td>
<td>Not known</td>
</tr>
<tr>
<td>Lee</td>
<td>29</td>
<td>19 / 10</td>
<td>25.4</td>
<td>39.4</td>
<td>Not known</td>
</tr>
</tbody>
</table>

Complications after PVE

Fifteen articles lacked a detailed description of complications encountered after embolization. From the other 29 studies (1248 patients), the complication rates are summarized in Table 7. The only study describing PVE-related mortality has been published by Giraudo et al. In a group of 146 patients, one patient died 20 days after PVE due to lethal pulmonary embolism. However, no embolization material was detected in the lung. A second patient, who was admitted with cholangitis, died of septic shock 39 days after PVE. All other studies reported no PVE-related mortality, resulting in a mortality rate of 0.1%.
Liver resection
The mean period between PVE and liver surgery was 36.9 days (range 21 – 84 days). The type of operative procedures are summarized in Table 8. In more than 70%, a right hemihepatectomy or extended hemihepatectomy was performed. From the originally planned liver resections after PVE, 19.8% was cancelled. This was mainly because of tumor progression or peritoneal spread seen during operation, but in some cases due to insufficient FRL volume, despite PVE.

Table 6. Influence of embolization material on the hypertrophy response

<table>
<thead>
<tr>
<th>Embolization material</th>
<th>Article</th>
<th>No. of patients</th>
<th>% Increase FRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVAc</td>
<td>Esschert</td>
<td>10</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>Libicher</td>
<td>10</td>
<td>26.4</td>
</tr>
<tr>
<td></td>
<td>Covey</td>
<td>100</td>
<td>24.3</td>
</tr>
<tr>
<td>Gelatin sponge</td>
<td>Fujii</td>
<td>30</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>Imamura</td>
<td>84</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>Kakizawa</td>
<td>14</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>Kim</td>
<td>17</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>Kusaka</td>
<td>18</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>Makuuchi</td>
<td>54</td>
<td>37.9</td>
</tr>
<tr>
<td></td>
<td>Nanashima</td>
<td>30</td>
<td>29.4</td>
</tr>
<tr>
<td></td>
<td>Sugawara</td>
<td>66</td>
<td>35.8</td>
</tr>
<tr>
<td>N-butyl cyanoacrylate</td>
<td>Baere</td>
<td>107</td>
<td>57.8</td>
</tr>
<tr>
<td></td>
<td>Barbaro</td>
<td>26</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Capussotti</td>
<td>31</td>
<td>48.5</td>
</tr>
<tr>
<td></td>
<td>Elias</td>
<td>68</td>
<td>59.1</td>
</tr>
<tr>
<td></td>
<td>Girudo</td>
<td>146</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>Sirichindakul</td>
<td>29</td>
<td>27.5</td>
</tr>
<tr>
<td></td>
<td>Broering</td>
<td>17</td>
<td>69.4</td>
</tr>
<tr>
<td>Fibrin glue</td>
<td>Liem</td>
<td>15</td>
<td>31.4</td>
</tr>
<tr>
<td></td>
<td>Nagino</td>
<td>105</td>
<td>27.4</td>
</tr>
</tbody>
</table>
In 52 patients (2.9%) surgery was not performed because of failure of PVE. In 35 patients (1.9%) the hypertrophy response was insufficient to perform the resection, although the embolization procedure was successful. In the other 17 cases, 10 did not technically succeed (0.6%) and 7 caused a complication leading to non-resectability (0.4%). These complications consisted of severe cholangitis, large abscesses and sepsis, portal venous or mesenterico-portal venous thrombosis.

Complications after surgery can be divided into major and minor complications. Major complications were defined as complications that required surgical treatment and/or lead to prolonged hospital stay. Minor complications were defined as complications that could be treated conservatively, not leading to prolonged hospital stay.

Table 7. Complications after PVE

<table>
<thead>
<tr>
<th>Minor complications</th>
<th>% of total of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>36.9</td>
</tr>
<tr>
<td>Elevation of transaminase</td>
<td>34.8</td>
</tr>
<tr>
<td>Abdominal discomfort / pain</td>
<td>22.9</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>2.0</td>
</tr>
<tr>
<td>ileus</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Major complications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal thrombosis</td>
<td>0.8</td>
</tr>
<tr>
<td>Migration of embolization material</td>
<td>0.6</td>
</tr>
<tr>
<td>Liver hematoma</td>
<td>0.4</td>
</tr>
<tr>
<td>Infection / abcess</td>
<td>0.4</td>
</tr>
<tr>
<td>Bile leakage</td>
<td>0.3</td>
</tr>
</tbody>
</table>

In 11 publications, a detailed description of the postoperative complications after resection was lacking. In the other 33 articles (1210 patients), the overall morbidity was 21.7%. Major and minor complications are given in Table 9. The overall mortality after liver resection was 3.3%. Primary liver failure (0.4%) or liver failure in combination with multiple organ failure (1.2%) caused death in 50% of the cases. Other causes were myocardial infarction (0.1%), sepsis (0.2%), abdominal/liver bleeding (0.2%), multiple organ failure (0.4%), cholangitis (0.1%) or unknown cause (0.4%).
Table 8. Surgical procedures

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right hemihepatectomy</td>
<td>774</td>
<td>43.2</td>
</tr>
<tr>
<td>Extended right hemihepatectomy</td>
<td>516</td>
<td>28.8</td>
</tr>
<tr>
<td>Left hemihepatectomy</td>
<td>21</td>
<td>1.2</td>
</tr>
<tr>
<td>Extended left hemihepatectomy</td>
<td>45</td>
<td>2.5</td>
</tr>
<tr>
<td>Right trisegmentectomy</td>
<td>38</td>
<td>2.1</td>
</tr>
<tr>
<td>Other (central resection, segmentectomy)</td>
<td>43</td>
<td>2.1</td>
</tr>
<tr>
<td>No resection/not described</td>
<td>354</td>
<td>19.7</td>
</tr>
</tbody>
</table>

Table 9. Complications after surgery

<table>
<thead>
<tr>
<th>Complication</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major complications</td>
<td>10.4%</td>
</tr>
<tr>
<td>Liver failure</td>
<td>5.5%</td>
</tr>
<tr>
<td>Portal thrombosis</td>
<td>0.1%</td>
</tr>
<tr>
<td>Bile leakage</td>
<td>3.1%</td>
</tr>
<tr>
<td>Abdominal/liver bleeding</td>
<td>1.0%</td>
</tr>
<tr>
<td>Cholangitis</td>
<td>0.2%</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>0.1%</td>
</tr>
<tr>
<td>Multiple organ failure</td>
<td>0.4%</td>
</tr>
<tr>
<td>Minor complications</td>
<td>11.3%</td>
</tr>
<tr>
<td>Ascitis</td>
<td>2.6%</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>2.9%</td>
</tr>
<tr>
<td>Abscesses</td>
<td>1.8%</td>
</tr>
<tr>
<td>Urine tract infection</td>
<td>0.9%</td>
</tr>
<tr>
<td>Wound infection</td>
<td>2.0%</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1.1%</td>
</tr>
</tbody>
</table>
Discussion

Since the first publication on PVE in a human being by Kinoshita in 1986, there are many articles published on this subject in the last decennia. Although redistribution of portal blood flow was initially thought to be the cause of hypertrophy, more recent studies aim that PVE induces an increase in hepatic growth factor (HGF) and transforming growth factor (TGF-α and -β), which contribute to the hypertrophy of the non-embolized lobe. The exact mechanism behind the atrophy of the embolized lobe and the hypertrophy of the FRL is however still unknown.

New techniques have been developed, and new embolization materials have been used and tested. The results of PVE and its role in the management of liver malignancies are mainly based on small or larger case series. There are no randomized controlled trials examining the efficacy of PVE. There is only 1 meta-analysis published on PVE. This meta-analysis mainly focused on the differences between the surgical transileocolic (TIPE) and the percutaneous transhepatic (PTPE) technique and their complications. The increase in FRL was significantly higher in PTPE than in TIPE. However, there was no difference in major complications. An advantage of the TIPE, is the combination with diagnostic laparoscopy and laparoscopic ultrasound, which can be performed in the same procedure and can provide additional information concerning the accurate staging of the malignancy, preventing unnecessary laparotomies for liver resection. In the context of a two-step procedure in which two partial liver resections are carried out with an interval of 4-6 weeks, open surgical techniques like portal vein ligation or the transileocolic PVE can be very useful. However, with the increasing availability of radiological intervention suites, the percutaneous transhepatic technique has become the standard technique for PVE. Another advantage of the percutaneous technique is a significant greater hypertrophy response after PVE compared to PVL and a shorter hospital stay.

The majority of PVE procedures have been performed by a percutaneous transhepatic technique (87.5%); in 62% by an ipsilateral and in 38% by contralateral approach. There are many factors that influence the choice of approach of the portal venous system, i.e. the anatomy of the liver in a specific patient, tumor burden, the preference of the operator, the experience of the operator with a specific technique and the use of a specific embolization material. Using the ipsilateral approach has the advantage of not puncturing the healthy FRL tissue and therefore, reducing the risk of complications like portal vein thrombosis, dissection, or subcapsular hematoma of the FRL. However, reverse-curved catheters or multiple lumen balloon occlusion catheters are usually necessary depending on the embolization material used. Additional embolization of the segment 4 branches is often easier working via the ipsilateral approach. The contralateral approach is easier in catheterization of the right portal branches and delivering the embolization material in the direction of the portal flow. This reduces the chance of migration of embolization material in the portal branches of the FRL.
This review could not extract enough data to evaluate the differences in complications of the ipsilateral or contralateral approach. However, studies of Ribero et al. and Di Stefano et al., evaluating complications of respectively the ipsilateral and contralateral approach, showed almost the same types of complications and no significant difference in complication rates.

As postoperative liver failure is an important complication after liver resection, with a high risk of mortality, it is of extreme importance to calculate the percentage of FRL in order to assure enough functional liver tissue left after resection. The importance of the size of the FRL is stressed by Ribero et al. They showed that both a small FRL and degree of hypertrophy (DH) are strongly associated with postoperative hepatic dysfunction. The most commonly used technique (69%) to estimate the volume of the FRL is to calculate the absolute volumes using CT volumetry. With this method, only volumetric information can be obtained. Urata et al. introduced an alternative method to calculate the total standardized liver volume (SLV) making use of an equation containing the BSA as a result of the relation between body weight and body length. No significant difference between standardized liver volume (SLV) and CT-estimated liver volume was seen. Functional information is obtained by the ICG plasma disappearance and retention rate test at 15 min. This technique, introduced in 1980, can accurately estimate post-resection remnant liver function. According to the literature, only few authors, mainly Japanese, have used this method to select patients for preoperative PVE. Another method of monitoring FRL function after PVE, is 99mTc-labelled mebrofenin hepatobiliairy scintigraphy (HBS) with single photon emission tomography (SPECT). With this technique functional information of the whole liver and the FRL is obtained. De Graaf et al. showed that the increase of FRL function exceeded the increase of FRL volume, suggesting that the necessary waiting time until resection could be shorter than indicated by volumetric parameters alone. Most studies use a FRL volume of 25-30% of the original liver volume as a threshold to select patients for PVE when no compromised liver function is expected. In patients with a compromised liver function, such as in post-chemotherapy steatosis, liver cirrhosis/ fibrosis and long lasting cholestasis, a threshold of 35-40% is preferred as minimum FRL volume. Worldwide there is a consensus on this indication. However, in patients with a compromised liver function, determining the minimally required FRL volume can be challenging. Quantitative, functional tests as HBS could play an important additional diagnostic role in these cases as these correlate functional information with volumetric data.

There is no consensus regarding the optimal waiting time between PVE and liver resection. We found a wide range of time-intervals between PVE and the follow-up CT-scan, i.e. 14 - 42 days, with a mean of 25.9 ± 10.1 days. A longer time interval allows extra growth of the FRL. However, volumetric data presented by Ribero et al. show that after the initial hypertrophy in the first 3 weeks, a plateau phase is reached. This is confirmed by the study of Nagino et al. Additionally, there is the issue of induction of
tumor growth by PVE. Clinical studies demonstrate that tumor progression after PVE is possible in both the embolized and non-embolized liver segments. However, accurate data regarding the risk of tumor progression after PVE are currently not available.65 The time between PVE and liver resection should be limited to avoid tumor growth. Furthermore, sequential transarterial chemo-embolization and PVE can be performed, particularly in patients with HCC, in order to avoid tumor growth. Post-PVE chemotherapy is another option in patients with CRM. Beal et al. reported a reduction in tumor size in 6 of the 10 patients having chemotherapy compared to tumor growth in 4 of the 5 patients without chemotherapy. However, they also observed less hypertrophy of the FRL in patients who received chemotherapy in the weeks between PVE and resection.26 A few large studies show no significant difference in increase of the FRL volume after PVE in patients who previously used or did not use chemotherapy.14,40,69

Many different embolization materials have been applied for PVE. The combination of n-butyl cyanoacrylate and lipiodol and PVA particles in combination with coils are non-resorbable materials which lead to a persistent occlusion of the portal branches preventing peripheral recanalization. As gelatin sponge is resorbable, portal recanalization is frequently seen, sometimes already 2 weeks after PVE.6,70 PVA particles are easy to use and provide permanent occlusion in the periphery. Little inflammatory reaction of the liver tissue is seen. The use of n-butyl cyanoacrylate requires more experience of the radiologist, because delivery must be very precise to prevent embolization of non-targeted branches. With the right delivering catheters, procedure time can be decreased. N-butyl cyanoacrylate induces a strong inflammatory reaction, making surgical resection sometimes more difficult. Ethanol is also an effective embolization material, but is not well tolerated by patients and therefore not very often used. Large clinical studies, directly comparing the effect of different embolization materials on the hypertrophy response are lacking. Comparison of the data in this review shows that the use of n-butyl cyanoacrylate seems to result in a higher % FRL volume increase.

Both the overall technical success of PVE (99.1%) and clinical success rate (97.4%) is very high. Only 1.6% of the patients could not undergo a liver resection because of insufficient hypertrophy. Suggested reasons for insufficient hypertrophy after successful PVE, are recanalization of the embolized portal branches, activation of underlying liver disease and the presence of major portal hypertension with portosystemic shunting.24 Only 0.4% of patients is unresectable because of PVE related complications, such as a large subcapular hematoma, portal thrombosis, biliary or infectious complications in the FRL after a contralateral procedure. Overall complication rates are higher, but these complications rarely needed treatment and they seldomly lead to unresectability.

In conclusion, preoperative PVE is an effective method to increase the FRL volume with a high technical and clinical success rate. Pre-existing liver damage due to cirrhosis, cholestasis, or chemotherapy seems to have no influence on the hypertrophy response. However, the use of n-butyl cyanoacrylate seems to result in a greater hypertrophy response compared to the other materials used.
References

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Volumetric and functional recovery of the remnant liver after major liver resection with prior portal vein embolization

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K.P. van Lienden
O.R. Busch
M. Heger
O.M. van Delden
D.J. Gouma
R.J. Bennink
J.S. Laméris
T.M. van Gulik
Abstract

Background: Portal vein embolization is an accepted method to increase the future remnant liver preoperatively. The aim of this study was to assess the effect of preoperative portal vein embolization on liver volume and function 3 months after major liver resection.

Materials and methods: This is a retrospective case-control study. Data were collected of patients who underwent portal vein embolization prior to (extended) right hemihepatectomy and of control patients who underwent the same type of resection without prior portal vein embolization. Liver volumes were measured by computed tomography volumetry before portal vein embolization, before liver resection, and 3 months after liver resection. Liver function was assessed by hepatobiliary scintigraphy before and 3 months after liver resection.

Results: Ten patients were included in the embolization group and 13 in the control group. Groups were comparable for gender, age, and number of patients with a compromised liver. The mean future remnant liver volume was 33.0±8.0% prior to portal vein embolization in the embolization group and 45.6±9.1% in the control group (p<0.01). Prior to surgery, there were no significant differences in future remnant liver volume and function between the groups. Three months postoperatively, the mean remnant liver volume was 81.9±8.9% of the initial total liver volume in the embolization group and 79.4±11.0% in the control group (p>0.05). Remnant liver function increased up to 88.1±17.4% and 83.3±14% respectively of the original total liver function (p>0.05).

Conclusion: Preoperative portal vein embolization does not negatively influence postoperative liver regeneration assessed 3 months after major liver resection.
Introduction

Portal vein embolization (PVE) has been widely accepted as an effective means to increase the future remnant liver volume (FRLV) in patients requiring extensive liver resection. The safety and efficacy of PVE have been confirmed by several studies and a recent meta-analysis. PVE induces atrophy of the ipsilateral liver segments with concomitant compensatory hypertrophy of the future remnant liver (FRL). Preoperative PVE is recommended when the FRLV is less than 30–40% of the total liver volume (TLV) as determined by computed tomography (CT) volumetry, depending on the presence of underlying liver disease (e.g., steatosis, cholestasis). Liver regeneration is generally assessed by CT volumetry. Liver volume, however, does not necessarily represent liver function during liver regeneration. Liver function can accurately be assessed by technetium-99m mebrofenin hepatobiliary scintigraphy (99mTc-mebrofenin HBS). The underlying mechanism of liver regeneration after partial liver resection or PVE is not fully understood. One suggested trigger for regeneration of the nonembolized liver lobes after PVE or resection is the instant increase in portal blood flow to the FRL. When right PVE is performed, the portal blood flow is preoperatively diverted to the left liver lobes. As a consequence, minimal changes in portal blood flow are induced at the time of partial liver resection and therefore, this trigger for posthepatectomy liver regeneration is lacking. Our hypothesis is therefore that preoperative PVE might hamper postoperative liver regeneration. The aim of this study was to evaluate the effect of preoperative PVE on postoperative liver volume and function 3 months after major liver resection.

Materials and methods

Patients

Eighteen patients underwent PVE of the right portal system prior to (extended) right hemihepatectomy at our institution between January 2005 and November 2007. Only those patients in whom a complete set of CT scans was obtained were included in the study, i.e., a four-phase CT scan prior to PVE, 3–4 weeks after PVE (before liver resection), and 3 months after liver resection (n=10). In all the patients, HBS was performed before PVE and in nine patients 3 months after liver resection. Patients who had undergone (extended) right hemihepatectomy without prior PVE in the same period and of whom a CT scan had been obtained prior to and 3 months after liver resection were included in the control group (n= 13). Twelve of the 13 patients underwent HBS prior to PVE, which was repeated 3 months after liver resection in 11 patients. Patient characteristics, including gender, age, and number of patients with a compromised liver were compared for both groups.
Indications for surgery in the control group were colorectal metastasis (n=5), hilar cholangiocarcinoma (n=4), hepatocellular carcinoma (n=1), and other metastases (n=3). In the PVE group, the indications were colorectal metastasis (n=5), hilar cholangiocarcinoma (n=1), hepatocellular carcinoma (n=3), and neuroendocrine tumor (n=1). Postoperative complications were subdivided into “minor” (grades I and II) or “major” (grades III, IV, V) according to the revised 2004 Clavien classification.

CT volumetry
Liver volumes were measured using CT. The total liver, the FRL, and tumor mass were manually delineated on each 5mm slide of the portal phase images. The TLV, tumor volume (TV), and FRLV were calculated using dedicated software (MxView 3.52, Philips Medical Systems, The Netherlands; Figure 1). The percentage FRLV before PVE was calculated by:

$$\% \text{FRLV}_{\text{pre-PVE}} = \left( \frac{\text{FRLV}_{\text{pre-PVE}}}{(\text{TLV} - \text{TV})_{\text{pre-PVE}}} \right) \times 100\%$$

To obtain the percentage, FRLV after PVE was computed by:

$$\% \text{FRLV}_{\text{pre-op}} = \left( \frac{\text{FRLV}_{\text{pre-op}}}{(\text{TLV} - \text{TV})_{\text{pre-PVE}}} \right) \times 100\%$$

The remnant liver volume (RLV) 3 months after liver resection was calculated as a percentage of the initial total functional liver volume (TLV-TV):

$$\% \text{RLV}_{\text{months}} = \left( \frac{\text{RLV}}{(\text{TLV} - \text{TV})_{\text{pre-PVE}}} \right)$$

Figure 1. CT cross section of the liver showing total liver (white delineation) and the future remnant liver (grey delineation). CT volumetry showed that the future remnant liver was markedly increased 3 weeks after portal vein embolization (pre-op, 507 ml) compared to before portal vein embolization (pre-PVE, 392 ml). Three months after partial liver resection, the remnant liver volume almost reached its original total liver volume.
Hepatobiliary scintigraphy

HBS was performed using $^{99m}$Tc-mebrofenin as previously described. Briefly, after injection of 85 MBq of $^{99m}$Tc-mebrofenin (Bridatec; GE-Amersham Health), dynamic images were acquired with a γ-camera (Diacam, Siemens, Milwaukee, WI, USA) for 60 min. During the first 10 min, 60 frames of 10 s were acquired (liver uptake phase) followed by 50 frames of 1 min (liver excretion phase). Total hepatic $^{99m}$Tc-mebrofenin uptake rate was calculated as described by Ekman et al. On preoperative scan, regions of interest (ROIs) were drawn around the total liver, the heart (serving as blood pool), and the total field of view. From these ROIs, three time–activity curves were generated. The total hepatic $^{99m}$Tc-mebrofenin uptake rate, representing total liver function (TLF), was calculated as percent per minute (of the injected dose) based on these three parameters. Calculations of the hepatic $^{99m}$Tc-mebrofenin uptake rate were performed using measured values obtained between 150 and 350 s post injection to ensure that hepatic uptake calculations were performed during a phase of homogenous distribution of the agent in the blood pool, before occurrence of the rapid phase of hepatic excretion. To compensate for differences in individual metabolic requirements, the TLF was divided by the body surface area and expressed as percent per minute per square meter.

Portal vein embolization

PVE was performed in patients in whom the estimated FRLV, based on CT volumetry, was <30% in case of normal liver parenchyma and <40% in patients with compromised liver parenchyma due to steatosis, cholestasis, or fibrosis. PVE was performed using the ipsilateral percutaneous transhepatic approach. After retrograde catheterization via a peripheral portal branch (segment 6 or 7), the right portal trunk and intrahepatic tributaries were occluded using a combination of polyvinyl alcohol particles (300–500 μm, Cook, Bloomington, IN, USA) and platinum coils of various sizes (Tornado embolization microcoil, Cook).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD). An independent sample t test was performed to assess the difference in future remnant liver volume and function between the two groups prior to surgery. A mixed analysis of variance was conducted to assess whether there were PVE and time differences in CT volumetry and HBS outcomes between the two groups after liver surgery. The correlation between variables was tested using the Pearson correlation coefficient r. All tests were two-tailed and differences were evaluated at the 5% level of significance.
Patient characteristics are shown in Table 1. There were no significant differences between the two groups with respect to gender, age, and number of patients with a compromised liver. The FRLV was based on the actual removed part of the liver. Prior to resection, the %FRLV was calculated taking into account the maximum volume of liver that would need to be resected to achieve complete removal of all lesions. In some patients, the extent of the resection was less than expected based on intraoperative findings, resulting in a higher %FRLVpre-PVE.

The %FRLVpre-PVE was 33.0±8.0% in the PVE group compared to a %FRLVpre-op 45.6±9.1% in the control group (p=0.002). Three to 4 weeks (mean 23 days) after PVE, the %FRLVpre-op increased to 41.6±9.5%, resulting in no significant difference between the two groups prior to liver resection (p=0.33). Liver scintigraphy showed a mean 99mTc-mebrofenin uptake rate in the total liver of 7.90± 1.5%/min/m² in the control group and 7.11±1.6%/min/m² in the PVE group before any intervention (p=0.24).

The increase in percentage remnant liver volume from preoperatively to 3 months after major liver surgery was not different between the two groups (p=0.81). Three months after surgery, the mean RLV in the PVE group was 81.9±8.9% of the initial total liver volume compared to 79.4±11.0% in the control group (p=0.57; Table 1; Figure 2). In
addition, the postoperative increase in liver function did not differ between both groups (p=0.471). Three months postoperatively, the RLF regained 88.1±17.4% of the original total liver function in the PVE group compared to 83.3±14% in the control group (p=0.50; Figure 3). No correlation was found between liver volume and function (r=0.13, p=0.59).

Figure 2. CT volumetry data. Mean percentage of (future) remnant liver volume (FRLV) in relation with initial total functional liver volume. Prior to PVE (pre-PVE), this percentage was significantly lower in the group requiring PVE (*p<0.01). Three to 4 weeks after PVE (pre-op), the FRLV increased with 8.7% in the PVE group, leading to comparable values in the two groups. Three months after partial liver resection (3m post-op), remnant liver volumes reached approximately 80% of initial total functional liver volume in both groups.

Figure 3. Uptake of 99mTc-mebrofenin by the total liver prior to any intervention and 3 months after partial liver resection. There were no significant differences in uptake between the PVE and the control groups at both time points. The remnant liver function reached 88.1% and 83.3%, respectively, of the original total liver function in both groups (p=0.50).
Discussion

The main goal of this study was to evaluate the influence of PVE on volumetric and functional liver regeneration after major liver resection. CT volumetry was performed prior to PVE and surgery. The increase of the %FRLV after PVE (%FRLV_{pre-PVE}−%FRLV_{pre-PVE}) was 8.7% in 23 days. In a recent meta-analysis, a mean increase of 11.9% was reported 29 days after PVE. However, results between the various studies are difficult to compare due to substantial differences in the time interval between PVE and subsequent CT volumetry and the different techniques of embolization. For example, Farges et al. observed an increase in FRL of 16% 4-8 weeks after PVE whereas Elias et al. reported an increase of 13% 1 month after PVE. Ribero et al. and Madoff et al. showed an increase of 8.8% and 7.7%, 2-8 and 2-4 weeks after PVE, respectively, using a calculation based on body surface area. Three months after partial liver resection, the remnant liver volume regenerated to approximately 80% of its original total volume in both groups. Liver function increased to 83% in the control group and to 88% in the PVE group. There was no correlation between volumetric and functional recovery, confirming the postulation that liver volume does not necessarily reflect liver function during liver regeneration. To our knowledge, there are no studies that compared postoperative liver volume increase and functional increase after partial liver resection in patients with and without prior PVE. Although there could have been a difference in initial regenerative response following liver resection, our results show comparable restoration rates of liver volume 3 months after (extended) hemihepatectomy in both groups. Most data on the process of hepatocyte regeneration have been obtained from animal or in vitro studies. The time course of liver regeneration after PVE and after partial liver resection appears to be similar as has been shown in a rat model. Although various mediators and pathways involved in liver regeneration have been described, the initial trigger of the entire process remains elusive. The instant change in portal blood flow after partial liver resection is believed to be a trigger for liver regeneration. Experimental studies have shown decreased posthepatectomy liver regeneration in rats receiving a portacaval shunt. When performing PVE prior to surgery, the change in portal blood flow is negligible in case of a standard right hemihepatectomy and less profound in case of an extended right hemihepatectomy because the portal blood had already been diverted to the left portal vein at the time of PVE. Our study shows that the liver regenerates up to 80% of its original total liver volume 3 months after major liver resection, in spite of prior PVE. One might speculate that instead of the change in portal blood flow, the change in arterial blood flow after hepatic resection induces liver regeneration. A study in rats showed that ligation of the hepatic artery alone did not affect liver regeneration. However, it is questionable whether the rat model is an appropriate surrogate model for studying the effects of altered hepatic arterial blood flow on liver regeneration or function. It is possible that the hypertrophy response of the
remnant liver is slower after prior PVE in the first weeks after liver resection, but this ultimately did not result in dissimilar liver volumes after 3 months.

**Conclusion**

PVE does not hamper the regenerative capacity of the FRL after partial liver resection. The remnant liver regenerates up to approximately 80% of its initial total liver volume and over 83% of its original total liver function 3 months after major liver resection with or without prior PVE.
References


Controversies in the use of portal vein embolization

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Abstract

Background: Portal vein embolization (PVE) has reached worldwide acceptance to increase future remnant liver (FRL) volume before undertaking major liver resection. The aim of this overview is to point out and discuss current controversies in the application of PVE.

Methods: Review of literature pertaining to techniques of PVE, complications, tumor proliferation, timing of resection, and hypertrophy response after PVE.

Results: Procedure-related complications after PVE include hematoma, hemobilia, overflow of embolization material, and thrombosis of portal vein branch(es) of the non-embolized lobe. Persistence of the embolized, atrophic lobe is usually not harmful. Embolization of the portal branches to segment 4 in addition to embolization of the right portal trunk is controversial and is advised only in selected cases. It remains undecided whether embolization of the portal venous system is more effective in inducing hypertrophy of the FRL than ligation of the portal vein. Accelerated tumor growth after PVE is a major concern and requires consideration of post-PVE chemotherapy. A waiting time of 3 weeks between PVE and liver resection is advised. Post hepatectomy regeneration is not hampered after preoperative PVE.

Conclusion: PVE is a useful preoperative intervention to increase volume and function of the FRL. Further progress awaits clarification of the mechanisms of the hypertrophy response induced by PVE in conjunction with new embolization materials and protective chemotherapy.
Introduction

Preoperative portal vein embolization (PVE) has become a well-established means to upsize the future remnant liver (FRL) in patients considered for extensive liver resection. Although the concept of portal vein occlusion inducing the liver atrophy-hypertrophy complex was described previously in experimental studies, Makuuchi was the first to perform preoperative PVE clinically. His first patient in whom PVE was carried out had a hilar cholangiocarcinoma requiring extended liver resection. Obviously, this was a serendipitous action because a substantial proportion of patients with hilar cholangiocarcinoma present with unilateral portal vein occlusion and a marked atrophy of the affected, ipsilateral side of the liver, while hypertrophy of the contralateral side may be impressive. Instead of the tumor blocking the portal vein branch, the same effect can be achieved using an intervention by which the portal vein branch is occluded. Although PVE now is a widely used interventional procedure to improve the outcome of major liver resection, there are still several controversies and issues which are addressed herein.

Do we really need portal vein embolization?

Although most centers now use PVE, the criteria for preoperative application of PVE are not well defined. PVE is considered when the FRL is found to be too small for sufficient postoperative function. In livers with normal parenchyma as is usually the case in patients with liver metastases, the minimum volume of remnant liver may be 25% based on CT volumetric studies to avoid post-resection liver failure. However, when there is liver parenchymal disease such as in cirrhosis, the minimum volume of remnant liver is rather set at 40 or even 50% depending on liver functional reserve. In a prospective clinical trial from the group of Beaujon Hospital in Paris, patients undergoing standard right hemihepatectomy were randomized to receive preoperative PVE or not. In patients with normal liver, the hypertrophy of the FRL induced by PVE had no beneficial effect on the postoperative course. However, in patients with chronic liver disease, the rate of postoperative complications was significantly reduced after preoperative PVE. These results suggest that PVE is particularly advantageous in patients requiring extended liver resection or in patients with diseased livers. There are also series of partial liver resection in literature showing excellent results of liver resection without using PVE, such as the series published by the Memorial Sloan Kettering group in New York reporting a mixed series of 1,803 patients undergoing partial liver resection. This series included patients with cirrhosis and showed an overall mortality of 3.1% and morbidity of 45%. Hence, as long as the true minimum volume of liver required for safe resection in a normal liver is debatable, the indication for performing PVE in normal livers remains controversial. On the other hand, in patients with normal liver requiring liver resection associated with a major gastrointestinal procedure, PVE is recommended. There is little discussion,
however, that in patients with chronic liver disease or injured livers as a result of cholestasis, steatosis or after chemotherapy, preoperative PVE should be considered to improve the safety of any substantial liver resection.

**What are the disadvantages of PVE?**

When evaluating a patient for major liver resection in whom the future remnant liver is calculated to be marginal, one may tend to stay on the safe side and apply preoperative PVE. Although PVE is considered a safe procedure, a recent meta-analysis of 1,088 patients who had successfully undergone PVE showed an overall morbidity rate of 2.2%, however, with no mortality. Complications reported due to the procedure are hematoma, hemobilia, septic complications, backflow of embolization material and thrombosis of the main portal vein or branch(es) of the portal venous system to the liver segments to be preserved. Obviously, the latter complication has important consequences for the resection plan, oftentimes rendering the patient technically unresectable. From an oncological point of view, enhancement of tumor cell proliferation in both the embolized and nonembolized lobes in the time after PVE are a concern, as addressed below in this overview.

Another uncertainty is the fate of the embolized liver segments when resection is not carried out because of tumor progression or extrahepatic metastases found at laparotomy. In the authors’ experience, there are no long-term adverse effects of the persisting embolized lobe in the presence of the nonembolized lobe, inasmuch as the atrophy-hypertrophy complex stabilizes and overall liver function is maintained. The situation is different, however, in patients with resectable hilar cholangiocarcinoma. These tumors typically require hilar resection with en bloc extended liver resection including the caudate lobe to achieve a R0 resection. Remnant liver volume is particularly critical in these patients because of concomitant cholestasis even when the biliary system on the side of the future remnant liver segments, usually segments 2 and 3, is adequately drained. In these patients, the remnant liver volume ideally is 35-40% often necessitating patients to undergo PVE in order to achieve this volume preoperatively. PVE, however, predetermines the side of the liver to be resected. This situation cannot be changed when on the basis of intraoperative findings during exploration, the resection strategy is reconsidered and the surgical approach is decided to be from the opposite side. Resecting the nonembolized liver and leaving the embolized, atrophic liver, is, of course, not an option. Even when the patient is found to be unresectable, persistence of the embolized liver segments may generate serious problems because the affected bile ducts are usually infected and incompletely drained. These liver segments are prone to develop troublesome abscesses after PVE, while the tumor progresses and additionally occludes the ipsilateral hepatic artery (Figure 1). Under these circumstances, the need for a palliative liver resection may be considered during explorative laparotomy.
What is the most effective technique: portal vein ligation or embolization?

The first question that arises is: What is the most effective method to interrupt portal perfusion of the part of the liver to be resected? Basically, the two modalities are ligation of the portal vein (PVL) or embolization of the portal venous system (PVE), usually of the right hemiliver. Ligation of the right portal vein is performed during laparotomy, when the left FRL is considered too small, or in a two-stage procedure, in which part of the resection on the left side is undertaken with concomitant ligation of the right portal vein, followed by a second resection (right hemihepatectomy) to complete the procedure after regeneration has taken place. Portal vein ligation in this setting is carried out by the surgeon during laparotomy. The surgeon can also choose to cannulate the ileocolic vein and pass a balloon catheter into the right portal venous trunk and embolize the portal system with particles or fibrin glue under fluoroscopic guidance. Alternatively, absolute alcohol can be injected into the right portal vein as a sclerosing agent prior to ligation, with the aim of obliterating the portal venous system in addition to occluding its origin. This combination, however, is reported to be accompanied by fibrosis and severe pain and, therefore, is not used very often anymore. In most centers, nowadays, PVE is performed using the percutaneous, transhepatic approach under local anesthesia. This procedure is undertaken by the interventional radiologist under ultrasound and fluoroscopic guidance. The transhepatic approach obviously requires expertise of an interventional radiologist dedicated to HPB procedures. Access to the portal venous system using the transhepatic approach is obtained either via the contralateral route or via the ipsilateral route. Although the ipsilateral route is technically more demanding, the advantage of this approach is the lower risk of injury or thrombosis of the portal vessels of the future remnant liver. A survey in The Netherlands showed that in...
2006 and 2007, 98 occlusions of the portal vein were performed. Approximately half of the procedures (48/98) were carried out as PVL, whereas the other half underwent transhepatic PVE (50/98). This reflects the initial, relative lack of radiologists being able to perform the procedure, while experience is now expanding and transhepatic PVE has become available in many of the specialized centers.

Intuitively, one would consider PVE more effective because it occludes the entire portal tree on the embolized side, whereas with PVL, only the entry of the portal venous trunk is occluded. A patent portal system distal to the site of ligation would easily enable collateral perfusion from the adjacent, nonoccluded liver segment(s) resulting in retrograde filling of the ipsilateral portal veins. In support of this hypothesis, Wilms et al.1 from Kiel applied PVE and PVL in a pig model and concluded that PVE was the more effective technique to increase the future liver remnant, owing to more durable occlusion of the portal venous branches. The short-range occlusion achieved by PVL resulted in retrograde filling of the portal system, most probably by arterial-portal connections located in the same liver lobes. However, recent (experimental) data show that this is still a controversial issue. In a retrospective clinical study from the group of Beaujon Hospital in Paris, Aussilhou et al.9 reported that right PVL was as effective as PVE to induce hypertrophy of the left liver remnant. However, in this study, PVL was undertaken in the setting of a two-stage liver resection. The combination of PVL with partial liver resection introduces an advantage because of postsectional liver regeneration augmenting post-PVL regeneration. In contrast, using a rat model of selective PVE or PVL, Furrer et al.10 from Zurich elegantly showed that hepatocyte proliferation after PVL was more pronounced than after PVE, suggesting that PVL is the more effective technique. The regenerative response after PVE was possibly hampered by a massive foreign body reaction around the microspheres used for embolization, draining the macrophages which are instrumental in starting hepatocyte proliferation in the non-embolized lobes. As long as the (patho)physiological events governing post-PVE or post-PVL regeneration are unclear, it is difficult to attest which is the superior technique. Percutaneous PVE obviously is a less invasive procedure than PVL requiring a laparotomy. However, reports are accumulating in which PVL is undertaken laparoscopically, hence combining a staging procedure with PVL in one minimally invasive session.11

Is embolization of the segment 4 portal branches advised in extended right hemihepatectomy?

Controversy exists concerning embolization of the portal branches to segment 4 in preparation of extended right hemihepatectomy in addition to embolization of the right portal trunk. Access to the portal tributaries to segment 4 has to be gained via the portal bifurcation and/or left portal trunk, increasing the risk of injuring the left portal vein. Another potential mishap is the backflow of embolization material into the left portal venous system leading to inadvertent embolization and thrombosis.
of the portal vessels of the FRL. In our experience, this complication occurred in one patient who, in spite of thrombectomy during exploration, was eventually considered too high risk for resection (Figure 2). Although Ribero et al.\textsuperscript{12} reported no difference in the incidence of complications after right PVE + segment 4 and right PVE alone, selective embolization of the portal branches to segment 4 is considered a difficult extension of PVE, obviously requiring an expert interventional radiologist. Even in experienced hands, partial embolization of the segment 4 portal branches may be preferred (only the branches to segment 4a) above embolization of the whole segment. A surgical approach encompassing PVL and selective ligation of the portal branches to segment 4 and segment 9 (and even to segment 1) may seem more secure, but has the disadvantage of scarring the hilar area which makes subsequent dissection at the time of resection more difficult. In case of tumor deposits in segment 4, as is usually the case when an extended right hemihepatectomy is elected, incomplete embolization of segment 4 carries the risk of stimulating tumor growth in the nonembolized areas, thus creating a dilemma.

Is tumor growth accelerated after PVE?
Potential promotion of tumor growth after preoperative PVE and consequent acceleration of tumor progression in the waiting time until resection are a major concern possibly limiting the use of PVE in patients with multiple liver metastases. This is an area of debate which has recently been extensively reviewed by de Graaf et al.\textsuperscript{13} Several authors reported increased proliferative activity of colorectal liver metastasis following PVE.\textsuperscript{14-16} Clinical evidence is, however, based on studies with small sample size. Kokudo et al.\textsuperscript{14} published the largest case series in which 19 patients undergoing PVE were compared to 29 patients resected without PVE (controls). Mean tumor volume, measured by CT volumetry, had significantly increased by 20.8% in
the 3-week interval after PVE. The proliferation rate of metastatic lesions which was based on histological assessment (Ki-67 labeling index) was significantly higher in the PVE group than in the control group undergoing only resection. Tumor progression precluding curative resection has been associated with rates ranging from 6.4 up to 33%.13,17 A recent meta-analysis reported that 11.3 of the 85% of the evaluable patients that had undergone PVE and subsequent exploratory laparotomy were unresectable due to intra- or extrahepatic tumor spread.1

Three important mechanisms influencing tumor growth after PVE have been recognized, i.e. changes in cytokines and/or growth factors, alterations in hepatic blood supply and enhanced cellular host response promoting local tumor growth after PVE. Growth factors such as IL-6, TNF-a and hepatocyte growth factor (HGF) are upregulated and play an essential role during liver regeneration after partial liver resection.18 The same growth factors have been implicated in stimulating growth of colorectal carcinoma cells in vitro. With respect to alterations in hepatic blood supply, compensatory, increased arterial perfusion, known as the hepatic arterial buffer response, occurs after reduction of portal blood flow.19 Because liver tumors are mainly vascularized by the hepatic artery, the hepatic arterial buffer response therefore potentially stimulates tumor growth in the embolized liver lobes. Other factors such as heat shock protein-70 (hsp70), heme oxygenase-1 (hmox-1) and plasminogen activator inhibitors (PAI-1) have been shown to facilitate growth and angiogenesis in solid tumors.20 The time to clinical outgrowth of micrometastases in the future remnant liver after PVE can also be viewed as a diagnostic window. Small metastases, not detectable on CT scan before embolization, may become visible after PVE, rendering the patient unresectable or requiring reconsideration of management (Figure 3). It may be assumed that if PVE had not been performed preoperatively, these metastases would have become apparent in the first few weeks after resection, as a result of the same growth-inducing factors associated with post-hepatectomy regeneration.

Although difficult to accurately estimate, the impact of tumor progression after PVE underscores the importance of minimizing the waiting time between PVE and resection, and of devising therapeutic strategies using chemotherapy to control tumor growth after PVE. Sequential hepatic transcatheter arterial chemo-embolization (TACE) after PVE as well as post-PVE chemotherapy have been used to prevent tumor progression. Several recent studies suggest that continuation of chemotherapy after PVE has no negative influence on the hypertrophy response or on the outcome after resection.14,21,22 The combination of TACE and PVE has the advantage of reducing post-PVE reperfusion through arterial-portal shunts, hence enhancing the regenerative response in the non-embolized lobes. An additional effect is the curbing of tumor progression by selectively cutting off arterial blood supply. Especially highly arterialized tumors such as hepatocellular carcinomas are likely to grow because of the compensatory increase of arterial blood flow after PVE. Sequential application of
TACE and PVE limits the risk of ischemia and infarction of the liver parenchyma, as has been shown in experimental as well as clinical studies. The Beaujon group in Paris reported a study in which PVE combined with TACE (mean time interval 3.6 weeks) was compared with PVE alone in patients with hepatocellular carcinoma and cirrhosis. The mean increase in FRL volume and the rate of hypertrophy were significantly higher in the group in which PVE was combined with TACE. Using this combination, complete tumor necrosis was achieved in 80% of the patients compared to 5% in the PVE group, also with a higher 5-year disease-free survival rate in the former group.

What is the optimal timing of resection after PVE?

Tumor progression after PVE creates a dilemma in terms of optimal waiting time until resection. The risk of tumor growth obviously demands an as short as possible waiting time. The time interval is mainly determined by the time required to attain the target FRL volume. According to a meta-analysis, the length of time after PVE to operation was 2-60 days. Usually, a period of 3-4 weeks is considered sufficient based on CT volumetry. Asian surgeons tended to wait shorter until operation, whereas in Europe and the US, waiting times were longer. Of note, however, is that the functional increase of remnant liver likely exceeds the volume increase of remnant liver mass. The recommended waiting time may therefore be shorter than based on volumetric...
The regeneration rate of the nonembolized liver segments typically shows an increase during the first 3 weeks after PVE, followed by a plateau phase with only slight additional increase of FRL volume (Figure 4). Patients showing slow growth of the FRL and those with a persistently small FRL volume after 3 weeks are unlikely to exhibit rapid regeneration beyond this time point; further extension of the waiting time, therefore, seems futile. In a study performed by Ribero et al. from M.D. Anderson Cancer Center in Houston, a low degree of hypertrophy as a measure of liver growth was identified as a predictor of poor clinical outcome after resection. In this regard, PVE may be used as a ‘stress test’ to assess the regenerative potential of the FRL in the most crucial period, i.e. the first 3 weeks after PVE. In our center, CT volumetry and $^{99m}$Tc-Mebrofenin hepatobiliary scintigraphy as a test of liver functional reserve are performed on the 21st day after PVE. If the increase in volume and function is insufficient, CT and $^{99m}$Tc-Mebrofenin hepatobiliary scintigraphy is repeated 14 days later. At that time, the patient is operated or resection is declined when FRL volume and/or function are considered insufficient.

Figure 4. Graph showing the regeneration rate of the non-embolized liver segments after PVE. Volume increase of future remnant liver is greatest during the first 3 weeks after PVE, after which a plateau phase follows with only slight additional increase of volume. FRL volume at baseline was 28.2%, and increased to 36.5 and 40% at 21 and 48 days after PVE.

**Does PVE render the posthepatectomy hypertrophy response less effective?**

The mechanisms underlying hypertrophy of the nonembolized liver segments after PVE are probably similar to the mechanisms triggering posthepatectomy hypertrophy of the remnant liver segments following liver resection. The question arises whether the hypertrophy response after liver resection and prior PVE is as efficient as after liver resection only. Considering that liver regeneration is an energy-consuming process, one might speculate that the resources responsible for posthepatectomy regeneration are to some extent exhausted after previous PVE. To examine this, we
performed CT volumetric studies in patients who underwent PVE prior to (extended) right hemihepatectomy, using patients as controls who underwent the same type of resection without prior PVE. CT volumetry 3 months after hemihepatectomy showed no significant difference in the increase of remnant liver volume after resection in patients who underwent (extended) right hemihepatectomy with or without prior PVE \(^2\) (Figure 5). Hence, the regenerative capacity of the liver is not hampered after PVE and subsequent major liver resection, showing the same hypertrophy response as after liver resection alone.

**Figure 5.** Graph showing increase of remnant liver volume at three months after (extended) right hemihepatectomy in 10 patients who had undergone PVE 3–4 weeks prior to resection (thin lines). Fat line represents the mean increase of remnant liver volume in 13 patients who had undergone (extended) right hemihepatectomy without preoperative PVE (control group). Posthepatectomy regeneration was not hampered after PVE as remnant liver volumes attained approximately 80% of initial total liver volume in both groups.

**Discussion**

PVE is increasingly used to preoperatively enlarge the FRL in patients with increased risk of postoperative liver failure. Although probably not in the same proportion, increased FRL volume translates into increased functional reserve of the liver remnant after resection. Preoperative diversion of the portal flow creates another advantage in terms of venous outflow of the liver remnant. Division of the portal branches of the part of the liver to be resected results in an immediate increase in flow through
the portal bed of the liver remnant. With extensive resection (70%), the volume of portal blood directed through the liver remnant is tripled, which requires immediate adaption of the portal venous bed to accommodate the larger volume of portal blood. This ‘small for size’ situation may lead to a relative venous outflow block and liver congestion which impede function and the capacity of the remnant liver to regenerate. Preoperative PVE allows for the FRL to preadapt to the increase in portal flow reducing the risk of the hyperperfusion syndrome and hepatic venous congestion directly after resection.

Inasmuch as we understand the mechanisms of regeneration after liver resection, we understand the biologic process of regeneration of the nonembolized lobe after PVE. It is not known which factor(s) set(s) off the cascade of cytokines and growth factors responsible for regeneration after PVE. The dramatic increase in portal blood flow through the remnant liver after partial liver resection is considered an important trigger for liver regeneration. Experimental studies have demonstrated that decreased portal flow in the remnant liver is associated with an impaired or delayed regenerative response. PVE results in a similar increase in portal perfusion to the FRL, which in turn is potentially responsible for post-PVE regeneration. However, one might speculate that posthepatectomy regeneration after prior PVE is less effective than posthepatectomy regeneration without prior PVE, because portal perfusion of the liver remnant after PVE and subsequent hemihepatectomy is largely unchanged. As dealt with above, the hypertrophy response after liver resection, however, is as efficient as without preceding PVE, suggesting other mechanisms to induce regeneration than only hemodynamic factors.

An interesting concept has been introduced by Lainas et al. concerning reversible PVE. In a model of PVE in monkeys, biodegradable gelfoam was used resulting in an initial, complete occlusion of the hemihepatic portal venous system, followed by revascularization of this system within 13 days, as the embolization material was gradually absorbed. The authors noted an efficient hypertrophy response of the nonembolized lobe in spite of the nonpermanent occlusion of the contralateral portal venous system, suggesting that a short period of portal occlusion suffices to initiate the process of regeneration in the nonembolized lobe. This approach obviously introduces some important advantages, as the embolized lobe, when not resected for whatever reason, is not left with decreased function. Secondly, inadvertent backflow of the embolization material to the nonembolized lobe will cause less harm and not preclude regeneration of the FRL. The concept of reversible PVE underscores the need for clinically relevant animal models to study PVE in conjunction with the mechanisms of PVE and new embolization materials.
## Conclusion

PVE is a useful preoperative intervention to increase volume and function of the FRL when planning extensive liver resection, especially in patients with compromised liver parenchyma or when associated with additional, major gastrointestinal procedures. The hypertrophy response after PVE has predictive value in regard with posthepatectomy outcome. Procedure-related complications after PVE (hematoma, hemobilia, sepsis, backflow of embolization material and thrombosis of portal vein branch(es) of the nonembolized lobe) should not be underestimated, and avoidance of adverse events requires expertise of interventional radiologists and liver surgeons. Because the overall liver volume and function after PVE remains unchanged, the persistence of the embolized, atrophic lobe usually does not generate an additional risk. Embolization of the portal branches to segment 4 in addition to embolization of the right portal trunk carries increased risk and is advised only in selected cases. It is as yet undetermined whether embolization of the portal venous system is more effective in inducing hypertrophy of the FRL than ligation of the portal vein. Accelerated tumor growth after preoperative PVE is a major concern and requires appropriate timing until resection in conjunction with effective chemotherapy around the procedure. Otherwise, the benefit of upsizing the liver may be counterbalanced by upsizing tumor load. A waiting time of 3 weeks is advised since beyond that time, additional increase of FRL volume is limited. Posthepatectomy regeneration is not hampered by preoperative PVE. The mechanisms of the hypertrophy response need to be elucidated in order to render PVE more effective, in conjunction with new embolization materials and new strategies such as reversible PVE.
References


Induction of tumor growth after preoperative portal vein embolization: is it a real problem?

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Abstract

Although preoperative portal vein embolization (PVE) is an effective means to increase future remnant liver (FRL) volume, little has been published on possible adverse effects. This review discusses the clinical and experimental evidence regarding the effect of PVE on tumor growth in both embolized and nonembolized liver lobes, as well as potential strategies to control tumor progression after PVE. A literature review was performed using MEDLINE with keywords related to experimental and clinical studies concerning PVE, portal vein ligation (PVL), and tumor growth. Cross-references and references from reviews were also checked. Clinical and experimental data suggest that tumor progression can occur after preoperative PVE in embolized and nonembolized liver segments. Clinical evidence indicating possible tumor progression in patients with colorectal metastases or with primary liver tumors is based on studies with small sample size. Although multiple studies demonstrated tumor progression, evidence concerning a direct increase in tumor growth rate as a result of PVE is circumstantial. Three possible mechanisms influencing tumor growth after PVE can be recognized, namely changes in cytokines or growth factors, alteration in hepatic blood supply and an enhanced cellular host response promoting local tumor growth after PVE. Post-PVE chemotherapy and sequential transcatheter arterial chemoembolization (TACE) before PVE have been proposed to reduce tumor mass after PVE. We conclude that tumor progression can occur after PVE in patients with colorectal metastases as well as in patients with primary liver tumors. However, further research is needed in order to rate this risk of tumor progression after PVE.
Preoperative portal vein embolization (PVE) is an accepted intervention in patients requiring major liver resection in whom the estimated future remnant liver (FRL) is too small to allow safe resection.\textsuperscript{1,2} PVE induces atrophy of the embolized, tumor-bearing liver lobe while compensatory hypertrophy of the nonembolized lobe occurs, thereby increasing FRL volume and function.\textsuperscript{4} Portal vein occlusion by either embolization (PVE) or ligation (PVL) has proven useful to reduce risk of postoperative liver dysfunction and enables resection in patients previously deemed unresectable due to a marginal FRL.\textsuperscript{7-14} FRL volume smaller than 25–30% of total preoperative liver volume is generally considered insufficient in patients with normal liver parenchyma.\textsuperscript{15-17} In patients with a compromised liver, the cutoff value for performing safe resection is more variable and PVE is usually undertaken when the FRL is smaller than 40% of total liver volume.\textsuperscript{10,12,16}

While experiences with PVE have accumulated, there is growing evidence that PVE stimulates not only the growth of the FRL but also affects tumor size in both embolized and nonembolized liver segments.\textsuperscript{18-20} This review discusses the clinical and experimental evidence regarding the effect of PVE on tumor growth in the nonembolized and embolized liver lobes, as well as potential strategies to control tumor progression after PVE.

The effect of PVE on tumor growth

Table 1 provides an overview of the studies describing tumor progression after preoperative PVE. Elias et al. were among the first to describe the potential of intrahepatic tumor enlargement after PVE.\textsuperscript{18} Their conclusion was based on five patients with known tumors in the nonembolized liver lobes. All patients had measurable tumors in the left (nonembolized) liver lobes before PVE, which provided the opportunity to measure tumor enlargement. In four of the five patients tumor size increased after PVE. One patient with impaired liver function showed no increase in tumor volume. The authors concluded that, in patients with normal liver parenchyma, the growth rate of metastases is more rapid than the hypertrophy of the surrounding liver parenchyma. Although the increase in tumor size in this study was impressive, no tumor growth rate before PVE was investigated, making it impossible to draw conclusions regarding direct stimulation of tumor growth by PVE. The study only demonstrates that tumor size can increase in the waiting period between PVE and resection. An increased growth rate of liver metastases as a result of portal vein diversion was however demonstrated in a murine model in which the portal vein to one side of the liver was ligated.\textsuperscript{21}

Tumor increase in the nonembolized segments is of special interest in patients with bilateral liver metastases. Although in these patients a right hemihepatectomy with simultaneous wedge resection in the left liver has been described after right PVE, the outgrowth of lesions in the left liver in the time between right PVE and resection poses a potential threat in respect with resectability.\textsuperscript{22} A two-staged resection in combination
Table 1. Studies describing the effect of preoperative PVE on tumor progression

<table>
<thead>
<tr>
<th>Study</th>
<th>Tumor type</th>
<th>Tumor location</th>
<th>No. of patients</th>
<th>Increase in tumor volume (%)</th>
<th>Increase in growth rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elias et al.18</td>
<td>Liver metastases</td>
<td>Nonembolized liver segments</td>
<td>5</td>
<td>215 % (30%, 970%)a</td>
<td>-</td>
</tr>
<tr>
<td>Hayashi et al.24</td>
<td>HCC</td>
<td>Embolized liver segments</td>
<td>6</td>
<td>-</td>
<td>263 % (200% - 746%)a</td>
</tr>
<tr>
<td></td>
<td>CCC</td>
<td>Embolized liver segments</td>
<td>2</td>
<td>-</td>
<td>116 % (100% - 132%)a</td>
</tr>
<tr>
<td>Kokudo et al.20</td>
<td>CRM</td>
<td>Embolized liver segments</td>
<td>15</td>
<td>20.8 %b</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CRM</td>
<td>Both embolized and nonembolized liver segments</td>
<td>3</td>
<td>3.0% (2.5% - 6.3%)c (embolized)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.7% (0.5% - 42.1%)c (nonembolized)</td>
<td>-</td>
</tr>
<tr>
<td>Barbaro et al.19</td>
<td>CRM</td>
<td>Embolized liver segments</td>
<td>6</td>
<td>84.4 % (62.4% - 562%)c</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Carcinoid tumor</td>
<td>Embolized liver segments</td>
<td>3</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

CRM colorectal metastasis, HCC hepatocellular carcinoma, CCC cholangiocarcinoma

*a* Median (minimum, maximum)

*b* Mean

with PVE can successfully be applied in some of these patients.23 In the first stage resection removal of all tumor mass located in the (future) nonembolized liver segments is required before the actual PVE (or PVL) in order to prevent rapid tumor enlargement after portal vein occlusion.23 Subsequently, major hepatectomy can be performed several weeks after the portal vein occlusion.

The time between PVE and follow-up computed tomography (CT) to recalculate FRL volume can also be viewed as a diagnostic window in which clinical outgrowth of micrometastases in the nonembolized lobe may occur. Small metastases in the nonembolized lobe, not detectable on a CT scan before embolization, may become visible after PVE as a result of the potential tumor-growth-stimulating environment provided by PVE. Such a finding will render the patient unresectable or will require reconsideration of management (Figure 1).

Several studies have described the effect of preoperative PVE on progression of colorectal metastases in the embolized liver segments. Kokudo et al. were the first to report increased proliferative activity of colorectal liver metastases.20 This is the largest case series concerning this issue, including 18 patients with prior PVE who were compared with 29 patients who were resected without PVE. One patient in the PVE group received preoperative intra-arterial chemotherapy. No use of neoadjuvant
Tumor Progression After PVE

Figure 1. Example of a patient with multiple colorectal metastases in the right liver lobe requiring an extended right hemihepatectomy. No lesions in segment 2 and 3 were visible (A). Due to a small RRL volume, PVE was performed. CT scan 3 weeks after PVE revealed multiple metastases in segments 2 and 3 excluding this patient from resection (B).

Chemotherapy was described in the other patients. Mean tumor volume, measured by CT volumetry, was significantly increased by 20.8% in the 3-week interval after PVE. However, the growth of liver tumors in the group without PVE was not assessed, and tumor growth rate before PVE was not measured. Instead, the proliferation of metastatic lesions measured by Ki-67 labeling index after PVE was compared with the proliferation rate of metastatic lesions in the control group undergoing only resection. Ki-67 labeling index was significantly higher in the PVE group. Although the distribution of well, moderately, and poorly differentiated adenocarcinoma was similar within the two groups, tumor size was significantly larger in the PVE group, which influences the Ki-67 labeling index. This study only demonstrates that colorectal liver metastases continue to grow in the embolized liver lobes after PVE. The evidence of direct tumor growth stimulation by PVE is however circumstantial. Barbaro et al. confirmed the increase of tumor volume after PVE in patients with colorectal metastases. In contrast, no increase in tumor volume was observed in patients with carcinoid metastases, suggesting that tumor characteristics are important for tumor progression after PVE. Again no evidence is provided in this study to demonstrate direct stimulation of tumor growth by PVE. Figure 2 demonstrates tumor enlargement in the embolized segments after PVE in a patient with colorectal metastases.

Data also exist that the growth of primary liver tumors including hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CCC) is influenced by PVE. In eight patients with primary liver tumors (six HCC, two CCC), rate of tumor growth after PVE was compared with growth rate of the same tumors in a period before embolization. Tumor growth rate accelerated from 0.59 cm³/day before PVE to 2.37 cm³/day after PVE, as measured by CT volumetry. This is the first study demonstrating...
induction of increased tumor growth by PVE as compared with the growth rate before PVE in the same patients.

In a recent study, Ribero et al. retrospectively analyzed 112 PVE patients. Changes in tumor size were measured in 80 patients, resulting in overall no increase in median tumor size within all patients after PVE. The change in 95% confidence interval after PVE indicates both an increase and a decrease in some patients. However, no information was provided regarding the percentage of patients with increased or decreased tumor size after PVE. Twenty-eight patients received chemotherapy prior to PVE and five patients received chemotherapy 2 weeks after PVE. No differentiation was made between patients with or without chemotherapy, and no tumor volumes were calculated. Additional studies are therefore required to precisely assess the risk of accelerated tumor growth in patients receiving PVE.

Besides reports of tumor progression after PVE, studies describe high percentages of patients with unresectable disease after PVE. In the study by Ribero et al. 10 out of the 112 patients (9%) did not undergo surgery after PVE because of extra- or intrahepatic disease progression. An additional 12 patients (11%) had unexpected extra- or intrahepatic disease that became evident at laparotomy. Similar percentages of tumor progression precluding curative resection are described, with percentages ranging from 6.4% to 33%, A recent meta-analysis assessing the results of preoperative PVE reported that 85% of the evaluable patients that had undergone PVE underwent laparotomy. Of these patients, 11.3% were unresectable due to intra- or extrahepatic tumor spread. The effect of tumor progression on disease-free and overall survival is currently elusive. In the study by Kokudo et al. disease-free survival at 2 and 4 years in patients who
had undergone PVE was significantly poorer than in those undergoing partial liver resection without prior PVE. However, the two groups were different with respect to preoperative tumor diameter and use of postoperative chemotherapy, making direct comparison impossible. In addition, no difference in overall survival was found. Azoulay et al. demonstrated similar survival rates after hepatectomy with or without previous PVE. Only resected patients were included in the survival analysis, and 33% of the patients with previous PVE were unresectable due to tumor progression. Survival rate in these patients was significantly poorer.

Little evidence exists regarding the effect of PVE on the induction of distant metastases. Breakdown and remodeling of the extracellular matrix takes place especially in the embolized, tumor-bearing liver lobes. Matrix metalloproteinases (MMPs) are most likely involved in this remodeling process similar to remodeling during liver regeneration. MMPs have been reported to promote metastatic behavior in several types of tumors, including colorectal cancer.

Mechanisms affecting tumor growth after PVE

Three possible mechanisms inducing tumor growth after PVE have been proposed, namely changes in cytokines and growth factors, alteration in hepatic blood supply, and enhanced cellular host response promoting local tumor growth.

Cytokines and growth factors

The mechanisms underlying the atrophy–hypertrophy complex induced after PVE remain largely undetermined. Growth factors such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and hepatocyte growth factor (HGF) are upregulated and play an essential role during liver regeneration after partial liver resection. The same growth factors have been implicated in stimulating growth of colorectal carcinoma cells in vitro. The HGF receptor is present in almost all human colorectal carcinomas. Treatment of human colon carcinoma cell lines with HGF stimulates cell growth and increases its metastatic potential. Experimental studies have shown an increase in IL-6, TNF-α, and HGF mRNA expression in the nonligated, hypertrophied liver lobes after PVL, suggesting a similar role of these factors as in post-hepatectomy liver regeneration.

Local elevation of these growth factors may stimulate colorectal metastases in the nonembolized liver segments. Interestingly, it has also been demonstrated that HGF and IL-6, although to a lesser extent, are upregulated in the ipsilateral liver lobes after PVL. In addition, increased tissue levels of HGF may increase plasma levels, thus stimulating the growth of hepatic tumors in the embolized lobe.

A recent experimental study demonstrated that PVL induced sinusoidal perfusion failure along with significant hypoxia during the initial few days after PVL, resulting in necrosis and apoptosis in the ligated liver tissue. However, most malignant tumors tolerate hypoxia quite well. In addition, hypoxia may induce cellular changes that can result in more aggressive phenotypes with increased potential for local invasive
growth, distant tumor spreading, and resistance to therapy. In the first days after PVL, negative regulators of hepatocyte proliferation, such as transforming growth factor-β (TGF-β) and interleukin-1 (IL-1), are strongly expressed in the atrophic lobes. TGF-β serves in normal tissue as a tumor suppressor by inhibiting cell proliferation. Many colorectal carcinomas, however, are resistant to TGF-β-induced growth inhibition. In advanced stages, TGF-β can even stimulate the proliferation of colon carcinoma cells. In the late phase after ligation, tissue remodeling takes place which is dominated by cell proliferation. Hence, tumor proliferation may be promoted in this late phase after PVE.

**Alteration in hepatic blood supply**

Increased hepatic arterial blood flow after embolization of the ipsilateral portal branch is another factor potentially stimulating tumor growth after PVE. The liver has a dual blood supply with about 75% being contributed by the portal vein and 25% by the hepatic artery. Compensatory increased arterial perfusion, known as the hepatic arterial buffer response, occurs after reduction of segmental portal blood flow. Clinical and experimental studies demonstrate a significant increase in hepatic arterial blood flow in the occluded liver lobes resulting from an increase in common hepatic arterial flow. After portal vein occlusion, sinusoidal perfusion is derived almost totally from arterial blood supply. Because liver tumors are mainly fed by the hepatic artery, the hepatic arterial buffer response potentially stimulates tumor growth in the embolized liver lobes. Although a very likely theory, no studies as yet have proven the involvement of the hepatic arterial buffer response in the induction of tumor growth.

**Cellular host response promoting local tumor growth**

A third mechanism implicated in tumor growth after PVE is the local cellular response evoked in the embolized atrophying liver lobes. Studies in animal models have demonstrated that portal vein occlusion induces an immediate early gene response in both ligated and nonligated liver lobes. Enhanced expression of several of these genes in the atrophying liver tissue such as heat shock protein-70 (hsp70), heme oxygenase-1 (hmox-1), or plasminogen activator inhibitors (PAI-1) have a cytoprotective effect and a role in tissue remodeling and repair. The same factors have been shown to facilitate growth and angiogenesis in solid tumors, including colon carcinomas. The production of these factors by the surrounding liver parenchyma may in this manner contribute to tumor progression. In addition, many tumors including HCC and colorectal carcinomas have the potential to express hsp70 themselves. In some tumors, expression of hsp70 has been related to cell proliferation, poor prognosis, and resistance to chemotherapy. Similar to the local parenchyma, tumor cells might upregulate hsp70 in response to occlusion of portal blood flow, thereby increasing their proliferative capacity.
Strategies to reduce tumor growth after PVE
Several therapeutic strategies have been described to prevent and even reduce tumor progression after PVE including sequential hepatic transcatheter arterial chem embolization (TACE) and PVE and post-PVE chemotherapy.

Sequential TACE and PVE
Studies from Japan reported that additional ipsilateral TACE before or after PVE improved the hypertrophy response of the FRL in patients with HCC and chronic liver disease.51,52 The rationale behind this combination was not only to improve the regenerative capacity after PVE by closing down arterial–portal shunts but also to reduce the risk of tumor progression secondary to the compensatory increase in arterial blood flow.51,53-55 Aoki et al. described a group of 17 HCC patients who underwent PVE 7-10 days after TACE.51 The combination generated sufficient hypertrophy of the nonembolized lobes within 2 weeks. No tumor progression was noted in the waiting time until resection as measured by CT volumetry. Examination of the resection specimen revealed almost complete tumor necrosis (90-100%) in ten patients (59%). In the other patients, extent of necrosis was 50-80%. Ogata et al. compared a group of HCC patients (n = 18) undergoing PVE 3 weeks after TACE with a group of HCC patients who underwent only right-sided PVE in the same period.54 Right hemihepatectomy was performed 4-8 weeks after PVE. Mean increase in FRL volume and rate of hypertrophy were significantly higher in the group in which PVE was combined with TACE. Using this combination, complete tumor necrosis was achieved in 80% of the patients compared with 5% in the PVE group. In addition 1-, 3-, and 5-year recurrence-free survival rates were higher in the combined group. This clearly demonstrates that TACE combined with PVE is effective in reducing tumor progression in HCC patients. A drawback of the combination of TACE with PVE is the risk of ischemic parenchymal damage. Vetelainen et al. demonstrated that simultaneous ligation of the hepatic artery and portal vein in rats resulted in massive liver cell necrosis with increased systemic inflammatory response and decreased liver function.56 An interval of 48 h between both procedures decreased the risk of liver injury. Nakao et al. reported, in a clinical study, that simultaneous hepatic arterial and portal venous embolization resulted in necrosis and infarction of the embolized tissue.53 Aoki et al. used intervals of 7-10 days in their clinical study and described a transient increase in liver damage parameters.51 Examination of the resection specimen showed minimal necrosis of the liver parenchyma in the majority of patients. Two patients however had segmental infarction in the embolized lobes. Ogata et al. used time intervals of 3-4 weeks which resulted in significant increase in aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels, however without decrease in liver function.54 The most suitable interval between ipsilateral arterial and portal embolization in the clinical setting remains uncertain as well as its effect in livers compromised by steatosis or previous chemotherapy.
Although TACE is most frequently used in patients with hepatocellular carcinoma, there are also reports indicating its use in other liver tumors including colorectal metastases. This suggests that sequential TACE with preoperative PVE could also be potentially beneficial in patients with colorectal metastases. To date, however, no study has reported the use of sequential TACE with preoperative PVE in this category of patients.

Post-PVE chemotherapy

With recent development of improved chemotherapeutic agents, an increasing number of patients with metastatic liver disease are treated with neoadjuvant chemotherapy. If preoperative PVE is required, chemotherapy is discontinued several weeks prior to embolization until surgery 3-4 weeks thereafter, because of its alleged negative influence on the hypertrophy response. This allows tumor progression to occur in the period in which chemotherapy is discontinued in addition to the possible tumor stimulating effects of PVE. Beal et al. reported in a retrospective study including 15 patients, of which 10 received post-PVE chemotherapy, that hypertrophy of the FRL did occur in the post-PVE chemotherapy group, although significantly less compared with the nonchemotherapy group. Tumor progression was seen in four of the five patients without post-PVE chemotherapy, whereas tumor reduction was seen in six of the ten patients with chemotherapy. The group of Belghiti recently demonstrated no significant difference in hypertrophy response nor in postoperative complications when chemotherapy was continued after PVE. They therefore recommended not to disrupt a successful chemotherapy course prior to or after portal vein occlusion in patients with colorectal metastases. These conclusions however, are based on a series of 20 patients, of whom only 10 received post-PVE chemotherapy.

Covey et al. recently confirmed that continuation of chemotherapy after PVE had no negative influence on the hypertrophy response in a series of 100 patients including 43 patients with post-PVE chemotherapy. Interestingly, significantly more patients were unresectable in the post-PVE chemotherapy group. The reasons for unresectability of each group were not given but, overall, 23% of the patients were not resected due to intra- and/or extrahepatic tumor progression. This difference can be caused by a selection bias between the two groups because tumor size and stage were also not provided.

Selzner et al. applied selective intrahepatic arterial (SIHA) chemotherapy combined with portal vein ligation for downstaging of colorectal liver metastases. SIHA chemotherapy has the advantage of a high response rate with a low rate of systemic toxicity. SIHA was started 3-7 days after PVL in 11 patients using a catheter positioned in the gastroduodenal artery with the tip at the junction with the hepatic artery. Chemotherapy involved serial administration of floxuridine for 2 weeks every 4 weeks. The volume of liver metastases decreased by 60% within 3 months after PVL. Although this study was performed in a highly selected group of unresectable patients, it shows that chemotherapy is able to reduce tumor growth after ipsilateral portal vein occlusion.
Discussion

PVE as a means to induce hypertrophy of the FRL is clearly established. Little has been reported, however, about the negative side-effects of PVE. Several studies have shown tumor progression in patients with colorectal metastases as well as in patients with primary liver tumors after PVE.\textsuperscript{18,20,24} Although the clinical studies clearly demonstrate that tumor progression is possible in both the embolized and nonembolized liver lobes, the evidence of direct stimulation of tumor growth by PVE is circumstantial. In addition the reports of increased tumor proliferation after PVE are based on limited case series. In a larger study, no increase in median tumor size after PVE was shown within the total group of 80 patients.\textsuperscript{13} However, to date, there is no information available on the percentage of patients with increased or decreased tumor size after PVE. Additional studies are therefore required to more precisely assess the risk of accelerated tumor growth in patients receiving PVE.

Tumor progression after PVE creates a dilemma in terms of optimal waiting time until resection. The risk of tumor growth obviously demands as short as possible waiting time. The time interval is mainly determined by the time required to attain sufficient FRL volume. Usually a period of 3-4 weeks is considered sufficient based on CT volumetry.\textsuperscript{14} Little is known concerning the improvement of FRL function after PVE. One study separately assessed biliary excretion of indocyanine green by the embolized and nonembolized liver lobes and concluded that the functional gain in the nonembolized lobes was of greater magnitude than the volumetric increase.\textsuperscript{6} Two additional studies from Japan confirmed that the increase in FRL function after PVE measured by technetium-99 m (\textsuperscript{99m}Tc)galactosyl-human serum albumin (GSA) scintigraphy exceeds the increase in volume in cirrhotic and noncirrhotic patients.\textsuperscript{3,65} This implies that the recommended waiting time until operation may be shorter than suggested by volumetric studies, which is more favorable in light of the risk of tumor progression after PVE.

The combination of TACE before PVE is effective in inducing tumor necrosis and thereby in inhibiting tumor progression after PVE.\textsuperscript{31,53,54} There is nevertheless, a risk of massive necrosis with serious complications. The time interval between the two procedures is therefore crucial to safely undertake the combination. More research is needed to define which patients benefit most from sequential TACE and PVE and to determine the optimal time interval between both procedures.

Neoadjuvant chemotherapy has become increasingly important in downsizing unresectable colorectal liver metastases and there are also data indicating its use in initially resectable cases.\textsuperscript{66} Continuation of chemotherapy after PVE is a concern as, during that time, regeneration of FRL has to take place. Since the inhibitory effect of chemotherapy on the hypertrophy response appears to be less than previously assumed, there seems to be a place for chemotherapy after PVE to control tumor growth in patients with colorectal metastases, particularly in those who previously
have shown to be good responders. Systemic chemotherapy may also have the advantage of controlling the progression of extrahepatic disease in the waiting time until resection. This is an area for controlled studies to further determine the role of chemotherapy after PVE.

The high percentage of patients reported to have unresectable disease after PVE, allegedly due to tumor progression, is another issue. No studies are available concerning the follow-up of unresectable patients after PVE. The described effect of PVE stimulating tumor growth raises the question of whether PVE reduces survival in comparison with unresectable patients who did not undergo PVE.

In conclusion, whereas PVE is an established method to increase the rate of patients with resectable liver tumors, several issues need to be further clarified. PVE allows tumor progression in both the embolized and nonembolized liver segments. Although clinical studies clearly demonstrate that tumor progression after PVE is possible, accurate data regarding the risk of tumor progression after PVE are currently not available. However, the possibility of tumor progression makes it important to minimize unnecessary waiting time between PVE and resection. Sequential TACE with PVE as well as post-PVE chemotherapy are promising strategies to control tumor progression after PVE.
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A rabbit model for selective portal vein embolization

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Abstract

**Background:** Portal vein embolization (PVE) is a technique to increase future remnant liver volume. A standardized animal model, resembling the clinical PVE procedure, is needed to clarify some of the unresolved issues surrounding PVE. For this purpose we developed a new rabbit model for PVE.

**Materials and methods:** Twenty female New Zealand white rabbits were allocated to two protocols, each containing two subgroups. Eighty percent of the liver portal venous system was embolized with polyvinylalcohol particles and coils (protocol 1: 300–500 mm particles and one coil; protocol 2: 90–180 mm combined 300–500 mm particles and three coils). In all rabbits CT-volumetry, ICG clearance test, blood sampling, and portography were performed prior to PVE and at d 7 and 14. Additional blood sampling and CT volumetry was done on d 3 and 7.

**Results:** PVE was technically feasible in the rabbit. CT-volumetry demonstrated a strong correlation with actual liver weight and volume measured at sacrifice. The hypertrophy response was highest at d 7 in both protocols, which was consistent with the amount of proliferating hepatocytes. Protocol 2 showed less revascularization of the portal venous system and demonstrated the highest hypertrophy response. Comparable to the clinical situation, only a small, transient increase in transaminases was observed. There were no changes in liver function parameters after PVE. Histopathologic findings in the rabbit livers were comparable to those found in human livers.

**Conclusion:** We successfully devised a rabbit model for PVE, which resembles the human clinical situation.
Introduction

In 1920, Rous and Larimore described their unforeseen observation that ligation of portal branches in a rabbit resulted in atrophy of the ligated liver lobes and a compensatory hypertrophy of the remaining organ. Although they recognized that this observation could explain the changes in liver morphology seen in liver lesions that cause disturbance of local portal flow, they did not foresee that this observation would lay the foundation for preoperative portal vein embolization (PVE). PVE was clinically introduced in 1986 to increase the number of patients eligible for liver resection. It induces atrophy of the embolized, tumor bearing liver segments while compensatory hypertrophy occurs in the nonembolized lobes, thereby increasing future remnant liver volume and function before major liver resection is undertaken. Although the beneficial effect of preoperative PVE for the induction of contralateral hypertrophy has been demonstrated by many studies, several issues remain uncertain or controversial, including the mechanisms underlying the atrophy-hypertrophy complex, the best occlusion technique (PVE or portal vein ligation (PVL)), the best embolization material, the effect of underlying liver disease on the hypertrophy response, and the effect of PVE on tumor progression.

The available information regarding the mechanisms behind the atrophy-hypertrophy complex is mostly derived from PVL models in the rat. Since a few years, there is growing evidence that the mechanisms behind liver regeneration after PVE might differ from those after PVL. A standardized animal model, resembling the clinical PVE procedure, is necessary to clarify some of the unresolved issues surrounding PVE and to subsequently improve the technique of PVE.

Recently, two new animal models for PVE were published. Furrer et al. described a rat model using embospheres as embolization material. Although an elegant model, the size of the rat has its limitations. Not all embolization materials can probably be used in this model, especially the use of coils is difficult. The procedure requires introduction of a relatively large needle in the central portal vein. In addition, the portal vein to the nonembolized segments was clamped during the embolization procedure. All these factors might influence the hemodynamics in the nonembolized liver lobes. Furthermore, noninvasive volumetric assessment of the different liver lobes is difficult in this model. Wilms et al. described a PVE model in a mini-pig, which is comparable with the human situation. Disadvantages are the costs and labour-intensiveness involved in experiments with mini-pigs.

The aim of this study was therefore to devise a new model for PVE in the rabbit, which resembles the clinical procedure.
Materials and methods

Animals
Experimental protocols were approved by the Institutional Animal Ethics Committee. Female New Zealand white rabbits (Harlan, Horst, The Netherlands) with a mean weight of 3.265 g were acclimatized for 2 weeks under standardized laboratory conditions in a temperature-controlled room with a 12 h-light/dark cycle and with access to standard chow and water ad libitum.

Figure 1. Anatomy of the rabbit liver (A). The rabbit liver is subdivided into four main lobes: these are the caudal liver (CL) lobe and three cranial liver lobes, comprising the left lateral (LL), left medial (LM), and right liver (RL) lobes. A portogram of the rabbit liver is depicted in (B). The portal vein branch to the caudate lobe takes off to the right. More cranially, the portal vein bifurcates into the right and left portal vein branches, after which the left portal vein divides into the medial and lateral segmental branches.

Experimental design
The rabbit liver is subdivided into four main lobes: a caudal liver lobe and three cranial liver lobes (Figure 1). We embolized 80% of the liver by embolizing the portal system of all cranial liver lobes. For this experiment, the combination of PVA particles and coils was used, since this is also used in the clinical setting in our institution. Rabbits (n=20) were allocated into two protocols. In protocol 1, PVE was performed using one PVA particle size combined with one coil. In protocol 2, the liver was embolized using two different sizes of PVA particles followed by three coils. Both protocols included two subgroups (n=5 per subgroup) in order to obtain liver tissue on different time points. In the first subgroup, CT volumetry was performed before PVE and on d 3 and 7 after PVE. Rabbits were sacrificed on d 7. In the second subgroup, CT volumetry was performed before PVE and on d 10 and 14 after PVE, followed by sacrifice on d 14.

Blood samples were drawn before PVE, as well as 3 h and 3 d after PVE. Additional blood samples were taken at the time of CT volumetry. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed as well established liver damage parameters by routine clinical chemistry. Prothrombine time (PT) and albumin (Alb) were used as indirect parameters of liver synthetic function, whereas plasma bilirubin
(bili) was used as an indirect measure of hepatic uptake and excretory function. In addition the indocyanine green (ICG) clearance test was performed as a quantitative dynamic liver function test prior to PVE and on the day of sacrifice. After sacrifice, liver weight was measured and liver biopsies of both embolized and the nonembolized liver lobe(s) were taken.

**Portal vein embolization**

Animals were anesthetized by intramuscular injection of 25.0 mg/kg ketamine (Nimatek; Eurovet, Bladel, The Netherlands) and 0.2 mg/kg dexmedetomidine (Dexdomitor; Orion Corp., Espoo, Finland). After subcutaneous injection of 0.03 mg/kg buprenorphine (Temgesic; Reckitt Benckiser Healthcare Ltd., Hull, UK) and 0.2 mg/kg Baytril (Bayer Healthcare, Berlin, Germany), the rabbit was placed in a supine position. Isoflurane 1%-2% (Forene; Abbott Laboratories, Kent, UK) with O2/air (0.5:0.5 L/min) was used to maintain anesthesia. Heart rate and arterial oxygen saturation were measured by pulse oximetry (Hewlett Packard M1165A model 56S; Andover, MA) throughout the procedure. The embolization was performed by an interventional radiologist with over 10 years experience. After midline laparotomy, a branch of the inferior mesenteric vein was cannulated with an 18 gauge catheter (Hospira Venisystems, Lake Forest, IL). A Renegade 3 Fr microcatheter (Boston Scientific, Place Natick, MA) with a Transend-ex 0.014 inch wire (Boston Scientific, Natick, MA) was subsequently introduced into the portal vein. A portogram was made to identify the individual portal branches (Figure 1). The microcatheter was positioned in the main portal branch supplying the cranial liver lobes after passing the portal branch to the caudal liver lobe. In protocol 1, a mixture of contrast (Visipaque; GE Healthcare, Waukesha, WI) with 300–500 mm PVA particles (Cook, Bloomington, IN) was injected until flow ceased, followed by the positioning of a platinum coil (6 mm, Tornado Embolization Microcoil; Cook, Bloomington, IN). In the second protocol, 90–180 mm PVA particles were used followed by 300–500 mm particles and three platinum coils (5 and 6 mm). Portography was repeated to confirm that the cranial liver lobes were deprived of portal blood flow. The inferior mesenteric vein was subsequently closed with a ligature. Rabbits were given Baytril 0.02 mg/kg subcutaneously once a day for 3 d postoperatively.

**CT volumetry**

After anesthesia, a multiphasic CT scan was performed using a 64-slice CT scan (Brilliance 64-channel; Philips, Eindhoven, The Netherlands). Rabbits were placed in supine position. After a blank series, a contrast enhanced scan was performed 15 s (arterial phase), 30 s (portal phase), and 45 s (venous phase) after contrast injection (4 mL Visipaque; GE Healthcare, Waukesha, WI), followed by 3 mL NaCl. 3D-reconstructions of the liver were made using reconstructed 2 mm axial slices (Figure 2). The total liver and the caudal liver lobe were manually delineated and total liver volume (TLV) and caudal liver volume (CLV) were calculated. CLV before PVE was expressed as percentage of TLV using the formula:
After PVE, %CLV was calculated using the formula:

\[
\%\text{CLV}_{\text{post-PVE}} = \frac{CLV_{\text{post-PVE}}}{CLV_{\text{pre-PVE}}} \times 100\% \]

Increase in CLV was calculated using the formula:

\[
\text{Increase in CLV} = \left( \frac{CLV_{\text{post-PVE}} - CLV_{\text{pre-PVE}}}{CLV_{\text{pre-PVE}}} \right) \times 100\% \]

**Indocyanine green clearance test**

A 22 gauge Venflon was placed in the ear vein and in the contralateral ear artery. Freshly prepared ICG was injected into the ear vein (12.5 mg ICG pulsion; Pulsion Medical Systems, München, Germany) in 5 mL sterile water. Blood samples were obtained before and 1, 2, 3, 4, 5, and 6 min after ICG injection. Plasma samples were diluted (250 mL plasma in 600 mL of 1% bovine serum albumin in 0.9% NaCl) and measured spectrophotometrically at 805 nm (Uvikon 850; Kontron Instruments, München, Germany). The ICG disappearance constant (k) was derived from the slope of the semilogarithmic decay curve. Accordingly, the ICG plasma disappearance rate (ICG-PDR, %/min) was calculated using the formula: \( \text{PDR} = k \times 100 \) \(^{15}\)

**Determination of liver weight and liver volume**

After sacrifice, the liver was weighed using a precision scale (Sartorius, Goettingen, Germany). Liver volume was measured using a measuring jug filled with water by subtracting the volume of the water alone from the volume of the water including the liver.

**Liver regeneration**

Immunostaining for Ki-67 was performed to identify proliferating hepatocytes. Sections were deparaffinized and endogenous peroxidase activity was blocked by incubation in 0.3% H2O2 in methanol. Subsequently, sections were boiled in 10 mM Tris/1 mM EDTA buffer (pH=9.0) for 20 min, and incubated for 1 h at room temperature with monoclonal mouse anti-rat Ki-67 antibody (MIB5; DAKO Cytomation, Glostrup, Denmark) with known cross reactivity for rabbit tissue. After incubation with anti-mouse poly-horseradish peroxidase (Immunologic, Duiven, The Netherlands) for 30 min, diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, MO) was used to label peroxidase complexes. Sections were counterstained with hematoxylin. Immunolabeled and hematoxylin-positive nuclei were quantified with Imagej software (NIH, Bethesda, MD).\(^{16}\) The mitotic index was defined as the percentage of Ki-67 positive hepatocytes averaged over 5 low power fields (10magnification) per slide.
Histopathologic evaluation
Histologic examination of the atrophic lobes of the rabbit liver demonstrated similar features as in biopsies taken from human atrophic liver segments after PVE (Figure 8A and B). PVA particles were clearly visible within the portal veins. A foreign body reaction characterized by multinucleated giant cells was found around the PVA particles. The liver parenchyma demonstrated sinusoidal dilatation with atrophic hepatocyte trabeculae. The areas of trabecular atrophy were unevenly distributed throughout the liver parenchyma. Diffuse infiltration of inflammatory cells was seen within the liver parenchyma, both perportal and centrilobular (although less pronounced in rabbit tissue than in human tissue). The hypertrophic liver segments were characterized by intact liver architecture in both rabbits and humans. There was little sinusoidal dilatation but not as pronounced as in the atrophic liver lobe. There was some influx of inflammatory cells in the periportal region, which was more prominent in the human liver tissue (Figure 8C). Overall, the histopathologic features of both the atrophic and hypertrophic liver parenchyma of the rabbit closely resembled the histology of the human liver after PVE.

Statistical analysis
Statistical analysis was performed with GraphPad Prism (Graph-Pad Software, San Diego, CA) and Statistical Package for Social Sciences (SPSS 16.02, SPSS Inc., Chicago, IL). CT volumetry data were compared using a mixed model analysis based on ranked data. Continuous data were compared by the Mann-Whitney U test. Correlation between variables was tested using the Spearman’s rank correlation coefficient. All statistical tests were two-tailed and differences were considered significant at a p value <0.05. Data were expressed as means ± SD.

Results
PVE procedure
One rabbit was excluded in protocol 1 because the coil migrated into the portal branch of the caudal lobe during the procedure. In all remaining rabbits, portography directly after embolization confirmed complete portal vein occlusion of the cranial liver lobes and normal flow in the caudal lobe (Figure 3A). In three out of the five rabbits in protocol 1, the portogram at d 7 revealed restoration of flow in the main portal branches to the cranial liver lobes distal of the coil and additional perfusion of the liver parenchyma, suggesting revascularization of the occluded portal venous system (Figure 3B). At d 14, three out of the four rabbits showed considerable parenchymal perfusion of the cranial liver lobes. In protocol 2, the portogram at d 7 showed minimal portal flow to the cranial liver lobes without parenchymal perfusion in two of five rabbits (Figure 3C). After 14 d,
also 2 of the five rabbits showed minimal revascularization at the site of the coils without parenchymal perfusion. However, one other rabbit demonstrated minimal parenchymal perfusion of the cranial lobes (Figure 3D). No formation of collateral vessels to the cranial liver lobes was observed.

Figure 2. Example of CT volumetry in the rabbit. (A) and (B) show a contrast enhanced CT scan. Because of the anatomical position, the caudal liver lobe (green) can be clearly distinguished from the other liver lobes. (C) depicts a three-dimensional CT scan reconstruction of the rabbit liver depicting total liver (yellow) and the caudal liver (green).

Validation of CT volumetry in the rabbit

The total liver volume measured by CT volumetry correlated well with actual liver volume and liver weight measured after sacrifice (Spearman’s r =0.77 and 0.84, respectively). In addition, caudal liver volume measured by CT volumetry showed a strong correlation with actual caudal liver volume and weight (Spearman’s r=0.85 and 0.93, respectively), indicating that CT volumetry is an accurate method for noninvasive measurement of liver volume in the rabbit (Figure 4). Liver volume measured by CT volumetry was, however, significantly larger than liver weight and liver volume measured after sacrifice.

Hypertrophy response after PVE

Before PVE, CLV expressed as percentage of total liver volume (%CLV) was 21.2% ± 2.0% and 21.8% ± 2.0%, for protocols 1 and 2, respectively (Figure 5A). When we
compared the %CLV between both groups, over time a significant difference was found between the two protocols showing that protocol 2 is more effective in increasing % CLV (p=0.013). When the separate time points were analyzed, a significant difference was found on d 10 and 14 (p=0.007 and p=0.002, respectively) in favour of protocol 2 (Figure 5B). There was a significant increase of CLV in both protocols after 3 and 7 d. Only little additional increase was observed after 10 and 14 d.

![Figure 3. Examples of portograms made after PVE.](image)

**Hepatocellular damage after PVE**

In protocol 2, a significant but mild elevation in AST level was found 3 h and 3 d after PVE compared with pre-PVE baseline values (p=0.008 and p=0.011, respectively). In protocol 1, a mild elevation was seen only after 3 d (p=0.008). Plasma ALT levels were significantly elevated in protocol 1 at 3 d (p=0.012) and in protocol 2 at 3 h, 3 d, and 7 d after PVE (p=0.008, p=0.008, and p=0.043, respectively). Plasma AST/ALT levels during the experiments are shown in Figure 6.

**Liver function after PVE**

No substantial changes in biochemical liver function tests as well as in the ICG-PDR were observed after PVE in both protocols (p > 0.05).
Liver regeneration

In both protocols a significant increase in proliferating cells within the nonembolized liver lobe was observed compared with the embolized liver lobes (Figure 7). At d 7, the percentage of proliferating cells in the hypertrophic lobe was significantly higher in protocol 2 compared with protocol 1 (p=0.032). At d 14, no significant difference was found.

Figure 4. Validation of CT-volumetry in the rabbit. There was a strong and significant correlation between total liver volume (TLV) measured by CT volumetry and actual liver volume (A) and liver weight (B). In addition, caudal liver volume (CLV) measured by CT volumetry showed a strong correlation with actual caudal liver volume (C) and weight (D) measured after sacrifice.

Histopathologic Evaluation

Histologic examination of the atrophic lobes of the rabbit liver demonstrated similar features as in biopsies taken from human atrophic liver segments after PVE (Figure 8A and B). PVA particles were clearly visible within the portal veins. A foreign body reaction characterized by multinucleated giant cells was found around the PVA particles. The liver parenchyma demonstrated sinusoidal dilatation with atrophic hepatocyte trabeculae. The areas of trabecular atrophy were unevenly distributed throughout the liver parenchyma. Diffuse infiltration of inflammatory cells was seen within the liver parenchyma, both periportal and centrilobular (although less...
pronounced in rabbit tissue than in human tissue). The hypertrophic liver segments were characterized by intact liver architecture in both rabbits and humans, which was more prominent in the human liver. There was little sinusoidal dilatation but not tissue (Figure 8C). Overall, the histopathologic features were pronounced as in the atrophic liver lobe. There was of both the atrophic and hypertrophic liver parenchyma of the rabbit closely resembled the histology of the human liver after PVE.

Figure 5. Increase in %CLV (A) and CLV (B) measured by CT volumetry. There was a significant difference in % CLV between the two protocols over time, showing that protocol 2 is more effective in increasing the % CLV (p=0.013) (A). When the separate time points were analyzed, a significant difference in % CLV was found on d 10 and 14 (A). In both protocols, the increase in CLV was most pronounced in the first week after PVE, with only little additional increase on d 10 and 14 (B).

Figure 6. Transient elevation of plasma ALT/AST levels after PVE. Statistically significant differences compared with pre-PVE baseline values are indicated by an asterisk.

Discussion

In this study we describe a rabbit model for PVE, which resembles the clinical situation. After initial pilot studies in which we developed the PVE procedure, pro-
Protocol 1 was performed using 300–500 mm PVA particles combined with one coil in the main branch to the cranial liver lobes. Application of PVA particles in combination with a coil is considered permanent. Protocol 1 was not successful in all animals as partial revascularization of the portal system to the cranial lobes was already observed after 7 d, resulting in less regeneration and less hypertrophy response. The coil was unable to prevent flow into the main portal branches and partial reperfusion of the liver parenchyma indicated that not all peripheral portal branches were occluded.

Figure 7. Liver regeneration measured by the percentage of Ki-67 positive hepatocytes (A) with representative histology (B), (C). Ki-67 staining demonstrated a significant increase (*) in proliferating cells within the caudal liver lobes (nonembolized) (B) compared with the atrophic liver lobes (C). At d 7 after PVE, the percentage of proliferating cells was significantly higher in protocol one compared to protocol 2 (#). At d 14, no significant difference was found.

by the PVA particles. Apparently, particle size and the number of coils are crucial for the efficacy of the PVE procedure. Therefore, in protocol 2, both 90–180 and 300–500 mm PVA particles in combination with three coils were used, showing no parenchymal perfusion of the cranial lobes after 1 wk. After 14 d however, three rabbits demonstrated flow in the main cranial portal branches, which resulted in only minimal perfusion of the liver parenchyma in one rabbit. Therefore, protocol 2 (using the combination of two PVA particle sizes, with three coils) is recommended for future research. The mechanism by which revascularization in the rabbit liver occurs may be due to lysis of the clot at the site of the coils. No evident collateral vessels were identified. Since portography in patients is usually not performed during follow-up after PVE, it is uncertain whether partial revascularization also occurs in the
human situation. Revascularization has been described after embolization of cerebral arteriovenous malformations using PVA particles as embolic material.17 Similar to the human situation, CT volumetry was useful in our rabbit model to assess liver growth noninvasively, and allowed us to perform repeated measurements within one rabbit, which obviously is a great advantage. Because of the anatomical position, the caudal liver lobe can be clearly distinguished from the other liver lobes. Liver volume measured by CT volumetry correlated strongly with actual liver weight and volume at time of sacrifice. Total liver volume measured by CT volumetry was, however, significantly larger than the liver weight at sacrifice. This is also observed in humans18 and can (partially) be explained by the fact that blood volume is not included after sacrifice, while it is included in vivo and measured by the CT scan.

Comparable with the clinical situation, only a small, transient increase in transaminases was observed post-PVE. Findings at histologic examination of the atrophic and hypertrophic liver lobes resembled those in humans and rabbits. As expected, Ki-67 staining showed a significant increase of proliferating cells in the caudal liver lobe, which was more pronounced at d 7 after PVE. This is in agreement with the findings of CT volumetry, which showed less volume increase in the second week.

Many different embolization materials are clinically used, including ethanol, fibrin glue, gelatine sponge particles, polyvinyl alcohol particles (PVA), embosphere particles, and various coils. Different responses regarding inflammation and the degree of hypertrophy have been observed with different embolization materials.19,20 Comparison between the different embolization materials is difficult in the clinical setting. A recent study by Lainas et al. demonstrated that although recanalization occurred, absorbable gelfoam was able to induce a significant hypertrophy response.21 The results from protocol 1 confirm that although less than protocol 2, significant hypertrophy does occur within the first week despite evidence of revascularization. As underscored by Lesurtel and Belghiti, the use of absorbable embolic agents has two advantages: First, the overflow of the embolization agent in the nonembolized lobe will not impair liver regeneration in these segments. Second, complete revascularization after embolization will reduce injury of the embolized liver lobes when they are eventually not resected.22 On the other hand, the risk of tumor progression after PVE makes it important to minimize the time required to attain sufficient hypertrophy of the nonembolized liver segments to allow safe resection.23 The most effective embolization technique is currently unknown and needs further investigation. Our standardized rabbit model can be used to compare different embolization materials and clarify this issue.

Controversy exists regarding the effect of PVE on tumor progression in both the embolized and nonembolized tumor lobes. A liver metastasis tumor model in the rabbit is well described using a VX2 cell line.24 This model can be combined with our PVE model to study the effect of embolization on tumor growth. In addition, liver steatosis and fibrosis can be introduced to study the effect of different parenchymal liver disease on the hypertrophy response.
Figure 8. Comparison of the histologic changes in the rabbit and human liver after PVE (H and E staining). Histologic examination of the atrophic liver lobe clearly demonstrates the PVA particles within the portal veins. A foreign body reaction (→) characterized by multinucleated giant cells is found around the PVA particles (A). The atrophic liver parenchyma demonstrates sinusoidal dilatation with atrophic hepatocyte trabeculae (B). The hypertrophic liver segments are characterized by intact liver architecture in both rabbits and humans. There is some influx of inflammatory cells in the periportal regions, which is more prominent in the human liver tissue (C).
Conclusion

The combination of two PVA particle sizes and three coils (protocol 2), creates a PVE model in the rabbit that resembles the human situation, taking into account features as histologic changes in the liver parenchyma and the hypertrophy response assessed by CT volumetry. This rabbit model provides an opportunity to perform investigations in a standardized animal model in order to improve the techniques currently used in PVE.
References

Rabbit model for PVE

Chapter 6
Portal vein embolization induces more liver regeneration than portal vein ligation in a standardized rabbit model

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Abstract

Background: Portal vein ligation (PVL) and portal vein embolization (PVE) are used to induce hypertrophy of the future remnant liver before major liver resection. The aim of our study was to compare the hypertrophy response of the liver after PVL versus PVE in a rabbit model.

Materials and methods: Twenty rabbits were divided into an embolization group (n = 10) and a ligation group (n = 10). Both groups were divided in 2 subgroups of 5 rabbits that were humanely killed after days 7 and 14. The portal vein branches to the 3 cranial liver lobes (80% of the liver) were occluded. Regeneration of the caudal liver lobe was measured using volumetry based on computed tomography on days 3, 7, 10, and 14. Immunohistochemistry for Ki-67 and RAM11 was performed to quantify proliferating cells and macrophages. In addition, tissue tumor necrosis factor-α and interleukin-6 were assessed.

Results: The caudal liver volume increased over time in both groups (p < .001), but this increase was greater after PVE than after PVL (p = .001) with a mean degree of hypertrophy of 15% ± 4% and 20% ± 2%, respectively. When comparing the groups on the separate time points, a difference was found on days 10 and 14 (p = .008 and p = .016, respectively). These data were confirmed by Ki-67 staining, which showed a greater number of proliferating hepatocytes on day 7 after embolization (p = .016). Cytokine analysis of liver tissue did not show significant differences between the ligation and embolization groups on days 7 and 14.

Conclusion: PVE is superior to PVL in terms of the extent of the hypertrophy response in this rabbit model.
Introduction

Portal vein ligation (PVL) and portal vein embolization (PVE) are used to induce hypertrophy of the future remnant liver (FRL) before major liver resection in patients with an otherwise too small FRL. PVL is an invasive procedure in which the portal vein is ligated during laparotomy. PVE is a minimally invasive technique that can be performed percutaneously as well as during laparotomy. Using both methods, the portal blood flow is blocked, but only after PVE are the peripheral portal vessels occluded completely by the embolization material. It is important to use a portal vein occlusion technique that provides adequate hypertrophy of the nonembolized lobe in as short a time interval as possible, because of potential tumor progression after the procedure. Clinical and experimental studies show opposite results regarding which embolization technique leads to a greater regeneration response. One prospective study in patients concluded that PVE is superior to PVL in terms of volume gain, lesser time to hypertrophy, lesser hospital stay, and fewer adhesions during major hepatectomy. Another retrospective clinical study showed that PVL is as effective as PVE in inducing hypertrophy of the FRL. In both studies, however, the patient characteristics were not comparable and the methods used were not standardized and could have affected the outcomes.

An animal model is useful to study the effects of PVE and PVL in a standardized fashion. Two animal studies have compared the effect of PVL and PVE on the hypertrophy response; however, these studies showed conflicting results. Furrer et al. performed a study in rats and concluded that PVL is superior to PVE in inducing a regenerative response of the remnant liver, because the amount of proliferating hepatocytes was significantly greater in the PVL compared with the PVE group. In contrast, Wilms et al. found that PVE in minipigs is the more effective technique to increase the FRL. Thus, the question remains of whether PVE or PVL is superior in inducing a liver regeneration response. To elucidate this question, we used our rabbit model in which PVE and PVL can be performed easily to study the hypertrophy response of both procedures. The rabbit liver is very suitable for this purpose because the rabbit liver consists of 3 cranial liver lobes and 1 caudal liver lobe. The caudal lobe accounts for approximately 20% of total liver volume (TLV), corresponding to the volume of FRL that would require portal vein occlusion in humans. Furthermore, like in the clinical situation, repeated computed tomography (CT) volumetry can be performed readily in the rabbit, because CT can easily identify the caudal liver lobe. The aim of this study was to compare the hypertrophy response of the liver after PVL or PVE in this rabbit model.
Materials and methods

Animals
The experimental procedures were approved by the animal ethics and welfare committee of the Academic Medical Center, University of Amsterdam, The Netherlands. Female New Zealand white rabbits with a mean weight of 3,336 g (range, 3,130-3,830; Harlan, France) were acclimatized for ≥7 days under standard laboratory conditions. They were individually housed with a 12-hour light-dark cycle and fed standard chow ad libitum.

Study design
Twenty rabbits were divided into a PVE group (n = 10) and a PVL group (n = 10). Both groups were divided in 2 subgroups of 5 rabbits. The first subgroup was humanely killed after 7 days and the other after 14 days to collect histologic specimens.

CT volumetry
All animals underwent a multiphase CT (Brilliance 64-channel; Philips, Eindhoven, The Netherlands) before PVE and PVL. The rabbits were anesthetized with intramuscular administration of ketamine (25 mg/kg body weight, Nimatek; Eurovet, Bladel, The Netherlands) and medetomidine (0.2 mg/kg body weight, Dexdomitor; Orion, Espoo, Finland), 0.8 mL total volume, and placed in supine position on the CT table. After acquisition of a baseline scan, 3 mL of contrast agent (Visipaque; GE Healthcare, Waukesha, WI) was injected in an ear vein followed by infusion of 4 mL of sterile physiologic saline (Baxter, Deerfield, IL). A scan was performed 15 (arterial phase), 30 (portal phase), and 45 (venous phase) seconds after infusion of contrast agent. In the first subgroup, CT was repeated on days 3 and 7 and in the latter subgroup on days 10 and 14, after which the rabbits were humanely killed. The total liver and the caudal liver lobe were delineated manually and TLV and caudal liver volume (CLV) were calculated by integrated software (Mx-View 3.52; Philips Medical Systems). CLV before PVE was expressed as percentage of TLV using the formula:

\[ \%_{\text{CLV}_{\text{pre-PVE}}}= \frac{\text{CLV}_{\text{post-PVE}}}{\text{TLV}_{\text{pre-PVE}}} \times 100\% \]

After PVE, %CLV was calculated using the formula:

\[ \%_{\text{CLV}_{\text{post-PVE}}} = \frac{\text{CLV}_{\text{post-PVE}}}{\text{TLV}_{\text{post-PVE}}} \times 100\% \]

The degree of hypertrophy was calculated by subtracting the %CLV_{pre-PVE} from the %CLV_{post-PVE} on a certain time point.
Portogram

A portogram was acquired before PVE or PVL, immediately after PVE or PVL, and before animal sacrifice (on days 7 and 14).

Procedures of PVE and PVL

Rabbits were anesthetized by intramuscular injection (1.3 mL) of ketamine (25 mg/kg) and dexdomitor (0.1 mg/kg). Before operation, buprenorphine (0.03 mg/kg body weight, Temgesic; Reckitt Benckiser Healthcare Limited, Hull, UK) and enrofloxacin (0.2 mg/kg body weight, Baytril; Bayer Healthcare, Berlin, Germany) were administered subcutaneously. The eyes were protected against drying out with eye cream (Oculentum simplex; Pharmachemie B.V., Haarlem, The Netherlands). The animal was placed in supine position and 1-2% isoflurane (Forene; Abbott Laboratories, Kent, UK) mixed with O2/air (0.5:0.5 L/min) was used to maintain anesthesia. For the PVL procedure, a midline laparotomy was performed, and the main portal branch to the cranial liver lobes was ligated just above the junction of the portal branch to the caudal liver lobe using a mersilene 4.0 ligature. For the PVE procedure, a branch of the inferior mesenteric vein was cannulated with an 18-gauge catheter (Hospira Venisystems, Lake Forest, IL) after a midline laparotomy. A Renegade 3 Fr microcatheter (Boston Scientific, Natick, MA) with a Transend-ex 0.014 inch wire (Boston Scientific) was inserted subsequently into the portal vein. The catheter was introduced into the portal main branch to the cranial liver lobes, bypassing the portal branch to the caudal liver lobe. A mixture of contrast agent with 90--180 mm polyvinylalcohol particles (Cook, Bloomington, IN) was injected until flow ceased, followed by the positioning of 3 platinum coils (5 and 6 mm, Tornado Embolization Microcoil; Cook). We chose to use this embolization material, because we and others already used this material in the clinical setting. The inferior mesenteric vein was closed subsequently with a ligature. The peritoneum was closed with a running Vicryl 4.0 suture and the skin with interrupted mersilene 3.0 U-sutures. The rabbits were given the antibiotics (0.02 mg/kg Baytril; Bayer Healthcare) subcutaneously once a day for 3 days postoperatively.

Assessment of liver damage and function

Blood samples were drawn before, 3 hours after, and 3 days after portal vein occlusion, and on the day of killing. An additional sample was drawn on days 10 and 14 in the survival subgroup. Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), bilirubin, albumin, and the prothrombintime were determined by routine laboratory assays. Before PVE or PVL and on the day of sacrifice, an indocyanine green (ICG) clearance test was performed to assess liver function. Two 22-gauge venflon catheters were placed in the ear vein and in the contralateral ear artery. Freshly prepared ICG was injected into the ear vein (12.5-mg ICG PULSION; PULSION Medical Systems, Munich, Germany; dissolved in 5 mL of sterile water). Blood samples were obtained before and 1-6 minutes after
ICG injection. Plasma samples were diluted (250 mL plasma in 600 mL of 1% bovine serum albumin in 0.9% NaCl) and measured spectrophotometrically at 805 nm (Uvikon 850; Kontron Instruments, Munich, Germany). The ICG disappearance constant (k) was derived from the slope of the semilogarithmic decay curve. Accordingly, the ICG plasma disappearance rate (PDR, %/min) was calculated using the formula: 

\[ PDR = k \times 100 \]

**Total liver weight and wet: dry weight ratio**

After killing, the total liver and the caudal liver lobe were weighed. To demonstrate that the increase in liver volume was not due to edema, the percentage of water was determined in caudal and left lateral liver lobe biopsies. The specimens were weighed directly after sacrifice (wet weight) and stored subsequently in a stove at 60˚C. After 4 weeks, the specimens were weighed again (dry weight). The percentage of water was calculated by the formula: 

\[ \text{Percent water} = \frac{\text{wet weight} - \text{dry weight} \times 100}{\text{wet weight}} \]

**Mediators of liver regeneration**

The cytokines tumor necrosis factor (TNF-α) and interleukin (IL)-6 were assessed by an enzyme-linked immunosorbent assay in homogenized liver tissue of the caudal and left lateral liver lobes using polyclonal TNF-α and IL-6 goat anti-rabbit antibodies (USCN Life, Wuhan, China) according to the manufacturer’s instructions.

**(Immunohistochemistry)**

Parafin sections of the caudal and left lateral liver lobes were fixed in buffered formalin, dehydrated in graded ethanol and xylene, and stained with hematoxylin and eosin (H&E). The H&E stained slides were scored for necrosis, inflammation, atrophy/sinusoidal dilatation, and edema using an ordinal scale: grade 0, none; grade 1, mild; grade 2, moderate; and grade 3, severe. All slides were scored by an experienced pathologist in a blinded fashion. Additionally, sections were immunostained with anti-Ki-67 antibodies (monoclonal mouse anti rat Ki-67 antigen, clone MIB-5; Dako Cytomation, Carpentaria, CA) and with antibodies against macrophages (monoclonal mouse anti-rabbit macrophage, clone RAM11; Dako Cytomation). The immunostained sections were counterstained with hematoxylin. Ki-67-and hematoxylin-positive cells were counted in 10 medium power fields (20magnification) per section using Image J software (National Institutes of Health, Bethesda, MD). The proliferation index was defined as the percentage of Ki-67-positive hepatocytes per total hepatocytes in the field of view. The pixels in 10 fields of view (20magnification) occupied by macrophages was determined by Image J software and expressed as percentage of the total amount of pixels in the field of view.

**Statistical analysis**

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS, Chicago, IL). CT volumetry data were compared using a linear mixed model analysis.
based on ranked data. The separate time points and other continuous data were compared by the Mann-Whitney U test. Correlation between variables was tested using the Spearman’s rank correlation coefficient. All statistical tests were 2 tailed, and differences were considered significant when p<0.05. Data were tested for normal distribution and equal variances and expressed as mean values ± standard deviations unless otherwise stated.

Results

Assessment of portal vein occlusion after PVE and PVL

In all rabbits, a portogram was performed before PVE and PVL, depicting portal perfusion of the whole liver. Directly after embolization or ligation, the portogram showed total occlusion of the portal blood flow to the cranial liver lobes in all rabbits (Figure 1). Total occlusion persisted in both groups 7 days post-PVE and PVL. On day 14, however, collateral formation was visible clearly in all 5 rabbits of the PVL group, resulting in extensive parenchymal perfusion of the cranial lobes. In the PVE group, partial revascularization was found of the main portal trunk to the cranial liver lobes in 3 of the 5 rabbits, but only a little parenchymal perfusion was observed. In the other 2 rabbits, the portal vein to the cranial liver lobes was still occluded completely.

PVE- and PVL-induced hypertrophy response

As demonstrated in concordance with the findings in a previous study,15 a strong positive correlation was found between liver volume based on CT volumetry and liver weight (Spearman’s r =0.91; p<.001). The %CLV before occlusion was 22% ± 2% in the PVE group versus 21% ± 3% in the PVL group (p = .19). The %CLV increased to 28% ± 4%, 33% ± 4%, 34% ± 3%, and 34% ± 6% on days 3, 7, 10, and 14 in the PVL group and to 29% ± 3%, 38% ± 3%, 41% ± 1%, and 42% ± 2% in the PVE group, respectively (p<.001 in both groups) and was greater after PVE than afterPVL(p= .001). The mean degree of hypertrophy after 14 days was 15% ± 4% in the PVL and 20% ± 2% in the PVE group (p=.016). When comparing the groups on the individual time points, there was already a difference in %CLV on day 7 in favor of the PVE group which reached significance on days 10 and 14 (p<.02).

The percentage of Ki-67-positive hepatocytes in the caudal liver lobe was greater in the PVE group (20% ± 6%) compared with the PVL group (7% ± 5%; p= .016) on day 7. On day 14, both groups showed the same amount of proliferating hepatocytes (Figure 2). Wet: dry ratios showed no differences in water content between the PVE and PVL group on both days, indicating that the enhanced volume gain in the PVE group was not from edema.

CT volumetry data showed that PVE led to a greater hypertrophy response than PVL. This finding is supported by the greater number of proliferating hepatocytes found in the PVE group.
Figure 1. Portography. Portograms are shown of a rabbit from the PVL (above) and the PVE group (below). Before the intervention the total portal tree is clearly visible. The narrowings of the portal vein in the PVL group (white arrowheads) are spasms of the vein as a consequence of the placement of the suture material for subsequent ligation. Directly after portal vein occlusion there is no portal blood flow to the cranial liver lobes visible anymore, indicating a successful procedure. At day 14, collateral formation was clearly visible in all PVL rabbits (black arrowheads) with marked parenchymal perfusion. In the PVE group 3 of the 5 rabbits showed little recanalization in the main trunk to the cranial liver lobes, but with no or (in this rabbit) minimal parenchymal perfusion.
Determination of liver damage and function
In both groups, parameters of liver damage in plasma showed a transient increase with a peak after 3 hours (LDH) or 3 days (AST, ALT), which returned to baseline values on day 7 (Figure 3). The H&E slides showed normal liver architecture in the caudal liver lobe after both procedures. In the PVE groups, the PVA particles were visible clearly within the portal veins of the atrophic liver lobe. A foreign body reaction characterized by multinucleated giant cells was present around these particles. No necrosis or edema was observed in either group. Diffuse infiltration of inflammatory cells (lymphocytes and granulocytes) and sinusoidal dilatation/atrophic trabeculae were observed in the atrophic lobes in the PVE and PVL group to a similar degree (Figure 4). Plasma bilirubin and albumin concentrations, prothrombin time, and ICG-PDR were measured to assess the synthetic and clearance functions of the liver. The bilirubin levels and prothrombin time were stable over time. Plasma albumin concentration and ICG-PDR showed a small decrease in the first week in both groups, which was restored in the second week (Figure 5).

Mediators of liver regeneration
Kupffer cells produce cytokines, which are important for liver regeneration. There were more Kupffer cells in the atrophic liver lobes of the PVE group compared with the PVL group on day 7 (p = .032; Figure 6). In contrast with the PVL group, there were more Kupffer cells present in the atrophic, embolized liver lobe compared with the hypertrophic, nonembolized liver lobe in the PVE group (p < .02 on days 7 and 14). There were no differences in IL-6 and TNF-α level between the PVE and PVL groups on days 7 and 14, respectively.
Figure 3. Liver damage. Plasma levels of LDH (A) and ALT (B) showed an increase after 3 days and 3 hours, respectively, in both groups, after which they returned to baseline values.

Figure 4. Histology. H&E slides of the atrophic liver lobe 7 days after portal vein embolization (PVE) and portal vein ligation (PVL) show sinusoidal dilatation after both procedures. The embolization material with foreign body reaction is clearly visible in the portal vein (arrow).
Figure 5. Liver function. Plasma albumin levels (A) and the ICG-PDR (B) showed a transient, mild decrease after both procedures and returned to baseline levels within 14 days.

Figure 6. Macrophages/Kupffer cells. At day 7 the RAM11-positive area was significantly larger in the atrophic liver lobe after PVE compared with PVL (*P = .032). In the PVE group, the area was larger in the atrophic compared with the hypertrophic liver lobe on days 7 and 14 (#P = .08 and .016, respectively).
Discussion

In this study, we compared the effects of ligation versus embolization of the portal vein on the hypertrophy response of the FRL in a rabbit model. The most effective occlusion technique is unknown currently. Every animal model has its strengths and weaknesses dictated by factors as species-dependent morphology and practical issues. The anatomy of the rabbit liver is unique in that the cranial and caudal lobes are separate, and the caudal lobe accounts for 20% of the TLV. These configurations make the rabbit liver very suitable to examine selective portal vein occlusion and the resulting regenerative response. In addition, a great advantage of our model is that each rabbit serves as its own control, because of the repeated CT volumetry measurements. This ability to repeat CT volumetry is comparable with the clinical situation and the results are statistically stronger. We showed that PVE induces a significantly greater hypertrophy response than PVL as assessed by CT volumetry and supported by liver weight measurements and the amount of proliferating hepatocytes. These results are comparable with the results of a study in minipigs of Wilms et al., in which the hypertrophy response was assessed by liver to body weight.

In our study, the amount of proliferating hepatocytes was significantly greater in the PVE group on day 7. In contrast to this result, Furrer et al showed in a rat model that the number of proliferating hepatocytes in the regenerating liver lobe was significantly less in the PVE group compared with the PVL and PH groups 48 hours after the intervention; this difference disappeared after 72 hours. In our study, the amount of proliferating hepatocytes was significantly greater in the PVE group on day 7. Although we performed the measurement on different time points, it is not likely to assume that more proliferating hepatocytes would occur in the PVE group at a later time point in this rat study.

Several factors may be considered to explain the difference in hypertrophy response after PVE and PVL. First, formation of collateral portal vessels leading to parenchymal portal reperfusion after PVL seems a likely explanation. This phenomenon was described previously by Denys et al and was confirmed by the abovementioned study in minipigs in which duplex ultrasonographic measurements showed that the portal branches were occluded for a greater time duration after PVE and that collateral formation seemed to be the cause of less effective regeneration in the PVL group. In our study, portal venous collateral formation was visible clearly 14 days after PVL with concomitant reperfusion of the parenchyma of the previously ligated liver lobes. In the PVE group, no collateral formation was seen, but minor recanalization of the main trunk of the portal vein to the cranial lobes was visible, which, however, did not lead to substantial parenchymal reperfusion of the embolized liver lobes. Interestingly, the hypertrophy response after PVE was already greater before collateral formation was visible in the PVL group. Therefore, there must be another mechanism for the hypertrophy response and the difference after PVE or PVL. This may be sought
in the cytokine response. Kupffer cells produce IL-6 and TNF-a, which are important mediators of liver regeneration. One of the hypotheses of Furrer et al. to account for the superiority of PVL over PVE was the observed entrapment of macrophages in the embolized liver lobes owing to a foreign body reaction caused by the embolization material. This process would lead to a decreased accumulation of macrophages and thus decreased production of cytokines in the regenerating liver lobe. In our study, we used a different embolization material. Nevertheless, we also found significantly more Kupffer cells in the atrophic, embolized liver lobes compared with PVL; however, in contrast to the study by Furrer et al., the regenerative response after PVE was greater than after PVL. We did not find a difference in IL-6 and TNF-a levels in the regenerating lobes of the PVE and PVL groups after 7 and 14 days postocclusion. It could be that we missed the peak of the cytokine response because this peak probably occurs earlier in rabbits.

Liver damage in our study was mild after PVE as well as after PVL as shown by plasma AST, ALT, and LDH levels and the absence of parenchymal necrosis. Liver function as assessed by plasma bilirubin and albumin levels, prothrombin time, and ICG clearance rate was not markedly affected. These findings are in accordance with other reports and confirm that both techniques can be performed safely, without a risk of liver failure.

Regarding the rabbit model used in our study, several remarks are in order. First, the rabbit liver consists of 4 main liver lobes connected by parenchymal bridges. The parenchymal contact between the occluded and nonoccluded lobes is, therefore, less extensive than in the human liver. The formation of a collateral portal venous flow is more likely to occur in the human liver because of the close contact of the right and left liver segments, which suggests that the hypertrophy response after PVL in humans would even be less. A limitation of this model is the different approach for PVE. In humans, the portal vein is usually catheterized via a percutaneous transhepatic approach, whereas in our rabbit model a laparotomy was used to access the portal vein. For reasons of comparing only the effects of PVL and PVE, it is preferable to perform a laparotomy in both instances in this model.

In conclusion, based on the results in our rabbit model, the regenerative response after PVE seems to be superior to PVL, at least in this rabbit model. Our findings may have important implications in man.
References

Liver regeneration after portal vein embolization using absorbable and permanent embolization materials in a rabbit model

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Abstract

**Background:** PVE is used to increase future remnant liver volume preoperatively. Application of temporary, absorbable embolization materials could be advantageous in some situations, provided sufficient hypertrophy is achieved of the non-embolized lobe. The aim of this study was to compare the safety and hypertrophy response following portal vein embolization (PVE) using two absorbable and three permanent embolization materials.

**Methods:** Six groups of rabbits (n=5) underwent PVE of 80% of the total liver volume using saline (sham), gelatin sponge, fibrin glue, polyvinyl alcohol particles with coils (PVAc), nbutylcyanoacrylate (nBCA), or polidocanol. The rabbits were sacrificed after 7 days. Portography, CT volumetry, Doppler ultrasound, laboratory liver function and damage parameters, (non-embolized) liver-to-body weight ratio, immunohistochemistry, and cytokine and growth factor tissue levels were assessed to examine the differences in the liver regeneration response.

**Results:** Polidocanol was discontinued because of toxic reactions in 3 rabbits. Gelatin sponge was the only material that was absorbed within 7 days and resulted in less hypertrophy of the non-embolized lobe compared to the other 3 materials. There were no significant differences in hypertrophy response between the other 3 embolization groups. CT volumetry data were supported by liver-to-body weight ratio and the amount of proliferating hepatocytes. The volume gain of the non-embolized lobe was proportional to the volume loss of the embolized liver lobes. The number of Kupffer cells in the embolized liver lobe was significantly higher in the fibrin glue, PVAc, and nBCA groups compared to the sham and gelatin sponge group. However, the levels of IL-6, TNF-α, HGF, and TGF-β1 were significantly lower.

**Conclusions:** Temporary occlusion using gelatin sponge for PVE resulted in significantly less hypertrophy response compared to the use of permanent embolization materials. Except for polidocanol, none of the embolization materials exhibited evident hepatotoxicity.
Introduction

Portal vein embolization (PVE) is a widely used method to increase the future remnant liver (FRL) before major liver resection. This is necessary when the amount of FRL is considered too small, thereby increasing the risk of postoperative liver failure. With PVE the portal vein branches of the to-be-resected liver lobe are occluded, causing atrophy of this liver lobe. This results in a release of regenerative factors that induces a compensatory hypertrophy response in the FRL.

There are two main methods to occlude the portal vein: by portal vein ligation (PVL) or by embolization. In a previous study we compared the effects of PVE and PVL in a rabbit model and concluded that PVE is superior to PVL in terms of the extent of the hypertrophy response. Many embolization materials are available, the majority of which causes a permanent occlusion of blood vessels. It is believed that permanent occlusion of the portal vein is more effective in inducing a hypertrophy response than transient occlusion. However, there are several clinically relevant drawbacks to the use of permanent embolization agents. Firstly, there is always a risk that the embolization material migrates to contralateral portal vein branches. When the material is absorbable, occlusion of non-targeted vasculature is reversible and therefore safer. Secondly, in patients in whom the embolized part of the liver is ultimately not resected, occlusion of the portal vein with an absorbable material would be advantageous over a permanent material in order to preserve/regain function of this part of the liver. Thirdly, reversible PVE for the induction of liver regeneration has potential use in living donor liver transplantation, in which the future graft in the donor could be increased without endangering residual liver function of the donor. These points underscore the potential benefit of using absorbable embolization agents for PVE.

Accordingly, there is a need to elucidate whether the hypertrophy response is dependent on the type of embolization material (permanent vs. absorbable) and to determine which material is most suitable for PVE with respect to the extent of liver regeneration and safety. A recent study on the effect of reversible PVE on liver regeneration in monkeys concluded that reversible PVE using gelatin powder efficiently induced a hypertrophy response. However, it is presently unclear which type of embolization material optimally induces liver regeneration. Consequently, this study investigated the extent of the hypertrophy response following PVE using 2 absorbable and 3 permanent embolization agents in a standardized rabbit model. In anticipation of potential clinical applicability of the embolization materials in liver surgery, the safety of the embolization agents was evaluated on the basis of post-PVE hepatocellular damage and liver function. Finally, regeneration-specific cytokines and growth factors as well as the cellular constituents responsible for their release were assayed in the atrophic and hypertrophic liver lobes.
Materials and methods

Animals
Experimental protocols were approved by the institute’s animal ethics and welfare committee. In total 38 female New Zealand White rabbits (Harlan, Gannat, France) with a mean weight of 3,108g (range 2,800-3,450g) were acclimatized for 2 weeks under standardized laboratory conditions in a temperature-controlled room with a 12-h light/dark cycle and access to standard chow and water ad libitum.

Experimental design
Six groups of 5 rabbits were planned for PVE, each group corresponding to a different embolization material. Prior to PVE, blood samples were drawn and CT volumetry and digital subtraction portography were performed as described below. The portal blood flow in the caudal and right liver lobe was quantified by Doppler ultrasound (ProSound 3500SX, Aloka, Tokyo, Japan).

PVE was performed as described below using 2 absorbable embolization materials and 3 permanent embolization materials. With respect to the former, fibrin glue (Tissucol, Baxter Healthcare, Deerfield, IL) or gelatin sponge (Spongostan, Ferrosan, Soeborg, Denmark) was used. The gelatin sponge was completely dissolved in sterile physiological saline (Baxter) by repetitively passing the gel foam shred-containing fluid from one 1-mL syringe to another via an interposed stopcock (BD Biosciences, San Jose, CA) while gradually closing the valve in the stopcock in order to produce a viscous fluid. For the permanent materials, a combination of polyvinyl alcohol particles (90-180 μm in diameter followed by 300-500 μm in diameter, Cook, Bloomington, IN) and 3 fibered platinum coils (4.0, 5.0, and 6.0 mm, Boston Scientific, Natick, MA) (PVAc) was used, or the infusion of n-butyl cyanoacrylate (nBCA) (Histoacryl, B. Braun Medical, Melsungen, Germany) or polidocanol (Aethoxysklerol 3%, Kreussler Pharma, Wiesbaden, Germany). It should be noted that PVE with polidocanol was discontinued due to the high level of toxicity; 2 of the first 3 animals died immediately after injection of polidocanol. The control group received sterile physiological saline as placebo embolization material (sham). Directly after PVE, digital subtraction portography was performed so as to confirm portal vein occlusion. Blood sampling, CT volumetry, and Doppler ultrasound were repeated on days 3 and 7 post-PVE. Digital subtraction portography was performed prior to sacrifice on day 7.

Additionally, 10 rabbits were added to the gelatin sponge and PVAc groups (n=5 per group) and sacrificed 24 hours after PVE in order to obtain liver tissue at the onset of the liver regeneration response.

Portal vein embolization
Animals were anesthetized by intramuscular injection of ketamine (25.0 mg/kg body weight, Nimatek, Eurovet, Bladel, the Netherlands) and medetomidine (0.2 mg/kg...
Comparison of PVE materials

Chapter 8

body weight, Dexdomitor, Orion, Espoo, Finland). Maintenance anesthesia consisted of 1-2% isoflurane (Forene, Abbott Laboratories, Kent, UK) mixed with O2: air (0.5:0.5 L/min). Buprenorphine (0.03 mg/kg body weight, Temgesic, Reckitt Benckiser Healthcare, Hull, Great Britain) and Baytril (0.2 mg/kg body weight, Bayer Healthcare, Berlin, Germany) were administered subcutaneously prior to the operation. The animals were placed in supine position. The eyes were protected from drying out using an eye cream (Oculentum simplex, Pharmachemie, Haarlem, the Netherlands). Heart rate and arterial oxygen saturation were measured by pulse oximetry (Hewlett Packard M1165A, model 56S, Andover, MA) on the hind leg throughout the operative procedure. After a midline laparotomy, a branch of the inferior mesenteric vein was cannulated with an 18-G catheter (Hospira Venisystems, Lake Forest, IL). A Renegade 3-Fr microcatheter (Boston Scientific) with a Transend-ex 0.36 mm x 182 cm guide wire (Boston Scientific) was subsequently introduced into the portal vein. Digital subtraction portography was performed with a mobile C-arm Exposcop 8000 (Ziehm Imaging, Nürnberg, Germany) to identify the individual portal vein branches. A schematic picture of the portal vein branches in the rabbit is shown in Figure 1A. After passing the portal branch to the caudal liver lobe, the microcatheter was positioned in the main portal branch supplying the cranial liver lobes. The portal branches were embolized by transcatheter infusion of the embolization agents. Subsequently, the catheter was flushed with sterile physiological saline or, in case of Histoacryl, with 5% glucose in order to prevent obstruction of the catheter. Following portographic confirmation of PVE, the catheter was removed and the mesenteric vein was closed with a ligature. The abdomen was closed in two layers. Baytril (0.02 mg/kg body weight) was administered subcutaneously once a day up to postoperative day 4.

CT volumetry

A multiphasic CT scan was performed with a 64-slice CT scanner (Brilliance 64, Philips, Eindhoven, the Netherlands) on anesthetized animals placed in supine position. After a baseline series, contrast solution (3 mL Visipaque, GE Healthcare, Waukesha, WI) was injected through a 22-G venflon catheter in the ear vein followed by a flush with 4 mL sterile physiological saline. A contrast-enhanced scan was performed at 15 s (arterial phase), 30 s (portal phase), and 45 s (venous phase) after injection of contrast solution. 3-D reconstructions of the liver were composed by superimposing sequential reconstructed 2-mm axial slices. The total liver and the caudal liver lobe were manually delineated and the total liver volume (TLV) and caudal liver volume (CLV) were calculated. Before PVE, CLV was expressed as a percentage of TLV (%CLV) using the formula:

\[
\%\text{CLV}_{\text{pre-PVE}} = \frac{\text{CLV}_{\text{pre-PVE}}}{\text{TLV}_{\text{pre-PVE}}} \times 100\%
\]
After PVE, the CLV was calculated by:

\[
\%CLV_{\text{post-PVE}} = \frac{CLV_{\text{post-PVE}}}{CLV_{\text{pre-PVE}}} \times 100\%
\]

The increase of the CLV was calculated by:

\[
\text{Increase} \text{ CLV} = \left( \frac{CLV_{\text{post-PVE}} - CLV_{\text{pre-PVE}}}{CLV_{\text{pre-PVE}}} \right) \times 100\%
\]

The degree of hypertrophy\(^1\) at designated time points was calculated by:

\[\text{Degree of Hypertrophy} = \%CLV_{\text{post-PVE}} - \%CLV_{\text{pre-PVE}}\]

The decrease of the atrophic liver volume (ALV), i.e., the cranial liver lobes, was calculated by:

\[\text{Degree of Atrophy} = \%ALV_{\text{post-PVE}} - \%ALV_{\text{pre-PVE}}\]

**Liver to body weight index**

After sacrifice the liver was weighed using a precision scale (Sartorius, Göttingen, Germany). The liver weight was divided by the body weight to correct for influences of body weight.

**Wet-to-dry weight ratio**

After sacrifice liver biopsies of the caudal and left lateral lobes were weighed (wet weight) and subsequently stored in a stove at 60°C. After 4 weeks, the specimens were weighed again (dry weight). The percentage of water was calculated by the formula: (wet weight – dry weight) x 100 / wet weight.

**Liver damage and function**

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined as well-established liver damage parameters. Prothrombin time and albumin were used as indirect parameters of liver synthesis function, whereas plasma bilirubin was used as an indirect measure of hepatic uptake and excretory function. All parameters were determined by routine clinical chemistry.

**Histological examination**

Liver tissue samples from an embolized (left lateral) and the non-embolized (caudal) liver lobe were fixed in buffered formalin, dehydrated in graded steps of ethanol and xylene, embedded in paraffin, and cut in 5-μm sections. The histological specimens were stained with hematoxylin and eosin (H&E). All H&E slides were scored by an experienced pathologist in a blinded fashion for necrosis, inflammation, atrophy/sinusoidal dilatation, and edema using an ordinal scale: grade 0, none; grade 1, mild;
grade 2, moderate; grade 3, severe. Additionally, sections were immunostained with dianinobenzidine (DAB)-conjugated anti-Ki-67 antibodies (monoclonal mouse anti-rat Ki-67 antigen, clone MIB-5, Dako Cytomation, Glostrup, Denmark) and with DAB-conjugated antibodies against macrophages (monoclonal mouse anti-rabbit macrophage, clone RAM11, Dako Cytomation) according to the manufacturer's instructions. The immunostained sections were counterstained with hematoxylin. Ki-67- and hematoxylin-positive cells were quantified in 10 fields of view per section (20× magnifications) using ImageJ software (Ki-67 plugin, NIH, Bethesda, MD). The proliferation index was defined as the percentage of Ki-67-positive hepatocytes per total hepatocytes in the field of view. The pixels in a field of view occupied by macrophages (RAM11-positive pixels) was determined by ImageJ software and expressed as a percentage of the total amount of pixels in the field of view.

**Cytokines and growth factors**

Several liver regeneration-specific cytokines and growth factors were quantified from liver tissue obtained from the caudal and left lateral liver lobe. The levels of interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), hepatocyte growth factor (HGF), and transforming growth factor beta 1 (TGF-β1) were measured in homogenized liver tissue using an ELISA kit for the respective antigen (USCN Life, Wuhan, China) according to the manufacturer’s instructions. Antibodies were diluted 4× in phosphate buffered saline (PBS). All samples were measured in duplicate and the concentrations were calculated from a standard curve. Protein concentrations were determined with a BCA Protein Assay kit (Pierce, Rockford, IL). Hepatic cytokine and growth factor content was normalized to protein content.

**Confocal microscopy**

Biopsies of the caudal and left lateral liver lobe were snap frozen in liquid nitrogen and stored at -80°C until histological processing. The sections were cryocut and equilibrated at room temperature for 30 min and fixed in ice cold acetone (-20°C) for 5 min. After drying in air, the sections were washed twice in PBS for 2 min. Subsequently, the sections were immunostained with anti-macrophage antibodies (1:500 dilution in PBS-1% bovine serum albumin (BSA), clone RAM11, Dako Cytomation) and either polyclonal goat anti-rabbit IL-6 or TNF-α antibodies (1:125 dilution in PBS-1%BSA, USCN Life) for 1 h at room temperature. The sections were washed 3× for 2 min in PBS after which the anti-macrophage and anti-cytokine primary antibodies were secondarily labeled with Cy3-conjugated donkey anti-mouse IgG (500 μg/mL, 1:50 dilution in PBS-1%BSA, Millipore, Billerica, MA) and Alexa488-conjugated chicken anti-goat IgG (H+L chains, 2 mg/mL, undiluted, Invitrogen, Carlsbad, CA), respectively, for 15 min in the dark. Control sections were incubated with the fluorophore-conjugated secondary antibody only to rule out unspecific binding and to set the background fluorescence.
intensity. The sections were washed 3× in PBS for 2 min, mounted (Vectashield, Vector Laboratories, Burlingame, CA), and stored in the dark at 4°C until used for confocal microscopy.

Confocal microscopy was performed with a Leica SP2 system equipped with an argon laser and OATB transmission filters (Wetzlar, Germany). Alexa488-labeled constituents were imaged at $\lambda_{ex} = 476$ nm, $\lambda_{em} = 498$-552 nm and Cy3-labeled constituents were imaged at $\lambda_{ex} = 561$ nm, $\lambda_{em} = 568$-627 nm. A Normanski filter set was used to generate differential interference contrast images with the 561-nm laser line.

**Statistical analysis**

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS, Chicago, IL). Tests were performed for equal variances (Levene’s test) and normality (Shapiro-Wilks test), in consequence to which statistical differences ($p<0.05$) were tested nonparametrically. Overall differences between groups were assessed by the Kruskal Wallis test. If the Kruskall Wallis indicated a significant difference between groups, separate Mann-Whitney U tests were used to compare the groups individually. In the latter we used a Bonferroni-Holm adjustment to correct for multiple testing with an adjusted alpha of 0.05 denoting the level of significance. Repeated measurements were analyzed using linear mixed model analysis based on rank-transformed data. A Mann-Whitney U test was used to analyze differences between the atrophic and caudal liver lobe. Correlation between variables was tested using the Spearman’s $\rho$ coefficient. Repeated measurement and correlation tests were two-tailed and differences were considered significant at a $p$-value of $<0.05$. Data are expressed as mean±SD unless otherwise stated.

**Results**

**Portal vein occlusion, liver damage, and liver function**

Digital subtraction portography performed before PVE did not reveal any notable anatomical variations in hepatic vasculature. Portography performed directly after PVE confirmed complete occlusion of the portal vein branches to the cranial liver lobes in all treatment groups (i.e., the gelatin sponge, fibrin glue, PVAc, and nBCA groups). On day 1 after PVE (animals included a posteriori), 3 out of 5 rabbits in the gelatin sponge group exhibited reperfusion of the portal vein to the cranial liver lobes, whereas this branch of the portal vein remained occluded in all PVAc animals. On day 7, the cranial segment of the portal vein had remained completely occluded in the fibrin glue, PVAc, and nBCA groups (from here onward collectively termed “long-term occluding embolization materials”). However, in the gelatin sponge group, recanalization of the portal vein was observed in all animals on day 7, leading to extensive parenchymal perfusion of the cranial liver lobes (Figure 1).
Figure 1. Representative portographs acquired 7 days after PVE. A schematic picture of the rabbit liver anatomy is shown in panel A (CL=caudal liver lobe, LL=left lateral liver lobe, LM=left medial liver lobe, and RL=right liver lobe). In (B), a radiographic image is shown of the total portal tree corresponding to the liver shown in A. Portal blood flow to the embolized cranial liver lobes was almost completely restored following PVE with gelatin sponge (C). In the fibrin glue (D), PVAc (E), and nBCA (F) groups, the portal vein to the cranial liver lobes did not fill with contrast fluid, indicating that the embolized branches were still occluded. The level of embolization is indicated by white arrows (D-F).

Doppler ultrasonography showed an increase in portal blood flow to the caudal liver lobe directly after PVE in all groups that had received an embolization agent, albeit the flow did not differ statistically from the control group. No portal blood flow was detected in the cranial liver lobes directly after PVE. Three and 7 days after PVE, portal blood flow in the cranial liver lobes was detected in all rabbits of the gelatin sponge group. In concordance with the portography findings, the cranial liver lobes of the fibrin glue, PVAc, and nBCA groups had remained deprived from portal blood flow (data not shown). Serum liver transaminases and LDH showed a transient increase after PVE in the 4 treatment groups with a concentration peak on day 1. AST levels on day 1 were significantly higher in all treatment groups compared to the sham group (p≤0.016) (Figure 2). The synthesis, uptake, and/or excretory functions of the liver, assessed by prothrombin time, albumin, and bilirubin were not significantly affected by the procedures in any of the groups (data not shown). Histopathological examination of H&E-stained liver biopsies of the left lateral and caudal liver lobes did not reveal necrotic regions in any of the groups, and no significant differences in scores were found for atrophy/sinusoidal dilatation and edema (data not shown).
Figure 2. Liver damage following PVE. Plasma AST, ALT, and LDH exhibited a transient increase that peaked 1 day (d1) after PVE. Only the AST levels on day 1 were significantly higher in all treatment groups compared to control (*, p≤0.016).

Figure 3. The degree of hypertrophy as determined by CT volumetry plotted as a function of time after PVE. The degree of hypertrophy of the caudal liver lobe is significantly higher in all treatment groups compared to the sham group on days 3 and 7 after PVE (# and *, respectively, p≤0.016). The degree of hypertrophy of the fibrin glue, PVAc, and nBCA groups was significantly higher compared to the gelatin sponge group 7 days after PVE (*, p≤0.016).

Liver regeneration response
CT volumetry data are presented in Table 1 for the caudal, hypertrophic liver lobe. The CLV increased significantly in the first 3 days after PVE in all 4 treatment groups and further increased from day 3 till 7 in the fibrin glue, PVAc, and nBCA groups. The degree of hypertrophy was significantly higher in all treatment groups compared to the sham group on day 3 (p≤0.016), whereas on day 7, the degree of hypertrophy was significantly higher for the long-term occluding embolization materials compared to the gelatin sponge and sham groups (p≤0.016) (Figure 3).

The CT volumetry data were supported by the liver-to-body weight index of the caudal liver lobes, which was also significantly higher for the long-term occluding
embolization materials compared to the gelatin sponge and sham groups on day 7 (p<0.016). The wet-to-dry weight ratio was not different between the groups (data not shown), precluding the possibility that edema caused the volume/weight gain.

In concordance with these findings, PVE performed with fibrin glue, PVAc, and nBCA induced significantly more hepatocyte proliferation in the caudal liver lobe compared to the absorbable gelatin sponge group as assessed by Ki-67 staining on day 7 (p<0.016) (Figure 4). Moreover, the number of proliferating hepatocytes was significantly higher on day 7 in the caudal, non-embolized liver lobe compared to the cranial, embolized liver lobe for the permanent occluding embolization materials (p<0.05).

In summary, long-term occlusion of the portal vein leads to a more profound hepatocyte proliferation in the non-embolized liver lobe and thus to a higher hypertrophy response compared to short-term occlusion.

Table 1. CT volumetry data of the caudal liver lobe

<table>
<thead>
<tr>
<th>Group</th>
<th>Measurement (mean±SD)</th>
<th>Measurement time point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-PVE</td>
</tr>
<tr>
<td>Sham</td>
<td>Absolute CLV [cm³]</td>
<td>17.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>%CLV</td>
<td>26.3 ± 1.4</td>
</tr>
<tr>
<td>Gelatin sponge</td>
<td>Absolute CLV [cm³]</td>
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<tr>
<td></td>
<td>%CLV</td>
<td>25.7 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>CLV increase [%]</td>
<td>-</td>
</tr>
<tr>
<td>Fibrin glue</td>
<td>Absolute CLV [cm³]</td>
<td>17.9 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>%CLV</td>
<td>22.6 ± 2.9</td>
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<tr>
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<td>PVAc</td>
<td>Absolute CLV [cm³]</td>
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<tr>
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<td>%CLV</td>
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<td>Absolute CLV [cm³]</td>
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<tr>
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<td>22.6 ± 1.9</td>
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<tr>
<td></td>
<td>CLV increase [%]</td>
<td>-</td>
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Figure 4. Extent of liver regeneration in the caudal and left lateral lobe expressed as the percentage of Ki-67-positive hepatocytes (A). In the fibrin glue, PVAc, and nBCA groups, significantly more proliferating hepatocytes were found in the hypertrophic, caudal liver lobe compared to the atrophic, left lateral liver lobe (#, p<0.05). Significantly more proliferating hepatocytes were present in the caudal liver lobes of the fibrin glue, PVAc, and nBCA groups, compared to the gelatin sponge and sham group (*, p<0.016). Ki-67-stained sections are depicted of the caudal (B) and cranial (C) liver lobe embolized with nBCA.

Figure 5. H&E (top row) and stained sections with a macrophage-specific antibody (RAM11, bottom row) of serially sectioned embolized portal vein segments of the left lateral liver lobe 7 days after PVE. Perilobular and periporal inflammation was predominantly observed in the fibrin glue group, whereas extensive inflammatory infiltration into the embolization material was observed in the gelatin sponge group (p=0.032 Mann-Whitney U test). RAM11 staining in the long-term occluding embolization material groups (PVAc and nBCA) was primarily confined to the vascular lumen.
Figure 6. Quantification of Kupffer cells in the caudal and left lateral lobes following PVE. Histological sections were stained with a Kupffer cell-specific antibody (RAM11) and analyzed with ImageJ software for the number of ‘positive pixels.’ The area filled with macrophages was significantly greater in the atrophic, left lateral liver lobe compared to the hypertrophic caudal lobe of the fibrin glue, PVAc, and nBCA groups (#, p<0.05). The differences between the atrophic liver lobes of the fibrin glue, PVAc, and nBCA groups was significantly greater compared to the gelatin sponge and sham groups (*, p<0.025).

Figure 7. Levels of IL-6 (A) and TNF-α (B) measured by ELISA in homogenized caudal liver tissue obtained 7 days after PVE, normalized to protein content. Confocal microscopy was performed on immunostained sections derived from the left lateral liver lobes on day 1 (C-F) and day 7 (G-J) after PVE. Representative images of TNF-α are shown from the gelatin sponge group. Kupffer cells were labeled with anti-macrophage antibodies secondarily labeled with Cy3-conjugated IgG, appearing red. TNF-α was labeled by antibodies raised against the respective epitope and secondarily labeled with Alexa488-conjugated IgG, appearing green. Kupffer cells (white arrowheads) expressed little TNF-α 1 day after PVE, whereas on day 7 TNF-α abundantly colocalized with Kupffer cells. Incubation with secondary antibodies only revealed no unspecific binding (K-N). Differential interference contrast (DIC) images were acquired to provide anatomical detail. CV=central vein, S=sinusoids.
Mechanistic features of the differential hypertrophy response

The hypertrophy response is believed to be triggered by the lobular atrophy induced by PVE in a proportional manner. Accordingly, correlation analysis was performed between the degree of atrophy and the degree of hypertrophy. A positive correlation was found on day 7 after PVE (Spearman’s $\rho=0.65$, $p=0.001$).

Furthermore, liver regeneration is known to be mediated by several cytokines released by activated Kupffer cells. Therefore, the amount of macrophages/Kupffer cells stained with a macrophage-specific antibody (RAM11) in liver tissue obtained on day 7 after PVE was visualized by light microscopy (Figure 5) and quantitated on the basis of the positively stained pixel fraction in the field of view (Figure 6).

Part of the macrophages in the portal fields were characterized as multinucleated giant cells, positioned in direct contact with the embolization materials. The RAM11-positive area in the left lateral, atrophic liver lobe was significantly greater than in the caudal, hypertrophic liver lobe for the fibrin glue, PVAc, and nBCA groups ($p=0.014$, $p=0.009$, and $p=0.004$, respectively), whereas no differences in macrophage/Kupffer cell density were found in the sham and gelatin sponge groups. Similarly, the RAM11-positive area in the left lateral liver lobe was significantly greater in the fibrin glue, PVAc, and nBCA groups compared to the atrophic lobe of the sham group ($p<0.025$).

Next, the intrahepatic levels of regeneration-triggering cytokines (IL-6 and TNF-α) were quantitated by ELISA in liver tissue obtained 7 days after PVE. Tissue levels of IL-6 and TNF-α in the caudal liver lobe of the fibrin glue, PVAc, and nBCA groups were not significantly different from the sham and gelatin sponge groups (Figure 7A,B).

Liver tissue acquired 1 day after PVE also revealed no significant differences in cytokine levels between the groups (data not shown). Additionally, histological sections of the left lateral liver lobes were fluorescently immunostained with the antibodies used in the ELISA assays in order to assess protein expression patterns and to determine the localization of the antigens. The left lateral lobes were chosen because these contained the cells that produced the cytokines (Figure 6). The left lateral liver lobes of day 1 samples contained fewer RAM11-positive cells than liver lobes excised 7 days post-PVE. Moreover, the RAM11-positive cells exhibited very little-to-no expression of either cytokine (Figure 7C-F), whereas the RAM11-positive cells in the day 7 liver samples abundantly expressed IL-6 and TNF-α (Figure 7G-J). Incubation of liver tissue with the fluorophore-conjugated secondary antibodies confirmed that no unspecific antibody binding had occurred (Figure 7K-N).

Lastly, the intrahepatic growth factor levels were quantified by ELISA in liver tissue samples obtained at day 7. HGF, which activates DNA synthesis, showed significantly lower levels in the caudal liver lobe of the fibrin glue, PVAc, and nBCA groups compared to the sham and gelatin sponge groups ($p=0.016$, data not shown). Similarly, the levels of TGF-β1, which is important in terminating liver regeneration, were significantly lower in the caudal liver lobes embolized with the long-term occluding materials compared to the gelatin sponge group ($p=0.016$, data not shown). No significant
differences in growth factor levels were found between the embolization groups on day 1 after PVE.

**Discussion**

In this study the use of three permanent (PVAc, nBCA, and polidocanol) and two biodegradable (fibrin glue and gelatin sponge) embolization materials for portal vein embolization was investigated in the context of the degree of hypertrophy and material safety. For these purposes, a validated rabbit model was used in which the hypertrophy response could be studied under controlled circumstances for a period of 7 days, corresponding to the end of post-PVE liver regeneration in this species. Polidocanol produced a lethally toxic reaction in 3 rabbits. The hepatotoxic effect of polidocanol has been described previously and therefore this material seems unsuitable for PVE. Fibrin glue, PVAc, and nBCA induced total occlusion of the portal vein branches up to 7 days after PVE, which was associated with a significantly greater hypertrophy response compared to the gradually degraded gelatin sponge. With the exception of polidocanol, the embolization agents inflicted minimal hepatocellular damage and were not found to impair liver function, confirming that the PVE materials tested in this study are appropriate for clinical application. Furthermore, the degree of hypertrophy was positively correlated with the degree of atrophy of the embolized liver lobe and was associated with increased inflammatory cell influx into the atrophic liver lobe. Neither the molecular triggers for liver regeneration, IL-6 and TNF-α, nor the proteins responsible for propagation (HGF) and termination (TGF-β1) of liver regeneration were found to be elevated in atrophic liver tissue. However, an elevated expression of cytokines was found in activated macrophages/Kupffer cells in the atrophic liver lobe.

Our study was set up according to the suggestions of Lesurtel et al. published in the Journal of Hepatology, who posited that the use of temporary embolization agents should be evaluated against permanent embolization materials and a sham group. Accordingly, the most important conclusion of this study was that permanent embolization materials induce the most prolific hypertrophy response. Although we showed that reversible PVE with gelatin also induced a hypertrophy response of the non-embolized liver lobe, as was recently demonstrated by Lainas et al. in monkeys, the hypertrophy response was significantly less compared to the permanent embolization materials.

Interestingly, fibrin glue, which is marketed as an absorbable embolization agent, was not absorbed after 7 days of PVE and yielded a hypertrophy response that was comparable to that after PVAc and nBCA. This effect is ascribable to differences in the rate of liver regeneration in rabbits versus humans. Aside from possible interspecies differences in fibrin degradation kinetics, the regeneration response is faster
In rabbits compared to humans and evidently reached a plateau before the fibrin glue was degraded. In human livers the fibrin glue is typically absorbed before liver regeneration plateaus at approximately 21 days post-PVE, as a result of which the extent of hypertrophy is reduced compared to that of permanent embolization agents. Consequently, fibrin glue should be classified as a permanent embolization material in this rabbit model and the implications of results should be interpreted accordingly. In the clinical setting, the plateau phase signifies the end of the waiting time between PVE and liver resection and therefore constitutes the most important time point. In light of the possibility of tumor growth during the time between PVE and resection, it is imperative to use an embolization material that induces the most profound hypertrophy response in the shortest time frame without inflicting excessive hepatocellular or systemic damage. We have shown that none of the permanent embolization materials, including fibrin glue, caused considerable hepatocellular/ histological damage. Additionally, liver synthetic function, and liver uptake and excretory function were preserved. In human livers, fibrin glue is absorbed before 3 weeks, i.e., before the time the plateau phase has been reached, and hence comprises an inferior embolization material compared to PVAc and nBCA for clinical use. Consequently, for the purposes of post-PVE resection procedures, PVAc and nBCA should be employed to induce the most extensive hypertrophy response in a minimum amount of time.

Additionally, we assessed several growth-promoting mediators of liver regeneration in liver tissue to explain the difference in hypertrophy response. Kupffer cells and recruited, activated macrophages are known to release cytokines and growth factors that trigger and propagate liver regeneration. The amount of macrophages was significantly higher in the embolized liver lobes of the fibrin glue, PVAc, and nBCA groups. However, the groups with the highest hypertrophy response exhibited lower intrahepatic IL-6, TNF-α, and HGF levels compared to the sham and gelatin sponge group, despite the fact that the former groups exhibited the greatest degree of hepatocyte proliferation. Although we did not investigate these contradictory findings any further, we hypothesize that these factors were not yet released on day 1 after PVE in these groups and that these factors had been extensively depleted after 7 days. On the other hand, it might also be that these factors do not play a prominent role in mediating liver regeneration after PVE. To our knowledge, no studies have been performed shedding light on this issue.

Embolization with long-lasting or permanent occlusion materials leads to a higher hypertrophy response than a temporary occlusion material. However, the use of an absorbable embolization material still can be advocated in cases where the portal vein ultimately needs to be patent, such as in living donor liver transplantations. After PVE with gelatin sponge, the hypertrophy response will lead to more hepatic function in the part of the liver that is going to be transplanted, albeit to a lesser extent than would be the case with permanent embolization materials. The remnant
donor liver will gradually regain portal blood flow before the hypertrophy plateau phase has been reached and sustains optimal functionality following the explantation procedure. However, an embolization material that is absorbed after 3 weeks at the earliest would be more ideal. PVE with such an embolization agent would result in a greater hypertrophy response with the added benefit of recovery of the portal blood flow to the embolized liver lobes before transplantation.

In conclusion, we found that the use of permanent or at least long-lasting embolization materials leads to a greater hypertrophy response of the FRL compared to an absorbable material. The clinical implication is that absorbable (gelatin-based) embolization materials should only be used for PVE when only little liver regeneration is needed or when the portal blood flow to the embolized liver lobes should preferably be restored.

Acknowledgements
We would like to thank Dr. Jan van Marle for assistance with the confocal microscope.
References

Short-term effects of combined hepatic vein embolization and portal vein embolization on the induction of liver regeneration

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M. Heger
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J.S. Lamèris
T.M. van Gulik
Abstract

Background: Because liver tumors may become irresectable in the waiting time between portal vein embolization (PVE) and liver surgery, alternative methods to optimize the hypertrophy response after PVE, particularly in terms of inducing maximum liver regeneration in minimum time, are desired. The aim of this study was to assess the effect of hepatic vein embolization (HVE) in addition to PVE on the liver hypertrophy response in a standardized rabbit model.

Materials and methods: Thirty New Zealand white female rabbits were allocated to a group according to the intervention: PVE alone, HVE alone, and a combination of HVE and PVE. Occlusion of the veins was assessed by a portogram and a contrast enhanced CT scan. The liver regeneration response of the caudal liver was assessed by CT volumetry, liver to body weight index, and the amount of proliferating hepatocytes.

Results: The caudal liver volume (CLV) increased significantly more in the PVE and combined PVE/HVE group compared to the HVE group, 3 and 7 days after the procedure (p<0.01). There were no significant differences in CLV increase or degree of hypertrophy between the PVE and the combined group. The caudal liver to body weight index was significantly higher in the PVE and combined group compared to the HVE group on day 7 (p<0.01). However, the index was also significantly higher in the combined PVE/HVE group compared to the PVE group (p=0.008). The caudal liver tissue of the PVE and combined group contained a significantly higher number of proliferating hepatocytes compared to the HVE group on day 7 (p<0.01).

Conclusion: The results of this study suggest that although histological and additional regenerative changes are seen, HVE in addition to PVE, has no significant effect on the hypertrophy response. The combination of HVE and PVE may therefore, have little use in a clinical setting.
Introduction

Since the first clinical application 25 years ago, portal vein embolization (PVE) has become a widely used method to increase the future remnant liver (FRL) before major liver resection. PVE is considered when the FRL is considered too small, thereby increasing the risk of postoperative liver failure. With PVE, the portal vein branches of the to-be-resected liver lobe are occluded, causing atrophy of this liver lobe. This results in a release of regenerative factors that in turn induces a compensatory hypertrophy response in the contralateral, non-embolized liver segments.

In most cases, the hypertrophy response following PVE reaches its plateau after 21 days. Thereafter, only little additional growth takes place. When the FRL volume, typically measured by CT volumetry 3 weeks after PVE, is ≥25% of the original total liver volume, partial liver resection is performed. However, the hypertrophy response is not always sufficient by this time. A drawback of PVE is the potential induction of tumor growth after PVE, leading to secondary irresectability. Therefore, much research effort is devoted to find alternative methods to optimize the hypertrophy response after PVE, particularly in terms of inducing maximum liver regeneration in minimum time.

Recently, a Chinese research group published on sequential, ipsilateral hepatic vein embolization (HVE) after PVE. In 12 patients who showed insufficient increase in volume of the FRL after PVE, the right hepatic vein was also embolized to induce additional hypertrophy. The additional HVE was performed 13.5 ± 4.2 days after PVE. Embolizing the ipsilateral hepatic vein blocks the hepatic outflow and, in combination with PVE, should decrease compensatory arterial hyperperfusion, thereby inducing additional hypertrophy of the non-embolized FRL. However, in this study, the increase in FRL could still be the result of ongoing hypertrophy caused by PVE alone. The hypertrophy response namely continues for at least 3 weeks in humans, before a plateau phase is reached. However, these findings conceived the idea that simultaneous PVE and HVE could possibly result in a greater hypertrophy response than PVE alone within the same time frame. To eliminate the influence of the time factor after PVE and to achieve the maximum hypertrophy result in a short follow-up period, we performed the PVE and HVE in one single procedure instead of sequentially. The aim of this study was to assess the effect of HVE in addition to PVE on the liver hypertrophy response in a standardized rabbit model.

Materials and methods

Animals

Experimental protocols were approved by the institute’s animal ethics and welfare committee. A total of 30 female New Zealand White rabbits (Harlan, Gannat, France) with a mean weight of 2,884 g (range 2,470-3,430 g) were acclimatized for 2 weeks under standardized laboratory...
conditions in a temperature-controlled room with a 12-h light/dark cycle and access to standard chow and water ad libitum.

Figure 1. Anatomy of the rabbit liver with the portal vein (A). Panel B shows a portogram after PVE, the coils are situated in the main portal vein branch to the cranial liver lobes (white arrow). The coils of additional HVE are visible on the portogram in panel C (black arrowhead).

Experimental design
A validated rabbit model was used for this study. The rabbit liver anatomy differs slightly from the human. It has 4 main lobes of which 3 are located cranially and 1 caudally. The 3 cranial lobes, which account for approximately 80% of the total liver, were embolized. The caudal lobe was spared and used as FRL to evaluate the hypertrophy response (Figure 1). Rabbits were divided into 3 groups of 10 rabbits according to the intervention: PVE alone, HVE alone, and a combination of HVE and PVE. All groups were subdivided into two subgroups (n=5 per subgroup), which were sacrificed 1 or 7 days after the intervention, respectively. These time intervals were chosen on the basis of previous studies, which showed that the hypertrophy response of the rabbit liver reaches a plateau phase 7 days after PVE. In the 7 days survival groups, CT volumetry was performed before embolization and 3 and 7 days after embolization for volumetric measurements and evaluation of revascularisation of the hepatic veins. Blood samples were drawn 3 hours and 1, 3, and 7 days after embolization. A portogram was performed before and directly after the intervention, as well as prior to sacrifice to confirm total occlusion of the portal vein. After sacrifice, liver tissue samples were excised and stored at -80°C or fixed in 4% formaldehyde.

Interventions
Animals were anesthetized by intramuscular injection of 25.0 mg/kg ketamine (Nimatek, Eurovet, Bladel, the Netherlands) and 0.2 mg/kg dexmedetomidine (Dexdomitor, Orion Corporation, Espoo, Finland). The eyes were protected from drying out using an eye cream (Oculentum simplex, Pharmachemie, Haarlem, the Netherlands). After subcutaneous injection of 0.03 mg/kg buprenorphine (Temgesic,
Reckitt Benckiser Healthcare, Hull, Great-Britain) and 0.2 mg/kg Baytril (Bayer Healthcare, Berlin, Germany), the rabbit was placed in supine position. Isoflurane 1-2% (Forene, Abbott Laboratories, Kent, UK) with O₂:air (1:0.7 L/min) was used to maintain anaesthesia. Heart rate and arterial oxygen saturation were measured by pulse oximetry (Hewlett Packard M1165A, model 56S, Andover, MA) on the hind leg throughout the procedure. Rabbits were given analgesic care by subcutaneous administration of 0.02 mg/kg Baytril once a day for 3 days postoperatively. Portal and hepatic vein embolizations were performed by an interventional radiologist (KPvL) with over 10 years of experience.

**Hepatic vein embolization**

The right internal jugular vein was exposed via a small incision in the neck. The jugular vein was cannulated with an 18 G catheter (Hospira Venisystems, Lake Forest, IL). Under fluoroscopic guidance using a mobile C arm (Oldelft Benelux, Veenendaal, The Netherlands), a Renegade 3 Fr microcatheter (Boston Scientific, Place Natick, MA) with a Transend-ex 0.014-inch wire (Boston Scientific, Place Natick, MA) was introduced into the 18 G catheter and guided through the heart and the inferior caval vein to the hepatic veins of the cranial liver lobes. A venogram was made against the flow direction. Sequentially, the microcatheter was positioned in the right, middle, and left hepatic vein, which were then embolized with multiple 3-7-mm coils (Boston Scientific, Place Natick, MA). The embolization of the veins started in the periphery of the vein and was continued more centrally to completely occlude the vein, without losing coils into the right atrium and the lung. Then the catheter was removed and the cannulated jugular vein was closed with a ligature.

**Portal vein embolization**

PVE was performed as described previously 11 prior to HVE in the combined group to avoid acute severe portal hypertension after HVE. Briefly, a branch of the superior mesenteric vein was cannulated with a venflon after a midline laparotomy. A microcatheter with a guidewire was subsequently introduced into the portal vein and a portogram was made to identify the individual portal vein branches. The microcatheter was positioned in the main portal branch supplying the cranial liver lobes after passing the portal branch to the caudal liver lobe. A mixture of contrast agent (Visipaque, GE Healthcare, Waukesha, WI) with 300-500-μm PVA particles (Cook, Bloomington, IN) was injected until flow ceased in the periphery. This procedure was followed by a more central positioning of 3 platinum coils close to the cranial portal main branch, without interfering with the caudal portal main branch. To confirm total occlusion of the cranial portal vein trunk, portography was repeated at the end of the procedure. Portography was concluded by ligation of the access branch of the superior mesenteric vein, after which the abdomen was closed.
**CT volumetry**

After induction anaesthesia, a multiphase CT scan was performed using a 64-slice CT scanner (Brilliance 64-channel, Philips Medical Systems, Eindhoven, The Netherlands). Rabbits were placed in supine position. After a blank series, a contrast-enhanced scan was performed 15 s (arterial phase), 30 s (portal phase), and 45 s (venous phase) after contrast agent injection (4 mL Visipaque), followed by 3 mL of sterile 0.9% NaCl solution. 3D-reconstructions of the liver were made using reconstructed 2-mm axial slices. The total liver and the caudal liver lobe were manually delineated and total liver volume (TLV) and caudal liver volume (CLV) were calculated by integrated software (MxView 3.52, Philips).

CLV before HVE was expressed as percentage of TLV using the formula:

\[
\%\text{CLV}_{\text{pre-embolization}} = \frac{\text{CLV}_{\text{pre-embolization}}}{\text{TLV}_{\text{pre-embolization}}} \times 100\%
\]

After HVE, %CLV was calculated using the formula:

\[
\%\text{CLV}_{\text{post-embolization}} = \frac{\text{CLV}_{\text{post-embolization}}}{\text{TLV}_{\text{pre-embolization}}} \times 100\%
\]

The increase in CLV was calculated using the formula:

\[
\text{Increase CLV} = \left( \frac{\text{CLV}_{\text{post-embolization}}}{\text{CLV}_{\text{pre-embolization}}} \right) \times 100\%
\]

The degree of hypertrophy = \(\%\text{CLV}_{\text{post-embolization}} - \%\text{CLV}_{\text{pre-embolization}}\)

**Biochemical parameters**

Whole blood was centrifuged at 3000g and platelet-poor plasma was isolated. Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were assessed as well-established liver damage parameters by routine clinical chemistry. Plasma gamma glutamyl transpeptidase and alkaline phosphatase were measured to assess the extent of bile duct congestion. In addition, plasma albumin was used as an indirect parameter of liver synthetic function, whereas plasma bilirubin was used as an indirect measure of hepatic uptake and excretory function.

**Caudal liver-to-body weight index**

After sacrifice the weight of the caudal liver lobe was measured using a precision scale (Sartorius, Göttingen, Germany). To obtain the caudal liver-to-body weight index, the liver weight was divided by the body weight.
Wet-to-dry weight ratio
Liver tissue samples of the caudal and left lateral liver lobe were weighed directly after sacrifice (wet weight), kept at 60°C for 4 weeks, and weighed again (dry weight). The wet-to-dry weight ratio was calculated by \[ \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100\% . \]

Histology
Liver tissue samples of the caudal and left lateral liver lobes were fixed in 4% formaldehyde, dehydrated in graded steps of ethanol and xylene, embedded in paraffin, and cut in 5-μm sections. Hematoxylin and eosin (H&E) staining was performed to evaluate the degree of necrosis, apoptosis, sinusoidal dilatation, and inflammation. Additionally, sections were immunostained with diaminobenzidine (DAB)-conjugated anti-Ki-67 antibodies (monoclonal mouse anti-rat Ki-67 antigen, clone MIB-5, Dako Cytomation, Glostrup, Denmark) according to the manufacturer’s instructions. The immunostained sections were counterstained with hematoxylin. Ki-67- and hematoxylin-positive cells were counted in 5 randomly selected fields of view per section (20× magnification) on microphotographs. The proliferation index was defined as the percentage of Ki-67-positive cells per total number of nuclei in the field of view.

Statistical analysis
Data were expressed as mean ± SD. Overall differences between the groups were analyzed with the Kruskal-Wallis test. If this test indicated a significant difference, 3 separate Mann-Whitney U tests were used for each comparison. To correct for multiple testing, a Bonferroni-Holm adjustment was made with an adjusted alpha of 0.05 denoting the level of significance.

Results
Survival
All rabbits survived the experiments without any clinical problems. No postoperative complications and no signs of illness were observed in the period after embolization. In one rabbit in the 1-day survival HVE group, a coil migrated via the heart into the left pulmonal artery directly after placement, however without any clinical consequences. A new coil was placed to occlude the hepatic vein.
**Degree of occlusion**

On portography, performed directly after PVE and before sacrifice, all rabbits that had undergone PVE or a combination of PVE and HVE showed complete occlusion of the portal vein, except for one rabbit in the combined group sacrificed on day 1, which showed persistent portal flow in part of the cranial liver lobe.

Occlusion of the hepatic vein was verified by CT. In most rabbits some small venous side branches were still patent, but in general, almost the complete venous outflow tract was adequately occluded. During sacrifice the position of the coils was checked in relation to the venous system. In all rabbits, the coils were found in the 3 main hepatic veins.

**Table 1. CT volumetry data**

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<th>Day 7</th>
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**Liver regeneration response**

Table 1 shows the changes in caudal liver volume after each procedure. The CLV increased more in the PVE and the PVE/HVE group compared to the HVE group 3 and 7 days after the procedure (p<0.01). The caudal liver volumes of the combined group were slightly larger than the PVE group, but there were no significant differences in CLV increase or degree of hypertrophy between the PVE and the combined group at any time point (Figure 2).

The caudal liver-to-body weight index supports these data, since this index was significantly higher in the PVE and PVE/HVE group compared to the HVE group on day 7 (p<0.01). However, the index was also significantly higher in the combined PVE/HVE group compared to the PVE group (p=0.008). On day 1, no significant differences in liver-to-body weight index between the 3 groups were observed.

The liver volume/weight gain could have been caused by edema formation in the
caudal lobe. The wet-to-dry weight ratio is a parameter that represents the amount of fluid in the liver tissue samples. There were no significant differences in the wet-to-dry weight ratio between the 3 groups on day 1 and 7. Therefore the significant difference in body weight index cannot be explained by edema.

In accordance with the results above, the caudal liver of the PVE and combined group contained a significantly higher number of proliferating hepatocytes compared to the HVE group on day 7 (p<0.01) (Figure 3). The number of proliferating hepatocytes in the PVE/HVE group was not significantly higher than in the PVE group.

Liver damage and liver function
Plasma AST, ALT, and LDH plasma levels showed transient elevation in the first hours to day, without significant differences between the groups. All levels returned to baseline level on day 7 (Figure 4). No changes in plasma gamma glutamyl transpeptidas, alkaline phosphatase, and bilirubin were observed directly after the procedure or in the follow-up period (data not shown).

Histology
H&E slides obtained from the caudal and cranial left lateral live lobes were evaluated by an experienced pathologist experienced in hepatic histopathology. A multinucleated giant cell reaction was seen around the embolization particles in the PVE animals as described before.11 There were no striking changes in liver architecture in the PVE group. In the cranial left lateral liver lobe, periportal and pericentral sinusoidal dilatation was observed in the PVE and combined group, while pericentral dilatation was seen particularly in the HVE group. Unexpectedly, marked changes were observed in the caudal liver lobe of the combined group. There was pericentral and periportal sinusoidal dilatation in the HVE group. However, in the combined
group, periportal sinusoidal dilatation in conjunction with atrophy of hepatocytes and local necrosis were clearly seen in all rabbits. Only little inflammation was observed. These observations apply for tissue samples obtained on days 1 and 7, although the necrosis was less pronounced in the former. One rabbit in the LVE group showed strong pericentral congestion and hepatocellular atrophy and necrosis in the lateral lobe on day 7, which could not be explained.

**Figure 3.** Ki-67 staining of the caudal lobe. There were significantly more proliferating hepatocytes in the combined and PVE group compared to the HVE group (*p<0.01, A). Slides of the three different groups showing the proliferating hepatocytes (brown staining, C-D).

**Discussion**

In this experimental study, we assessed the value of HVE in addition to PVE for the increase of the FRL. The combined PVE and HVE was well tolerated by the animals. Clinically as biochemically, there were no signs of substantial liver damage, portal hypertension, or bile retention. Hepatic vein embolization in a rabbit is a challenging
intervention, because of the risk of losing coils into the pulmonary system, while working against the current. The results were good, as evidenced by the follow-up CT scans. Although some small patent venous side branches were seen, all three major veins were completely occluded in all rabbits.

The liver receives dual blood supply, i.e., from the portal vein as well as the hepatic artery. Supplying about 75% of the liver's blood pool, the portal vein carries venous blood drained from the splanchnic system. Besides PVE, HAE may be employed to reduce blood flow in a normal hepatic sinusoid. However, there are studies showing that this combined technique of PVE and HAE does not lead to an increased hypertrophy response. In this study, we showed that there is also no additional effect of HVE to PVE on the liver regeneration rate. Although these interventions were performed sequentially by Hwang et al., we think that the additional value of HVE is nihil. We have doubts concerning their conclusion that the additional liver regenerative response after HVE is explained by the HVE. HVE was performed 1-2 weeks following PVE and the increase in FRL could still be the result of ongoing hypertrophy caused by PVE alone. Particularly as we know that hypertrophy response continues for at least 3 weeks in humans, before a plateau phase is reached.

The results of this study suggest that although histological and additional regenerative changes are seen, HVE in addition to PVE, has no significant effect on the hypertrophy response. The combination of HVE and PVE may therefore, have little use in a clinical setting.
References

Part I

Summary, conclusions and future perspectives

Clinical and experimental studies on preoperative portal vein embolization
Summary and conclusions

The first part of this thesis concerns liver regeneration after portal vein embolization (PVE). The use of PVE over the past 20 years was reviewed and its effect on tumor growth and (postresectional) hypertrophy response discussed. The effects of several portal vein occlusion techniques were evaluated in experimental studies using a rabbit model of PVE.

Chapter 1 is a historical review of how the concept of portal vein occlusion was defined. As early as in the late 19th century, James Cantlie foresaw that the potential of one half of the liver to hypertrophy when the other half is deprived of its blood supply, could be used to the advantage of hepatic resection. It would take another 85 years, however, before the first clinical, preoperative PVE was carried out.

The use of PVE in the clinical setting over the past 20 years (1990-2011) was reviewed in Chapter 2. After critical evaluation, 44 publications were included for this review comprising 1791 patients. The mean hypertrophy rate of the FRL after PVE was 37.9 ± 0.1%. In 52 patients (2.9%), surgery was not performed because of failure of PVE. Major complications were seen in 2.5% and the mortality rate was 0.1%. A meta-analysis of several subgroups could not be performed because of the small number of articles and the inhomogeneity of the subgroups. However, a head-to-head comparison could be made. In conclusion, preoperative PVE appeared to be an effective method to increase the FRL volume with a high technical and clinical success rate. Pre-existing liver damage due to cirrhosis, cholestasis, or chemotherapy seemed to have no influence on the hypertrophy response. However, the use of n-butyl cyanoacrylate seemed to result in a greater hypertrophy response compared to the other materials used.

In Chapter 3, a retrospective case-control study was performed to assess the effect of preoperative PVE on liver volume and liver function 3 months after major liver resection, as measured by CT volumetry and hepatobiliary scintigraphy. Data were collected of 10 patients who underwent PVE prior to (extended) right hemihepatectomy and of 13 comparable control patients who underwent the same type of resection without prior PVE in the same time period. The future remnant liver volume prior to intervention was significantly less in the PVE group compared to the control group. Prior to surgery (and after PVE in the embolization group) there were no longer significant differences in future remnant liver volume and function between the groups. Three months postoperatively, there were still no significant differences in mean remnant liver volume and function between the groups. The remnant liver regenerated up to approximately 80% of its initial total liver volume and over 83% of its original total liver function, 3 months after major liver resection with or without prior PVE. From this study, we therefore concluded that preoperative
PVE does not hamper the regenerative capacity of the future remnant liver after partial liver resection.

Chapter 4 discussed several controversies and issues concerning PVE. PVE is considered when the FRL is found to be too small for sufficient postoperative function. However, the criteria for preoperative application of PVE are not well defined. Especially the true minimum volume of liver required for safe resection in a normal liver is debatable, rendering the indication for performing PVE in normal livers controversial. Embolization of the portal branches to segment 4 in addition to embolization of the right portal trunk is controversial and is advised only in selected cases. Clinical and experimental data suggest that tumor progression can occur after preoperative PVE in embolized and nonembolized liver segments, which is a major concern. The regeneration rate of the non-embolized liver segments typically shows an increase during the first 3 weeks after PVE, followed by a plateau phase with only slight additional increase of FRL volume. Therefore a waiting time of 3 weeks between PVE and liver surgery is advised. Finally, PVE does not seem to hamper postresectional liver regeneration.

Chapter 5 is a review of the evidence regarding the effect of PVE on tumor growth. Although clinical studies clearly demonstrated that tumor progression after PVE is possible, accurate data concerning increase of tumor growth rate after PVE were lacking. Three possible mechanisms inducing tumor growth after PVE have been proposed, namely changes in cytokines and growth factors, alteration in hepatic blood supply, and enhanced cellular host response promoting local tumor growth. Sequential TACE with PVE as well as post-PVE chemotherapy were discussed as promising strategies to control tumor progression after PVE.

Chapter 6 describes the development of a rabbit model for PVE to study the hypertrophy response of the liver after this intervention. The combination of two PVA particle sizes and three coils created a PVE model in the rabbit that resembles the human situation, taking into account features as the amount of embolized liver tissue, histologic changes in the liver parenchyma, and the hypertrophy response assessed by CT volumetry. This rabbit model provides an opportunity to perform investigations in a standardized animal model in order to improve the techniques currently used in PVE.

In Chapter 7 the hypertrophy response after portal vein ligation and PVE was compared in the rabbit model. The conclusion was that PVE is superior to PVL in terms of the extent of the hypertrophy response. In the rabbit model, we also compared the use of several embolization materials for PVE in Chapter 8. Polidocanol was discontinued because of toxic reactions in 3
rabbits. Gelatin sponge was the only material that was absorbed within 7 days and this resulted in less hypertrophy of the non-embolized lobe compared to fibrin glue, polyvinyl alcohol particles with coils, and n-butyl cyanoacrylate. No other mechanism than recanalisation of the unilateral portal venous system was found to explain the differences in liver regeneration.

In Chapter 9 the value of hepatic vein embolization (HVE) in addition to PVE was assessed. HVE alone showed no hypertrophy response of the non-embolized liver lobe. A combination of HVE and PVE did not result in a greater or earlier hypertrophy response than PVE alone. The results of this study suggest that simultaneous, unilateral embolization of the hepatic and portal vein is not to be recommended.

**Future perspectives**

Many issues concerning PVE still remain unexplored. A major concern is potential acceleration of tumor growth after PVE. The rabbit model including a tumor engrafted in the liver, will provide the appropriate model to confirm this phenomenon and subsequently, to find solutions to tackle this problem. PVE results in compensatory hyperperfusion of the ipsilateral hepatic artery branch, which promotes growth especially of hypervascular tumors as hepatocellular carcinoma. Sequential embolization of the hepatic artery, feeding the tumor, and embolisation of this artery offers an attractive strategy to limit tumor progression subsequent to PVE. There also is a need for absorbable embolization materials which occlude the portal vein long enough to result in sufficient hypertrophy response, but which are absorbed shortly after occlusion thereby obviating permanent atrophy of the embolized segments.

Finally, the underlying mechanisms of the hypertrophy response after PVE, consisting of a growth factor and cytokine response, need to be unravelled. These issues require further experimental and clinical research in the near future.
Samenvatting en conclusies

Het eerste deel van dit proefschrift betreft lever regeneratie na vena portae embolisatie (VPE). De toepassing van VPE in de laatste 20 jaar en haar effect op tumorgroei en (postresectie) hypertrofie respots was besproken. Het effect van verschillende occlusie technieken werd geëvalueerd in experimentele studies, waarbij gebruik werd gemaakt van een konijnenmodel.

Hoofdstuk 1 is een historische samenvatting van waar het concept van vena portae occlusie vandaan komt. Al eind 19e eeuw, voorzag James Cantlie dat de mogelijkheid tot hypertrofie van één helft van de lever nadat de andere helft van zijn bloedvoorziening afgesloten was, gebruikt kon worden ter verbetering van lever resectie. Het zou nog 85 jaar duren, voordat de eerste klinische preoperatieve VPE uitgevoerd werd.

Het gebruik van VPE in de kliniek in de laatste 20 jaar (1990-2011) werd bekeken in Hoofdstuk 2. Na kritische evaluatie, werden 44 publicaties voor deze review geïncludeerd met in totaal 1791 patiënten. De gemiddelde hoeveelheid hypertrofie van de toekomstige restlever was 37.9 ± 0.1%. Lever resectie werd niet uitgevoerd in 52 patiënten (2.9%) in verband met falen van de embolisatie. Grote complicaties werden beschreven in 2.5% van de patiënten en de mortaliteit was 0.1%. Een meta-analyse van verschillende subgroepen kon niet worden uitgevoerd in verband met het kleine aantal en de inhomogeniteit van de artikelen in de subgroepen. Een head-to-head vergelijking kon echter wel worden gedaan. Concluderend bleek VPE een effectieve methode om het FRL volume te vergroten met een hoog technisch en klinisch succes percentage. Pre-existente leverschade door cirrhose, cheolestase of chemotherapie leek geen invloed te hebben op de hypertrofie respons. Het gebruik van n-butyl cyanoacrylaat leek tot een grotere regeneratie respons te leiden dan de andere gebruikte embolisatie materialen.

In Hoofdstuk 3 werd een retrospectieve case-control studie verricht om het effect van preoperatieve VPE op het uiteindelijke volume, gemeten met CT volumetrie, en de functie, gemeten met hepatobiliaire scintigrafie, van de lever 3 maanden na lever resectie te beoordelen. Gegevens van patiënten die een resectie van de rechter helft van de lever hadden ondergaan, met (n=10) en zonder (n=13) voorafgaande VPE in dezelfde periode, werden verzameld. Het volume van de toekomstige restlever was significant lager in de VPE groep dan in de controle groep vóór VPE. Na VPE en vóór de operatie waren er geen significante verschillen meer in volume en functie van de toekomstige restlever tussen beide groepen. Drie maanden na de lever resectie was er ook geen verschil in volume en functie tussen de groepen. De restlever groeide naar ca. 80% van zijn oorspronkelijke volume en meer dan 83% van zijn oorspronkelijke lever functie 3 maanden na de lever resectie met of zonder voorafgaande VPE. We
concludeerden dan ook dat preoperatieve VPE de postoperatieve lever regeneratie niet belemmert.

In Hoofdstuk 4 werden verschillende discussies en kwesties over VPE besproken. VPE wordt overwogen als de toekomstige restlever te klein wordt geacht om aan de postoperatief benodigde functie te kunnen voldoen. De criteria voor de toepassing van VPE zijn niet goed omschreven. In het bijzonder het minimaal benodigde volume van een goed functionerende restlever om een veilige lever resectie uit te kunnen voeren, is onduidelijk. De embolisatie van segment 4 in aanvulling op de embolisatie van de rechter takken van de vena portae is controversieel en wordt alleen in geselecteerde gevallen geadviseerd. Klinische en experimentele data suggereren dat tumorgroei kan optreden als gevolg van VPE. De mate van regeneratie van de niet-geëmboliseerde lever segmenten laat een toename zien in de eerste 3 weken, waarna een plateau fase volgt met weinig aanvullende groei. Op basis hiervan wordt een wachtijd van 3 weken geadviseerd tussen VPE en de lever resectie. Als laatste lijkt VPE de postoperatieve lever regeneratie niet te belemmeren.

Hoofdstuk 5 is een samenvatting van bewijs van het effect van VPE op tumorgroei. Ook al lieten klinische studies zien dat er weldegelijk tumorgroei na VPE mogelijk is, er was geen duidelijk bewijs dat de mate van tumorgroei toeneemt door VPE. Drie mogelijke mechanismen voor toename van tumorgroei werden gegeven, namelijk verandering in cytokines en groeifactoren, verandering in bloedtoevoer en versterkte cellulaire respons waardoor lokale tumorgroei wordt gestimuleerd. Achtereenvolgens TACE en VPE almede post-VPE chemotherapie werden beschreven als veelbelovende strategieën om tumor groei na VPE te onderdrukken.

Hoofdstuk 6 beschreef de ontwikkeling van een konijnen model voor VPE om de hypertrofie respons van de lever na deze interventie te bestuderen. Het gebruik van de combinatie van polyvinylalcohol partikels in 2 maten en 3 coils leidde tot een VPE model dat de humane situatie dicht benaderd qua hoeveelheid geëmboliseerde lever, histologische veranderingen in de lever en hypertrofie respons gemeten met CT volumtrie. Dit konijnenmodel maakt het mogelijk om onderzoek naar VPE te doen in een gestandaardiseerd model om de VPE technieken te verbeteren.

In Hoofdstuk 7 werd de hypertrofie respons na ligatie en embolisatie van de vena portae vergeleken. De conclusie was dat embolisatie tot een grotere hypertrofie respons leidt dan ligatie.

Het gebruik van verschillende embolisatie materialen voor VPE werd vergeleken in Hoofdstuk 8. Het gebruik van polidocanol werd vroegtijdig gestopt in verband met een toxische reactie in 3 konijnen. Gelatine spons was het enige materiaal dat binnen 7
dagen werd geabsorbeerd. Dit leidde tot minder hypertrofie van de niet geëmboliseerde leverlobben vergeleken met het gebruik van fibrinelijn, polyvinylalcohol partikels met coils en n-butyl cyanoacrylaat. Er werd geen andere oorzaak gevonden voor dit verschil in lever regeneratie dan recanalisatie van het portale systeem.

In Hoofdstuk 9 werd de toegevoegde waarde van embolisatie van de levervene (HVE) in combinatie met VPE bestudeerd. HVE alleen leidde niet tot een hypertrofie respons van de niet geëmboliseerde leverlobben. Een combinatie van VPE en HVE resulteerde niet in een grotere hypertofierespons dan VPE alleen. We concludeerden dan ook dat het gebruik van de gelijktijdige combinatie van VPE en HVE niet wordt aanbevolen.

Toekomstig onderzoek

Veel kwesties rondom VPE blijven onduidelijk. Een grote zorg is de potentiële toename van tumorgroei na VPE. Een konijnenmodel met levertumor levert een geschikt model op om dit fenomeen aan te tonen en vervolgens oplossingen te vinden om dit probleem te voorkomen. VPE leidt tot een compensatoire hyperperfusie van de ipsilaterale tak van de lever arterie, welke de tumor voedt. Embolisatie van een deel van deze arterie zou een mooie oplossing zijn om tumor progressie na VPE te voorkomen. Het zou wenselijk zijn als we een embolisatie materiaal vinden dat de vena portae lang genoeg concludeert om voldoende hypertrofie respons te genereren, maar welke kort daarna wordt geabsorbeerd zodat permanente atrofie van de geëmboliseerde segmenten wordt voorkomen. Als laatste is het nog steeds onduidelijk welk mechanisme ten grondslag ligt aan de hypertrofie respons. Om deze kwesties op te helderen is meer klinisch en experimenteel onderzoek nodig.
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