Radiological aspects of portal vein embolization
van Lienden, K.P.

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Radiological aspects of portal vein embolization

Krijn Peter van Lienden
Radiological aspects of portal vein embolization

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Prometheus is a Titan, a culture hero, who in Greek mythology is credited with the creation of man from clay and the theft of fire for human use, an act that enabled progress and civilization of mankind. He is known for his intelligence and often associated with technical developments.

The punishment of Prometheus as a consequence of the theft is a major theme of his mythology, and is a popular subject of both ancient and modern art. Zeus, king of the Olympian gods, sentenced him to eternal torment for his transgression. The immortal Prometheus was bound to a rock, where each day an eagle (Ethon) was sent to feed on his liver, only to have it grow back to be eaten again the next day. This punishment had to last forever but in some stories Prometheus is freed at last by the hero Hercules. This story suggests that the ancient greek already had knowledge of the regenerative capacity of the liver.

The studies described in this thesis were performed at the Department of Radiology and the Department of Surgery (Surgical Laboratory), Academic Medical Center, University of Amsterdam, the Netherlands.

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Radiological aspects of portal vein embolization

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aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
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ten overstaan van een door het college voor promoties ingestelde commissie,
in het openbaar te verdedigen in de Aula der Universiteit
op vrijdag 7 december 2012, te 13:00 uur

door

Krijn Peter van Lienden

geboren te Ede
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Promotor:  Prof. dr. T.M. van Gulik
            Prof. dr. J.S. Laméris

Co-promotor:  Dr. O.M. van Delden

Overige leden:  Prof. dr. M.A.A.J van den Bosch
                Prof. dr. O.R.C. Busch
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                Dr. J.B.C.M. Puylaert
                Prof. dr. J.A. Reekers

Faculteit der Geneeskunde
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Chapter 1

General introduction and outline of this thesis
General introduction

For patients with a primary liver malignancy or with metastatic liver disease, surgical resection is the only curative option. (1) Additional techniques such as radio frequency ablation allow even more patients to undergo resection especially in bilobar disease. (2) Extended liver resections however bear the risk of postoperative liver failure, particularly when the future remnant liver (FRL) is small or when patients have a compromised liver due to cirrhosis, cholestasis, steatosis or chemotherapy. (3, 4) Postoperative liver failure remains difficult to treat and results in a mortality rate of approximately 80% of patients. (5)

Therefore, preoperative risk assessment in patients considered for extensive liver resection is of utmost importance. The minimal required volume of the future remnant liver (FRL) is considered to be 25-30% in patients with otherwise normal liver parenchyma and 40% in patients with a compromised liver. To date, the most common method of preoperative evaluation of the remaining liver volume is CT-volumetry. (6)

Although CT volumetry enables accurate measurement of FRL-volume, it provides no information regarding the quality of the liver parenchyma in terms of functional capacity and therefore, does not reflect liver function. (7) Quantitative liver function tests are therefore necessary, to have a better understanding of the functional FRL-volume in the work-up for liver resection. The indocyanine green (ICG) clearance test is the most widely used dynamic liver function test. A tricarbocyanic green dye is used which, after intravenous injection, is exclusively removed by the liver and excreted into the bile. (8) More recently an alternative liver function test has been developed in our Institution using Technetium-99-m-labelled Luminodiaceticacid (IDA) analogues which are excreted in the same way as ICG, using the same biliary transporters. (9) 99mTc-mebrofenin hepatobiliary scintigraphy (HBS) correlates well with the ICG clearance test and provides additional 3-D information on segmental liver function, which proves useful in planning the liver resection. (10)

When the anatomical or functional FRL volume is too small, there are several techniques to induce hypertrophy, increasing the volume of the FRL, after which liver resection is enabled.

The mythological story of Prometheus demonstrates that the ancient Greeks already had knowledge about the regenerative capacity of the liver.

In 1920 Rous and Larimore observed in rats, that ligation of portal branches caused atrophy of the ligated liver and compensatory hypertrophy of the non-ligated remaining liver, hence demonstrating the induced regenerative capacity of the liver. (11) In 1965, portal vein ligation (PVL) was reported in humans as part of a two-stage extended hepatectomy. (12) Kinoshita et al. reported the first preoperative portal vein embolization (PVE) in a human being in 1986 (13). Since then, numerous reports have shown the efficacy of inducing compensatory hypertrophy of the FRL after PVE or PVL in preparation of surgical resection (14-16).

Several techniques of portal vein occlusion have been reported, including intraoperative portal branch ligation (17-19) and trans-ileocolic PVE (20-22), and the percutaneous transhepatic ipsilateral (23, 24) or contralateral (25, 26) PVE techniques. Occlusion of the portal
venous blood flow to the liver segments to be resected, induces atrophy of the ipsilateral and compensatory hypertrophy of the contralateral, non-embolized liver segments, resulting in increase of FRL-volume. This regeneration response is generated via a complex interaction of cytokines, growth factors and metabolic networks.

The question arises however, what is the most effective augmentation technique when it comes to the extent of the induced hypertrophy response. In addition to the different techniques, different embolization materials are used clinically, e.g. polyvinyl alcohol particles (PVA), coils, gelatin sponge, n-butyl cyanoacrylate and lipiodol, fibrin glue or combinations of these agents. The efficacy of the different embolization materials on the hypertrophy response in percutaneous portal embolization is also discussed in literature.

Since patients are increasingly included in neoadjuvant chemotherapy regimens, the influence of chemotherapy on the hypertrophy response of the FRL is also under debate. Some authors report that chemotherapy does not affect the hypertrophy response at all (27), whereas others conclude that chemotherapy has a negative influence on the rate of hypertrophy.(28,29) The role of cholestasis in regeneration of the FRL after PVE also remains controversial. It is stated by some authors that longstanding cholestasis, as is usually encountered in patients with hilar cholangiocarcinoma, impedes the hypertrophy response of the FRL, emphasizing the need for pre-procedural biliary drainage.(30) This notion however, has not been confirmed in other studies.(31) Finally, patients with pre-existing liver cirrhosis have an impaired hypertrophy response and a higher risk of post-resectional liver failure.(32) The role of cholestasis in regeneration of the FRL after PVE also remains controversial. It is stated by some authors that longstanding cholestasis, as is usually encountered in patients with hilar cholangiocarcinoma, impedes the hypertrophy response of the FRL, emphasizing the need for pre-procedural biliary drainage.(30) This notion however, has not been confirmed in other studies.(31)

In these patients, PVE seems to have a positive influence on post resectional hypertrophy.(33)

The technical success rate of PVE in literature approaches nearly 100%. The clinical success rate however is approximately 85%. Reasons of unresectability are local tumor progression, distant metastases, newly developed metastases in the FRL or insufficient hypertrophy response, despite technically successful PVE.(35) To decrease the percentage of patients who have an insufficient hypertrophy response, new techniques are being developed. New embolization materials are used, causing more periportal tissue reaction, and hence inducing a stronger hypertrophy response. Also sequential embolization techniques are developed in which embolization of the portal vein is followed by embolization of the hepatic vein or hepatic artery with an interval of several weeks. (36,37)

Although PVE nowadays is an accepted method to increase the resectability rate of patients with liver tumors, there is a major drawback which is a major concern. Several studies describe enhanced tumor growth after PVE (38,39) as a result of cytokines, growth factors and an increased arterial blood supply, although the exact mechanisms of this phenomenon are still largely unknown. Growth of tumor may be accelerated, while micrometastases in the non-embolized remnant liver may also develop or progress. The potential boost of tumor proliferation, therefore, creates a dilemma in terms of optimal waiting time until resection. A possible solution might be found in super selective embolization of the hepatic artery prior to PVE, as it probably prevents compensatory hyperperfusion of the tumor through the hepatic artery, thereby curbing tumor growth. It has also been
shown that sequential hepatic artery embolization and ipsilateral portal vein embolization supports the hypertrophy response of PVE. (40)

Although the technique of liver augmentation using portal vein embolization already exists for decades, there are still many questions that need to be elucidated.

Aim of this thesis
In this thesis, the value of functional scintigraphic liver imaging in addition to regular CT-volumetry, is assessed, especially for risk assessment in preparation of extended liver resection.

Based on experimental and clinical studies, answers will be given to questions such as, which augmentation technique is superior in inducing liver regeneration, which factors influence the hypertrophy response and which modifications to standard PVE can be devised, to increase the hypertrophy response of the non-embolized liver lobe, at the same time preventing increase of tumor growth.

Outline of this thesis
Since the first report on clinical application of portal vein embolization, many articles on this subject have been published. Chapter 2 provides a systematic review of the literature on portal vein embolization over the last 20 years (1990 and 2011). The technical and clinical success rates are discussed, as well as the influence of the used embolization materials on the hypertrophy response. Special interest is devoted to the effect of liver cirrhosis, steatosis, cholestasis and pre-operative chemotherapy on the hypertrophy response of the future remnant liver.

In chapter 3, the outcomes are presented of patients who underwent portal vein embolization in our institution, prior to (a planned) liver resection. Especially the effect of predamaged liver parenchyma (cirrhosis, steatosis, cholestasis and chemotherapy) on the hypertrophy response is assessed in this chapter.

After initial reports of portal vein ligation to induce hypertrophy of the non-ligated liver segments, intra-operative and percutaneous embolization methods have been developed. In literature there is still discussion on which technique is more effective in inducing hypertrophy of the FRL. In chapter 4, we therefore evaluated the difference in hypertrophy response after PVL and PVE in a standardized rabbit model of PVE(41). In patients who underwent PVL, as part of a two-stage liver resection, a revascularized portal system was seen on CT-scan three weeks after PVL. To examine portal revascularisation after PVL, intra-operative portograms were made in patients who had undergone portal occlusion, at the time of laparotomy. The results are presented in chapter 5, in which twenty-one patients are described who underwent PVL or PVE. In all patients who had undergone PVL, collateral flow was seen through an intrahepatic, left-to-right portal venous collateral system. This process of portal
revascularization explains the inferiority of PVL in the hypertrophy response in patients and confirms the results in the rabbit model of chapter 4.

The use of absorbable embolization materials for PVE could be advantageous, especially in patients who finally appear unresectable. For optimizing the technique of portal vein embolization, the use of different embolization materials is described in chapter 6. The use of absorbable versus permanent embolization materials, and the respective effect on the hypertrophy response was evaluated in a standardized rabbit model of PVE.

In patients who clinically fail PVE because of insufficient hypertrophy response, sequential embolization of portal vein and hepatic artery or portal vein and hepatic vein, have been described in literature. In chapter 7, our standardized rabbit model is used to evaluate the additional short-term effect on hypertrophy response of hepatic vein embolization in combination with portal vein embolization. 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 possibility of performing an extended liver resection is dependent on the volume of the remnant liver. As CT-volumetry gives no functional information, $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy (HBS), as functional modality, is compared to the volumetric data of the standard CT-scan. The additional value of $^{99m}$Tc-mebrofenin-HBS in estimating the risk of postoperative liver failure, especially in patients with uncertain liver function, is evaluated.

In chapter 9, $^{99m}$Tc-mebrofenin-HBS is combined with single photon emission computed tomography (SPECT). This enables the measurement of functional liver volume in a 3-dimensional manner and makes adequate, functional segmental delineation possible. The additional value of $^{99m}$Tc-mebrofenin-SPECT for the measurement of segmental liver function and liver functional volume is evaluated in this chapter.

Chapter 10 discusses the use of $^{99m}$Tc-mebrofenin-HBS with SPECT in a group of patients who underwent portal vein embolization prior to extensive liver resection. A comparison of the future remnant liver-volume (FRL-V) and functional future remnant liver (FRL-F) before and after PVE is made. The additional value of FRL-F over FRL-V in determining the optimal waiting time between PVE and surgical resection is evaluated, against the background of performing a safe resection.

There is increasing evidence that PVE not only stimulates growth of the FRL, but also increases tumor size because of growth factors and cytokines released in the process of liver regeneration. The challenge for future use of PVE is to limit the growth of tumor while inducing a maximum hypertrophy response in the non-embolized liver lobe. Therefore, in chapter 11, we devised an animal model in rabbits, in which the rate of tumor growth could be assessed in relation with PVE, closely resembling the clinical situation. In this rabbit tumor-model, PVE is performed using the same methods and imaging protocol used in patients undergoing PVE. The combination of a VX2 liver tumor in this rabbit model allows us, in addition, to explore the effects of PVE on tumor kinetics and at the same time, to assess the hypertrophy response of the non-embolized liver lobe.
After we studied this issue in a rabbit model, we evaluated in chapter 12, a series of patients with colorectal metastases after PVE and liver resection. Not only the hypertrophy response of the FRL and tumor mass were investigated, but also the outcomes of resection and survival during follow-up.

A conceivable treatment option, to prevent increased tumor growth in patients who have to undergo PVE, is embolization of the hepatic artery branches supplying the tumor-bearing liver segments. In chapter 13, the same VX2 liver tumor rabbit model is used to examine the influence of hepatic arterial embolization on tumor growth and the atrophy/hypertrophy response of the embolized and non-embolized liver lobes, respectively.

Finally in chapter 14, the results of the studies performed in this thesis are summarized and discussed.
References


34. Elias D, de BT, Roche A et al. (1999) During liver regeneration following right portal embolization the growth rate of liver metastases is more rapid than that of the liver parenchyma. Br J Surg 86:784-88.


Chapter 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. Lameris
T.M. van Gulik
O.M. van Delden
Abstract

**Purpose:** Review of literature on the indications, technique and outcome of portal vein embolization (PVE).

**Methods:** A systematic literature search on outcome of PVE from 1990 to 2011 was performed in Medline, Cochrane, and Embase databases.

**Results:** Finally, 44 articles were selected, including 1791 patients with a mean age of 61 ± 4.1 years. Overall technical success rate was 99.3%. The mean hypertrophy rate of the FRL after PVE was 37.9 ± 0.1%. In 70 patients (3.9%), surgery was not performed because of failure of PVE (clinical success rate 96.1%). In 51 patients (2.8%) the hypertrophy response was insufficient to perform liver resection. In the other 19 cases, 12 did not technically succeed (0.7%) and 7 caused a complication leading to unresectability (0.4%). In 6.1% resection was cancelled because of local tumor progression after PVE. Major complications were seen in 2.5% and the mortality rate was 0.1%. A head-to-head comparison shows a negative effect of liver cirrhosis on hypertrophy response. The use of n-butyl cyanoacrylate seems to have a greater effect on hypertrophy, but the difference with other embolization materials did not reach statistical significance. No difference in regeneration is seen in patients with cholestasis or chemotherapy.

**Conclusion:** Preoperative PVE has a high technical and clinical success rate. Liver cirrhosis has a negative effect on regeneration but cholestasis and chemotherapy do not seem to have an influence on the hypertrophy response. The use of n-butyl cyanoacrylate may result in a greater hypertrophy response compared to the other embolization materials used.
Introduction

Liver resection is still in many cases 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 patients with normal livers, but is 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 the first preoperative PVE in a human being in 1986[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 for 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 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 addition to the different techniques, different embolization materials are used clinically, e.g. polyvinyl alcohol particles (PVA), coils, gelatin sponge, n-butyl cyanoacrylate and lipiodol, or fibrin glue.

Many clinical 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. They focused especially on the differences between various access techniques (transhepatic vs. transileocolic) regarding the ensuing hypertrophy response and surgical outcome[18]. However, with the growing availability of radiological intervention suites, during the last decades, the percutaneous transhepatic technique has become the standard technique for PVE. In addition, many new articles on PVE have been published since Abulkhir’s report.

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

Materials and 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 which was used, time between PVE and CT/surgery, results, and complications of PVE, as well as results of liver surgery.

After the initial search, 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 and Data extraction.

All included studies were evaluated for 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[19].

The main points of interest included (1) patient characteristics (number of patients, age, sex, type of liver tumor, liver fibrosis, chemotherapy), (2) indication for PVE (minimal percentage FRL based on CT volumetry data or indocyanine green (ICG) clearance), (3) embolization technique (transileocolic, transhepatic ipsilateral, transhepatic contralateral) and embolization material used (polivinyl alcohol particles (PVAc), gelatin sponge, n-butyl cyanoacrylate, fibrin glue, ethanol, coils, vascular plug, or a combination), (4) data on CT volumetry, (5) follow-up (PVE success rate (successful occlusion of the portal vein), clinical
success rate, post-PVE complications and morbidity), and (6) surgical outcome (percentage and type of resection, postoperative complications and mortality).

Articles were valid and used for data extraction if the above mentioned points were described clearly.

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 [5,9,11,12,14-17,20-22,24-46,50-58,69] 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 could not be extracted from the articles. The mean age was 61 ± 4.1 years. The underlying pathology is summarized in Table 1.

Figure 1. Flowchart showing selection of papers for analysis
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 in most studies. Three studies[20-22] used the ICG plasma disappearance rate or retention rate at 15 minutes[23]. 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 were 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 were used to calculate the percentage FRL [5,9,11,12,14-17,24-47].

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

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

\[
\text{TELV} = (\text{total liver volume} - 706.2) \times \text{surface body area} + 2.4
\]

as previously described[48], or using the standardized FRL (sFRL) which was calculated by dividing FRL-V (measured by CT volumetry) by total liver volume (\text{calTL-V}) which was calculated using a formula described by Vauthey et al.[49]:

\[
\text{calTL-V} = -794.41 + 1267.28 \times \text{BSA}, \text{ with } \text{BSA} = \sqrt{\text{height (cm)} \times \text{weight (kg)}} / 3600
\]

<table>
<thead>
<tr>
<th>Table 1. Underlying pathology</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal metastasis</td>
<td>709 (39.6%)</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>518 (28.9%)</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>

VE technique

PVE is performed using a transileocolic or transhepatic approach. The transileocolic approach requires 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 (Table 2).
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, as the primary embolization-material, were used the most in the evaluated studies (59.5%), often in combination with other materials.

### 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 (12.4%)</td>
</tr>
<tr>
<td>Transhepatic ipsilateral contralateral</td>
<td>963 (53.8%)</td>
</tr>
<tr>
<td></td>
<td>605 (33.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Embolized branches:</th>
<th>No. of procedures (%)</th>
</tr>
</thead>
<tbody>
<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>

### 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>71</td>
<td></td>
</tr>
<tr>
<td>Gelatin sponge + thrombine + urografine</td>
<td>102</td>
<td>26.3</td>
</tr>
<tr>
<td>Gelatin sponge + urografine</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Gelatin sponge + polidocanol</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Gelatin sponge + amplatzer</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>Ethoxysclerol/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>
Success rate of PVE procedure and its effect on the hypertrophy response

The mean time-interval between PVE and the follow-up CT-scan was 25.9 ± 10.1 days (range 14-42 days).

The mean technical success rate of the PVE procedures was 99.3% (range 86.6 %-100%). Reasons for failure were the impossibility of cannulating the portal system[17,35,43,50], because of altered portal anatomy caused by the tumor mass or unexpected thrombosis of the portal system due to tumor progression/invasion[17,26,50,51].

The clinical success rate (successful PVE procedure, inducing enough hypertrophy of the FRL to allow resection) however was 96,1%.

In 70 patients (3.9%), surgery was not performed. In 51 patients (2.8%) the hypertrophy response was insufficient to perform the resection, although the embolization procedure was successful. In the other 19 cases, 12 did not technically succeed (0.7%) 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.

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):

   \[
   \text{% FRL volume increase} = \frac{\text{FRL}_{post-PVE} - \text{FRL}_{pre-PVE}}{\text{FRL}_{pre-PVE}} \times 100\%
   \]

2. The difference between the percentage FRL, before and after embolization (in literature referred to degree of hypertrophy (DH)):

   \[
   DH = \% \text{FRL}_{post-PVE} - \% \text{FRL}_{pre-PVE}
   \]

When available, the percentage FRL volume increase was extracted from the article, otherwise it was calculated from the available data.

The mean increase of the FRL volume was 37.9 ± 0.1% (20.5-69.4%).

Atrophy of the embolized lobe

Embolization of the liver not only causes hypertrophy of the non-embolized lobe, but also atrophy of the embolized lobe.

Only 10 studies[16,25,26,35,37,46,50-53], including 593 patients, contained all data on total liver volumes, FRL volumes and the volumes of the embolized lobe, pre- and post-PVE. From these studies we could calculate the percentage of atrophy of the embolized liver (EL), using the following equation:

\[
\text{% atrophy} = \frac{\text{EL}_{post-PVE} - \text{EL}_{pre-PVE}}{\text{EL}_{pre-PVE}} \times 100\%
\]
In these studies the influence of the tumor volume was not taken into account. The mean percentage of atrophy of the embolized liver in these studies was -12.3% (range -24.5 – 0.0 %), measured 29 days after PVE (range 14-42).

Influence of different 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. Insufficient data were available to make a strong statistical 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 of the effect of chemotherapy and cirrhosis/fibrosis on the hypertrophy response, enough studies are available for a head-to-head comparison (Table 4 and 5). Comparing the data, chemotherapy seems to have no influence on the hypertrophy response. However patients with pre-existing chronic liver disease (cirrhosis or fibrosis) show less hypertrophy response than patients with a normal liver. Statistical significance is not given in these studies.

<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>Covey¹⁴</td>
<td>100</td>
<td>43 / 57</td>
<td>22</td>
<td>26</td>
<td>Not known</td>
</tr>
<tr>
<td>Nafidi⁴³</td>
<td>20</td>
<td>13 / 7</td>
<td>45.8</td>
<td>41.2</td>
<td>NS</td>
</tr>
<tr>
<td>Ribero⁵⁵</td>
<td>112</td>
<td>28 / 80</td>
<td>9.0 (DH)</td>
<td>8.5 (DH)</td>
<td>NS</td>
</tr>
<tr>
<td>De Baere¹⁶</td>
<td>107</td>
<td>97/10</td>
<td>56.6-71.2</td>
<td>83.6</td>
<td>NS#</td>
</tr>
</tbody>
</table>

NS=not significant in the studies. DH=degree of hypertrophy.  
# A significant difference in hypertrophy response was seen in patients who received chemotherapy with platin agents

<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>Cotroneo²⁸</td>
<td>31</td>
<td>7 / 24</td>
<td>32.1</td>
<td>44.2</td>
<td>Not known</td>
</tr>
<tr>
<td>Farges³²</td>
<td>27</td>
<td>14 / 13</td>
<td>24.4</td>
<td>41.6</td>
<td>Not known</td>
</tr>
<tr>
<td>Ko³⁹</td>
<td>51</td>
<td>22 / 29</td>
<td>38.4</td>
<td>46.0</td>
<td>Not known</td>
</tr>
<tr>
<td>Lee⁴⁰</td>
<td>29</td>
<td>19 / 10</td>
<td>25.4</td>
<td>39.4</td>
<td>Not known</td>
</tr>
</tbody>
</table>

Table 6 shows only the studies which used a single embolization material. There seems to be a trend that the use of the permanent occluding n-butyl cyanoacrylate results in a greater % FRL volume increase compared to gelatin sponge, fibrin glue, and PVA.

Complications after PVE

Fifteen articles[9,11,12,16,25,32,41-44,53-57] lacked a detailed description of complications encountered after embolization. From the other 29 studies (1179 / 1248 patients), the complication rates are summarized in Table 7.
In 0.4%, major complications after PVE led to non-resectability of the patient. These complications consisted of severe cholangitis, large abscesses and sepsis, portal venous or mesenterico-portal venous thrombosis.

### 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>PVA + coils/vascular plug</td>
<td>Esschert³¹</td>
<td>10</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>Libicher⁴¹</td>
<td>10</td>
<td>26.4</td>
</tr>
<tr>
<td></td>
<td>Covey¹⁴</td>
<td>100</td>
<td>24.3</td>
</tr>
<tr>
<td>Gelatin sponge</td>
<td>Fuji¹¹</td>
<td>30</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>Imamura³⁴</td>
<td>84</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>Kakizawa³⁶</td>
<td>14</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>Kim³⁸</td>
<td>17</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>Kusaka¹²</td>
<td>18</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td>Makuuchi²⁰</td>
<td>54</td>
<td>37.9</td>
</tr>
<tr>
<td></td>
<td>Nanashima⁵³</td>
<td>30</td>
<td>29.4</td>
</tr>
<tr>
<td></td>
<td>Sugawara²²</td>
<td>66</td>
<td>35.8</td>
</tr>
<tr>
<td>N-butyl cyanoacrylate</td>
<td>Baere¹⁶</td>
<td>107</td>
<td>57.8</td>
</tr>
<tr>
<td></td>
<td>Barbaro²⁵</td>
<td>26</td>
<td>53.0</td>
</tr>
<tr>
<td></td>
<td>Capussotti⁹</td>
<td>31</td>
<td>48.5</td>
</tr>
<tr>
<td></td>
<td>Elias³⁰</td>
<td>68</td>
<td>59.1</td>
</tr>
<tr>
<td></td>
<td>Giraudo¹⁷</td>
<td>146</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>Sirichindakul⁵⁷</td>
<td>29</td>
<td>27.5</td>
</tr>
<tr>
<td></td>
<td>Broering⁴⁷</td>
<td>17</td>
<td>69.4</td>
</tr>
<tr>
<td>Fibrin glue</td>
<td>Liem⁵⁸</td>
<td>15</td>
<td>31.4</td>
</tr>
<tr>
<td></td>
<td>Nagino¹⁵</td>
<td>105</td>
<td>27.4</td>
</tr>
</tbody>
</table>

### 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>
<tr>
<td>Major complications</td>
<td></td>
</tr>
<tr>
<td>Portal thrombosis</td>
<td>0.8</td>
</tr>
<tr>
<td>Embolization of non-target vessels</td>
<td>0.6</td>
</tr>
<tr>
<td>Liver hematoma</td>
<td>0.4</td>
</tr>
<tr>
<td>Infection / absces</td>
<td>0.4</td>
</tr>
<tr>
<td>Intra-abdominal bile leakage</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The only study describing PVE-related mortality has been published by Giraudo et al.[17] In a group of 146 patients, one patient died 20 days after PVE due to lethal pulmonary embolism. No embolization material was detected in the lung. A second patient developed
cholangitis, and died of septic shock 39 days after PVE. All other studies reported no PVE-related mortality, resulting in an overall mortality rate of 0.1%.

Liver resection

In total, 20.0% (358/1791) of the originally planned liver resections after PVE were cancelled.

<table>
<thead>
<tr>
<th>Surgical procedures</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>Tri-segmentectomy right</td>
<td>36</td>
<td>2.0</td>
</tr>
<tr>
<td>Other (central resection, segmentectomy)</td>
<td>41</td>
<td>2.3</td>
</tr>
<tr>
<td>No resection</td>
<td>358</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Seven studies (327 patients) lacked a detailed description of the cause of cancellation. In the other 37 studies (1464 patients), 18.7% of the planned resections were cancelled. In 6.1% because of local intrahepatic tumor progression or newly developed metastases in the FRL, making resection impossible. In 8.1% because of extrahepatic tumor spread (peritoneal metastases, mesenteric lymph node metastases or lung metastases) and in 4.5% by other causes (insufficient hypertrophy of FRL despite PVE, complications of PVE leading to non-resectability, patients refusing further treatment, pre-operative mortality).

The mean period between PVE and liver surgery was 36.9 days (range 21 – 84 days). The types of operative procedures are summarized in Table 8. In more than 70%, a right hemihepatectomy or extended hemihepatectomy was performed.

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

In 11 publications [9,12,14,15,26,39-42,50,58], 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%).

Discussion
Since the first publication on clinical PVE by Kinoshita in 1986 [4], many articles have been published on this subject. The exact mechanisms bleeding to atrophy of the embolized lobe and hypertrophy of the FRL are still unknown. Recent studies have shown that, in addition to the redistribution of portal blood flow 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[59,60].

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 is mainly based on small or larger case series, while no randomized controlled trials on the efficacy of PVE have been conducted. Only 1 meta-analysis has been published on PVE [18]. This meta-analysis mainly focused on the differences between the surgical transileocolic (TIPE) and the percutaneous transhepatic (PTPE) technique, demonstrating a significantly higher increase in FRL in PTPE than in TIPE. There were no differences in major complications [18]. However, with the increasing availability of radiological intervention suites, the percutaneous transhepatic technique has become the standard technique for PVE. Percutaneous PVE can be performed by an ipsilateral or contralateral approach. Using the ipsilateral approach (53.8% of the cases in this review) has the advantage of not puncturing the healthy FRL tissue thereby 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 when using the ipsilateral approach. The contralateral approach (33.8%) 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.[55] and Di Stefano et al.[62], evaluating complications of the ipsilateral and contralateral
approach, respectively, showed almost the same types of complications and no significant difference in complication rates.

The selection of patients for PVE is traditionally based on CT volumetry. Most studies use a FRL volume of 25-30% of the original liver volume as 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 liver damage, liver cirrhosis/fibrosis and long lasting cholestasis, a threshold of 35-40% is preferred as minimum FRL volume. Worldwide there is consensus on these indications. Functional information can be obtained by the ICG plasma disappearance or retention rate test at 15 min. This technique, introduced in 1980, can accurately estimate post-resection remnant liver function[23]. According to the literature, only few authors, mainly Japanese, have used this method to select patients for preoperative PVE. More recently developed quantitative liver function tests such as $^{99}$Tc-labelled mebrofenin hepatobiliairy scintigraphy HBS[63] and $^{99}$Tc-galactosyl-human serum albumin (GSA) scintigraphy could play an important role in a more accurate selection of patients for PVE.

It is important to calculate the percentage of FRL volume following PVE, in order to assure enough functional liver tissue to be left after resection. The importance of the size of the FRL is stressed by Ribero et al.[55]. They showed that both a small FRL and limited degree of hypertrophy (DH) are strongly associated with postoperative hepatic dysfunction. The percentage of FRL volume can be calculated using the absolute volumes by CT volumetry or by relating FRL volume (measured by CT volumetry) to a standardized liver volume based on BSA [48,49]. Monitoring FRL function after PVE is difficult as there are only few liver function tests that can measure the specific increase of the FRL. $^{99}$Tc-labelled mebrofenin HBS with single photon emission tomography (SPECT) [63] and $^{99}$Tc-GSA scintigraphy can be used for this purpose[54,64,65]. 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 only.

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 [55] 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. [15].

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 so far, accurate data regarding the risk of tumor progression after PVE are currently not available [66]. In this study 6.1% of planned liver resections are cancelled because of local intrahepatic tumor progression after PVE. This can be regarded as complication of the treatment, causing irresectability. A direct causality seems obvious and is described in literature, however is not yet proven. An additional 8.1% of the resections are cancelled, because of extrahepatic tumor spread (peritoneal metastases and distant metastases). To restrict tumor growth, the time between PVE and liver resection
should be limited. Furthermore, sequential transarterial chemo-embolization and PVE can be performed, particularly in patients with HCC [67], in order to limit 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]. Other studies showed no significant difference in hypertrophy response or in postoperative complications when chemotherapy was continued [68]. A few large studies evaluated in this review, show no significant difference in increase of the FRL volume after PVE in patients who previously did or did not receive chemotherapy [14,16,43,55]. De Beare et al. however described a significant lower hypertrophy response in patients who received chemotherapy with platin agents. Restricted by the limited number of articles and their inhomogeneity, evaluation was only possible by head-to-head comparison.

The same applies to the effect of pre-existing liver damage (liver cirrhosis and fibrosis) on hypertrophy response after PVE. Comparison of relevant studies show an impaired hypertrophy response compared to normal livers, however statistical significance has not been demonstrated. Farges et al. [32] stated that patients with cirrhotic livers and a normal hypertrophy response had less post operative complications. On the other hand could failure of increase of the FRL, be considered an indicator of inability of regeneration of liver parenchyma and liver resection should be avoided.

Many different embolization materials have been applied for PVE. The combination of n-butyl cyanoacrylate and lipiodol and the combination of PVA particles with coils are mostly used. Both are non-absorbable materials which lead to persistent occlusion of the portal branches, preventing peripheral recanalization. As gelatin sponge is absorbable, 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 of the portal venous system. Little inflammatory reaction of the liver tissue is seen when using PVA. 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. Using the appropriate delivering catheters, procedure time can be decreased. N-butyl cyanoacrylate induces a strong inflammatory reaction, rendering surgical resection sometimes technically more difficult [6]. Large clinical studies, comparing the effect of different embolization materials on the hypertrophy response are lacking. The data in this review suggest that the use of n-butyl cyanoacrylate results in a higher % FRL volume increase.

Finally, both the overall technical success of PVE (99.3%) and clinical success rate (96.1%) of PVE are very high. Only 2.8% 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 [32]. Only 0.4% of patients appear unresectable because of PVE related complications, such as a large subcapsular hematoma, portal thrombosis, or biliary or infectious complications.
in the FRL after a contralateral procedure. Overall complication rates are higher, but these complications rarely need treatment and they rarely lead to unresectability.

In conclusion, preoperative PVE is an effective method to increase FRL volume with a high technical and clinical success rate. The complication rate is low, but local tumor progression after PVE is an imminent cause of unresectability. Pre-existing liver damage due to cirrhosis seems to have a negative effect on the hypertrophy response. Chemotherapy however does not seem to have any influence on the hypertrophy response, except for platin agents. The use of n-butyl cyanoacrylate may result in a greater hypertrophy response compared to the other embolization materials used.
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Chapter 3

Outcomes of portal vein embolization and extensive resection in predamaged livers

K.P. van Lienden
L.T. Hoekstra
C.S. van Doorn
R.J. Bennink
O.M. van Delden
D.J. Gouma
J.S. Lameris
T.M. van Gulik

Submitted CVIR
Abstract

**Objectives:** To assess efficacy and safety of portal vein embolization (PVE) in relation to pre-existing liver cirrhosis, steatosis, cholestasis, chemotherapy, and clinical outcomes.

**Methods:** Between January 2005 and July 2011, 56 consecutive patients underwent PVE, at least three weeks before extensive liver resection. Volumes of total liver, tumour and future remnant liver (FRL) were analyzed. Outcomes were assessed in relation to pre-existing liver cirrhosis, cholestasis and chemotherapy.

**Results:** All patients underwent successful embolization. A serious adverse event occurred in one patient (1.7%), consisting of contralateral portal vein thrombosis, rendering the patient unresectable. The mean increase of the FRL was 51% (0-305%). Insufficient hypertrophy response precluding surgical resection was seen in only one patient (1.7%). There were no significant differences in hypertrophy response of FRL after PVE between patients with and without chemotherapy (p=0.51), fibrosis/steatosis (p=0.43) or patients with and without cholestasis (p=0.58) and there are no significant differences in regeneration three months after liver resection.

**Conclusions:** PVE is a safe and efficient technique in patients with compromised liver function due to fibrosis, cholestasis or liver damage after chemotherapy. There were no significant differences in post-PVE hypertrophy response nor in post-resectional liver regeneration between patients with predamaged and normal livers.
Introduction

In patients with metastatic liver disease or with primary hepatic or biliary malignancy, surgical resection is the only option to achieve long-term survival. As almost half of these patients need at least a hemihepatectomy to ensure margin-negative resection, many patients are found unresectable because of an anticipated, small future remnant liver (FRL). The function and volume of the FRL are important determinants for predicting postoperative liver failure, which is a life-threatening complication after resection.[1]

Makuuchi et al. clinically introduced in 1990 the technique of portal vein embolization (PVE) to induce liver hypertrophy, rendering patients with a small FRL resectable.[2] Since then, many studies have demonstrated the augmenting effect and safety of this procedure. In patients with normal liver parenchyma, the minimum volume of the FRL is considered to be at least 25% to avoid liver failure after major liver resection. However, in predamaged livers (fibrosis/cirrhosis, steatosis, cholestasis, post-chemotherapy), the minimum volume of the FRL is rather chosen at 40%.[3]

PVE increases the opportunities for patients to undergo resection and allows surgeons to perform more extensive liver resections, exploring the limits of what is technically possible. Since patients are increasingly included in neoadjuvant chemotherapy regimens, the influence of chemotherapy on the hypertrophy response of the FRL is under debate.[4,5] Some authors reported that chemotherapy does not affect the hypertrophy response at all [4,5,6,7], whereas others concluded that chemotherapy negatively influenced the rate of hypertrophy.[8,9]

The role of cholestasis in regeneration of the FRL after PVE also remains controversial. It is stated by some authors that longstanding cholestasis, as is often encountered in patients with cholangiocarcinoma, impedes the hypertrophy response of the FRL, emphasizing the need for pre-procedural biliary drainage.[10,11] This notion however, has not been confirmed in other studies.[6,12]

The aim of this study was to assess our results of PVE in patients with compromised liver function and to compare these with the results in patients with normal liver function, including regeneration of the liver remnant 3 months after resection.

Materials and methods

Patients

Between January 2005 and July 2011, 56 consecutive patients underwent PVE prior to liver resection. Patient characteristics, indications for PVE, volumetric changes, hypertrophy response, complications after PVE, surgical outcome and surgical complications were evaluated.

The influence of pre-existing liver cirrhosis/fibrosis, steatosis, cholestasis and chemotherapy on the hypertrophy response was examined in particular. Data concerning cirrhosis, fibrosis and steatosis were extracted from pathology reports, radiology reports and patient records.
All patients with extensive intrahepatic cholestasis caused by cholangiocarcinoma or colorectal liver metastasis (CRLM) were evaluated separately. More than half of the patients with CRLM received pre-operative chemotherapy according to locale treatment strategies (mostly 5-FU/Leucovorin combined with Oxaliplatin or Irinotecan). As data concerning interruption of chemotherapy between PVE and resection were inconsistent, these were not taken into account.

**PVE**

All procedures were performed using the percutaneous, ipsilateral approach as described by Madoff[12] to prevent complications of the contralateral, usually the left portal vein and left liver lobe. The procedure was performed under conscious sedation by midazolam (Midazolam, Actavis 5mg/ml, Actavisgroup PTC ehf, Iceland), fentanyl (Fentanyl 50 microgram/ml, Bifarma pharmaceuticals, Hameln, Germany) and local infiltration of the skin with lidocaine (Lidocaine HCL 2%, B. Braun AG, Melsungen, Germany). After ultrasound-guided puncture of an anterior branch of the right portal vein, a 5 French sheath was inserted. Following portography, all right branches of the portal vein were selectively catheterized using a reverse curved catheter, and embolized with PVA particles (300-500 μg, Cook Incorporated, Bloomington, United States of America) and multiple 6 to 12 mm platinum coils (Tornado Embolization Coils, Cook Incorporated, Bloomington, USA). The procedure was completed with a check portogram to confirm total occlusion of the right portal system and normal flow through the left, future remnant portal system. Finally, the puncture tract was closed with a gelfoam plug (Spongostan Standard, Ferrosan A/S, Soeborg, Denmark). In patients with cholestasis, complete percutaneous or endoscopic drainage of the obstructed bile ducts was performed prior to PVE.

Technical success was defined as complete occlusion of the right portal venous system at the end of the embolization procedure. Clinical success was defined as an uncomplicated procedure and adequate hypertrophy response after a minimum of 3 weeks after PVE, making resection possible.

**Biochemical follow-up after PVE**

Biochemical tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AF), γ-glutamyltransferase (γGT) and total bilirubin were performed before PVE, immediately following PVE, and 1, 2 and 21 days after PVE. These tests were repeated 6 hrs after surgery, and on day 1, 2, 3, 4, 5, 6, 7, 8 and 14 after resection.

**Measurement of liver volume**

A multiphase computed tomography (Mx 8000 or Brilliance, Philips, Eindhoven, the Netherlands) with intravenous injection of contrast medium (Ultravist-300, Bayer Schering Pharma, Bayer BV, Mijdrecht, the Netherlands) was performed in all patients before PVE,
approximately three weeks after PVE and three months after surgery, to calculate the maximum hypertrophy response after PVE and after resection. The portal phase or the 4-minutes late enhancement phase of the CT-scan was used to visualize the right, middle and left branches of the hepatic vein, as well as the portal vein, to delineate the individual liver segments according to Couinaud.

CT-data were post-processed and evaluated on a MxView – Independent Multi-Modality Diagnostic Workstation (Version 3.52 B2, August 2002, Philips Medical Systems, Eindhoven, the Netherlands). Five millimetre Multi Planar Reconstructions were made. Segmental anatomy of the left and right liver segments, as well as the tumour were manually delineated after which total liver volume (TLV), future remnant liver volume (FRLV) and tumour volume (TV) were calculated.

The percentage FRL before and three weeks after PVE were calculated, using the following equation:

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

Liver hypertrophy after PVE was defined as

\[
1 - \frac{\text{FRL prePVE}}{\text{FRL postPVE}} \times 100 \%
\]

The same calculation was made with the FRL volumes after surgery:

\[
1 - \frac{\text{FRL 3months after surgery}}{\text{FRL postPVE}} \times 100 \%
\]

Measurement of liver function using hepatobiliairy scintigraphy

Hepatobiliary scintigraphy (HBS) was performed with \(^{99m}\text{Tc}\)-labeled 2,4,6 trimethyl-3-bromo aminodiacetic acid (\(^{99m}\text{Tc}\)-mebrofenin [Bridatec]; GE Healthcare) to evaluate liver function and to calculate function of the FRL, as described previously.[13] Images are obtained in supine position, with a large-field-of-view (FOV) SPECT/CT camera (Infinia II; GE Healthcare), equipped with low-energy high-resolution collimators, positioned over the liver and heart region. Firstly, a dynamic acquisition (36 frames of 10 s/frame, 128 matrix) immediately after the intravenous administration of 200 MBq of \(^{99m}\text{Tc}\)-mebrofenin was obtained for calculation of the hepatic uptake function. Subsequently, a fast SPECT acquisition was performed (60 projections of 8 s/projection, 128 matrix) centered on the peak of the hepatic time–activity curve, which was used for the 3-dimensional assessment of liver function and calculation of functional liver volume. Immediately after SPECT, a low-dose non–contrast-enhanced CT scan was obtained for attenuation correction and anatomic mapping on the same gantry, without moving the patient. Finally, a second dynamic acquisition (15 frames of 60 s/frame, 128 matrix) was obtained to evaluate biliary excretion. Data were processed on a workstation (MultiModality; Hermes Medical Solutions).

The scintigraphy was performed approximately 14 days before and three weeks after PVE. It was also performed, three months after surgery. Scintigraphy, combined with CT-volumetry, was used to determine the volumetric and functional reserve of the liver. Patients were considered to have sufficient residual liverfunction with an uptake of at least 2.7%/min./m² and a functional FRL of 25-40%.
Liver resection
All patients elected for PVE were scheduled for either hemihepatectomy (3 or 4 Couinaud segments) or extended hemihepatectomy (4 or 5 Couinaud segments). Surgery was performed if the FRL was at least 25% in patients with normal liver parenchyma and 40% in patients with predamaged livers.

Time of surgery, postoperative course and complications were assessed. The Clavien classification was used for evaluation of complications.[14] Morbidity and mortality were compared in 56 patients who had undergone liver resection without PVE in the same period.

Statistic analysis
SPSS Statistics version 17.0 and GraphPad Prism Version 5.0 was used for statistical analysis. Increase of FRL-volume for each diagnosis was analyzed using the Wilcoxon signed rank test. To compare FRL growth in patients with and without compromised liver, as well as in patients with and without chemotherapy, the Wilcoxon Mann-Whitney U-test was used. A difference with a $P$ value < 0.05 was considered significant.

Results
PVE was performed in 56 patients, 36 men and 20 women, with a mean age of 60 ± 12 yrs (range 31-78). The majority of patients (n=40) were diagnosed with CRLM, in which 18 patients had bilobar disease. A compromised liver, defined by pre-existing fibrosis, steatosis, previous chemotherapy or long-standing cholestasis, was documented in 22 patients. A detailed description of the patient characteristics is given in Table 1.

**Table 1:** patient characteristics.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>36/20</td>
</tr>
<tr>
<td>Age at PVE (yr)</td>
<td>60 (31-78)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Colorectal metastasis</td>
<td>40</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>8</td>
</tr>
<tr>
<td>Hepatocellular adenoma</td>
<td>4</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Neuroendocrine tumour</td>
<td>1</td>
</tr>
<tr>
<td>Gallbladder carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Compromised liver</td>
<td>22/56</td>
</tr>
<tr>
<td>Previous Chemotherapy</td>
<td>26/56</td>
</tr>
</tbody>
</table>
Technical and clinical success of PVE

The technical success rate of the procedure was 100%. Clinical success was achieved in 54 of 56 patients (96.3%). In one patient, portal vein thrombosis of the (left) FRL was found at surgery 4 weeks after PVE, causing unresectability despite exploration of the left portal vein and thrombectomy. This was the only patient in whom a contralateral portal venous access was chosen. After this event, all subsequent patients were managed by ipsilateral right portal access. In one patient PVE was considered to have failed because there was no hypertrophy response at all, despite technical success of the procedure. Therefore, resection in this patient was deferred.

PVE related complications

One patient developed skin rash during the procedure, caused either by the contrast medium or by pre-procedural antibiotics. Embolization of the portal vein could be continued after administration of an antihistamine drug, clemestine (Taveyyl, Novartis Consumer Health BV, Breda, Netherlands) and subsequently was successful. Except for some self-limiting, mild abdominal discomfort, no other complications were seen in the remaining 54 patients. There was no PVE related mortality.

Follow-up of liver function after PVE

An initial elevation in levels of AST, ALT, AF, γGT and total bilirubin was seen, which returned to almost normal in two days. No significant differences in liver enzymes were observed between any of the groups.

Liver volume

In all 56 patients, CT-scans were performed to calculate liver volumes pre and post PVE. The CT-scans were made with a mean of 34 ± 29 days before PVE and a mean of 24 ± 10 days after PVE.

Increase of FRL

As shown in table 2, FRL-volumes significantly increased after PVE as well as after resection (both p<0.0001), compared to pre-PVE volumes. Also the percentage FRL significantly increased from 28.4 ± 8 % to 41.0 ± 9 % (p<0.0001). The FRL volume increased with a mean of 51 ± 50 %.

<table>
<thead>
<tr>
<th>Table 2: liver volumes before PVE, after PVE and after surgery.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean volumes</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Before PVE</td>
</tr>
<tr>
<td>After PVE</td>
</tr>
<tr>
<td>After surgery</td>
</tr>
</tbody>
</table>

TLV: Total liver volume; RLV: right liver volume (total liver volume – tumour volume – FRL)
The volume of the embolized segments to be resected (RLV) corresponding with the right liver lobe, decreased significantly after PVE showing a mean decrease of 16% (range 0 - 66%), as well as the percentage of right liver volume (both \( p<0.05 \)). Total liver volume did not change significantly (\( p=0.17 \)).

**Cholestasis**
Comparison of patients with cholestasis (\( n=7 \)) and without cholestasis (\( n= 49 \)) showed no significant differences in FRL-volumes before PVE (\( p=0.74 \)) and after PVE (\( p=0.76 \); Table 3). Neither were there any significant differences in increase of FRL after PVE (\( p=0.58 \)).

**Table 3:** increase of FRL-V in patients with and without cholestasis.

<table>
<thead>
<tr>
<th></th>
<th>No cholestasis (( n=49 ))</th>
<th>Cholestasis (( n=7 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRL volume (ml) before PVE</td>
<td>473 ± 180</td>
<td>454 ± 104</td>
</tr>
<tr>
<td>Percentage FRL (%) before PVE</td>
<td>28 ± 8</td>
<td>25 ± 8</td>
</tr>
<tr>
<td>FRL volume (ml) after PVE</td>
<td>716 ± 290</td>
<td>717 ± 222</td>
</tr>
<tr>
<td>Percentage FRL (%) after PVE</td>
<td>41 ± 9</td>
<td>36 ± 8</td>
</tr>
<tr>
<td>Increase after PVE (%)</td>
<td>55 ± 54</td>
<td>63 ± 57</td>
</tr>
</tbody>
</table>

**Chemotherapy**
Comparison of patients receiving (\( n=26 \)) or not receiving (\( n=30 \)) chemotherapy before PVE, showed no significant differences in FRL-V before PVE (\( p=0.12 \)) nor after PVE (\( p=0.46 \)). The increase of FRL in mL/days was also not significantly different (\( p=0.51 \); Table 4).

**Table 4:** increase of FRL-V in patients with and without chemotherapy.

<table>
<thead>
<tr>
<th></th>
<th>No chemotherapy (( n=30 ))</th>
<th>Chemotherapy (( n=26 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRL volume (ml) before PVE</td>
<td>590 ± 307</td>
<td>467 ± 159</td>
</tr>
<tr>
<td>Percentage FRL (%) before PVE</td>
<td>30 ± 9</td>
<td>28 ± 8</td>
</tr>
<tr>
<td>FRL volume (ml) after PVE</td>
<td>816 ± 387</td>
<td>698 ± 249</td>
</tr>
<tr>
<td>Percentage FRL (%) after PVE</td>
<td>41 ± 11</td>
<td>41 ± 8</td>
</tr>
<tr>
<td>Increase after PVE (%)</td>
<td>45 ± 47</td>
<td>54 ± 60</td>
</tr>
</tbody>
</table>

**Compromised liver**
There were no significant differences between patients with a compromised liver (consisting of steatosis, fibrosis/cirrhosis or a combination (\( n=12 \)) and patients with a non-compromised liver (\( n=44 \)) as regards FRL-volumes before PVE (\( p=0.33 \)); FRL-volumes after PVE (\( p=0.53 \)) and increase of the FRL in time after resection (\( p=0.57 \); Table 5).
Hepatobiliary scintigraphy

Hepatobiliary scintigraphy was performed in 51 patients, approximately 14 ± 21 days before and at least three weeks after PVE (mean of 21 ± 5 days). Before PVE, total liver uptake was 16 ± 9.5 % per minute. The FRL showed an uptake of 27 ± 7 %, representing the functional fraction of this part of the liver. A control scintigraphy was performed at least three weeks after PVE in 51 patients. Total liver uptake was then 14 ± 3 % per minute whereas the functional contribution of the FRL had significantly increased to 42 ± 14 % (p=0.027).

Total liver uptake did not change significantly (p=0.27). The uptake by the left liver segments did increase significantly after right PVE, showing a mean of 4.21 ± 3 % per minute before PVE, and a mean of 5.66 ± 2 % per minute after PVE (p<0.0001). Hence, PVE resulted in a significant increase in function of the hypertrophic FRL, three weeks after PVE.

Patients with cholestasis, showed a significantly lower total liver uptake pre- and post-PVE as compared to patients with normal livers (p=0.002), but there were no significant differences in liver function of the FRL pre- (p=0.37) and post-PVE (p=0.63).

There were also no significant differences in uptake of the non-embolized, left liver segments before PVE (p=0.96) or after PVE (p=0.46) in patients after chemotherapy as compared to normal livers. HBS showed a lower increase in uptake of the FRL three weeks after PVE in patients with a compromised liver (9.7%) as compared to patients with a normal liver (54.9%), but this difference is not statistically significant.

Surgery

Of the initial 56 patients, six patients were deemed unresectable on the post-PVE CT-scan. Five patients because of tumor progression (two of them also developed metastases in the future remnant, left liver) and one patient showed insufficient hypertrophy of the FRL. Of the remaining 50 patients, 5 patients were found to be unresectable at laparotomy because of portal vein thrombosis in the left portal vein (n=1, as described above), metastases discovered in the FRL, not detected on preoperative CT-scan (n=1), gross tumor invasion in the bifurcation of the portal vein and segment four (n=1) and extrahepatic metastases found during exploration (n=2). A sixth patient, with a gallbladder carcinoma, proved to have no liver metastasis at laparotomy, and therefore a cholecystectomy sufficed.

<table>
<thead>
<tr>
<th></th>
<th>Non-compromised liver (n=44)</th>
<th>Compromised liver (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRL volume (ml) before PVE</td>
<td>478 ± 186</td>
<td>588 ± 303</td>
</tr>
<tr>
<td>Percentage FRL (%) before PVE</td>
<td>29 ± 8</td>
<td>29 ± 9</td>
</tr>
<tr>
<td>FRL volume (ml) after PVE</td>
<td>711 ± 294</td>
<td>808 ± 353</td>
</tr>
<tr>
<td>Percentage FRL (%) after PVE</td>
<td>42 ± 9</td>
<td>42 ± 9</td>
</tr>
<tr>
<td>Increase after PVE (%)</td>
<td>52 ± 59</td>
<td>44 ± 47</td>
</tr>
</tbody>
</table>

Table 5: Increase of FRL-V in patients with and without compromised liver.
The types of resection in the 44 patients undergoing resection is summarized in table 6. The mean duration of the operation was 327 ± 123 minutes. Postoperative complications were seen in 23 patients (46%). Most patients showed minor complications (Clavien grade II-IIIa), such as self-limiting bile leakage, hypertension, pulmonary embolism, intra-abdominal abscess, or urinary tract infection. Major complications (Clavien grade IIIb-IVb) occurred in 4 patients (leakage of the hepatico-jejunostomy (n=1), abdominal wound dehiscence (n=1), transient liver failure (n=2)).

Three patients died of liver failure within 30 days after surgery (mortality 6.8%). One patient with CRLM had an uncompromised liver but developed liver failure despite pre-operative FRL of 37.5% after right PVE. A specific cause for the postoperative liver failure and encephalopathy was not found. The second patient who had undergone a right hemihepatectomy including segment 1 for hilar cholangiocarcinoma developed postoperative portal vein thrombosis of the liver remnant, causing liver failure. This patient had a pre-operative FRL of 29.7% on CT scan and a functional FRL of 53% (uptake of 10.5%/min./m²). The third patient had undergone a right hemihepatectomy for CRLM, leaving a FRL of 29.3% after PVE. The procedure was complicated by massive intraoperative and postoperative bleeding, and the patient died two weeks later of multiple organ failure.

In the same period 56 patients underwent liver resection without pre-operative PVE. The procedures consisted of 8 right hemihepatectomies, 2 left hemihepatectomies, 1 right extended hemihepatectomy, 1 left extended hemihepatectomy, 28 multiple segment resections, 13 metastasectomies and 3 hilar resections. The 30 days mortality in this group was 3.6%.

Table 6: overview of liver resections.

<table>
<thead>
<tr>
<th>Type of resection</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right hemihepatectomy</td>
<td>16</td>
</tr>
<tr>
<td>Extended right hemihepatectomy</td>
<td>10</td>
</tr>
<tr>
<td>Right hemihepatectomy + metastatectomy</td>
<td>12</td>
</tr>
<tr>
<td>Extended right hemihepatectomy + metastasectomy</td>
<td>4</td>
</tr>
<tr>
<td>Segment/metastasis/hilar resection</td>
<td>2</td>
</tr>
<tr>
<td>No resection possible</td>
<td>12</td>
</tr>
</tbody>
</table>

Follow-up after surgery

AST levels in the blood initially increased after surgery with peak concentrations on day 1 until day 2 (NS). After three days, these values returned to normal. ALT levels also increased after surgery (NS). In one patient, the AST levels progressively increased up to 3000 U/L in the first days after resection with concomitant liver failure, which finally became fatal (Figure 1,2).

Postoperative liver enzyme levels were not significantly correlated with FRL-V. There were no significant differences in levels of AST, ALT, AF, γGT and total bilirubin within 14 days after surgery.
Growth of the remnant liver after surgery

CT-scans 3 months after resection were available in 30 of the 44 resected patients. The mean volume of the remnant liver had increased to $1487 \pm 495 \text{ml}$ which was significantly larger than the volume of FRL after PVE ($741 \pm 315 \text{ml}$ ($p<0.0001$)). Table 7 and Figure 3 show the future remnant liver volumes and percentages before and after PVE, and the eventual liver volumes 3 months after resection.

There were no significant differences in the remnant liver volumes three months after surgery in patients with or without cholestasis (mean volume of $1844 \pm 456 \text{ml}$ versus $1333 \pm 285 \text{ml}$, $p=0.11$), in patients with or without chemotherapy (mean volume $1429 \pm 367 \text{ml}$ versus $1546 \pm 398 \text{ml}$, $p=0.66$) and in patients with or without a compromised liver (mean volume $1334 \pm 368 \text{ml}$ versus $1605 \pm 465 \text{ml}$, $p=0.23$).

**Table 7**: volumes and increase of FRL before and after PVE, and after resection.

<table>
<thead>
<tr>
<th></th>
<th>FRL volume (mL)</th>
<th>FRL (%)</th>
<th>Increase of FRL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before PVE</td>
<td>$509 \pm 238$</td>
<td>$28.5 \pm 8$</td>
<td></td>
</tr>
<tr>
<td>After PVE</td>
<td>$741 \pm 315$</td>
<td>$41 \pm 9$</td>
<td>$51 \pm 51$</td>
</tr>
<tr>
<td>After surgery</td>
<td>$1487 \pm 495$</td>
<td>$100$</td>
<td>$105 \pm 67$</td>
</tr>
<tr>
<td>After surgery</td>
<td></td>
<td>$207 \pm 124$ [compared to volume before PVE]</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1**: Concentration of AST after surgery.

**Figure 2**: Concentration of ALT after surgery.

**Figure 3**: Regeneration of the FRL, after PVE and after resection.
14 patients underwent hepatobiliary scintigraphy approximately three months after surgery (87 ± 8 days), in conjunction with CT-volumetric assessment. The mean percentage uptake of the FRL per minute was 11.3 ± 4%, compared to 5.7 ± 2% three weeks after PVE. The total liver uptake and function of the liver remnant after surgery was significantly increased compared to the uptake of the FRL before PVE (p=0.001) and after PVE (p=0.002). Differences in regeneration of the liver remnant three months after surgery between normal and compromised livers could not be established because of the small number of patients.

Discussion

Our findings that PVE proved to be a safe and efficient procedure to induce hypertrophy of the FRL is in line with many previous studies. [11,15,16,17,18,19] In literature, many techniques have been described and many types of embolization materials are used. In our experience, an ipsilateral puncture and the use of PVA particles and coils is a safe technique. The only patient in our study treated by contralateral approach unfortunately became unresectable because of subtotal portal vein thrombosis in the FRL. Giraudo [7], de Baere [9] and Elias[20] all described large groups treated by contralateral access and only Giraudo [7] experienced this complication only once.

Post procedural fever was not seen in our patients as has been described many times in patients embolized with N-butyl-cyanoacrylate (NBCA). Elias[20] described such an inflammatory response in 90% of patients. Covey et al[5] also reported pyrexia in 45% of patients embolized with PVA particles. All cases were transient and self-limiting.

The influence of pre-existing compromised liver on regeneration and the hypertrophy response as in patients with cirrhosis, fibrosis or steatosis, is unclear. Previous studies reported a significantly poorer response to PVE [21,22], although other reports showed no significant difference in regeneration compared to uncompromised livers. [7,6,23] The same applies to patients who received chemotherapy. Some studies report an impaired hypertrophy response [8,9], whereas others mention no significant differences. [4,5,6,7,24,25] Our study shows no significant differences in increase of FRL volumes in patients with compromised livers (p=0.77) or in patients without cholestasis (p=0.19). In all patients with cholestasis, adequate drainage of the biliary system was performed prior to undertaking PVE, to facilitate post-PVE regeneration and to avoid infectious biliary complications as cholangitis. The regenerative responses after PVE and subsequently after liver resection were not significantly different compared to patients with uncompromised livers, probably owing to this policy.

Many studies find no significant differences in growth of the FRL when comparing patients with and without chemotherapy. De Baere et al., however, reported a lesser hypertrophy response in patients who had received platin agents, whereas the use of other chemotherapeutic agents did not influence hypertrophy of the FRL. [9] No differences in hypertrophy response in patients with and without chemotherapy were reported by Nafidi [25]. Beal et al. [8] evaluated a group of 15 patients after PVE who all had received pre-PVE
chemotherapy, and compared patients who continued chemotherapy with patients who stopped chemotherapy in the regeneration period between PVE and resection. No significant growth of tumour volume was seen, but a significant reduction in hypertrophy response was observed in patients receiving chemotherapy after PVE. In our series, no significant differences in hypertrophy response or in post-operative liver regeneration were seen in patients with or without chemotherapy. It therefore seems that chemotherapy given before PVE does not affect the hypertrophy response of the FRL, nor has a limiting influence on surgical treatment options. Continuing chemotherapy after PVE therefore, potentially avoids PVE-related tumour progression without significantly influencing the hypertrophy response, although this notion awaits further clinical assessment.

We evaluated the results of PVE not by volumetric data only, but also by quantitative assessment of function of the FRL, obtained by $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy (HBS) as has been validated by de Graaf et al.[26] Functional uptake of the left liver segments did increase after PVE ($p<0.0001$) whereas the uptake rate of the embolized, right liver segments decreased. Three months after surgery, the functional uptake had increased with 194% compared to the uptake of the FRL before surgical resection, demonstrating the inexhaustive power of the liver cells to regenerate. The uptake function was ultimately almost 84% of total liver function before resection (mean $11.3 \pm 3.6$% per minute). In our experience, HBS is a valuable tool in the preoperative work-up to improve risk assessment in patients requiring extensive liver resection. De Graaf concludes that functional assessment using HBS is of more value than morphological evaluation using CT volumetry, especially in patients with a compromised liver. [26]

Major hepatectomies still have significant morbidity and mortality, ranging from 4 to 8%, depending on the extensiveness and complexity of the procedure. [27,28] Postoperative liver failure is associated with increased morbidity and mortality and its pathogenesis is related to the amount of functional liver mass remaining after resection. [1,29] Three patients died within 30 days after surgery because of progressive liver failure. One of them had an un-compromised liver with post-PVE FRL of even 37.5%. The other two patients, also with sufficient post-PVE FRL volume of almost 30%, developed liver failure because of septic complications culminating in multiple organ failure.

The high mortality rate (6.8%) reported in our study is in part explained by patient selection as all patients subjected to preoperative PVE had increased tumor burden, and required more complex procedures. A relatively high proportion of patients in our series had hilar cholangiocarcinoma requiring large resections in combination with biliary anastomoses, carrying an increased risk of postoperative morbidity, liver failure and mortality, the latter reported up to 10%. After introduction of PVE in our institution, an increased number of patients who would initially have been considered unresectable, did undergo resection in spite of extensive liver tumor(s). These patients obviously had an increased operative risk. For comparison, the overall postoperative mortality in patients undergoing liver resection without PVE in the same period in our institution (n=56), was 3.6%.
PVE is safe and efficient in patients with normal livers, as well as patients with compromised livers or patients receiving preoperative chemotherapy. There were no significant differences in the hypertrophy response in patients with pre-existing liver cirrhosis/fibrosis, steatosis, cholestasis, or after chemotherapy. Also post-resectional liver regeneration was not influenced by these factors.
References


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

J.W. van den Esschert
K.P. van Lienden
W. de Graaf
M.A.W. Maas
J.J.T.H. Roelofs
M. Heger
T.M. van Gulik
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.

**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-a 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 20% ± 2%, and 15% ± 4% 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, Nimetek; 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:

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

After PVE, %CLV was calculated using the formula:

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

The degree of hypertrophy was calculated by subtracting the %CLVpre-PVE from the %CLVpost-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. Therabbits 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 prothrombin time 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:
\[
\left(\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}}\right) \times 100
\]

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.

(Immuno)histochemistry
Paraffin 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 (20× magnitude) 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 (20× magnitude) 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.

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.
PVE-and PVL-induced hypertrophy response

As demonstrated in concordance with the findings in a previous study, 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 $22\% \pm 2\%$ in the PVE group versus $21\% \pm 3\%$ in the PVL group ($P = .19$). The %CLV increased to $28\% \pm 4\%$, $33\% \pm 4\%$, $34\% \pm 3\%$, and $34\% \pm 6\%$ on days 3, 7, 10, and 14 in the PVL group and to $29\% \pm 3\%$, $38\% \pm 3\%$, $41\% \pm 1\%$, and $42\% \pm 2\%$ in the PVE group, respectively ($P < .001$ in both groups) and was greater after PVE than after PVL ($P = .001$). The mean degree of hypertrophy after 14 days was $15\% \pm 4\%$ in the PVL and $20\% \pm 2\%$ 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 ($P \leq .02$).

The percentage of Ki-67–positive hepatocytes in the caudal liver lobe was greater in the PVE group ($20\% \pm 6\%$) compared with the PVL group ($7\% \pm 5\%$; $P = .016$) 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.

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).

**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.

**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-a level between the PVE and PVL groups on days 7 and 14, respectively.

**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).
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.\textsuperscript{14} 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.\textsuperscript{13} 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.\textsuperscript{19} 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-\(\alpha\), which are important mediators of liver regeneration.\textsuperscript{20} One of the hypotheses of Furrer et al.\textsuperscript{13} 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.\textsuperscript{13} the regenerative response after PVE was greater than after PVL. We did not find a difference in IL-6 and TNF-\(\alpha\) 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\textsuperscript{14,21} 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

Chapter 5

Intrahepatic left to right porto-portal venous collateral vascular formation in patients undergoing right portal vein ligation

K.P. van Lienden
L.T. Hoekstra
R.J. Bennink
T.M. van Gulik
Abstract

**Background:** We investigated intrahepatic vascular changes in patients undergoing right portal vein ligation (PVL) or portal vein embolization (PVE), in conjunction with the ensuing hypertrophy response and function of the left liver lobe.

**Methods:** Between December 2008 and October 2011, 7 patients underwent right PVL and 14 patients PVE. CT-volumetry to assess future remnant liver (FRL) and functional hepatobiliary scintigraphy were performed in all patients before and 3 weeks after portal vein occlusion. In 18 patients an intra-operative portography was performed to assess perfusion through the occluded portal branches.

**Results:** In all patients after initially successful PVL, reperfused portal veins were seen on CT scan three weeks after portal occlusion. This was confirmed in all cases during intra-operative portography. Intra hepatic porto-portal collaterals were identified in all patients in the PVL group and in one patient of the PVE group. In all other PVE patients, complete occlusion of the embolized portal branches was seen on CT scan and on intra-operative portography. The median increase of FRL volume after PVE was 41.6 (range 10-305) %, and after PVL only 8.1(range 0-102)% (p=0.179). There were no differences in FRL-function between both groups.

**Conclusions:** Preoperative PVE and PVL are both methods to induce hypertrophy of the FRL in anticipation of major liver resection. PVL seems less efficient in inducing hypertrophy of the non-occluded left lobe, compared to PVE. This is most likely caused by the formation of intrahepatic porto-portal neo-collateral vessels, through which the ligated portal branches are reperfused within 3 weeks.
Introduction

Beside portal vein embolization (PVE), the most common method to enlarge the future remnant liver (FRL) before extensive liver resection, is portal vein ligation (PVL). Both techniques are extensively described in literature. PVE is a minimally invasive procedure, in which the right portal venous system can be occluded by ipsilateral or contralateral percutaneous puncture under local anesthesia. Different embolization materials have been described, of which the majority of agents causes permanent occlusion.\(^1\),\(^2\),\(^3\) Complications consist of hematoma, septic complications and dislocation of embolization material in portal branches of liver segments to be preserved.\(^4\) PVL requires a surgical approach under general anaesthesia and is mostly performed, as part of a two-stage procedure in resection of bilobar colorectal metastases in which typically during the first stage, the primary tumor is resected along with the lesser liver resection on the left side, usually a left-lateral resection of segments 2 and 3. At the same time, the right portal vein is ligated to induce hypertrophy of segment 4. Following 3-6 weeks, after sufficient volume increase has been determined, a completion right hemihepatectomy is performed in the second stage.\(^5\) The mechanism underlying both methods is occlusion of part of the portal venous system, to induce a hypertrophy response of the non-occluded liver segments.

There is however much discussion regarding the efficiency of both techniques. With PVE, the entire portal tree of the embolized side is permanently occluded, whereas occlusion caused by ligation of the portal vein is presumably short-lived, because of retrograde filling of the ligated portal branches through collateral supply from adjacent non-ligated segments. So far, it is unclear whether arterio-portal or porto-portal collaterals are responsible for this process of revascularization. Krupski et al described in a pig model, an uniform pattern of collaterals with subsequent complete recanalization of the formerly occluded portal vein, distal from the ligation.\(^6\) These collaterals had a cavernous aspect and reduced the portal blood flow.

To our knowledge, porto-portal collaterals were described in humans after portal vein ligation only by Denys et al.\(^7\) These collaterals were described as intrahepatic porto-portal collaterals arising from segment 4 and connecting with the portal venous branches of the adjacent segment 8 within the ligated portal system.

In this study we investigate the intrahepatic vascular changes in 7 patients after PVL along with the differences in hypertrophy response and function compared with 14 patients who underwent PVE in the same period.

Materials and methods

Between December 2008 and October 2011, 20 patients underwent PVE or PVL prior to extensive liver resection. One patient who initially showed no hypertrophy response after PVL, underwent PVE in second intention. In the majority of these patients (n=18), a control portography was performed intra-operatively at the time of resection, to evaluate blood
flow in the embolized or ligated portal branches. In the remaining two patients, the pre-operative CT scan performed to evaluate the volume of the FRL, was also used to evaluate liver vascularisation following PVE or PVL.

Patient characteristics, indications for PVE or PVL, volumetric changes, hypertrophy response, complications after PVE and PVL, and vascularisation of the embolized liver segments were evaluated.

PVE

All procedures in 14 patients were performed using the percutaneous ipsilateral approach as described by Madoff. After ultrasound-guided puncture of an anterior branch of the right portal vein, a 5 French sheath was inserted. Following portography, all right branches of the portal vein were selectively catheterized using a reverse curved catheter, and embolized with PVA particles (300-500 μg, Cook Incorporated, Bloomington, United States of America) and multiple 6 to 12 mm coils (Tornado Embolization Coils, Cook Incorporated, Bloomington, USA). The procedure was completed with a control portogram to confirm total occlusion of the right portal system and normal flow through the left future remnant portal system. Finally, the puncture tract was closed with a gelfoam plug (Spongostan Standard, Ferrosan A/S, Soeborg, Denmark).

PVL

In all 7 patients who had undergone PVL, PVL was electively performed as part of a multi-stage treatment plan in which right PVL was undertaken during initial resection of the primary tumor (n=2); during resection of metastasis in the left liver lobe in anticipation of subsequent right hemihepatectomy (n=2); during explorative laparotomy (n=2) and during an originally planned right hemihepatectomy in which the FRL appeared smaller and more cirrhotic than expected (n=1). In this patient, liver resection was performed 5 weeks after right PVL.

In all patients the portal bifurcation was dissected during intra-operative exploration and the origin of the right portal main branch was suture ligated and divided. In one patient, the portal branches arising from the left portal vein to segment 4 were also dissected and ligated.

Definition of technical and clinical success

After portal vein occlusion, technical success was defined as complete occlusion of the target branches at the end of the procedure. Clinical success was defined as adequate hypertrophy response of the FRL after portal occlusion, allowing liver resection.

Measurement of liver volume

A multiphase computed tomography (Mx 8000 or Brilliance, Philips, Eindhoven, the Netherlands) with intravenous injection of contrast medium (Ultravist-300, Bayer Schering Pharma, Bayer BV, Mijdrecht, the Netherlands) in the portal phase was performed in all
patients before PVE or PVL, and approximately three weeks after PVE or PVL to calculate the maximum hypertrophy response after occlusion of the portal vein. CT-data were processed and evaluated on an MxView – Independent Multi-Modality Diagnostic Workstation (Version 3.52 B2, August 2002, Philips Medical Systems, Eindhoven, The Netherlands). The segmental anatomy of the left and right liver segments, as well as the tumor, were manually delineated according to the Couinaud classification after which total liver volume (TLV), FRL-volume (FRLV) and tumour volume (TV) were calculated. The percentage FRL was calculated before and three weeks after PVE and PVL using the following equation:

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

Liver hypertrophy after PVE was defined as:

\[
1 - \frac{FRL_{prePVE}}{FRL_{postPVE}} \times 100\%
\]

Measurement of liver function with hepatobiliairy scintigraphy

Hepatobiliary scintigraphy (HBS) was performed with \(^{99m}\text{Tc}\)-labeled 2,4,6 trimethyl-3-bromo aminodiacetic acid (\(^{99m}\text{Tc}\)-mebrofenin [Bridatec]; GE Healthcare) including SPECT to evaluate liver function, and to calculate function of the FRL, as described previously. Images are obtained with a large-field-of-view (FOV) SPECT/CT camera (Infinia II; GE Healthcare) equipped with low-energy high-resolution collimators. Firstly, a dynamic acquisition, immediately after the intravenous administration of 200 MBq of \(^{99m}\text{Tc}\)-mebrofenin, was obtained for calculation of the hepatic uptake function. Subsequently, a fast SPECT acquisition was performed centred on the peak of the hepatic time–activity curve, which was used for the 3-dimensional assessment of liver function, and calculation of functional liver volume. Immediately following SPECT, a low-dose non-contrast-enhanced CT scan was obtained for attenuation correction and anatomic mapping on the same gantry, without moving the patient. Finally, a second dynamic acquisition was obtained to evaluate biliary excretion. Data were processed on a workstation (MultiModality; Hermes Medical Solutions). The scintigraphy was performed approximately 14 days before and three weeks after PVE or PVL.

Intra-operative portography

After laparotomy, the hepatoduodenal ligament was dissected and the main portal vein was identified. A 18G venflon needle was inserted into the portal vein main branch after a purse-string suture (5-0 Prolene) allowing advancement of the catheter in distal direction. Subtraction portographies were performed in several directions after manual injection of 10 ml of contrast material (Ultravist-300, Bayer Schering Pharma, Bayer BV, Mijdrecht, the Netherlands). The degree of occlusion of the ligated or embolized portal branches and the presence of collateral flow was evaluated.
Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS 18.0). CT volumetry data were compared using a mixed model analysis based on ranked data. The independent sampled T-test was used for continuous data. Non-parametric data were compared by the Wilcoxon Mann-Whitney U test. All statistical differences were considered significant at a p-value of \( \leq 0.05 \). Data were expressed as means ± SD, unless otherwise stated.

Results

Patients

In 20 patients (12 male and 8 female, mean age 59.6 ± 10.2 years), 21 procedures have been performed to occlude the right portal vein. Seven patients initially underwent a PVL. In one patient, ligation of the right portal vein caused no hypertrophy response at all. The CT-scan, performed 4 weeks after ligation, showed a completely patent right portal vein, despite adequate ligation, without any increase of the FRL volume. In this patient, additional PVE was performed 6 weeks after PVL. In the other 13 patients, PVE was primarily performed. There were no significant differences in patient characteristics. Also, the number of patients receiving chemotherapy or radiotherapy prior to the procedure, or the occurrence of compromised livers, was not significantly different between both groups. (Table 1)

Indications for liver resection consisted of colorectal liver metastasis (CRLM, n=17), cholangiocarcinoma (CC, n=2) or hepatocellular carcinoma (HCC, n=1).

<table>
<thead>
<tr>
<th>Table 1: Patient characteristics</th>
<th>PVE (n=14)</th>
<th>PVL (n=7)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60.2±11.6</td>
<td>59.4±7.6</td>
<td>0.849</td>
</tr>
<tr>
<td>Male/ Female</td>
<td>8:6</td>
<td>4:3</td>
<td>1.000</td>
</tr>
<tr>
<td>BMI</td>
<td>24.6 (19.9-32.0)</td>
<td>22.5 (19.9-24.6)</td>
<td>0.232</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>5/14 (36%)</td>
<td>1/7 (14%)</td>
<td>0.317</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>2/14 (14%)</td>
<td>0 (0%)</td>
<td>0.305</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>4/14 (29%)</td>
<td>1/7 (14%)</td>
<td>0.429</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>9/14 (64%)</td>
<td>5/9 (71%)</td>
<td>0.868</td>
</tr>
</tbody>
</table>

Success rate portal vein occlusion

All PVL and PVE procedures were technically successful. Except for mild abdominal discomfort in some cases (n=4), no other complications were seen in the PVE group. The ligation procedures were also conducted without complications. All PVE procedures were clinically successful showing adequate hypertrophy response of the FRL, three weeks after PVE. In one patient of the PVL group, no hypertrophy response was seen at all on CT scan 4 weeks after PVL, resulting in a clinical success rate of 86% in the PVL group. Six weeks after
PVL, an additional PVE was performed inducing enough hypertrophy response to make liver resection possible. (Figure 1)

CT scan and liver volumetry

CT scans were performed with an average of 25 (range 1-79) days before PVE and 35 (range 25-41) days prior to PVL. Control CT scans were performed with an average of 22 (range 18-40) days after PVE and 22 (range 20-38) days after PVL. There were no significant differences between the groups. Liver volumes are listed in Table 2. There were no significant differences in base-line liver volumes and percentage FRL between the PVE and PVL groups.

![Figure 1. A. Controle CT after PVL. Normal patency of the main portal vein (large arrow) Despite adequate ligation (small arrow), reperfusion of the right portal system (arrowhead). B. Patent right portal system after percutaneous puncture for embolization with PVA particles.](image)

In the PVE group, portal occlusion led to a significant increase of the FRL from 25.8±7.5% to 37±6.4% (p=0.001). The median percentage of increase of the FRL was 41.6 (range 10.4-304.6) %.

In the PVL group, the FRL significantly increased from 27.2±7.0% to 36.1±5.0% (p=0.016). The median percentage of increase of the FRL after PVL was 8.1 (range 0-102) %. Although the percentage of increase of the FRL after PVL is obviously less than after PVE, this is statistically not significant (p=0.179).

Evaluation of the vascular structures after PVE demonstrated complete occlusion of the embolized right portal branches in all cases but one, were flow was seen in a segment 7 branch of the right portal system. Complete patency was seen of the hepatic artery, hepatic veins and left portal vein branches. Evaluation of portal occlusion on CT-scan was however, difficult since severe artefacts caused by coils in the proximal segmental branches of the right portal vein hampered definition of vascular structures.

Although all PVL procedures were technically successful, the right portal branches were patent in 100% of patients on CT scan three weeks after PVL. The site of central ligation
of the right portal system could be identified in all cases. In one patient, CT scan revealed ligation of the right anterior portal branches only, although ligation of the complete right portal system was planned. This ligated sectorial right portal branch also showed retrograde flow distal to the ligature. In the latter case, partial right portal vein ligation however caused sufficient hypertrophy of the left liver lobe to allow liver resection. Intrahepatic collateral formation from the left to the right hemi liver could be identified on CT scan in 5/7 (71%) of patients after PVL.

No extrahepatic collaterals were seen.

<table>
<thead>
<tr>
<th>Table 2. Liver volumes before and after PVE / PVL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver volumes</td>
</tr>
<tr>
<td>Pre-intervention:</td>
</tr>
<tr>
<td>Total liver volume (ml)</td>
</tr>
<tr>
<td>Tumor volume (ml)</td>
</tr>
<tr>
<td>FRL volume (ml)</td>
</tr>
<tr>
<td>% FRL</td>
</tr>
<tr>
<td>Post-intervention:</td>
</tr>
<tr>
<td>Total liver volume (ml)</td>
</tr>
<tr>
<td>Tumor volume (ml)</td>
</tr>
<tr>
<td>FRL volume (ml)</td>
</tr>
<tr>
<td>% FRL</td>
</tr>
<tr>
<td>Increase (%)</td>
</tr>
</tbody>
</table>

Hepatobiliary scintigraphy and functional liver volumetry

In the PVE-group, HBS was performed 5 (range 1-21) days before, and 22 (range 18-40) days after PVE. In the PVL group HBS was performed 3 (range 1-41) days before, and 23 (range 20-35) days after ligation. There were no significant differences between the groups.

Before intervention, total uptake of the complete liver was 15.8 (range 5.7-25.0) %/min in the PVE group and 17.2 (range 11.6-19.6) in the PVL group. The percentage functional-FRL was 24.8 ± 4.5 % in the PVE group and 27.0±1.9 in the PVL group. The estimated FRL-function before intervention was 3.6(1.4-7.0) %/min/m² in the PVE group and 4.9(3.0-5.7) %/min/m² in the PVL group. After portal vein occlusion, total uptake of the complete liver was 13.7 (8.9-18.3) %/min in the PVE group and 17.6 (9.4-22.6) %/min in the PVL group. The percentage functional-FRL increased from 24.8 ± 4.5 to 35.7 ± 9.9% in the PVE group and from 27.0 ± 1.9 to 38.8 ± 7.6 % in the PVL group. The estimated FRL-function after intervention increased to 4.5 (3.4-7.3) %/min/m² in the PVE group and 5.6 (4.2-9.0) %/min/m² after PVL. The increase in functional volume of the FRL was 44.0% after PVE and 43.7% after PVL (p=0.003).

Data are listed in table 3. No significant differences between the groups were found.
Portography before resection

Of all patients who underwent PVE, one patient was found unresectable because of tumor progression as evidenced on the post-PVE CT-scan. Therefore no portography was performed. Complete occlusion of the right portal system was however observed in this patient on CT-scan performed 3 weeks after PVE.

In the other 13 patients, complete occlusion of the total right portal system after PVE was seen in 11 (84.6%) patients on portography performed during laparotomy before liver resection. In one patient, normal hepatopetal flow was seen in the portal branch of segment 7, which was missed during the initial portal vein embolization procedure. In the second patient with incomplete occlusion, a right segment 8 portal branch was patent and filled with contrast through left to right collateral flow from segment 4 to segment 8, although the portal branch was centrally, adequately occluded with coils. (Figure 2) Both patients had a normal hypertrophy response.

In the patient who underwent PVE in addition to PVL, no intra-operative portography was performed. In all other patients who primarily underwent PVL, normal flow was seen through the left portal venous system. Although adequate right central occlusion was demonstrated, complete or partial patency of the ligated right portal system was seen in all patients after PVL. All portograms confirmed the re-established, right portal venous perfusion as demonstrated on the pre-resection CT-scans. Porto-portal collateral flow was seen from segment 4 to segment 5 or 8 (Figure 3). During resection, no extrahepatic collaterals were found.

<table>
<thead>
<tr>
<th>Liver-uptake</th>
<th>PVE</th>
<th>PVL</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-intervention:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total liver uptake (%/min)</td>
<td>15.8 (5.7-25.0)</td>
<td>17.2 (11.6-19.6)</td>
<td>0.741</td>
</tr>
<tr>
<td>% FRL</td>
<td>24.8 ± 4.5</td>
<td>27.0 ± 1.9</td>
<td>0.193</td>
</tr>
<tr>
<td>Estimated FRL function (%/min/m²)</td>
<td>3.6 (1.4-7.0)</td>
<td>4.9 (3.0-5.7)</td>
<td>0.229</td>
</tr>
<tr>
<td><strong>Post-intervention:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total liver uptake (%/min)</td>
<td>13.7 (8.9-18.3)</td>
<td>17.6 (9.4-22.6)</td>
<td>0.122</td>
</tr>
<tr>
<td>% FRL</td>
<td>35.7 ± 9.9</td>
<td>38.8 ± 7.6</td>
<td>0.482</td>
</tr>
<tr>
<td>Estimated FRL function (%/min/m²)</td>
<td>4.5 (3.4-7.3)</td>
<td>5.6 (4.2-9.0)</td>
<td>0.160</td>
</tr>
<tr>
<td>Increase functional FRL (%)</td>
<td>44.0</td>
<td>43.7</td>
<td>0.976</td>
</tr>
</tbody>
</table>
Discussion

In literature, different techniques for portal vein occlusion are described. Right portal vein ligation can be performed during staging laparotomy or during a two-stage procedure in which local tumor resection on the left side is combined with right portal vein portal ligation prior to right hemihepatectomy. This procedure was already described in 1965. Alternatively, PVE can be performed during laparotomy using an ileocolic venous approach although this approach is less popular among surgeons.

Since the introduction of percutaneous PVE, the need for invasive techniques as PVL is strongly reduced. In our institution, PVL procedures are now only performed in selected cases.
as in two-stage resection for bilobar tumors. PVE by the percutaneous transhepatic approach is an effective method to induce hypertrophy of the FRL, with only minor complications.4

The exact mechanism behind the hypertrophy response is not exactly known, but for many years it has been believed that redirecting the flow through partial portal vein occlusion caused hypertrophy of the un-affected liver lobe. Goto et al described in 1990 that the hypertrophy rate of the non-embolized hepatic segments after embolization, was predictable from the extent of increase in portal blood flow in the non-embolized liver.11 This is in line with the theory that hypertrophy of the non-occluded lobe is influenced by increased supply of hepatotrophic substances as growth factors, nutrients and gastrointestinal hormones.12 There is also evidence from experimental models that indicate the hemodynamic influences

Figure 3. Control portogram during laparotomy, performed before liver resection, demonstrates complete reperfusion of the ligated right portal venous system through collateral flow from segment 4 to segment 8 (white arrows).
after portal vein ligation, to be the crucial factor in regulating volume of the un-affected liver after resection.\textsuperscript{13,14}

Currently the theory is exposed, that the hypertrophy response is regulated by the amount of parenchymal loss. Liver mediating cytokines, including TNF-\textit{\textalpha}, interleukin IL-1\textbeta and IL-6, are released from Kupffer cells and activate hepatocytes to proliferate.\textsuperscript{15} Furrer et al\textsuperscript{15} showed in a rat-model that hepatocyte proliferation after PVL was more pronounced than after PVE, suggesting that PVL is the more effective technique. Wilms et al\textsuperscript{12} however, studied PVL and PVE in a pig model, and concluded that PVE was more effective in increasing the future liver remnant, owing to more durable occlusion of the portal venous branches. This was also demonstrated by our group (vd Esschert et al\textsuperscript{16}) in a rabbit PVE-model, in which beside a greater increase of the FRL volume after PVE, also a greater amount of proliferating hepatocytes was demonstrated, without any difference in cytokine levels between PVE and PVL.

Human studies comparing the hypertrophy response after PVE and PVL, are also ambiguous. Aussilhou et al\textsuperscript{17} reported no significant difference in hypertrophy response between PVE and PVL. 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 post-resectional liver regeneration is probably augmenting post-PVL regeneration. Broering et al\textsuperscript{18} on the other hand, reported a significantly better result in hypertrophy response after PVE compared to PVL. Also, the time between portal occlusion and operation, to achieve the necessary hypertrophic reaction was significantly longer in patients after PVL. In this study however, there was a large proportion of PVE procedures in which additional segment 4 branches were embolized. This may cause an overrating of the hypertrophy results in comparison with PVL in which only the right portal branches are ligated.

None of the PVE patients in our study, underwent additional embolization of segment 4. In one patient of the PVL group, segment 4 branches arising from the left portal vein were selectively ligated. There were obviously substantial differences in hypertrophy response of the FRL in the PVL and PVE groups, however these were not statistically significant, as may be explained by the small number of patients in the PVL group. The percentage functional FRL was significantly increased after portal vein occlusion whereas no significant differences were seen between the PVE-group and PVL-group. The reason for this may be that increase in function evolves well ahead of increase in volume after portal vein occlusion, as was previously demonstrated by de Graaf et al\textsuperscript{8}.

In literature, the difference in hypertrophy response between PVE and PVL is in part attributed to the formation of collaterals after PVL. The first to describe these collaterals in humans after PVL, were Denys et al\textsuperscript{7} in 1999. In their case report, intrahepatic porto-portal collaterals from segment 4 branches were shown to drain into segment 8 branches, causing complete reperfusion of the ligated right system. Hypertrophy response of the FRL, was only achieved after embolization of these collaterals.

Few animal studies have been published on this topic. Wilms et al\textsuperscript{12} in a pig model of PVE reported revascularization of the ligated portal branches in all cases, by intrahepatic arterio-
portal collaterals coming from adjacent non-occluded liver segments. This was confirmed by Ferko et al.\textsuperscript{19} who reported patent intrahepatic portal branches after truncal portal vein occlusion caused by rapidly developed hepatopetal collaterals in mini-pigs.

Our study provides the first evidence of collateral flow and reperfusion of the ligated portal venous system in a group of patients after PVL. As soon as 3 weeks after PVL, complete patency of the ligated portal branches was seen on all CT-scans. This was also confirmed by the portograms performed intra-operatively, showing intrahepatic collateral connections between the branches of segment 4 and the branches of the adjacent right segments 5 and 8. No extrahepatic collaterals were detected. This phenomenon could very well explain the difference in hypertrophy response between PVE and PVL. This theory was particularly demonstrated in one patient who showed no hypertrophy response after PVL but finally showed increase of the FRL volume after complete secondary percutaneous embolization with PVA particles distal of the central ligation.

The lack of statistical significance does not support the conclusion of this study as probably caused by the relatively small sample sizes. Because PVE has become the method of choice in inducing hypertrophy of FRL before extensive liver resection, the number of PVL-procedures has become limited. We demonstrated neo-collateral vessel formation after PVL and a trend of restricted hypertrophy corroborating the notion of PVE being superior to PVL.

In summary, we conclude that PVE and PVL are both useful methods to induce hypertrophy of the FRL before major liver resection. PVL seems less efficient in inducing hypertrophy, compared to PVE. This is most likely caused by the formation of intrahepatic porto-portal neo-collateral vessels, through which the ligated portal branches are reperfused in spite of an appropriate ligation procedure.
References


Chapter 6

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

J.W. van den Esschert
K.P. van Lienden
L.K. Alles
A.C. van Wijk
M. Heger
J.J.T.H. Roelofs
T.M. van Gulik

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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 2 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. First, 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. Second, 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. Third, 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.
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 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 × 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:

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

After PVE, the CLV was calculated by:

\[
\%CLV_{\text{post-PVE}} = \frac{CLV_{\text{post-PVE}}}{TLV_{\text{pre-PVE}}} \times 100\%
\]
The increase of the CLV was calculated by:

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

The degree of hypertrophy\(^{13}\) 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) × 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.

In addition, 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) 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 $\lambda_{\text{ex}} = 476$ nm, $\lambda_{\text{em}} = 498-552$ nm and Cy3-labeled constituents were imaged at $\lambda_{\text{ex}} = 561$ nm, $\lambda_{\text{em}} = 568-627$ 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).

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 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).

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).
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 group 7 days after PVE (*, p ≤ 0.016).

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>
<td>15.8 ± 1.8</td>
</tr>
<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>
</tr>
<tr>
<td></td>
<td>CLV increase [%]</td>
<td></td>
</tr>
<tr>
<td>PVAc</td>
<td>Absolute CLV [cm³]</td>
<td>17.3 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>%CLV</td>
<td>22.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>CLV increase [%]</td>
<td></td>
</tr>
<tr>
<td>nBCA</td>
<td>Absolute CLV [cm³]</td>
<td>17.1 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>%CLV</td>
<td>22.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>CLV increase [%]</td>
<td></td>
</tr>
</tbody>
</table>

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).
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.

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 ρ = 0.65, p = 0.001).

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.
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).

**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 periportal 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).
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.
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.
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 inter-species 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.
References

Chapter

Short term effects of combined hepatic vein embolization and portal vein embolization for the induction of liver regeneration in a rabbit model

K.P. van Lienden
J.W. van den Esschert
M. Rietkerk
M. Heger
J.J.T.H. Roelofs
J.S. Laméris
T.M. van Gulik

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Abstract

**Background:** Alternative methods to optimize the hypertrophy response after portal vein embolization (PVE) are desired. In this study we assessed the effect of hepatic vein embolization (HVE) in addition to PVE on the liver hypertrophy response in a standardized rabbit model.

**Methods:** Thirty rabbits were allocated to a group according to the intervention: PVE alone, HVE alone, and a combination of HVE and PVE. The liver regeneration response of the non-embolized, 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). 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:** Although histological and additional regenerative changes are seen, HVE in addition to PVE, has no additional short term effect on the hypertrophy response. The combination of HVE and PVE may therefore, have little use in a clinical setting.
Introduction

Portal vein embolization (PVE) is now a widely used method to increase the future remnant liver (FRL) before major liver resection (1,2). PVE is considered when the FRL is considered too small, thereby increasing the risk of postoperative liver failure (3). With PVE, the portal vein branches of the liver lobe to-be-resected are occluded, causing atrophy of this liver lobe. Liver mediating cytokines, including TNF-α, interleukin IL-1β and IL-6, are released from Kupffer cells and activate hepatocytes to proliferate, inducing a compensatory hypertrophy response in the contralateral FRL (4-6). In most cases, the hypertrophy response following PVE reaches its plateau after 21 days(7). Thereafter, only little additional growth is seen. When the FRL volume, 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 induction of tumor growth after PVE, potentially leading to secondary unresectability(8). Therefore, a technique should be developed that leads to a more rapid and reliable increase in FRL without tumour induction.

Recently, a Chinese research group published on sequential, ipsilateral hepatic vein embolization (HVE) after PVE in humans. In 12 patients who showed insufficient increase in volume of the future remnant liver after PVE, the right hepatic vein was also embolized to induce additional hypertrophy (9;10). The additional HVE was performed 13,5 ± 4.2 days after PVE. Embolizing the ipsilateral hepatic vein, theoretically should block the hepatic outflow and in combination with PVE, should decrease compensatory arterial hyperperfusion, thereby inducing additional hypertrophy of the non-embolized, future remnant liver. However, in this study, the increase in FRL could still be the result of ongoing hypertrophy caused by PVE alone. Nevertheless, 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.

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. 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.

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 2,470-3,430g) were acclimatized for 2 weeks under standardized laboratory conditions.

Experimental design

A validated rabbit model was used for this study (11,12) The liver of the rabbit has four main lobes of which three are located cranially and one is located caudally. Only the three
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. (Figure1)

Rabbits were divided into 3 groups of intervention, comprising 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. Previous studies showed that the hypertrophy response of the rabbit liver reaches a plateau-phase 7 days after portal vein occlusion(11;12). Therefore we chose in this design only a 1 and 7 days survival group.

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). After subcutaneous injection of 0.03mg/ kg buprenorphine (Temgesic, Reckitt Benckiser Healthcare Limited, Hull, Great-Britain) and 0.2mg/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 (1:0.7L/min) was used to maintain anaesthesia. Rabbits were given the analgesic drug Baytril, 0.02mg/kg subcutaneously once a day for 3 days postoperatively. Portal and hepatic vein embolizations were performed by an interventional radiologist (KPvL) with over 10 years experience.

Hepatic Vein Embolization

The right jugular vein was cannulated with an 18 gauge 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 subsequently introduced into the 18G catheter and guided through the heart into the hepatic veins of the cranial liver lobes. A venogram was made against the flow direction. The microcatheter was sequentially 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. At the end of the procedure the catheter was removed and the cannulated jugular vein was closed with a ligature.
Portal Vein Embolization

PVE was performed prior to HVE in the combined group. The PVE-procedure was performed as described previously(11). Briefly, a branch of the superior mesenteric vein was cannulated with a venflon® after midline laparotomy. After introduction of a microcatheter in the portal vein, a portogram was made, visualizing the portal anatomy. Then the microcatheter was positioned in the main portal branch supplying the cranial liver lobes. A mixture of contrast (Visipaque, GE Healthcare, Waukesha, WI) with 300-500 μm PVA particles (Cook, Bloomington, IN) was injected until flow ceased in the periphery, 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 trunc, 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 of anaesthesia, a multiphase 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 sec (arterial phase), 30 sec (portal phase) and 45 sec (venous phase) after contrast injection (4mL Visipaque, GE Healthcare, Waukesha, WI), followed by 3 ml 0.9% NaCl. 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 (Mx-View 3.52, Philips Medical Systems, Eindhoven, The Netherlands)

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

\[
\%CLV_{pre-embolization} = \frac{CLV_{pre-embolization}}{TLV_{pre-embolization}} \times 100\%
\]

After HVE, %CLV was calculated using the formula:

\[
\%CLV_{post-embolization} = \frac{CLV_{post-embolization}}{TLV_{pre-embolization}} \times 100\%
\]

Increase in CLV was calculated using the formula:

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

The degree of hypertrophy = \%CLV_{post-embolization} - \%CLV_{pre-embolization}
Biochemical parameters

In all blood samples 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 amount of bile duct congestion. In addition, plasma albumin was used as 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, this weight was divided by the body weight in order to exclude the influence of 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 \( \frac{\text{wetweight} - \text{dry weight}}{\text{wetweight}} \times 100\% \).

Histology

Liver tissue samples of the caudal and left lateral liver lobes were fixed in 4% formaldehyde, embedded in paraffin, and cut in 5-μm sections. Hematoxylin-eosin (H&E) staining was performed to evaluate architectural changes. 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× magnifications) on microphotographs. The proliferation index was defined as the percentage of Ki-67-positive cells per total cells 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

No postoperative complications and no signs of sickness in the period after the embolization were observed.

In one rabbit of the 1-day survival HVE group, a coil migrated during the procedure via the heart into the left pulmonary artery directly after placement, however without any clinical consequences. A new coil was placed that occluded the hepatic vein.

Degree of occlusion

On portography, performed directly after PVE and before sacrifice, all rabbits that underwent 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.

On CT scan, in most rabbits some small venous side branches were still patent, but in general, almost the complete venous outflow tract of the cranial lobe was adequately occluded. During sacrifice the position of the coils was checked in relation to the venous system to identify possibly missed and non-embolized venous branches. In all rabbits, the coils were found in the 3 main hepatic veins.

Liver regeneration response

Table 1 shows the changes in caudal liver volume after each procedure. The 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).

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 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). On day 1, no significant differences in body weight index between the 3 groups were present yet. Because the volume/weight gain could have been caused by edema formation in the caudal liver lobe, the wet-to-dry weight ratio was 107

Chapter 7

The effect of combined hepatic vein and portal vein embolization in a rabbit model
assessed as a parameter representing 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 sacrificed on day 7, nor on day 1. Therefore edema does not account for the significant difference in body weight index. Also calculating the total body weight gain/loss of all rabbits during the experiment, no significant differences were seen between the groups (p=0.083).

In accordance with the results as mentioned above, the caudal liver of the PVE and combined group contained a significantly higher number of proliferating hepatocytes in the Ki-67 stained slides, compared to the HVE group on day 7 (p<0.01). The number of proliferating hepatocytes of the PVE/HVE group was higher than the PVE group, only this difference was not significant (Figuur 3).

### Table 1. CT volumetry data.

<table>
<thead>
<tr>
<th></th>
<th>Before the intervention</th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVE CLV [%]</td>
<td>22.8±1.9</td>
<td>20.1±0.9</td>
<td>23.6±1.2</td>
</tr>
<tr>
<td>Increase of CLV [%]</td>
<td>N.A.</td>
<td>-10.2±5.5</td>
<td>5.6±7.0</td>
</tr>
<tr>
<td>Degree of hypertrophy [%]</td>
<td>N.A.</td>
<td>-2.7±1.2</td>
<td>0.79±1.5</td>
</tr>
<tr>
<td>PVE CLV [%]</td>
<td>22.4±0.5</td>
<td>29.8±0.8</td>
<td>40.1±1.1</td>
</tr>
<tr>
<td>Increase of CLV [%]</td>
<td>N.A.</td>
<td>33.6±4.5</td>
<td>79.8±8.4</td>
</tr>
<tr>
<td>Degree of hypertrophy [%]</td>
<td>N.A.</td>
<td>7.5±0.9</td>
<td>17.7±1.5</td>
</tr>
<tr>
<td>HVE+PVE CLV [%]</td>
<td>18.8±0.6</td>
<td>26.9±1.3</td>
<td>38.2±2.1</td>
</tr>
<tr>
<td>Increase of CLV [%]</td>
<td>N.A.</td>
<td>43.4±7.5</td>
<td>103.6±10.5</td>
</tr>
<tr>
<td>Degree of hypertrophy [%]</td>
<td>N.A.</td>
<td>8.1±1.3</td>
<td>19.4±2.0</td>
</tr>
</tbody>
</table>

(HVE= hepatic vein embolization, PVE= portal vein embolization, CLV= caudal liver volume, NA= not applicable)

![Figure 2](image)

**Figure 2.** Increase of the caudal liver volume. HVE alone gives no hypertrophy response. A combination of HVE and PVE does not lead to a significant greater hypertrophy response than PVE alone.
Liver damage

Biochemical results
Plasma AST, ALT, and LDH plasma levels showed transient elevation in the first hours to days, without significant differences between the groups. All levels returned to baseline levels 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 a pathologist experienced in liver pathology. Around the particles in the PVE groups, a multinucleated giant cell reaction was seen 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, whereas in the HVE group, pericentral dilatation was seen. Unexpectedly, substantial 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 the hepatocytes and local necrosis were clearly seen in all rabbits. Only little inflammation was observed. These observations apply for tissue samples obtained on day 7, just as on day 1, although necrosis was less pronounced in the latter.

**Discussion**

Besides PVE, there are other ways to reduce blood flow in a normal hepatic sinusoid. One of the strategies could be hepatic artery embolization (HAE). However, there are studies showing that the combination of PVE and HAE, compared to PVE alone, does not lead to an increased hypertrophy response(13). A recent study comparing HAE with PVE, also concluded that PVE was significant superior inducing hepatic hypertrophy (14).

In this experimental study, we assessed the value of HVE in addition to PVE. To eliminate the influence of the time factor after PVE and to achieve the maximum hypertrophy result in a short follow-up time, we performed PVE and HVE in one single procedure instead of sequentially. The results were satisfactory, as evidenced by the follow-up CT scans. Although some small patent venous side branches were seen, all three major hepatic veins of the cranial lobe were completely occluded in all rabbits. The effect of HVE alone on hypertrophy of the FRL was negligible. Although flow changes in the embolized lobes were present, and changes were seen on the histological slides, this did not result in increase of the FRL volume.

The combination of PVE/HVE and PVE alone resulted in a significantly greater hypertrophy response than HVE alone, as measured by CT volumetry, caudal liver to body weight index, and amount of proliferating hepatocytes.

**Figure 4.** Liver damage. There was a transient elevation of plasma AST levels in all groups without significant differences between the groups (0, 3h, and day 1 n=10/group; day 3 and 7 n=5/group).
The hypertrophy response after PVE/HVE was also significantly higher than after PVE alone as shown by the increased caudal liver to body weight ratio. The increase of CLV and the amount of proliferating hepatocytes showed the same trend, although the differences were not significant after 1 week.

The differences in liver volume and weight cannot be explained by edema formation, while no differences in wet-to-dry weight ratio were observed. Therefore, additional embolization of the hepatic veins after PVE had no significant additional effect on liver volume in the period of strongest hypertrophy response.

The histological changes and the significant increase in caudal liver-to-body weight ratio suggest that there is an additional effect on liver regeneration, only this effect did not translate into significant increase of volume of the FRL. There may be a long term effect, but this was beyond the scope of the present study as our interest focused on the short term effects of combined HVE/PVE embolization on the hypertrophy response.

As in most animal studies, one of the limitations is human extrapolation of the results. Although there are some differences, the rabbit liver shows great similarities in pathophysiological and anatomical aspect, demonstrated in previous publications (11). The regeneration response in the rabbit is faster, but the response curve is similar, therefore results can be extrapolated to the human situation (11,15)

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


Assessment of future remnant liver function using hepatobiliary scintigraphy in patients undergoing major liver resection

W. de Graaf
K.P. van Lienden
S. Dinant
J.J.T.H. Roelofs
O.R.C. Busch
D.J. Gouma
R.J. Bennink
T.M. van Gulik
Abstract

**Background:** $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy was used as a quantitative method to evaluate liver function. The aim of this study was to compare future remnant liver function assessed by $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy with future remnant liver volume in the prediction of liver failure after major liver resection.

**Methods:** CT volumetry and $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy were performed prior to major resection in 55 high risk patients, including 30 patients with parenchymal liver disease. Liver volume was expressed as percentage of total liver volume or as standardized future remnant liver volume. Receiver operating characteristic (ROC) curve analysis was performed to identify a cut-off value for future remnant liver function in predicting postoperative liver failure.

**Results:** Postoperative liver failure occurred in nine patients. A liver function cut-off value of 2.69 %/min/m² was calculated by ROC curve analysis. $^{99m}$Tc-Mebrofenin hepatobiliary scintigraphy demonstrated better sensitivity, specificity, positive and negative predictive value compared to future remnant liver volume. Using $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy one cut-off value suffices in both compromised and non-compromised patients.

**Conclusion:** Preoperative $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy is a valuable technique to estimate the risk of postoperative liver failure. Especially in patients with uncertain quality of the liver parenchyma, $^{99m}$Tc-mebrofenin HBS proved of more value than CT volumetry.
Introduction

Major liver resection may result in a small postoperative remnant liver, thereby increasing the risk of postoperative liver failure, especially in patients with parenchymal disease [1]. Posthepatectomy liver failure is the most frequent cause of mortality after liver resection. Although the causes of liver failure are multifactorial, postoperative remnant liver function is one of the main contributing factors.

Preoperative CT-volumetry, in which liver volume is used as an indirect measurement of liver function, is widely used to identify patients who should be excluded from a planned liver resection or to select patients who will benefit from preoperative portal vein embolization (PVE) [1-5]. Future remnant liver (FRL) volume (FRL-V) is expressed as a percentage of total liver volume (%FRL-V)[3], or as standardized FRL (sFRL), in which FRL-V is calculated as percentage of total liver volume based on body surface area (BSA)[4,6]. sFRL recognizes patient characteristics (body weight/BSA), but has only been validated in patients with healthy livers. In patients with a normal liver parenchyma, an %FRL-V or sFRL larger than 25-30% of total preoperative liver volume is considered sufficient for a safe resection [3,4,7-9], whereas in patients with a compromised liver (e.g. fibrosis, steatosis or cholestasis) a %FRL-V or sFRL of more than 40% is preferred[10]. The separate cut-off values indicate the necessity to assess the quality of the liver parenchyma in order to perform an accurate and safe preoperative risk analysis using CT volumetry. Preoperative liver biopsy is currently the most reliable method to assess the quality of the liver parenchyma. Biopsies are not routinely performed due the potential unequal distribution of parenchymal damage[11] and the risk of complications[12,13]. As a result, the quality of the liver parenchyma frequently remains unknown, rendering preoperative risk analysis by CT volumetry less reliable.

For accurate preoperative risk analysis, additional tests of liver function are required. Dynamic 99mTc-mebrofenin hepatobiliary scintigraphy (HBS) was developed as a quantitative method for evaluating total and regional liver function, including FRL function [14,15]. The hepatic uptake of 99mTc-mebrofenin is similar to the uptake of organic anions such as bilirubin[16]. After the hepatic uptake, 99mTc-mebrofenin is excreted into the bile canaliculi without undergoing biotransformation during its transport through the hepatocytes. Although 99mTc-mebrofenin is not metabolized, the uptake and intracellular transit are similar to various endogenous and exogenous substances including bilirubin, hormones, drugs and toxins. In a recent publication, we demonstrated that 99mTc-mebrofenin HBS has potential to predict postoperative liver failure in a patient population including both minor and major liver resections [17]. The advantage of using 99mTc-mebrofenin HBS is the fact that the same cut-off value can be used for both patients with a compromised or normal liver parenchyma, which makes the test applicable in patients with an uncertain quality of the liver parenchyma. However, it remains uncertain if 99mTc-Mebrofenin HBS is sufficiently accurate to predict liver failure in a population containing high risk patients requiring major hepatic resection.
This study compares preoperative FRL function assessed by HBS with FRL-V, expressed as %FRL-V and sFRL, in the prediction of postoperative liver failure after major liver resection in high risk patients.

Patients and methods

Patients

Between May 2000 and November 2006, 213 patients underwent a partial hepatectomy. Of all patients undergoing major liver resection (three or more Couinaud segments), both CT volumetry and HBS were preoperatively performed in 71 patients. Sixteen patients were excluded from the study, because of preoperative PVE (n=15) or partial portal vein thrombosis (n=1) in the time period between HBS and CT volumetry. Hence, a group of 55 patients was retrospectively analyzed with the approval of our Institutional Review Board with waiver of informed consent. Table 1 summarizes the types of resection performed. Patients with a preoperative suspicion of hilar cholangiocarcinoma underwent an (extended) hemihepatectomy combined with hilar resection and caudate lobe resection. In cholestatic patients, preoperative biliary drainage was performed more than 6 weeks prior to surgery using endoscopic retrograde cholangiopancreatography or percutaneous transhepatic drainage.

<table>
<thead>
<tr>
<th>procedure</th>
<th>Number of patients</th>
<th>Percentage</th>
<th>Weight resection specimen (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extended right hemihepatectomy</td>
<td>14</td>
<td>25,50%</td>
<td>975 ± 247</td>
</tr>
<tr>
<td>Right hemihepatectomy</td>
<td>26</td>
<td>47,20%</td>
<td>936 ± 396</td>
</tr>
<tr>
<td>Extended left hemihepatectomy</td>
<td>1</td>
<td>1,80%</td>
<td>443</td>
</tr>
<tr>
<td>Left hemihepatectomy</td>
<td>14</td>
<td>25,50%</td>
<td>348 ± 120</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>100,00%</td>
<td></td>
</tr>
</tbody>
</table>

Pre- and peri-operative factors associated with postoperative morbidity and mortality were analyzed (Table 5). Histopathology of the resection specimen was assessed by an experienced pathologist taking into account features of cholestasis, steatosis, fibrosis and chronic inflammation.

Postoperative complications were recorded according to the modified classification of surgical complications proposed by Clavien[18]. In-hospital complications were recorded as well as complications requiring hospital readmission within 3 months related to the operation. Minor complications included grade 1 and 2 complications. Major complications were defined as grade 3 and severe complications as grade 4 and grade 5 complications. Liver failure was defined as bilirubin plasma levels > 50μmol/l, and/or prothrombin time index < 50% [19], elevated plasma ammonia levels combined with signs of hepatic encephalopathy and/or hepatorenal syndrome, requiring intensive care treatment.
Surgical technique

Surgery was performed under low central venous pressure (CVP< 4cmH2O). Liver parenchymal transsection was performed using Cavitron Ultrasonic Surgical Aspirator (CUSA, Valley Lab, Boulder, Co, USA). Pringle manoeuvre was applied in 29 patients (54%) to reduce intraoperative blood loss, with a mean ischemic period of 37 ± 13 min. Intermittent clamping was applied in 8 patients (15%).

Scintigraphic imaging and data acquisition

HBS was performed using $^{99m}$Tc-mebrofenin as previously described[14,15]. Briefly, after injection of 85 MBq of $^{99m}$Tc-mebrofenin (Bridatec; GE-Amersham Health), dynamic images were obtained with a $\gamma$-camera (Diacam, Siemens, Milwaukee, USA) for 60 min. During the acquisition of these dynamic images, the isotope is accumulated in the liver parenchyma. A region of interest (ROI) is drawn around the entire liver (red line) and around the mediastinum (blood pool) (yellow line). A third ROI is drawn around the future remnant liver (green line). A blood pool corrected liver-uptake time-activity curve is shown in panel B. The hepatic $^{99m}$Tc-mebrofenin uptake is calculated as an increase of $^{99m}$Tc-mebrofenin uptake (y-axis) per minute over a time period of 200 sec (X-axis). Panel C demonstrates the use the anterior projection of the liver on the CT volumetry image as a guideline for delineating the FRL on the HBS image (D).

**Figure 1:** An example is shown of summed HBS images from 150-300 sec after i.v. injection of $^{99m}$Tc-mebrofenin (A). A region of interest (ROI) is drawn around the entire liver (red line) and around the mediastinum (blood pool) (yellow line). A third ROI is drawn around the future remnant liver (green line). A blood pool corrected liver-uptake time-activity curve is shown in panel B. The hepatic $^{99m}$Tc-mebrofenin uptake is calculated as an increase of $^{99m}$Tc-mebrofenin uptake (y-axis) per minute over a time period of 200 sec (X-axis). Panel C demonstrates the use the anterior projection of the liver on the CT volumetry image as a guideline for delineating the FRL on the HBS image (D).
first 10 min, 60 frames of 1 sec 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.[20]. On preoperative HBS, 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, 3 time-activity curves were generated (Figure 1). Total hepatic $^{99m}$Tc-mebrofenin uptake rate, representing total liver function (TL-F) was calculated as %/min (of the injected dose) based on these 3 parameters. Calculations of hepatic $^{99m}$Tc-mebrofenin uptake rate were performed using measured values obtained between 150 and 350 sec 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, TL-F was divided by BSA and expressed as %/min/m$^2$. For determination of FRL uptake, a ROI was drawn around the FRL by two independent investigators, blinded for the results, according to the performed resection and inter-observer variation was calculated. The round ligament was used as the border between segment three and four. Cantlie’s line, projected on the liver surface as a plane between the middle of the gallbladder fossa (visible in the late phase of the scintigraphy) and the inferior caval vein, was used as a border between the right and left liver lobes. In addition, the anterior projection of the liver on the CT volumetry was used as a guideline for delineating the FRL on the HBS images (Figure 1). FRL uptake function (FRL-F) was calculated by dividing counts within the delineated FRL by the total liver counts and multiplying this factor with total liver $^{99m}$Tc-mebrofenin uptake (TL-F) and expressed as %/min/m$^2$. In 33 patients a postoperative HBS was performed within 3 days after the operation to measure actual remnant liver function.

CT-volumetry

Contrast enhanced CT scans were generated with a helical scanner (Philips, Eindhoven, The Netherlands). Manual 3D reconstructions of the liver were made using reconstructed 5 mm thick axial slices from 2-3 mm original slices. The total liver as well as tumour(s) and the FRL were manually outlined using portal and hepatic veins as landmarks for segmental division. Integrated software (Mx-View 3.52, Philips Medical Systems) was used to calculate total liver volume (TL-V), tumor volume (TV) and FRL volume (FRL-V). All delineations were made by an experienced radiologist. FRL-V was expressed as percentage of TL-V using the formula:

$$%FRL-V = \frac{FRL-V}{(TL-V-TV)} \times 100\%$$

The non-tumorous total liver volume ($^{NTL-V}$) was calculated by excluding the tumour volume from the TL-V.

Standardized FRL measurements

FRL-V was determined using CT volumetry, while total liver volume ($^{calTL-V}$) was calculated using a formula based on BSA[6]:

$$^{calTL-V} = -794.41 + 1267.28 \times BSA$$
The standardized FRL (sFRL) was calculated as the percentage between FRL-V and calculated TL-V.

Preoperative risk assessment

Receiver operator characteristics (ROC) curve analysis was used to calculate the optimal cut-off value for FRL-F in predicting postoperative liver failure. Cut-off values were determined based on the following assumptions: The chance that liver failure would develop while the test result was above the cut-off value needed to be as low as possible. Secondly, a test result below the cut-off value, should accurately select high risk patient who might benefit from PVE. Based on literature, cut-off values for %FRL-V and sFRL were set at 30% for patients with normal liver parenchyma[9] and 40% for patients with a compromised liver[10]. Positive predictive values (PPV), negative predictive values (NPV), as well as sensitivity and specificity were determined for each method.

Statistical analysis

Statistical analysis was performed with GraphPad Prism (GraphPad Software, San Diego, CA) and Statistical Package for Social Sciences (SPSS 12.02, Chicago, IL). ROC curve analysis was used to identify a cut-off value for FRL-F in predicting postoperative liver failure. Univariate analysis of preoperative and intraoperative variables was performed by the independent t-test for continuous parameters and by Pearson’s χ² tests and Fisher’s exact test for categorical data. Correlation between variables was tested using the Pearson correlation coefficient r. Continuous data were compared by independent sample t-test and expressed as mean ± standard deviation (SD). All statistical tests were two-tailed and differences were considered significant at a P-value of ≤0.05.

Results

Patient characteristics

CT-volumetry and ⁹⁹ᵐTc-mebrofenin HBS were performed in 55 patients (male 26, female 29, mean age 59±13 y). Indications for liver resection are shown in Table 2. Thirty patients were diagnosed with a compromised liver parenchyma based on the histopathological evaluation of the resection specimen by an experienced pathologist, including cirrhosis (n=2), severe fibrosis (n=3), steatosis (> 30% of the hepatocytes affected) (n=3), severe cholestasis (n=8), chronic inflammation (n=3), or a combination of these diseases (n=11).

Liver function and liver volume

TL-F was significantly lower in patients with parenchymal liver disease (7.4 ± 1.4 %/min/m²) as compared to patients with healthy liver parenchyma (8.5 ± 1.7 %/min/m², P = 0.007),
NTTL-V. was significantly larger in patients with compromised livers (1037.1 ± 208.0 mL/m² vs. 877.0 ± 143.3 mL/m², \( P = 0.001 \)) (Figure 2).

According to the type of resection performed, FRL-F was calculated for each individual patient by two independent observers. The interobserver agreement was excellent (Pearson \( r = 0.97 \)) and Bland-Altman analysis revealed almost no bias between the two observers (mean bias of 0.00058 with 95% limit of agreement between -0.835 and 0.836). Preoperative FRL-F correlated strongly with actual postoperative remnant liver function determined within 3 days after surgery (Pearson \( r = 0.83, P < 0.0001 \)) (Figure 3).

Liver weight of the resection specimen revealed a strong correlation (Pearson \( r = 0.91, P < 0.0001 \)) with its volume assessed by CT volumetry, confirming the CT measurements.

FRL-V correlated well with FRL-F (Pearson \( r = 0.72, P = 0.0001 \)) in patients with normal livers. In contrast, patients with a compromised liver demonstrated only a moderate correlation between FRL-V and FRL-F (Pearson \( r = 0.61 P < 0.0003 \)). The slope coefficient of the linear regression curve indicated that FRL-V is associated with significantly (\( P = 0.0015, \) ANCOVA test) reduced FRL-F in compromised livers as compared to normal livers (Figure 4).

**Table 2: Indications for liver resection**

<table>
<thead>
<tr>
<th>Indications for liver resection</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver metastasis</td>
<td>14</td>
</tr>
<tr>
<td>Hilar cholangiocarcinoma</td>
<td>19</td>
</tr>
<tr>
<td>Intrahepatic cholangiocarcinoma</td>
<td>3</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>6</td>
</tr>
<tr>
<td>Benign biliary strictures</td>
<td>7</td>
</tr>
<tr>
<td>Benign lesions</td>
<td>6</td>
</tr>
</tbody>
</table>

**Figure 2:** Total hepatic \(^{99m}\text{Tc}\)-mebrofenin uptake according to parenchymal status

Patients with parenchymal liver disease had significantly less liver (uptake) function (grey box, 7.4 ± 1.4 %/min/m²) as compared to patients with healthy liver parenchyma (white box, 8.5 ± 1.7 %/min/m², \( P = 0.007 \)) (A). Total liver volume was significantly higher in patients with compromised livers (1037.1 ± 208.0 mL/m² vs. 877.0 ± 143.3 mL/m², \( P = 0.001 \)) (B).
Postoperative Complications

In 42 of the 55 (76%) patients, one or more complications occurred following liver resection (Table 3). Minor and major complications were evident in 14 patients (25%) and 13 patients (24%), respectively. Fifteen patients (27%) developed severe complications requiring ICU treatment and the mortality rate was 15%. Patients with severe complications had significantly lower FRL-F as compared to patients with no complications ($P=0.0043$), minor complications ($P=0.0028$) or major complications ($P=0.0046$).

Nine patients (16%) developed postoperative liver failure, of which 8 patients died. In 4 patients, liver failure was evident within one week after the operation. Five patients developed liver failure within several weeks after the operation in conjunction with signs of sepsis. Evidence of a compromised liver was seen in 8 patients (89%) and in 7 patients an extended hemihepatectomy had been performed. The FRL-F was significantly lower in patients with postoperative liver failure (2.18 %/min/m² vs. 4.32%/min/m², $P=0.0001$).

**Table 3.** Postoperative complications. Grade 1 needed no therapy except analgetics, diuretics, anti-emetics and physiotherapy. Grade 2 complications required pharmacological treatment. Grade 3 complications required surgical, endoscopic or radiological intervention (grade 3a under local anesthetics, grade 3b under general anesthetics). Grade 4 complications included life-threatening complications requiring ICU management (grade 4a with single organ dysfunction, grade 4b with multi organ failure). Grade 5 complications resulted in death.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Number (n)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>n=13</td>
<td>No complications</td>
</tr>
<tr>
<td>Grade 1*</td>
<td>n=5</td>
<td>Minor complications</td>
</tr>
<tr>
<td>Grade 2*</td>
<td>n=9</td>
<td>Minor complications</td>
</tr>
<tr>
<td>Grade 3a</td>
<td>n=12</td>
<td>Major complications</td>
</tr>
<tr>
<td>Grade 3b</td>
<td>n=1</td>
<td>Major complications</td>
</tr>
<tr>
<td>Grade 4a</td>
<td>n=5</td>
<td>Severe complications</td>
</tr>
<tr>
<td>Grade 4b</td>
<td>n=2</td>
<td>Severe complications</td>
</tr>
<tr>
<td>Grade 5</td>
<td>n=8</td>
<td>Severe complications</td>
</tr>
</tbody>
</table>

*One patient could have multiple grade 1 or 2 complications*
Preoperative and intra-operative parameters associated with liver failure

Univariate analysis revealed that elderly patients \( (P=0.043) \), small \%FRL-V \( (P=0.024) \), small sFRL \( (P=0.012) \), small FRL-F \( (P=0.001) \), resection type \( (P=0.001) \), prolonged operating time \( (P=0.0018) \) increased blood loss \( (P=0.0018) \) during the operation and the presence of a compromised liver parenchyma \( (P=0.024) \) were significantly associated with postoperative liver failure (Table 3). Due to a small sample size in the liver failure group \( (n=9) \), no multivariate analysis was performed.

Preoperative prediction of postoperative liver failure

ROC analysis revealed that a cut-off value for FRL-F of 2.69 \%/min/m\(^2\) was able to identify patients who developed postoperative liver failure with a sensitivity of 89\% and a specificity of 87\% (Figure 5). The risk of postoperative liver failure in patients with a FRL-F above 2.69 \%/min/m\(^2\) was 2.4\% (with a NPV of 97.6\%, and a likelihood ratio for a negative test result of 0.12). The PPV was 57.1\% with a likelihood ratio for a positive test result of 6.8. Table 4 summarizes the sensitivity, specificity, PPV, NPV and likelihood ratios of the different tests. For an accurate use of \%FRL-V and sFRL, two cut-off values were used and patients were divided in patients with a normal liver parenchyma and patients with a compromised liver parenchyma based on the histopathology of the resection specimen. Using \(^{99}\)Tcmefrofenin HBS, one cut-off value sufficed in both compromised and non-compromised
<table>
<thead>
<tr>
<th>Demographics</th>
<th>Patients with liver failure (n=9)</th>
<th>Patients without liver failure (n=46)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male: female</td>
<td>07:02</td>
<td>19:27</td>
<td>0.069 ¶</td>
</tr>
<tr>
<td>Age</td>
<td>67.1 ± 6.0 (58-67)</td>
<td>57.1 ± 13.7 (18-78)</td>
<td>0.027*</td>
</tr>
<tr>
<td>BMI</td>
<td>25.1± 2.1</td>
<td>24.0 ± 3.6</td>
<td>0.33*</td>
</tr>
<tr>
<td>FRL volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%FRL-V (%)</td>
<td>35.0 ± 22.0</td>
<td>49.7 % ± 17.8</td>
<td>0.013*</td>
</tr>
<tr>
<td>sFRL (%)</td>
<td>35.2 ± 9.2</td>
<td>49.2 % ± 3.6</td>
<td>0.018*</td>
</tr>
<tr>
<td>FRL-F (%/min/m²)</td>
<td>2.2 ± 0.6</td>
<td>4.3 % ± 1.6</td>
<td>0.001*</td>
</tr>
<tr>
<td>Co-morbidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes (yes/no)</td>
<td>02:07</td>
<td>05:41</td>
<td>0.32 ¶</td>
</tr>
<tr>
<td>Chronic Hepatitis (yes/no)</td>
<td>02:07</td>
<td>03:43</td>
<td>0.18 ¶</td>
</tr>
<tr>
<td>Vascular disease(yes/no)</td>
<td>03:06</td>
<td>09:37</td>
<td>0.39 ¶</td>
</tr>
<tr>
<td>Compromised liver (yes/no)</td>
<td>08:01</td>
<td>22:24</td>
<td>0.024 ¶</td>
</tr>
<tr>
<td>Resection type:</td>
<td></td>
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<td></td>
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<tr>
<td>Left hemihepatectomy</td>
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<td>13</td>
<td></td>
</tr>
<tr>
<td>right hemihepatectomy</td>
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<td>25</td>
<td>0.001 ¥</td>
</tr>
<tr>
<td>extended hemihepatectomy</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Pre-operative laboratory values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>51.4 ± 19.1</td>
<td>48.2 ± 32.4</td>
<td>0.24*</td>
</tr>
<tr>
<td>ALT</td>
<td>57.9 ± 27.2</td>
<td>65.6 ± 65.6</td>
<td>0.55*</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>19.9 ± 14.9</td>
<td>14.2 ± 12.0</td>
<td>0.20*</td>
</tr>
<tr>
<td>AF</td>
<td>265.2 ± 204.6</td>
<td>280.1 ± 260.5</td>
<td>0.76*</td>
</tr>
<tr>
<td>GGT</td>
<td>409.9 ± 272.7</td>
<td>392.7 ± 605.7</td>
<td>0.13*</td>
</tr>
<tr>
<td>albumin</td>
<td>39.4 ± 5.8</td>
<td>39.5 ± 5.9</td>
<td>0.84*</td>
</tr>
<tr>
<td>prothrombin time</td>
<td>13.0 ± 1.5</td>
<td>13.1 ± 0.90</td>
<td>0.63*</td>
</tr>
<tr>
<td>Intra-operative parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>5200 cc ± 2673</td>
<td>3025 ± 2464</td>
<td>0.021*</td>
</tr>
<tr>
<td>Operating time (min.)</td>
<td>507.4 ± 135.1</td>
<td>382.3 ± 131</td>
<td>0.011*</td>
</tr>
<tr>
<td>Pringle manoeuvre yes/intermittent/ no</td>
<td>03:02:03</td>
<td>26:06:14</td>
<td>0.62 ¥</td>
</tr>
<tr>
<td>Pringle time (min)</td>
<td>35.0 ± 5.0</td>
<td>36.7 ± 13.5</td>
<td>0.96*</td>
</tr>
<tr>
<td>Intermittent total ischemia time (min)</td>
<td>40.0</td>
<td>47.6</td>
<td>0.5*</td>
</tr>
</tbody>
</table>

Abbreviations: AST, aspartate aminotransferase; ALT, alanine amino transferase; AF, alkaline fosfatase; GGT, gamma glutamyl transferase; BMI, body mass index * Mann-Whitney U test; ¥ χ² test; ¶ Fisher’s exact test
patients. Assuming that, of all the patients, the quality of the liver parenchyma was preoperatively known, sensitivity, specificity, positive and negative predictive values were still better for FRL-F compared to %FRL-V and sFRL.

Discussion

Accurate measurement of liver function before liver resection is crucial in the assessment of resectability, especially in patients requiring major liver resection. The availability of preoperative PVE to induce hypertrophy of the FRL has further increased the importance of preoperative assessment of regional hepatic function [7,21-24]. In the present study, dynamic planar $^{99m}$Tc-mebrofenin HBS was used to measure liver function. This technique can be implemented in every hospital with a nuclear medicine department, is easy to perform and has a small inter-observer variability. More importantly, preoperative estimated function of the future remnant liver (FRL-F) correlates strongly with actual postoperative liver function [14], indicating that dynamic planar $^{99m}$Tc-mebrofenin HBS is an accurate method to assess FRL-F.

In this study, we compared FRL-F measured by $^{99m}$Tc-mebrofenin HBS with two parameters based on CT volumetry, which are widely accepted parameters to determine the possible extent of resection[1-4],[3,5]. Patients with a compromised liver had a significantly lower liver function compared to patients with normal liver parenchyma, whereas their liver volume was significantly larger. FRL-V showed a strong relation with FRL-F in patients with normal liver parenchyma. In contrast, FRL-V and FRL-F only moderately correlated in patients with compromised liver parenchyma in whom FRL-V was associated with reduced FRL-F. The impact of different parenchymal diseases such as steatosis, cholestasis and fibrosis on liver function and liver volume is unknown and may vary among individuals. In addition, parenchymal damage is often not equally distributed [11], which can partially explain the moderate correlation between FRL-V and FRL-F in patients with compromised livers. ROC curve analysis yielded an FRL-F cut-off of 2.69 %/min/m² for the prediction of postoperative liver failure. This cut-off value is comparable to the cut-off value determined in a patient population including both minor and major resections [17].

A reliable preoperative test should primarily establish whether patients with a FRL-F above the critical threshold can be safely resected. One patient developed liver failure despite a FRL-F above 2.69 %/min/m² (Table 6). This cirrhotic patient developed massive necrosis after left hemihepatectomy, due to an obliterated right hepatic artery and a compromised portal venous system. When CT-volumetry would have been used as selection criterion for operation, two patients developed liver failure despite a %FRL-V of more than 40% (Table 6). Standardized FRL wrongly predicted a safe resection in three patients (Table 6). Although the formula generally used to calculate TL-V based on BSA is used for all patients, it is derived from patients with normal liver parenchyma. In our study, patients with a compromised liver had significantly larger liver volumes resulting in a relatively larger FRL-V in relation to their BSA. As a consequence, there is an overestimation of liver function in these patients.
Secondly, a preoperative test should be accurate in selecting high risk patients who might benefit from PVE, without treating patients unnecessarily. Despite having a FRL-F below the critical value of 2.69 %/min/m², 43% of these high risk patients did not develop liver failure. In literature, a similar percentage was reported when using CT volumetry for the prediction of postoperative hepatic dysfunction[8]. Additional negative predictive factors, including high BMI, significant intraoperative blood loss and prolonged operating time, were described in patients with hepatic dysfunction, underlining the multifactorial cause of postoperative liver failure. In our study univariate analysis revealed that, besides small FRL volume and function, increased intraoperative blood loss, prolonged operating time, a compromised liver parenchyma and older age were associated with liver failure. Unfortunately, a multivariate analysis was not possible in our study due to the small number of patients with postoperative liver failure. Cut-off values for the prediction of postoperative complications and hepatic dysfunction have been reported using CT volumetry [3,4,7-9], ICG

<table>
<thead>
<tr>
<th>Outcome parameter</th>
<th>FRL-F</th>
<th>%FRL-V</th>
<th>sFRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut-off value</td>
<td>2.69 %/min/m²</td>
<td>Normal liver &lt; 30%</td>
<td>Normal liver &lt; 30%</td>
</tr>
<tr>
<td>Compromised liver</td>
<td>40%</td>
<td>67%</td>
<td></td>
</tr>
<tr>
<td>Normal liver</td>
<td>30%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Overview of the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) as well as likelihood ratio’s for FRL-F, %FRL-V and sFRL in the prediction of postoperative liver failure

Table 6. Overview of the results of the 3 different preoperative tests in patients with liver failure. The marked values indicate a false negative result of the test.

<table>
<thead>
<tr>
<th>Liver parenchyma</th>
<th>FRL-F [%/min/m²]</th>
<th>%FRL-V [%]</th>
<th>sFRL [%]</th>
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<tbody>
<tr>
<td>1 normal</td>
<td>2.17</td>
<td>46</td>
<td>57</td>
</tr>
<tr>
<td>2 compromised</td>
<td>2.52</td>
<td>38</td>
<td>24</td>
</tr>
<tr>
<td>3 compromised</td>
<td>2.67</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>4 compromised</td>
<td>1.56</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>5 compromised</td>
<td>2.22</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>6 compromised</td>
<td>1.41</td>
<td>29</td>
<td>41</td>
</tr>
<tr>
<td>7 compromised</td>
<td>2.17</td>
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</tr>
<tr>
<td>8 compromised</td>
<td>1.51</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>9 compromised</td>
<td>3.36</td>
<td>88</td>
<td>101</td>
</tr>
</tbody>
</table>

Assessment of future remnant liver function using hepatobiliary scintigraphy
clearance test [25], galactose elimination capacity [26] and $^{99m}$Tc-GSA scintigraphy[27-30]. These cut-off values were however, mostly not based on accurate risk calculations and no or inappropriate multivariate analyses had been performed.

Morbidity and mortality rates reported in our study were high, which is explained by the patients selected for this study. We only included patients undergoing major liver resection of which the majority (55%) had parenchymal liver disease. A relatively high proportion (39%) of patients had undergone resection on the suspicion of hilar cholangiocarcinoma, including 6 patients who had developed postoperative liver failure. These patients require large resections and biliary anastomoses, with increased risk of postoperative morbidity and mortality, reported up to 10-20% [31-33]. The overall postoperative mortality in patients operated for benign lesions or liver metastasis in our institution is 2% [34]. In addition, none of the patients included in this study had undergone PVE. In some patients who developed postoperative liver failure, PVE would be indicated in retrospect, however in these patients the performed resection was larger than anticipated because of unexpected intra-operative findings. Patients included in this study may be different from patient populations in other clinical practices in which most patients have non-compromised livers. However, the fact that postoperative morbidity and mortality were considerable, did add necessary power to the study in which risk assessment was the primary goal. Further research is however warranted for subgroup analysis of different patient populations.

The main advantage of HBS lies in the fact that liver function is measured, taking into account the presence of underlying parenchymal liver disease. Hence, one cut-off value for the prediction of liver failure suffices in all possible patients regardless of the quality of the liver parenchyma. In contrast, volumetric assessment of the FRL requires two distinct cut-off values for patients with a compromised or non-compromised liver, assuming that the quality of the liver parenchyma is known. Especially in patients with uncertain quality of liver parenchyma, preoperative HBS is therefore of more value than %FRL-V or sFRL. The results of our study have led us to use HBS routinely, in addition to CT volumetry, in all patients considered for major liver resection Preoperative PVE is performed when FRL-F is lower than 2.69%/min/m$^2$ or %FRL-V is less than 30%. Although around 40% of these patients will not develop liver failure, the risk of a potentially lethal complication outweighs the relatively low complication rate observed after PVE [35]

**Conclusion**

HBS is a simple technique that can be implemented in every hospital with a nuclear medicine department. It is a valuable technique to estimate the risk of postoperative liver failure in high risk patients undergoing major liver resection. Especially in patients with uncertain quality of the liver parenchyma, $^{99m}$Tc-mebrofenin HBS is of more value than CT volumetry since only one cut-off value can be used in both normal and compromised livers. Therefore, additional HBS can improve risk assessment in patients requiring extensive liver resection.
References


Chapter

99mTc-mebrofenin hepatobiliary scintigraphy with spect for the assessment of hepatic function and liver functional volume before partial hepatectomy

W. de Graaf
K.P. van Lienden
T.M. van Gulik
R.J. Bennink

Abstract

Background: Preoperative evaluation of future remnant liver (FRL) function is crucial in the determination of whether a patient can safely undergo liver resection. Although dynamic ⁹⁹mTc-mebrofenin hepatobiliary scintigraphy (HBS) is used to measure FRL function, 2-dimensional planar images lack the ability to assess segmental liver function. Modern SPECT/CT cameras combine dynamic ⁹⁹mTc-mebrofenin HBS with additional SPECT and the anatomic information of the CT scan. The aim of this study was to evaluate the additional value of ⁹⁹mTc-mebrofenin SPECT for the measurement of segmental liver function and liver functional volume.

Methods: Preoperative CT volumetry and ⁹⁹mTc-mebrofenin HBS with SPECT were performed in 36 patients undergoing liver resection. In 18 patients, postoperative ⁹⁹mTc-mebrofenin HBS with SPECT was performed within 3 d after operation. Dual-head dynamic acquisitions were used to calculate FRL function using anterior and geometric mean (Gmean) datasets. Total and FRL functional liver volumes were measured by SPECT.

Results: Because of the anatomic position of the liver, the anterior projection resulted in an underestimation of FRL function in patients undergoing left hemihepatectomy. In patients with normal liver parenchyma, total functional liver volume was comparable to total liver volume measured by CT volumetry, indicating that ⁹⁹mTc-mebrofenin SPECT is an accurate method to measure hepatic volume. In compromised livers, compared with normal livers, FRL function per cubic centimeter of liver volume was significantly less. In addition, liver function was not distributed homogeneously, with the segments to be resected relatively more affected. FRL function, measured by a combination of SPECT and dynamic HBS, was able to accurately predict actual postoperative remnant liver function.

Conclusion: The Gmean dataset is recommended for the assessment of hepatic function by dynamic planar ⁹⁹mTc-mebrofenin HBS. The combination of SPECT data with the dynamic uptake function measured by planar HBS provides valuable visible and quantitative information regarding segmental liver function and is an accurate measure for FRL function.
Introduction

The preoperative evaluation of future remnant liver (FRL) function is crucial for the identification of patients with an increased risk of postoperative liver failure before major liver resection or living donor liver transplantation. Major liver resection can cause insufficient postoperative remnant liver function leading to postoperative liver failure, particularly in patients with parenchymal liver disease (1). The presence of parenchymal liver disease is of growing interest because of the rising number of patients presenting with steatosis or pretreatment with neoadjuvant chemotherapy (2).

FRL volume (FRL-V), measured by CT volumetry, is used as an indirect measurement of liver function (3–5) and is currently the established method to determine whether a patient can safely undergo liver resection. Multiphasic contrast-enhanced CT (CeCT) scans have the advantage of high-resolution diagnostic images that enable accurate measurements of segmental liver volume using portal and hepatic veins as landmarks for segmental division. These types of scans, however, provide no information on the quality of the liver parenchyma in terms of functional capacity and may, therefore, not reflect liver function (6). When using CT volumetry, 2 distinct cutoff values for patients with compromised (due to steatosis, cholestasis, or fibrosis) or noncompromised livers are required for accurate preoperative risk assessment; therefore, CT volumetry is reliable only when the quality of the liver parenchyma is known.

Dynamic $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy (HBS) has the ability to measure total liver function (TL-F) and FRL function (FRL-F) (6–8). It incorporates the presence of underlying parenchymal liver disease, as indicated by significantly less liver function in patients with a compromised liver (6). $^{99m}$Tc-mebrofenin HBS has the advantage that a single cutoff value for the prediction of liver failure suffices in patients with either normal or compromised liver parenchyma. In patients with unknown quality of liver parenchyma, preoperative dynamic HBS is therefore more valuable than CT volumetry in selecting those with increased risk of postoperative liver failure (6,8).

In previous studies, a single-head $\gamma$-camera was used for dynamic HBS, which permits anterior or posterior projections of the liver only (7,9). Because of the anatomic position of the liver, the left hemiliver is situated more anteriorly, potentially leading to an overestimation of segmental left liver function in the anterior projection. The increasing availability of dual-head rotating $\gamma$-cameras enables simultaneous data acquisition of the anterior and posterior projections, from which a geometric mean (Gmean) dataset can be calculated, thereby reducing the attenuation bias. In addition, fast 3-dimensional SPECT can be performed.

Although dynamic $^{99m}$Tc-mebrofenin HBS has the possibility to measure regional liver function, the 2-dimensional planar images lack the ability to assess liver function on the segmental level. With a SPECT/CT camera, the functional data from $^{99m}$Tc-mebrofenin SPECT can be combined with the anatomic information from the CT scan, enabling the measurement of segmental liver function using the CT scan as reference for accurate delineation of liver segments. In addition to segmental liver function, $^{99m}$Tc-mebrofenin SPECT could be used to
measure hepatic volume. Liver volume calculated with $^{99m}$Tc-mebrofenin SPECT represents the functional volume because only parts of the liver with functional $^{99m}$Tc-mebrofenin uptake are included in the volume, and regions with minimal or no uptake are excluded.

The aim of this study was to assess the additional value of $^{99m}$Tc-mebrofenin SPECT with low-dose CT for the measurement of segmental liver function and liver functional volume in patients undergoing partial liver resection.

**Material and methods**

**Patients**

Between July 2004 and September 2007, 117 patients underwent a partial hepatectomy. Inclusion criteria for this study were liver resection of 2 or more Couinaud segments, preoperative CeCT scan, and $^{99m}$Tc-mebrofenin HBS combined with SPECT. Patients with preoperative portal vein embolization were excluded. Hence, a group of 36 patients was retrospectively analyzed. Types of resection are summarized in Table 1. Histopathology of the resection specimen was assessed by an experienced hepatopathologist, taking into account features of fibrosis, cholestasis, steatosis, and chronic inflammation. On the basis of the histology, patients were divided into 2 groups: 1 with a normal liver parenchyma and 1 with a compromised liver parenchyma.

**CT Volumetry**

Four-phase CeCT scans were obtained with a multislice helical scanner (Philips). Three-dimensional reconstructions of the liver were made from reconstructed 5-mm-thick axial slices using the portal phase. The total liver, tumor masses, and FRL were manually delineated using portal and hepatic veins as landmarks for segmental division (Fig. 1A). Integrated software was used to calculate total liver volume (TL-V), tumor volume (TV), and FRL-V. The nontumorous total liver volume ($^{NTTL}$-V) was calculated by excluding the TV from the TL-V. The FRL-V, expressed as a percentage of the $^{NTTL}$-V, was calculated using the formula:

$$\%FRL-V = \frac{FRL-V}{^{NTTL}-V} \times 100\%$$

Different imaging techniques. Anterior projection of CeCT reconstruction (A) was used as guideline for delineating FRL on planar dynamic $^{99m}$Tc-mebrofenin HBS images (B). Portal and hepatic veins were used as landmarks for delineation of FRL CeCT scans (C and D). On SPECT image, FRL was manually outlined on CTlow scans linked to SPECT images (E and F). Delineated FRL of CeCT scans was used as constant reference (D).

**HBS Imaging**

HBS was performed with $^{99m}$Tc-labeled (2,4,6 trimethyl-3-bromo)iminodiacetic acid ($^{99m}$Tc-mebrofenin [Bridatec]; GE Healthcare). Patients were positioned supine on the imaging table, with a large-field-of-view (FOV) SPECT/CT camera (Infinia II; GE Healthcare) positioned over the liver and heart region. The SPECT/CT camera was equipped with low-energy high-
resolution collimators. First, a dynamic acquisition (36 frames of 10 s/frame, 128 matrix), which was used for the calculation of the hepatic uptake function, was obtained immediately after the intravenous administration of 200 MBq of $^{99m}$Tc-mebrofenin. Subsequently, a fast SPECT acquisition was performed (60 projections of 8 s/projection, 128 matrix), centered on the peak of the hepatic time–activity curve, which was used for the 3-dimensional assessment of liver function and calculation of functional liver volume. Immediately after SPECT, a low-dose non–contrast-enhanced CT (CT$_\text{low}$) scan was obtained for attenuation correction and anatomic mapping on the same gantry, without moving the patient. Finally, a second dynamic acquisition (15 frames of 60 s/frame, 128 matrix) was obtained to evaluate biliary excretion. Data were processed on a workstation (MultiModality; Hermes Medical Solutions). In 18 patients, postoperative $^{99m}$Tc-mebrofenin HBS with SPECT/CT$_\text{low}$

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Demographics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male: female</td>
<td>17:19</td>
</tr>
<tr>
<td>Age</td>
<td>58.5 ± 12.5.0 (33-78)</td>
</tr>
<tr>
<td>BMI</td>
<td>25.1± 2.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis</th>
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</thead>
<tbody>
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<td>Cholangiocarcinoma</td>
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<tr>
<td>Liver meastases</td>
<td>11</td>
</tr>
<tr>
<td>Benign biliary strictures</td>
<td>4</td>
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<tr>
<td>Hepatocellular carcinoma</td>
<td>3</td>
</tr>
<tr>
<td>Cyst adenoma</td>
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</tr>
<tr>
<td>Adenoma</td>
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<tr>
<td>Caroli syndrome</td>
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<thead>
<tr>
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<td>Fibrosis</td>
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</tr>
<tr>
<td>Biliary fibrosis</td>
<td>4</td>
</tr>
<tr>
<td>cholestasis</td>
<td>2</td>
</tr>
<tr>
<td>Steatosis</td>
<td>2</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>2</td>
</tr>
<tr>
<td>Combined disease</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Resection type:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisegmentectomy</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>Trisegmentectomy</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Left hemihepatectomy</td>
<td>10 (28%)</td>
</tr>
<tr>
<td>Extended left hemihepatectomy</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Right hemihepatectomy</td>
<td>14 (39%)</td>
</tr>
<tr>
<td>Extended right hemihepatectomy</td>
<td>6 (16%)</td>
</tr>
</tbody>
</table>
was performed within 3 d after the operation to measure actual remnant liver function and functional volume.

Calculations of Dynamic HBS Parameters
The first dual-head dynamic acquisition was used to calculate the hepatic $^{99m}$Tc-mebrofenin uptake rate using the dataset of the anterior projection and the $G_{\text{mean}}$ dataset. $G_{\text{mean}}$ was calculated using the formula

$$G_{\text{mean}} = \sqrt{\text{anterior} \times \text{posterior}}$$

in which anterior is
the data from the anterior projection and posterior from the posterior projection. Regions of interest (ROI) were drawn around the liver, heart, large vessels within the mediastinum (serving as blood pool), and total FOV (indicative of total body activity). ROIs were saved to make sure that identical ROIs were used for the anterior and Gmean datasets. Generation of 3 different time–activity curves was based on ROIs of the liver, blood pool, and total FOV. With these 3 parameters, the liver uptake rate was calculated as described by Ekman et al. (10) and expressed as percentage per minute (%/min). The hepatic $^{99\text{m}}$Tc-mebrofenin uptake rate was calculated using scanned radioactivity values acquired between 150 and 350 s after injection, to ensure that calculations were made during a phase of homogeneous distribution of the agent in the blood pool and before the rapid phase of hepatic excretion. To compensate for differences in individual metabolic requirements, total liver $^{99\text{m}}$Tc-mebrofenin uptake rate (%/min), representing TL-F, was divided by the body surface area and expressed as %/min/m². For comparison of parameters within the same patient, original data (%/min) were used. TL-F was calculated for both the anterior and the Gmean datasets.

For the FRL (uptake) function (FRL-F), an ROI based on the performed resection was drawn around the FRL by 2 independent investigators. The round ligament was used as the border between segments 3 and 4. Cantlie’s line, projected on the liver surface as a plane between the middle of the gallbladder fossa (visible in the late phase of the scintigraphy) and the inferior caval vein, was used as a border between the right and the left liver lobes. In addition, the anterior projection of the CT volumetry was used as a guideline for delineating the FRL (Figs. 1A and 1B). FRL-F was calculated by dividing the summed counts (150–350 s after injection) within the delineated FRL by the total liver counts within the same time frame and multiplying this factor by the total liver $^{99\text{m}}$Tc-mebrofenin uptake rate expressed as %/min or %/min/m². FRL-F was calculated for both the anterior ($^{\text{Anterior}}$FRL-F) and the Gmean ($^{\text{Gmean}}$FRL-F) datasets.

To determine whether the anterior dataset overestimated the function of the left liver segments because of its anatomic position, patients were divided into a group undergoing (extended) right hemihepatectomy (including segments 4–8), with the left hemiliver as FRL ($n = 20$), and a group undergoing a left hemihepatectomy (including segments 2–4) ($n = 11$). FRL-F was expressed as the ratio of TL-F for both Gmean ($^{\text{Gmean}}$FRL-F ratio) and anterior data ($^{\text{Anterior}}$FRL-F ratio) using identical ROIs. The difference in uptake rate between the anterior and the Gmean datasets was calculated. Positive values indicate an overestimation of the anterior data, and negative values indicate an underestimation, compared with Gmean data.

**Calculation of SPECT Parameters**

After the uptake phase, $^{99\text{m}}$Tc-mebrofenin is excreted into the bile. The SPECT acquisition was centered around the peak of the hepatic time–activity curve, because the amount of radioactivity within the liver is relatively stable during this phase. In some patients with a fast hepatic uptake, biliary excretion was already visible during the SPECT phase. Accumulation of radioactivity in the small bile ducts results in voxels with relatively high counts, disturbing
threshold-based calculations of total and regional liver function and volume. Therefore, the activity within the extrahepatic bile ducts was manually removed using a masking tool. For the intrahepatic bile ducts, 3 ROIs were drawn around typical bile ducts to determine the minimal and maximal voxel count within the bile ducts. Subsequently, 3 ROIs were placed in surrounding normal liver tissue to measure the average voxel count value of liver tissue. The intrahepatic bile ducts were automatically replaced by the average count density of normal liver tissue using these values. Subsequently, an outline extraction method (with a threshold of 30% of the maximal voxel count value) was applied to automatically outline the liver and calculate total functional liver volume (TL-FV). The FRL was manually outlined on the CT<sub>low</sub> linked to the SPECT images. The delineated FRL of the CeCT scan was used as a constant reference (Figs. 1D and 1E). FRL functional volume (FRL-FV) was subsequently calculated using the same threshold. FRL-FV, expressed as percentage of TL-FV, was calculated using the formula:

\[
\%FRL-FV = \frac{FRL-FV}{TL-FV} \times 100\%
\]

The percentage of counts within the FRL (SPECT%FRL-C) is calculated by dividing the counts within the FRL by the total counts within the entire liver using the formula:

\[
SPECT\% FRL - C = \frac{FRL\ counts}{Total\ liver\ counts} \times 100\%
\]

SPECT%FRL-C reflects the function of the FRL relative to the TL-F. For the actual calculation of the FRL-F using the SPECT data (SPECTFRL-F), this percentage was multiplied by the total liver 99m<sup>Tc</sup>-mebrofenin uptake rate as measured by the Gmean dataset of the dynamic HBS. To compensate for the size differences between individual FRLs, the SPECTFRL-F was divided by the FRL-V, as measured by the CeCT.

**Statistical Analysis**

Statistical analysis was performed with GraphPad Prism, version 4.01 (GraphPad Software) and Statistical Package for Social Sciences (version 14.02; SPSS Inc.). Correlation between variables was tested using the Pearson correlation coefficient. For the comparison between patients with a compromised and patients with a noncompromised liver, the slope coefficient of the linear regression curve was calculated. Continuous data were compared by independent-sample t test or paired t test and expressed as mean ± SD. All statistical tests were 2-tailed, and differences were considered significant at a P value less than or equal to 0.05.

**Results**

**Patient Characteristics**

In this study, 36 patients were included. Patient characteristics are shown in Table 1. Twenty-one patients had a compromised liver based on the histopathology of the resection specimen including severe fibrosis (n = 2), biliary fibrosis (n = 4), severe cholestasis (n = 2),
steatosis (>30% of the hepatocytes affected) \((n = 2)\), chronic inflammation \((n = 2)\), or a combination of these diseases \((n = 9)\).

Dynamic HBS

In patients undergoing a right-sided hemihepatectomy (left hemiliver as FRL), the \(\text{AnteriorFRL-F} \) ratio—compared with the \(\text{GmeanFRL-F} \) ratio—resulted in an indisputable overestimation of the FRL, whereas an underestimation was seen in patients undergoing a left hemihepatectomy (Fig. 2A). When the FRL-F was calculated, the \(\text{AnteriorFRL-F} \) was significantly smaller than the \(\text{GmeanFRL-F} \) in patients undergoing a left hemihepatectomy (Fig. 2B). In patients undergoing a right hemihepatectomy, the relative overestimation as indicated by the \(\text{AnteriorFRL-F} \) ratio was partially compensated by the significantly smaller total liver function, as calculated by the anterior dataset \((7.71\% \pm 1.19\%/\text{min/m}^2)\) versus the Gmean dataset \((8.76\% \pm 1.23\%/\text{min/m}^2)\) \((P < 0.0001\) paired \(t\) test), resulting in only a small, but significant, difference between FRL-Fs of the 2 datasets (mean difference of 0.1%/min/m\(^2\), \(P < 0.0001\) paired \(t\) test).

**Figure 2.** FRL-F, calculated by dynamic 99mTc-mebrofenin HBS using anterior and Gmean datasets. In patients undergoing right-sided hemihepatectomy (with left hemiliver as FRL), AnteriorFRL-F ratio demonstrated clear overestimation of FRL, and underestimation was seen in patients undergoing left hemihepatectomy (A). However, when FRL-F was calculated, anterior projection demonstrated only clear underestimation in patients undergoing left hemihepatectomy (B).

TL-F was significantly less in compromised, compared with noncompromised, livers for both the anterior \((7.4\%/\text{min/m}^2\) vs. \(8.7\%/\text{min/m}^2\)) and the Gmean data \((8.7\%/\text{min/m}^2\) vs. \(9.9\%/\text{min/m}^2\)). Patients with a normal liver parenchyma demonstrated a slightly better correlation between FRL-V and FRL-F than did patients with a compromised liver (anterior: Pearson \(r = 0.81\) vs. Pearson \(r = 0.73\); Gmean: Pearson \(r = 0.85\) vs. \(r = 0.81\)). Moreover, the slope coefficient of the linear regression curve indicated that FRL-V was associated with a significantly reduced FRL-F in compromised livers versus noncompromised livers \((P = 0.008, \text{ANCOVA test})\).

In 18 patients, a postoperative HBS was performed within 1–3 d after the operation to measure actual remnant liver function \((\text{ActualRL-F})\). Although FRL-F measured by Gmean
dataset correlated strongly with ActualRL-F, it slightly underestimated the ActualRL-F with 0.62%/min/m² (P = 0.009) (Table 2).

FRL-F, calculated by dynamic $^{99m}$Tc-mebrofenin HBS using anterior and Gmean datasets. In patients undergoing right-sided hemihepatectomy (with left hemiliver as FRL), AnteriorFRL-F ratio demonstrated clear overestimation of FRL, and underestimation was seen in patients undergoing left hemihepatectomy (A). However, when FRL-F was calculated, anterior projection demonstrated only clear underestimation in patients undergoing left hemihepatectomy (B).

Morphologic Liver Volume and Functional Liver Volume

The TL-FV (measured by SPECT) was compared with morphologic NTTL-V (measured by CeCT volumetry). The TL-FV had a strong and significant correlation with the NTTL-V (Pearson r = 0.85). The values of NTTL-V and TL-FV were similar in patients with a normal liver parenchyma, indicating the accuracy of $^{99m}$Tc-mebrofenin SPECT for the measurement of hepatic volume (1,432.2 ± 315.9 vs. 1,481.2 ± 301.5, mean difference; 49 mL, P = 0.198). However, in patients with a compromised liver parenchyma TL-FV was significantly less than NTTL-V (1,787.9 ± 391.5 vs. 2,000.1 ± 522.0, mean difference; 212 mL, P = 0.006).

In contrast to liver function, NTTL-V was significantly larger in patients with a compromised liver than in patients with a noncompromised liver (1,022 ± 225 mL/m² vs. 853 ± 163 mL/m²), whereas TL-FV did not significantly differ between these 2 patient groups (824 ± 168 mL/m² vs. 916 ± 171 mL/m²).

Focusing on the volume of the FRL, a strong correlation was found between FRL-FV and FRL-V (Pearson r = 0.95), with a similar correlation coefficient for both patients with a compromised and patients with a noncompromised liver parenchyma and a similar slope coefficient of the linear regression curve (P = 0.77). In addition, the absolute values of FRL-FV were comparable to the values of the FRL-V in both patient groups, indicating that the presence of a compromised liver had not affected the FRL-FV.

The %FRL-V is a clinically important tool to decide on the resectability of candidates for partial hepatectomy. Analyzing the 2 patient groups separately, %FRL-FV was comparable to %FRL-V in patients with a normal liver parenchyma (56.9% ± 18.9% vs. 57.2% ± 18.5%, P =

<table>
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<th>Preoperative parameter</th>
<th>Postoperative parameter</th>
<th>Pearson r</th>
<th>difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gmean FRL-F</td>
<td>actual RL-FV*</td>
<td>0.96</td>
<td>-0.62 %/min/m²</td>
<td>0.009*</td>
</tr>
<tr>
<td>SPECT FRL-F</td>
<td>actual RL-FV*</td>
<td>0.94</td>
<td>-0.47 %/min/m²</td>
<td>0.068</td>
</tr>
<tr>
<td>CT FRL-Fun</td>
<td>actual RL-FV*</td>
<td>0.96</td>
<td>-2.56 %/min/m²</td>
<td>&lt; 0.0001*</td>
</tr>
</tbody>
</table>

* Measured by Gmean.
† P < 0.05.

Although all parameters demonstrated strong correlation with ActualRL-F, only SPECTFRL-F demonstrated no significant difference in actual values between preoperatively estimated and actual remnant liver function.
0.84, paired t test), whereas %FRL-FV was significantly larger than %FRL-V in compromised patients (58.6% ± 22.2% vs. 55.0% ± 19.4%, \(P = 0.006\)), suggesting that the presence of a compromised liver parenchyma had a greater impact on the functional volume of the segments to be resected than on the FRL.

Postoperative SPECT was performed on 18 patients to measure \(\text{Actual}_\text{RL-FV}\). Although the correlation between FRL-FV and \(\text{Actual}_\text{RL-FV}\) was strong (Pearson \(r = 0.90\)), the \(\text{Actual}_\text{RL-FV}\) was significantly larger than the preoperative prediction (mean difference, 188.5 mL; \(P = 0.0012\), paired t test).

Regional Liver Function by SPECT

The combination of SPECT and CT images provided valuable visual information on the distribution of function within the liver. The absence of \(^{99m}\text{Tc-mebrofenin}\) uptake in liver tumors made them clearly visible (Fig. 1E). For the regional distribution of liver function, the FRL counts were expressed as a percentage of total liver counts (\(\text{SPECT\%FRL-C}\)). Although \(\text{SPECT\%FRL-C}\) correlated strongly with the %FRL-V (Pearson \(r = 0.95\)), a discrepancy of more than 10% between the 2 parameters was seen in 9 patients, 7 of whom had a compromised liver. The latter suggests that in these patients, liver function was not distributed homogeneously over the liver volume because of disturbances of regional liver function such as biliary obstruction or tumor compression on the surrounding liver tissue and vessels. The differences in regional liver function of these 9 patients are depicted in Figure 3. In 8 of these patients (89%), the function within the segments to be resected was more affected than the function within the FRL.

In patients with a compromised liver, \(\text{SPECT\%FRL-C}\) was significantly larger than %FRL-V (60.0% ± 24.3% vs. 55.0% ± 19.4%, \(P = 0.011\)), whereas no difference was found between \(\text{SPECT\%FRL-C}\) and %FRL-FV, measured with the outline extraction method.

The \(\text{SPECT\%FRL-C}\) provides information on the distribution of function within the liver volume, but it does not represent the actual regional function of the liver. Therefore, the \(\text{SPECT}_\text{FRL-F}\) was calculated by multiplying the \(\text{SPECT\%FRL-C}\) by the total liver \(^{99m}\text{Tc-mebrofenin}\) uptake rate, as measured by the dynamic HBS. This \(\text{SPECT}_\text{FRL-F}\) correlated strongly with \(\text{Actual}_\text{RL-F}\). Moreover, there was no significant difference between \(\text{SPECT}_\text{FRL-F}\) and \(\text{Actual}_\text{RL-F}\) (mean difference, 0.47%/min/m\(^2\); \(P = 0.068\), paired t test), indicating that \(\text{SPECT}_\text{FRL-F}\) accurately predicted \(\text{Actual}_\text{RL-F}\).

The FRL-F was also calculated using %FRL-V as measured by CeCT volumetry multiplied by the total Gmean uptake function from the dynamic HBS (\(\text{CT}_\text{FRL-F}\)). Although the correlation between \(\text{CT}_\text{FRL-F}\) and \(\text{Actual}_\text{RL-F}\) was strong (Pearson \(r = 0.96\)), the \(\text{CT}_\text{FRL-F}\) significantly underestimated the \(\text{Actual}_\text{RL-F}\), with a mean difference of 2.56%/min/m\(^2\) (\(P < 0.0001\), paired t test).

In parallel, \(\text{SPECT-FV}_\text{FRL-F}\) was calculated using %FRL-FV multiplied by the Gmean uptake function from the dynamic HBS. This result also underestimated the \(\text{Actual}_\text{RL-F}\), although to a lesser extent, with a mean difference of 2.32%/min/m\(^2\) (\(P < 0.0001\), paired t test).
Figure 3. Regional differences in liver function within 9 patients with discrepancy of more than 10% between SPECT%FRL-C and %FRL-V.
CCC 5 cholangiocarcinoma; CRM 5 colorectal metastases; segm 5 segment.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Impaired function</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hilar CCC* (klatskin 3)</td>
<td>segm 7,8</td>
<td>Cholestasis with dilated bile ducts compromised</td>
</tr>
<tr>
<td>2 hilar CCC* (klatskin 3)</td>
<td>Segm 2,3</td>
<td>Cholestasis without dilated bile ducts compromised</td>
</tr>
<tr>
<td>3 hilar CCC* (klatskin 3)</td>
<td>Segm 7</td>
<td>Cholestasis with dilated bile ducts compromised</td>
</tr>
<tr>
<td>4 Intrahepatic bile stones</td>
<td>Segm 2,3</td>
<td>Cholestasis with dilated bile ducts compromised</td>
</tr>
<tr>
<td>5 Metastasis endometrial carcinoma</td>
<td>Diffuse left liver mainly segm 6 distal of tumor</td>
<td>Tumor compression on surrounding tissue and vessels normal</td>
</tr>
<tr>
<td>6 hilar CCC* (klatskin 3)</td>
<td>Segm 2,3</td>
<td>Cholestasis with dilated bile ducts compromised</td>
</tr>
<tr>
<td>7 hilar CCC* (klatskin 3)</td>
<td>Segm 2,3</td>
<td>Cholestasis with dilated bile ducts. Tumor invasion left portal vein compromised</td>
</tr>
<tr>
<td>8 CRM*</td>
<td>Segm 5,6,7,8</td>
<td>Tumor compression on surrounding tissue and bile ducts compromised</td>
</tr>
<tr>
<td>9 CRM*</td>
<td>Segm 2,3 (FRL)</td>
<td>unknown normal</td>
</tr>
</tbody>
</table>

*CCC, cholangiocarcinoma; *CRM, Colorectal metastases; Segm = segment

Finally, we investigated the function per volume of the FRL, enabling the comparison between the group of patients with parenchymal liver disease and the group with a normal liver parenchyma while compensating for the individual difference in FRL volumes. \( \text{SPECTFRL-F/cm}^3 \) liver volume was significantly smaller in patients with a compromised liver.

In patients with a normal liver, the \( \text{SPECTFRL-F/cm}^3 \) liver volume within the segments to be resected and within the FRL was similar, confirming a homogeneous distribution of liver function. Nevertheless, in compromised livers, function per cubic centimeter of volume was
significantly less in the segments to be resected than in the FRL, confirming the unequal distribution of liver function and indicating that the resected segments suffer from more functional loss than the FRL.

**Discussion**

In this study, we demonstrated the clinical value of $^{99m}$Tc-mebrofenin SPECT for the measurement of segmental liver function and liver functional volume in patients undergoing partial liver resection.

In previous studies, liver function was calculated with dynamic HBS using the anterior projection of the liver (6–9). This study clearly demonstrated that, because of the anatomic position of the liver and hence different photon attenuation of liver segments, the data from the anterior projection resulted in a relative overestimation of the left hemiliver and an underestimation of the right hemiliver, when data were expressed as a ratio of TL-F. On the other hand, when the function of the FRL was calculated, a clear discrepancy between $\text{Anterior FRL-F}$ and $\text{Gmean FRL-F}$ existed in patients undergoing left hemihepatectomy, whereas only a small discrepancy existed in patients undergoing right hemihepatectomy.

These discrepancies can be explained by the fact that the right-sided liver segments contribute to approximately two thirds of total liver volume (11), and underestimation of these segments by the anterior projection translates to a significantly smaller TL-F in the anterior projection. For the calculation of $\text{Anterior FRL-F}$ in patients undergoing right hemihepatectomy, the relative overestimation of the left hemiliver as indicated by the $\text{Anterior FRL-F}$ ratio is consequently compensated by the smaller TL-F. This compensation is relevant because it has been demonstrated that patients undergoing right (extended) hemihepatectomy, especially, are at risk of the development of postoperative liver failure (12,13). Although it is possible...
to use the anterior projection in these patients, it is recommended that the Gmean data for the dynamic $^{99m}$Tc-mebrofenin HBS be used in the future.

Modern dual-head rotating $\gamma$-cameras enable additional SPECT for the assessment of liver function and liver functional volume within 1 test. The timing of the SPECT is a challenge when a dynamic tracer that is first taken up by the liver and subsequently excreted into the bile is used. We have centered the SPECT around the peak of the hepatic time–activity curve, because the amount of radioactivity within the liver is relatively stable during this phase. In some patients with a fast hepatic uptake, biliary excretion was already visible during the SPECT phase. Accumulation of radioactivity in the small bile ducts results in voxels with relatively high counts, disturbing calculation of total and regional liver function and functional volume. Therefore, we decided to remove the extrahepatic bile ducts by masking on transverse slices. The intrahepatic bile ducts were replaced with the mean voxel count of the liver parenchyma, because true bile duct volume is almost negligible but can be substantial in the SPECT reconstruction because of the relatively low spatial resolution of the SPECT and the relatively high voxel count. This makes $^{99m}$Tc-mebrofenin SPECT more complex than $^{99m}$Tc-labeled galactosyl human serum albumin ($^{99m}$Tc-GSA) SPECT, another well-known liver function test showing no early biliary excretion (14–16). $^{99m}$Tc-GSA scintigraphy is a receptor-mediated scintigraphy that is not available for clinical use in Europe and the United States.

The outline extraction method with specific threshold levels is frequently used to calculate functional liver volume from SPECT data (16,17). NTTL-V, measured by CeCT volumetry, and TL-FV, measured by $^{99m}$Tc-mebrofenin SPECT, were similar in patients with a normal liver parenchyma, indicating that $^{99m}$Tc-mebrofenin SPECT is an accurate method to measure hepatic functional volume. As expected, a compromised liver translated into a significantly smaller TL-FV than NTTL-V. Interestingly, the FRL–FV was not significantly different from the FRL-V, indicating that mainly the segments to be resected were affected, leading to loss of functional volume.

A drawback of the outline extraction method is the fact that it does not provide any information regarding the distribution of function within the delineated volume. SPECT images combined with CT images provided valuable visual information on the distribution of function within the liver. For the quantification of the distribution of liver function, we calculated the SPECT%FRL-C. In patients with a compromised liver, the SPECT%FRL-C was significantly larger than the %FRL-V, as determined by CeCT, clearly demonstrating that in patients with a compromised liver, function was not distributed equally among the various liver segments; the segments to be resected were more affected than was the FRL-F. In our patient populations, the proportion of FRL-F is consequently relatively better than suggested by the CeCT scan. Patients in our study were categorized as patients with a normal or a compromised liver on the basis of the histopathology of the resection specimen.

The SPECT count ratio provides information on the distribution of function within the liver, but it does not incorporate the actual regional function of the liver. The actual segmental liver function was, therefore, calculated by multiplying the SPECTFRL count ratio with the total uptake function measured by the Gmean of the dynamic HBS. Preoperative SPECT FRL-F was
able to accurately predict the actual postoperative remnant liver function. FRL-F measured by dynamic planar HBS resulted in only a small underestimation of the postoperative remnant liver function. The combination of %FRL-V acquired from CeCT volumetry with the TL-F from the HBS resulted in a clear underestimation of postoperative remnant liver function. CT volumetry is sometimes combined with TL-F measured by indocyanine green (ICG) clearance test (18,19). Because the ICG clearance test also represents global liver function, this could result in a wrong representation of the FRL-F in a liver with potentially inhomogeneous distribution of function.

To compensate for the size of the FRL, $^{\text{SPECT}}$FRL-F was divided by FRL-V measured by the CeCT volumetry. Although FRL-V and FRL-FV were similar for patients with either compromised or normal livers, the function within the FRL-V was significantly smaller in compromised livers. In addition, $^{\text{SPECT}}$FRL-F/cm$^3$ of liver volume was significantly smaller in the resected segments, confirming that liver function was not distributed homogeneously and that the segments to be resected were more affected.

The SPECT/CT camera used in our study allowed only a low-resolution non-contrast-enhanced CT scan, primarily developed as an anatomic reference and for attenuation correction. This low-dose CT is not suitable for use with intravenous contrast and is not a diagnostic tool. We therefore used the separately performed, diagnostic CeCT scan as a reference for the delineation of the FRL. Modern SPECT/CT scans have the ability to combine the SPECT acquisition with a diagnostic multislice CT scan. This advantage makes it possible to combine a 4-phase CT scan with HBS in which the SPECT data can be used as an additional functional phase, creating a 5-phase SPECT/CT for multimodality preoperative investigation of both liver morphology and liver function. This would enable a safe and accurate prediction of FRL volume and function, forming the basis for clinical decision making in the future.

Conclusion

When using the dynamic $^{99m}$Tc-mebrofenin HBS, it is recommended that the Gmean dataset be used. Although $^{99m}$Tc-mebrofenin SPECT is quite complex because of the rapid biliary excretion of $^{99m}$Tc-mebrofenin, it does provide valuable visible and quantitative information on total and segmental liver function. The combination of SPECT data with the dynamic uptake function measured with the planar HBS results in a complete and accurate prediction of the postoperative remnant liver function.
References


Chapter

Increase in future remnant liver function after preoperative portal vein embolization

W. de Graaf
K.P. van Lienden
J.W. van den Esschert
R. J. Bennink
T.M. van Gulik

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Abstract

**Background:** Preoperative portal vein embolization (PVE) is performed in patients with insufficient future remnant liver (FRL) to allow safe resection. Although many studies have demonstrated an increase in FRL volume after PVE, little is known about the increase in FRL function. This study evaluated the increase in FRL function after PVE using $^{99m}$Tc-labelled mebrofenin hepatobiliary scintigraphy (HBS) with single photon emission computed tomography (SPECT) and compared this with the increase in FRL volume.

**Methods:** In 24 patients, computed tomography volumetry and $^{99m}$Tc-labelled mebrofenin HBS with SPECT were performed before and 3–4 weeks after PVE to measure FRL volume, standardized FRL and FRL function. A hypothetical model was used to assess safe resectability after PVE. The limit for safe resection for FRL function was set at an uptake of 2.69 %/min/m$^2$. For FRL volume and standardized FRL, 25 or 40% of total liver volume was used, depending on the presence of underlying liver disease.

**Results:** After PVE, FRL function increased significantly more than FRL volume. The correlation between the increase in FRL volume and FRL function was poor. Using the hypothetical model, seven patients did not achieve a sufficient increase in FRL function to allow safe resection 3–4 weeks after PVE, compared with 12 and nine patients based on FRL volume and standardized FRL respectively.

**Conclusion:** The increase in FRL function after PVE is more pronounced than the increase in FRL volume, suggesting that the necessary waiting time until resection may be shorter than indicated by volumetric parameters.
Introduction

Future remnant liver (FRL) volume, measured by computed tomography (CT) volumetry, is currently the standard method for determining whether a patient can safely undergo liver resection. Although there are no formal guidelines, a FRL volume (expressed as a percentage of total liver volume, or as standardized FRL) larger than 25–30% is considered sufficient in patients with normal liver parenchyma, whereas more than 40% is preferred in patients with parenchymal disease. A large proportion of livers are deemed unresectable owing to insufficient FRL volume.

Preoperative portal vein embolization (PVE) is used to increase the number of resectable patients. It induces atrophy of the embolized, tumour-bearing liver segments, while compensatory hypertrophy occurs in the non-embolized lobe, thereby increasing FRL volume and, supposedly, FRL function. PVE reduces the risk of postoperative liver insufficiency and enables resection of livers previously considered unresectable owing to a marginal FRL. CT volumetry is the established method of assessing the increase in FRL volume after PVE.

Little is known about the improvement in FRL function after PVE, because few liver function tests have the ability to measure FRL function selectively. Quantitative liver function tests, such as the indocyanine green (ICG) clearance test and the galactose elimination capacity test, measure overall liver function and are used widely as preoperative predictors of postoperative outcome. CT volumetry is sometimes combined with total liver function measured by the ICG clearance test to calculate FRL function; however, this is potentially inaccurate in livers with an inhomogeneous functional distribution, which is the case after PVE. Therefore, these tests are probably not suitable for assessing the relative increase in FRL function after PVE.

Clinical studies from Japan have demonstrated that the functional increase in FRL measured by 99mTc-labelled galactosyl–human serum albumin (99mTc-GSA) scintigraphy exceeds the increase in volume after PVE. This implies that the recommended waiting time until operation may be shorter than suggested by volumetric studies, providing a potential benefit in light of the risk of tumour progression after PVE. These results, however, have not been confirmed using other quantitative liver function tests.

99mTc-labelled mebrofenin hepatobiliary scintigraphy (HBS) with single photon emission CT (SPECT) has recently been validated as a quantitative method for evaluating liver function as well as liver functional volume. 99mTc-mebrofenin HBS measures both total liver (uptake) function as well as FRL function, and can be used before surgery to predict postoperative liver failure. 99mTc-GSA scintigraphy and 99mTc-mebrofenin HBS are both nuclear imaging techniques, but are based on different principles. 99mTc-GSA scintigraphy measures the binding of 99mTc-GSA to its receptor, which is expressed on functional hepatocytes. 99mTc-mebrofenin HBS measures the kinetic process of uptake and excretion of 99mTc-mebrofenin by hepatocytes. The aim of this study was to compare...
the increase in FRL function, measured by $^{99}$mTc-mebrofenin HBS, with the increase in FRL volume, measured by CT volumetry, after preoperative PVE.

Methods

Between 2005 and 2008, 28 patients underwent PVE before liver resection. In 24 patients, $^{99}$mTc-mebrofenin HBS and CT volumetry were performed before, and 3–4 weeks after PVE. These patients were analysed with the approval of the institutional review board and after informed consent had been obtained. Post-PVE $^{99}$mTc-mebrofenin HBS and CT volumetry were performed on the same day. The FRL was delineated according to the planned resection. When FRL hypertrophy proved insufficient, the interval until resection was extended by 2 weeks.

The histopathology of the resection specimens and/or perioperative biopsies was assessed by an experienced pathologist, taking into account cholestasis, steatosis, fibrosis and chronic inflammation. A compromised liver was defined as: severe fibrosis (marked portoportal bridging or cirrhosis); steatosis of more than 30%; moderate to severe cholestasis; or a combination of diseases, that is mild fibrosis (fibrous expansion of portal areas or incomplete portoportal septa) combined with mild cholestasis and/or steatosis (5–30%) in combination with mild or moderate inflammation.

Portal vein embolization

PVE was indicated when the anticipated FRL volume was less than 25–30% of total liver volume. In patients with biliary obstruction or an expectedly compromised liver, a cut-off value of 40% was applied. All patients underwent embolization of the right portal system. In 23 patients, a percutaneous transhepatic ipsilateral approach was used as described previously (22). In one patient, a contralateral approach was employed. The branches of the right portal trunk were embolized using polyvinyl alcohol particles (300–500 nm; Cook Medical, Bloomington, Indiana, USA) and coils (Tornado® Embolization Microcoil; Cook Medical). Additional embolization of segment IV was performed in two patients.

Computed tomography volumetry

Multiphase contrast-enhanced CT was carried out with a multislice helical scanner (Philips Medical Systems, Eindhoven, The Netherlands). Total liver, tumour mass(es) and FRL were delineated manually by an experienced radiologist in collaboration with a hepatobiliary surgeon according to the anticipated resection (19) (Fig. 1a,e). Integrated software (Mx-View 3.52; Philips Medical Systems) was used to calculate total liver volume (TLV), tumour volume (TV) and FRL volume (FRL-V) (Fig. 1b,f). Percentage FRL volume (%FRL) before PVE was calculated using the formula:

$$%FRL - V_{pre-PVE} = \frac{FRL-V_{pre-PVE}}{(TLV- TV)_{pre-PVE}} \times 100\%$$
%FRL-V after PVE was calculated by dividing FRL volume after PVE by pre-PVE total liver volume. Pre-PVE total liver volume was used because atrophy of the embolized liver segments can influence individual post-PVE total liver volume, affecting %FRL volume independently of FRL increase.

**Standardized future remnant liver measurements**

Standardized FRL (sFRL) is a frequently used alternative way of expressing FRL volume (measured by CT volumetry) as percentage of calculated total liver volume (calTLV), determined using a formula based on body surface area (BSA) (23):

\[
\text{calTLV} = -974.41 + 1267.28 \times \text{BSA}
\]

where \( \text{BSA} = \sqrt{\text{height (cm)} \times \text{weight (kg)}} / 3600 \)

**Scintigraphic imaging and data acquisition**

HBS was performed with 99mTc-labelled (2,4,6 trimethyl-3-bromo) iminodiacetic acid (99mTc-mebrofenin, Bridatec; GE Healthcare, Eindhoven, The Netherlands), as reported previously (18). Briefly, a dynamic acquisition (36 frames at 10 s/frame, 128 matrix) was made using an Infinia™ II SPECT/CT camera (GE Healthcare) immediately after intravenous administration of 200 MBq 99mTc-mebrofenin, which was used for the calculation of hepatic uptake function. Subsequently, a fast SPECT acquisition was performed (60 projections at 8 s/projection, 128 matrix) followed by low-dose non-contrast-enhanced CT for attenuation correction and anatomical mapping on the same gantry without moving the patient. Finally, a second dynamic acquisition was performed to evaluate biliary excretion. Data were processed on a Hermes workstation (Hermes Medical Solutions, Stockholm, Sweden).

**Calculation of dynamic planar hepatobiliary scintigraphy parameters**

Hepatic 99mTc-mebrofenin uptake rate (per cent per minute) was calculated on an anterior and geometric mean (Gmean = \( \sqrt{\text{anterior} \times \text{posterior}} \)) data set using time–activity curves from the liver, the heart/large vessels within the mediastinum (serving as blood pool) and around the total field of view, with scanned radioactivity values acquired between 150 and 350 s after injection (18). To compensate for differences in individual metabolic requirements, total liver 99mTc-mebrofenin uptake rate (per cent per minute), representing total liver function, was divided by the BSA and expressed as %/min./m². FRL (uptake) function (FRL-F) was calculated by dividing the summed counts (150–350 s after injection) within the delineated FRL by the total liver counts within the same time frame, and multiplying this factor by total liver 99mTc-mebrofenin uptake rate expressed as %/min./m² (Fig. 1c,g). Total liver function and FRL function were calculated using both the anterior and Gmean data sets. The anterior data set was used because it has been correlated with clinical outcome after partial liver resection (19,21), whereas Gmean is a more precise parameter (18). The uptake...
Figure 1. Examples of images produced by the different techniques a–d before and e–h after portal vein embolization (PVE). a,e Portal and hepatic veins were used as landmarks for delineation of the future remnant liver (FRL) on contrast-enhanced computed tomography (CT) images. b,f The anterior projection of the CT reconstruction was used as a guideline for delineating the FRL on c,g planar dynamic $^{99m}$Tc-labelled mebrofenin hepatobiliary scintigraphy (HBS) images. In addition, the round ligament was used as the border between segments III and IV. The line, projected on the liver surface as a plane between the middle of the gallbladder fossa (visible in the late phase of the scintigraphy) and the inferior caval vein, was used as a border between the right and left liver lobes. The delineated FRL on the contrast-enhanced CT images was used as a constant reference for delineation of the FRL on d,h single photon emission CT (SPECT) images.
of mebrofenin per litre of FRL liver tissue was calculated by dividing the FRL function (not corrected for BSA) by FRL volume and expressed as %/min./L.

Calculation of single photon emission computed tomography parameters

SPECT acquisition was centred around the peak of the hepatic time–activity curve because the amount of radioactivity within the liver is relatively stable during this phase. SPECT was used for three-dimensional assessment of liver function and calculation of functional liver volume (18). Briefly, an outline extraction method (with a threshold of 30 per cent of the maximal voxel count value) was applied automatically to outline the liver and calculate total functional liver volume. In patients with a fast hepatic uptake, accumulation of radioactivity in the small bile ducts disturbs the calculation of total and regional liver function and volume. Therefore, the activity within the extrahepatic bile ducts was removed manually and the intrahepatic bile ducts were automatically replaced by the average count density of normal liver tissue. Subsequently, the FRL was outlined manually on the CT images linked to the SPECT images (Fig. 1d, h), with the contrast-enhanced CT image as a constant reference (Fig. 1a, e).

Before PVE, FRL functional volume (FRL-FV) was expressed as a percentage of total liver functional volume (%FRL-FV). As with CT volumetry, %FRL-LV after PVE was calculated by dividing FRL functional volume after PVE by pre-PVE total functional liver volume.

For calculation of actual SPECT FRL function (SPECTFRLF), the percentage of counts within the FRL was multiplied by the total liver 99mTc-mebrofenin uptake rate as measured by the Gmean data set of the dynamic HBS.

Hypothetical model for resectability

In this study, liver resection was not performed exactly 3–4 weeks after PVE in all patients, and some patients had a less extensive resection than anticipated, making it impossible to correlate FRL function, measured at 3 weeks after PVE, with actual clinical outcome. To determine whether the more pronounced increase in FRL-F could eventually be translated into a shorter time interval between PVE and resection, a hypothetical model was derived to assess resectability 3 weeks after PVE.

Patients were divided into those with a normal liver parenchyma and those with a compromised liver parenchyma based on preoperative diagnostic findings. Patients with hilar cholangiocarcinoma and preoperative chemotherapy were considered to have a compromised liver parenchyma. In six patients the presence of parenchymal liver disease was assessed by preoperative biopsy. In five patients magnetic resonance spectroscopy was used for non-invasive assessment of steatosis. Thirteen patients were considered to have a compromised liver before operation, two of whom were subsequently found to have normal liver parenchyma on histopathological evaluation of the resection specimen or perioperative biopsy. Eleven patients were considered to have normal liver parenchyma, of which two ultimately had evidence of compromised liver parenchyma.
The limit for safe resection for %FRL-V and sFRL was set at 25% in patients with anticipated normal liver parenchyma (1,24) and 40% for those with a compromised liver parenchyma (2,25,26). For FRL-F, a cut-off of 2.69 %/min./m² was used as the limit for safe resection, using the anterior data set of the dynamic HBS. A cut-off value for FRL function of 2.69 %/min./m² was able to identify patients with an increased risk of postoperative liver failure in a study of patients undergoing major liver resection (19).

Statistical analysis

Continuous data were expressed as mean(s.d.) and compared by independent-samples t-test or paired t-test. Correlation between variables was tested using the Pearson correlation coefficient. All statistical tests were two-tailed and differences were considered significant at \( P \leq 0.05 \). Statistical analysis was performed with GraphPad®Prism 4.01 (GraphPad Software, San Diego, California, USA) and SPSS® version 14.02 (SPSS, Chicago, Illinois, USA).

Results

Patient characteristics

Patient characteristics are shown in Table 1. The PVE procedure was uncomplicated in 23 patients. One patient, in whom segment IV was additionally embolized, developed a thrombus in the left portal vein which became apparent during surgery and the planned extended right hepatectomy was abandoned.

Laparotomy was performed in all patients. Five further patients did not undergo liver resection, four because of intrahepatic or extrahepatic tumour progression; in the fifth patient a non-infiltrating gallbladder carcinoma was identified on exploration for which a

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<td><strong>Demographics</strong></td>
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cholecystectomy sufficed. Of all patients who underwent liver resection, one developed remnant liver failure and eventually died 1 month after extended hemihepatectomy from postoperative portal vein thrombosis. Two patients had transient liver insufficiency.

Thirteen patients had evidence of a compromised liver parenchyma in the resection specimen or perioperative biopsy, including biliary fibrosis (1), severe cholestasis (4), steatosis (5) or a combination of these (3). In one patient, no liver tissue was available for histological evaluation.

Increase in liver volume and liver functional volume after portal vein embolization

$^{99m}$Tc-mebrofenin HBS and CT volumetry were performed 23.0(4.9) days after PVE. Total liver volume and total functional liver volume (measured by SPECT) were not significantly affected by PVE ($P = 0.622$ and $P = 0.160$ respectively). The %FRL volume measured by CT volumetry increased from 25.2(7.2)% before PVE to 33.7(8.2)% after embolization ($P < 0.001$). The %FRL functional volume measured by SPECT increased from 25.1(5.4)% to 34.0(8.1)% ($P < 0.001$) (Fig 2). There was no significant difference between %FRL volume and %FRL functional volume before ($P = 0.940$) or after ($P = 0.678$) PVE. Standardized FRL increased from 27.6(10.4) to 36.8(11.5)%.

As a result of PVE, FRL volume and FRL functional volume increased by 35.8(14.7)% and 35.7(12.3)% respectively. For compromised and normal livers, the increase in both FRL volume (37.5(13.8)% versus 34.7(16.8)% respectively; $P = 0.666$) and FRL functional volume (34.0(12.0)% versus 38.7(14.7)%; $P = 0.382$) was similar.

Increase in liver function after PVE measured by planar dynamic hepatobiliary scintigraphy

Total liver function before PVE was significantly less in compromised compared with normal livers for both the anterior data set (5.7(1.7) %/min/m$^2$ versus 7.8(1.2) %/min/m$^2$; $P = 0.005$) and the Gmean data set (7.1(2.5) %/min/m$^2$ versus 8.9(1.2) %/min/m$^2$; $P = 0.048$). Total liver function was not significantly changed by PVE ($P = 0.601$ and $P = 0.085$ for anterior and Gmean respectively).

The effect of PVE on FRL function is shown in Fig.3. FRL function was increased by 51.9(25.7)% (anterior data set) and 54.5(22.8)% (Gmean data set), with a similar increase in patients with compromised versus normal liver parenchyma ($P = 0.392$ and $P = 0.609$ respectively). Overall, the increase in FRL function was significantly greater than the increase in FRL volume ($P < 0.001$, anterior data set; $P = 0.003$, Gmean data set). The uptake of $^{99m}$Tc-mebrofenin in the FRL increased from 9.2(3.3) %/min/L liver tissue before PVE to 10.3(3.1) %/min/L after PVE based on the anterior data ($P < 0.001$), and from 8.5(3.2) to 9.7(3.1) %/min/L based on the Gmean data ($P = 0.001$). In the majority of patients, PVE resulted in a more pronounced increase in FRL function than in FRL volume (18 of 24 for anterior data, 17 of 24 for Gmean). Based on the anterior data set, the FRL function increase exceeded the FRL volume increase in both normal and compromised livers ($P = 0.047$ and $P = 0.027$).
Gmean data set showed a significant difference in favour of FRL function for compromised liver ($P = 0.004$), but not for normal liver ($P = 0.083$).

The increase in FRL volume resulting from PVE correlated poorly with the increase in FRL function in patients with a normal liver ($r = 0.15, P = 0.068$) as well as in those with a compromised liver parenchyma ($r = 0.53, P = 0.062$), indicating that the functional increase of the FRL was at least partially independent of the volumetric increase.

Figure 2. Effect of portal vein embolization (PVE) on mean(s.d.) percentage future remnant liver (FRL) volume and percentage FRL functional volume. *$P = 0.940$, †$P = 0.678$ for comparison of percentage FRL volume versus functional volume (paired $t$ test).

**Figure 3.** Effect of portal vein embolization (PVE) on mean(s.d.) future remnant liver (FRL) function, measured by dynamic hepatobiliary scintigraphy (HBS), based on anterior and Gmean data sets (both $P < 0.001$ after versus before PVE; paired $t$ test). b Mean(s.d.) increase in FRL function measured by HBS versus FRL volume measured by computed tomography volumetry in the anterior data set. *$P < 0.001$ versus FRL volume (paired $t$ test). The Gmean data set showed the same pattern.

Gmean data set showed a significant difference in favour of FRL function for compromised liver ($P = 0.004$), but not for normal liver ($P = 0.083$).

The increase in FRL volume resulting from PVE correlated poorly with the increase in FRL function in patients with a normal liver ($r = 0.15, P = 0.068$) as well as in those with a compromised liver parenchyma ($r = 0.53, P = 0.062$), indicating that the functional increase of the FRL was at least partially independent of the volumetric increase.

**Increase in liver function after portal vein embolization measured by SPECT**

Although planar dynamic HBS parameters have been validated extensively in the clinical setting, $^{\text{SPECT}}_{\text{FRLF}}$ recently proved to be a better predictor of postoperative remnant liver function(18). Similar to the planar dynamic HBS parameters, PVE induced a significantly greater increase in $^{\text{SPECT}}_{\text{FRLF}}$ compared with FRL volume ($P = 0.001$) and FRL functional volume ($P = 0.002$) (Fig 4a). In 19 of the 24 patients (79%), the increase in $^{\text{SPECT}}_{\text{FRLF}}$...
exceeded the increase in FRL volume. The increase in SPECTFRLF was similar in compromised and normal liver (62.1(35.7)% versus 66.1(37.9)%; $P = 0.799$). Again, no significant correlation was found between increases in FRL volume and SPECTFRLF in normal liver ($r = 0.05, P = 0.889$). A poor correlation was found for compromised liver parenchyma ($r = 0.58, P = 0.0363$) (Fig 4b). The uptake of $^{99m}$Tc-mebrofenin in the FRL increased from 7.8(2.4) %/min/L liver tissue before PVE to 9.2(3.0) %/min/L after embolization ($P = 0.001$).

Hypothetical model for resectability
To determine whether the significantly more pronounced increase in FRL function could eventually be translated into a shorter time interval between PVE and resection, the hypothetical model described above was used. One patient with severe steatosis and a large adenoma, which was difficult to delineate from the surrounding tissue, underwent preoperative PVE despite a percentage FRL volume just above 40%. When using the cut-off value for FRL function, three patients would not have needed preoperative PVE. Seven patients did not achieve a sufficient increase in FRL function to allow safe resection 3 weeks after PVE, compared with 12 and nine patients based on FRL volume and standardized FRL respectively (Fig 5). These data indicate that the waiting time to resection may be shorter than indicated by the volumetric parameters.
Discussion

Although the increase in FRL volume after PVE has been investigated in many studies, few have described the increase in FRL function (14,15,27). In this study the increase in FRL function induced by PVE was measured by $^{99m}$Tc-mebrofenin HBS and compared with the increase in FRL volume measured by CT volumetry.
The liver has multiple roles including metabolic, synthetic and detoxifying functions. The ideal liver function test representing the multiple aspects of liver function has not yet been identified. Several quantitative liver function tests have been developed, each reflecting a separate component of the broad spectrum of liver functions. Although $^{99m}$Tc-mebrofenin is not metabolized, it is taken up by organic anion transporting polypeptides and subsequently excreted into the bile by multidrug resistance proteins(28). It follows a path of intracellular transit that is similar to that of various endogenous and exogenous substances, including bilirubin, hormones, drugs and toxins, and therefore represents an important liver function. Clinically, preoperative $^{99m}$Tc-mebrofenin HBS has been correlated with the ICG clearance test, and accurately predicts postoperative liver function and outcome after liver resection(19,21). $^{99m}$Tc-mebrofenin HBS with SPECT therefore offers a valuable clinical liver function test.

Among studies that have investigated the improvement in FRL function after PVE, one investigated the biliary excretion of ICG in patients with multiple biliary drains, which enabled the assessment of ICG excretion in the non-embolized and embolized liver segments separately(27). The functional gain in the non-embolized lobes was of greater magnitude than the volumetric increase. This method is, however, suitable only for patients with percutaneous biliary drains and is not applicable in the general patient population. Three Japanese studies used non-invasive dynamic $^{99m}$Tc-GSA SPECT as a liver function test and showed that FRL function increased to a greater extent than FRL volume in patients with and without cirrhosis(14-16). The present study has confirmed that the increase in FRL function is more pronounced than the increase in FRL volume after PVE. The uptake function in the FRL per litre of liver tissue increases after PVE. The increased liver function per gram or millilitre of liver tissue has been described previously during liver regeneration after partial hepatectomy in both humans and rats(29,30).

Combination of the ICG clearance test and the %FRL volume derived by CT volumetry has been described for the assessment of FRL function after PVE(8). This method, however, is based on the assumption that the increase in FRL volume is related to the increase in FRL function, and that liver function is distributed homogeneously within the liver volume. However, only a weak correlation between the increase in FRL volume and FRL function was found, and so combination of the ICG clearance test with the %FRL volume derived from CT volumetry may not accurately measure FRL function after PVE. This indicates the importance of additional quantitative functional assays that specifically measure FRL function after PVE.

In this study, the hypertrophic response of the FRL (both liver volume and liver function) after PVE was similar for patients with a compromised liver and those with a normal liver parenchyma. However, no patients with liver cirrhosis were included. Controversy exists regarding the effect of parenchymal liver disease on the hypertrophic response after PVE. Several large single-centre studies have shown a similar increase in FRL volume in patients with parenchymal liver disease(25,31). Others, however, have demonstrated that patients with cirrhosis in particular have a smaller hypertrophic response(32).

Tumour progression has been reported after PVE in patients with colorectal metastases or primary liver tumours(17). In addition, high rates of unresectable disease have been
described after PVE, ranging from 6.4 to 33 % (17). In the present study, in four of the 21 (19%) patients with malignant disease, the tumour was unresectable after PVE as a result of intrahepatic or extrahepatic tumour progression. Tumour progression after PVE creates a dilemma in terms of optimal waiting time until resection. The possible risk of tumour growth obviously demands a waiting time as short as possible. The interval is mainly determined by the time required to attain sufficient FRL volume or FRL function. The reported waiting time to resection after PVE varies considerably, from 2 weeks to several months, with most of the hypertrophy occurring within the first 3 weeks (9,33).

To determine whether the significantly greater increase in FRL function could eventually be translated into a shorter interval between PVE and resection, a hypothetical model was used to assess resectability 3 weeks after PVE. The model was based on cut-off values associated with the limits for performing safe liver resection. This study was not designed to correlate FRL function with actual clinical outcome. Three weeks after PVE, more patients reached sufficient FRL function to allow safe resection compared with standardized FRL and FRL volume, indicating that the waiting time until resection may be shorter than indicated by volumetric parameters. This model is hypothetical and requires correlation with actual postoperative outcome.
References


Chapter 11

Enhanced tumor growth after portal vein embolization in a rabbit VX2 tumor model

L.T. Hoekstra
K.P. van Lienden
J. Verheij
C.M. van der Loos
T.M. van Gulik

Submitted CVIR
Abstract

Objectives: To assess tumor growth and liver regeneration after portal vein embolization (PVE) in a rabbit hepatic tumor model.

Background data: Preoperative PVE is employed to increase future remnant liver volume through induction of hepatocellular regeneration. This event, however, potentially enhances tumor growth.

Methods: Two weeks after subcapsular implantation of a VX2 carcinoma in the cranial liver lobe, New Zealand White rabbits were allocated to a control group or PVE group (n=5/group). In the PVE group, the portal vein branch to the cranial liver lobes (80%) was embolized using particles and coils (PVE-group), leaving the caudal liver lobe (20%) free. In the tumor control group, the liver was only mobilized. CT volumetry was performed on days 3, 7, 10, and 14. Tumor growth rate (TGR), hepatocellular proliferation rate, and liver damage parameters were assessed before PVE, and on day 1, 3, 7, 10, and 14.

Results: Portography confirmed complete occlusion of the portal vein branch to the cranial liver lobes in all PVE-rabbits. The hypertrophy response and proliferation rate in the non-embolized liver lobes were significantly higher in the PVE group, which was confirmed by liver to body-weight index assessment. TGR was increased in both groups, with a significantly larger increase in the PVE-group over time (day 14: mean 34.4±4.3mL/day vs control: 24.1±7.2mL/day).

Conclusions: Tumor growth (TGR) was significantly increased after PVE in the rabbit tumor model. This finding supports the notion that PVE potentially enhances tumor growth, along with regeneration of the non-embolized liver lobe.
Introduction

Liver resection is the most effective treatment for primary or metastatic liver tumors. In advanced tumors, the extent of liver resection is restricted by the minimum volume of liver remnant required to provide sufficient postoperative liver function. Insufficient future remnant liver (FRL) is a reason why patients are considered unresectable. Preoperative portal vein embolization (PVE) is an option to increase FRL volume through induction of regeneration of the hepatocellular mass of the FRL. Following occlusion of the right or left main branch of the portal vein, atrophy of the embolized liver segments occurs while hypertrophy of the contralateral, non-embolized liver lobe is induced. PVE has shown to reduce the risk of liver failure after resection and consequently increases the number of patients who are eligible for liver resection.

Although increasingly used, the mechanisms of PVE largely remain unknown. Resection is usually performed 3-6 weeks after PVE, but the exact time optimum remains controversial. There is increasing evidence that PVE not only stimulates growth of the FRL but also increases tumor size because of growth factors and cytokines released in the process of liver regeneration. Furthermore, after unilateral reduction of portal blood flow following PVE, there is a compensatory increase in blood perfusion through the hepatic artery (hepatic arterial buffer response). As liver tumors are mainly fed by arterial blood supply, these mechanisms altogether potentiate local tumor growth after unilateral embolization of the portal vein.

The challenge for future use of PVE is to limit the growth of tumor while inducing a maximum hypertrophy response in the non-embolized liver lobe. Therefore, we devised an animal model in rabbits, in which the rate of tumor growth can be assessed in relation with PVE, resembling the clinical situation. In this rabbit model, PVE is performed using the same methods and imaging protocol used in patients undergoing PVE. The combination of a VX2 liver tumor in this rabbit model allows us in addition, to explore the effects of PVE on tumor kinetics. The aim of our study is, therefore, to determine the extent and kinetics of induced tumor growth after PVE in a rabbit VX2 liver tumor model, along with assessment of the hypertrophy response of the non-embolized liver lobe.

Materials and methods

Animals

The study protocol was approved by the Institutional Animal Ethics Committee of the Academic Medical Center of the University of Amsterdam. Ten female New Zealand White rabbits (Harlan, Charles River, France) with a mean weight of 2987±149g were acclimatized for 2 weeks under standardized laboratory conditions. The VX2 celline was obtained from Utrecht Medical Center (Utrecht, The Netherlands) for tumor implantation. VX2 tumor cell suspension was injected into the thigh muscles of the hind limb of a donor rabbit. Three weeks later, the solid tumor was harvested from the donor rabbit. In the PVE-tumor-
group, four tumor fragments of 0.5x0.5 mm were injected superficially in the subcapsular area of the left medial liver lobe using a 16-gauge angiocatheter. After removal of the angiocatheter, the liver capsule was manually compressed, followed by closure of the abdomen in two layers. Two weeks after implantation of the VX2 carcinoma, the tumor had acquired sufficient mass to be used for the experiments. Anaesthesia for tumor implantation or for PVE/laparotomy procedures, was induced by intramuscular injection of a mixture of nimatek (25.0 mg/kg body weight, Eurovet, Bladel, the Netherlands) and medetomidine (0.2 mg/kg body weight, Orion, Espoo, Finland).

**Experimental design**

The rabbit liver is subdivided into four main lobes: these are the caudal liver lobe and three cranial liver lobes, comprising the left lateral, left medial, and right liver lobes, each supplied by branches of the portal venous system (Figure 1). The cranial liver lobes accounting for 80% of total liver volume, are isolated from the caudal liver lobe (20%), making the rabbit liver suitable for selective occlusion of the portal vein to the cranial liver lobes, thereby inducing a compensatory hyperplasia in the caudal lobe.

Ten New Zealand White rabbits with tumor bearing livers, were divided into two groups: a control tumor group without PVE (control, n=5), and a group undergoing embolization of the portal vein to the cranial liver lobes (PVE, n=5). PVE was performed on day 0 with PVA particles 90-180 μm combined with 300-500 μm particles and 3 or 4 platinum coils, since this has shown to result in the greatest increase in volume of the non-embolized, caudal liver lobe.9,10 Furthermore, the same embolization materials are also successfully used in clinical PVE. Details about the method of PVE in rabbits have been described elsewhere.9,10

Portography was performed with a mobile C-arm Exposcop 8000 (Ziehm Imaging, Nürnberg, Germany) at laparotomy before PVE, directly after PVE and on day 14 to confirm complete occlusion of the embolized portal vein. In the control group, the liver and an access branch to the inferior mesenteric vein were mobilized without embolization, after midline laparotomy. The abdomen was closed in two layers, similar to the PVE-group.

All animals received Buprenorphine (0.03 mg/kg body weight, Reckitt Benckiser Healthcare, Hull, Great Britain) subcutaneously before surgery. Enrofloxacin (0.02 mg/kg body weight, Baytril, Bayer Healthcare, Berlin, Germany) was administered subcutaneously before operation and postoperatively from day 1 till day 3.

**CT volumetry**

A CT-scan was made on the day of tumor implantation, which is 14 days before embolization or laparotomy alone. Tumor growth and hypertrophy of the caudal, non-embolized liver lobe were determined in all groups by CT-volumetry following PVE or laparotomy, and on day 3, 7, 10 and 14, after which the rabbits were sacrificed. Anaesthesia was maintained with medetomidine 0.2 mg/kg and nimatek 25.0 mg/kg. The rabbits were resting in a supine position, followed by an injection of contrast solution (3 mL Visipaque, GE Healthcare, Waukesha, WI) in the ear vein, whereafter 4 mL sterile physiological saline was flushed. A
contrast-enhanced multiphasic CT-scan was performed using a 64-slice CT scan (Brilliance 64-channel, Philips, Eindhoven, The Netherlands) for the arterial phase (15s), portal phase (30s), and venous phase (45s). On each section of the CT scan, the total liver, the caudal liver lobe and the tumor(s) were delineated manually. Volumes of the total liver (TLV), the caudal liver lobe (CLV) and the tumors (TV) were calculated.

Tumor growth rate (TGR) after PVE or laparotomy was calculated by the formula: \( \frac{TV_{dx}}{TV_{d0}} \), in which \( dx=x \) days after the procedure, and \( d0= \) tumor volume two weeks after tumor implantation. The ratio of the caudal, non-embolized liver lobe (\( \%CLV \)), was calculated according to the following formula:

\[
\%CLV = \frac{CLV \times 100}{(TLV-CLV)}
\]

Atrophy was calculated as: \( \% \) cranial liver lobe = \((\text{TLV-CLV})-\text{TV}_{\text{cranial}}\) \( \times 100 / \text{TLV} \)

Wet-to-dry weight ratio

Caudal and left lateral lobe biopsies were weighed after sacrifice (wet weight). The specimens were stored for 4 weeks in a stove at 60°C; hereafter the biopsies were weighed again (dry weight). The wet-to-dry weight ratio was calculated by the formula: \( \text{wet weight – dry weight) \times 100 / \text{wet weight} } \)

Liver to body weight index

The total liver and caudal liver lobes were weighed after sacrifice by means of a precision scale (Sartorius, Göttingen, Germany). The body weight can influence the total liver weight; therefore, caudal liver lobe weights were divided by the body weight.

Mediators of liver regeneration

The cytokines interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-\( \alpha \)), as well as the growth factors hepatic growth factor (HGF), and transforming growth factor beta 1 (TGF-\( \beta \)) were determined in homogenized liver tissue of the left lateral and caudal liver lobe after sacrifice on day 14, using an ELISA kit for the respective antigen (USCN Life, Wuhan, China). All antibodies were washed out 4x with phosphate buffered saline (PBS, 1%BSA). Cytokines were assessed using polyclonal TNF-\( \alpha \) and IL-6 goat anti-rabbit antibodies (USCN Life, Wuhan, China). Measurements were repeated and concentrations were calculated from a standard curve. A BCA Protein Assay kit (Pierce, Rockford, IL) was used for evaluation of protein concentrations. All values were normalized to protein content.

Biochemical assessments

Liver function and damage parameters were assessed by routine clinical chemistry. Blood samples were obtained before tumor implantation, and after two weeks of tumor growth, before PVE or laparotomy. Three hours after the procedure, and on post-operative day 1, 3, 7, 10 and 14 blood samples were also obtained. AST (aspartate aminotransferase),
ALT (alanine aminotransferase), AP (alkaline phosphatase), γGT (gamma-glutamyl transpeptidase), and bilirubin were determined as liver damage parameters. Liver synthesis function was determined by measurements of prothrombin time and albumin.

**Histology**

In all groups, biopsies from the left lateral, embolized lobe and the caudal, non-embolized liver lobe were taken at sacrifice. Tissue samples were routinely fixed in 4% formalin (48 hours) and processed to paraffin tissue blocks. 4 μm sections were cut and stained with haematoxylin and eosin (H&E). H&E slides were blindly evaluated by an experienced liver pathologist.

Steatosis was estimated as the percentage of involved hepatocytes: grade 0 (absent; <5%), grade I (mild; 5-33%), grade II (moderate; 33-66%), or grade III (severe; >66%).

Portal inflammation was arbitrarily graded as follows: 0 (absent), 1 (mild), 2 (moderate), or 3 (severe). Sinusoidal dilation as: 0 (absent), 1 (mild; involving ≤ one-third of the (centro-) lobular area), 2 (moderate; involvement ≤ 2/3 of the parenchyma), or 3 (severe; involving ≥ 2/3 of the liver parenchyma). Intralobular inflammation and lytic necrosis were graded as: 0 (not present), 1 (<2 foci per x10 objective), 2 (2-4 foci per x10 objective), 3 (5-10 foci per x10 objective), and 4 (>10 foci per x10 objective). Portal edema was scored as the percentage of the portal tracts involved: 0 (not present), 1 (<25%), 2 (25-50%), 3 (50-75%), and 4 (>75%). The presence of areas with confluent necrosis of the parenchyma was scored as: 0 (absent), 1 (affecting <25% of the parenchyma), 2 (affecting 25-50% of the parenchyma), 3 (affecting 50-75% of the parenchyma), and 4 (affecting >75% of the parenchyma). The presence or absence of embolization material with concomitant giant cell reaction was also evaluated.

Cytokeratin 8+18 / Ki67 sequential alkaline phosphatase double staining was performed to evaluate hepatocyte proliferation in normal, non-tumorous liver parenchyma of the embolized cranial liver lobes and the non-embolized caudal liver lobe. The double staining combined a general hepatocyte marker cytokeratin 8/18, clone K8.8 + DC10 (Abcam, Cambridge, UK) stained in red (Vector Red, Vector Laboratories, Burlingame, CA, USA) with the proliferation marker Ki67 (mouse anti-rat, clone MIB5) (Dako, Glostrup, Denmark) in blue (Vector Blue) and a weak hematoxylin counterstain. Five multispectral data sets per case were acquired using a Nuance™ camera system (Caliper Life Science, Hopkinton, MA) from 420-720 nm at intervals of 20 nm. To analyze the percentage of Ki67 positive hepatocytes, spectral data sets were analyzed with the segmentation and machine-learning Inform™ 1.2 software (Caliper Life Science) similar to that described by Al-Kofahi et al. This software allows cell-by-cell analysis of all multispectral data sets for the co-expression of cytoplasmic cytokeratin 8/18 and nuclear Ki67 within all hepatocytes, thus excluding the proliferation of leucocytes and other cell types.

**Statistical analysis**

Data are tested for normal distribution, and equal variances. Values are expressed as means ± SD, unless otherwise stated. Differences in tumor volume, and CLV were analyzed by
using a repeated ANOVA model, and the Kruskal-Wallis test as appropriate. Furthermore, ANOVA with the linear mixed model for repeated measurements data was also performed for different liver biochemistry levels. The separate time points were analyzed by means of the two-tailed unpaired Student’s t-test for parametric continuous data. The Mann-Whitney U test was used for non-paramateric data. Histological specimens were evaluated using the Fisher’s Exact test and Chi-square test where appropriate. Since most of the histology scores are ordinal (there is a ranking in the categories), the linear by linear association test was also used, which is identical to the Fisher’s Exact test. Statistical significance was accepted when p<0.05. The data were analyzed by statistical software (SPSS 18.0.0; SPSS, Chicago, Illinois, USA) and GraphPad Prism (Graph-Pad Software, San Diego, CA).

Results

The VX2 cells were successfully implanted into the left medial liver lobes of all rabbits (n=10). Following implantation, the fragments grew as one tumor, which enlarged rapidly. Two weeks after the implantation of the VX2 carcinoma in the liver, the solid tumor was clearly visualized on CT imaging, and could be used for the experiments.

Portal vein embolization

Portograms performed directly after embolization and at sacrifice showed complete occlusion of the portal vein branch to the cranial liver lobes in all PVE-rabbits (n=5; Figure 1).

Hypertrophy response of non-embolized caudal liver lobe

As expected, the volume increase of the caudal liver lobe was significantly higher in the PVE-group, compared to the control-group (Figure 2). An increase in CLV was seen until day 7, and only little additional increase was observed until day 10 and 14. When considering all measurement points, significant differences were found between the PVE- and control-group for the caudal lobes (p<0.001). When the time points were analyzed one-by-one, significant differences were also seen from day 3 until day 14 (p<0.01). Before PVE or laparotomy alone, there were no differences in %CLV between both groups.

In the PVE-group, the rate of Ki67 positive hepatocytes in the caudal liver lobe was higher (median 7.0, range 4.0-10.0%) than in the embolized, cranial liver lobes (median 0.3, range 0.1-1.2%; p=0.037). In the control group, the caudal and cranial liver lobes showed the same amount of proliferating hepatocytes (p=0.606). The rate of Ki67 positive hepatocytes in the caudal, non-embolized liver lobes in the PVE-group was higher (median 7.0, range 4.0-10.0%) compared to the caudal liver lobes of the control group (median 1.8, range 1.4-2.2%; p=0.083) whereas a lower rate of Ki67 positive hepatocytes was found in the atrophic, embolized cranial liver lobes (median 0.3, range 0.1-1.2% vs control: median 1.4, range 0.8-8.2%; p=0.007). The wet-to-dry weight ratios of liver biopsies were not significantly different between the groups (data not shown), excluding the possibility that edema was the cause of the volume increase.
The liver-to-body weight index of the caudal liver lobes at sacrifice were consistent with the CT volumetry data. The mean liver-to-body weight index of the caudal lobe of the PVE-group was 0.0118±0.0018 mL/g versus 0.0042±0.0001 mL/g (p=0.009) in the control group. No significant differences were found between or within both groups as regards the cytokines IL-6 and TNF-α, or the growth factors HGF and TGF-β1 in both cranial and caudal liver lobes (data not shown).

Tumor growth as measured by CT volumetry
Tumor sizes two weeks after implantation (before starting the procedures) were different in both groups, with a mean volume of 2.50±0.5 mL in the control group, compared to a
volume of 0.58±0.1 mL (p=0.009) in the PVE-group. Two weeks after PVE or laparotomy alone, tumor volumes were still larger in the control group (p<0.01). This is not surprising, since tumor growth is usually exponential. Therefore, a larger tumor load at the beginning of the analysis also resulted in a larger tumor load at the end. TGR is therefore, a better marker for tumor growth over time. Tumor growth rate (TGR) data are shown in figure 3. TGR was slightly increased in the first week, and further increased from day 7 until day 14 in both groups, showing significantly higher values in the PVE-group.

Biochemistry

The liver damage parameters AST and ALT were significantly higher in PVE-rabbits, with peak concentrations on day 1, remaining high until day 3, and declining thereafter (Figure 4). When considering all measurement points, no significant differences were seen in AST-levels between the PVE- and control-group (p=0.07), although a significant difference was observed for ALT (p=0.013). When we analyzed the time points separately, the AST values were significantly different as of 3 hours after PVE until day 7, and the ALT levels from day 1 until day 7. There were no differences in synthesis functions of the liver, as assessed by prothrombin time. Albumin was significantly decreased in the PVE group on day 3 (mean albumin PVE-group 39.6±1.1 vs 49.6±1.1g/L; p=0.009), and on day 7 (PVE: 38.4±1.1 vs 49.6±1.1g/L; p=0.009).

Figure 2. Volume increase rate of CLV as measured by CT volumetry, over 14 days after PVE. The mean %CLV is significantly higher in the PVE-group from day 3 until day 14 (*p<0.01), compared to the control group.

Figure 3. Tumor growth rate (TGR) following PVE/control. A larger increase was determined in the PVE-group over time with a mean TGR of 34.4±4.3 on day 14 in the PVE-group vs 24.1±7.2 (p<0.05) in the control group.
46.0±0.8g/L vs control: 48.6±0.4g/L, p=0.007). Hereafter, these values returned to normal. When considering all measurement points, there was no significant difference between the groups for albumin levels (p=0.097). On day 10, bilirubin-levels were higher in the PVE-group than in the control group (median PVE 2.0±0.5 vs control: 1.0±0.0; p=0.014). Over time there was also a significant difference in plasma bilirubin values (p=0.002).

Histology

No steatosis was found in any animal in cranial or caudal liver lobes. Portal and lobular inflammation, focal lytic necrosis, and portal edema were mild in both liver lobes in the two groups (NS, within or between groups). Mild or moderate sinusoidal dilation was seen in both groups in cranial and caudal liver lobes (NS, within or between groups) in terms of extent as reported by others in humans; the width of the sinuses was however, not evaluated. Confluent necrosis of liver parenchyma in both groups was <25% (range 0-50%) for both cranial and caudal liver lobes (NS, within or between groups). A multinucleated giant cell reaction was observed around the portal vessels with the PVA particles in 4 out 5 embolized liver lobes, as evidence of a foreign body reaction.

Figure 4. Liver damage parameters after PVE and control animals. Plasma AST values showed a significant, transient increase with peak concentrations on day 1 following PVE in panel A (*p<0.05). ALT-values were also significantly elevated in the first week after PVE (#p<0.05), and returned to normal after 7 days (panel B).
Discussion

A small-for-size future remnant liver is an important reason why patients are considered unresectable. PVE is a treatment that is performed preoperatively to induce hypertrophy of the non-embolised FRL. Several clinical studies reported an increase in tumor growth after PVE in patients with primary or secondary liver tumors.\textsuperscript{3,5-7,15-17} The concerns that PVE potentially enhances tumor proliferation is confirmed by our study, in which TGR was significantly increased after PVE over time in a rabbit tumor model. However, there are no reports comparing tumor growth in patients with or without PVE with comparable tumor loads, before and after (the time of) PVE. In this study, we aimed to assess tumor progression before and after PVE, although there also is natural growth of tumor with the lapse of time. Therefore, a control group without PVE was included in our study.

Several animal studies reported changes in tumor volumes in embolized or ligated liver lobes as ascribed to upregulated cytokines, transcription factors, and regulatory factors. One of these studies showed that liver metastases in rats were smaller in embolized liver lobes (PVE) but larger in the ligated liver lobes (PVL), compared to sham laparotomy.\textsuperscript{18} Bretagnol et al. reported a decrease in tumor growth in the embolized lobe of rats after PVE.\textsuperscript{19} Another study performed in a mice tumor-model showed that tumor growth had doubled after PVL compared to 70% hepatectomy.\textsuperscript{20} These results are conflicting and furthermore, only models with small animals have been used. The rabbit liver is more suitable for the purpose of these studies, because PVE can be performed easily in a liver tumor model in which the caudal liver lobe accounting for approximately 20% of total liver volume, is isolated from the embolized, cranial liver lobes. Furthermore, CT-scans were used for determination of CT volumetry in rabbits, which resembles the clinical situation. The isolated, caudal liver lobe in the rabbit is easily distinguishable from the rest of the liver on CT-scan, which makes the model more appropriate than rat- or mice-models.

The VX2 celline was used for the experimental model in this study, having several advantages. The VX2 carcinoma is a rapidly growing, hypervascular tumor, mainly vascularized by the hepatic artery, similar as to human liver tumors. The tumor becomes large enough to be evaluated on CT images, and therefore, provides a perfect tumor model for our experiments. Moreover, the tumor is implanted into the subcapsular area of the liver, whereas in most rat and mice tumor models, the tumor cells are injected into the spleen, which may lead to immunological bias. Our model very much resembles the clinical setting of PVE. The cranial liver lobes corresponding to 80% of total liver volume are embolized, while the caudal, non-embolized liver lobe consists of only 20% of the total volume of the liver, which mirrors the situation in patients in whom the FRL is considered small-for-size.

TGR in our study was highest in the second week after PVE, however, this finding is clinically less relevant. In humans, the volume increase rate of the FRL is highest during the first 3 weeks following PVE, after which it plateaus.\textsuperscript{1} Liver resection is often planned from three weeks after PVE for this reason. The hypertrophy response in the rabbit model is
highest at day 7 with only little additional increase by day 10 and 14\(^9\), suggesting that the regenerative process after PVE is more efficient in rabbits.

There are some limitations to this study. CT volumetry was used to measure volumes of the total liver, the non-embolized liver lobe, and tumor. As mentioned, tumors were small two weeks after implantation (between 0.6 and 2.5 mL), which could introduce a bias when delineating the contours on each serial section of CT scans. However, the images were enlarged during delineation, rendering the outcomes more accurate. Furthermore, CT images were analysed by two experienced, independent researchers in a blinded fashion. TGR could however, not be determined before PVE since the tumors were not visible on CT scan on the day of tumor implantation.

As mentioned above, tumor volumes after two weeks of implantation were significantly higher in the control-group compared to PVE-rabbits (2.5±0.5mL vs 0.58±0.1mL, resp.). There are several explanations for this different outcome. Firstly, smaller tumor fragments may have been implanted in the PVE-rabbits, although we tried to avoid implanting different fragment sizes. Secondly, tumor growth could have been more rapid in the two weeks before intervention in the control group, despite the same VX2 celline was used and, laboratory conditions and operations were standardized. We assume that growth of the experimental tumor is exponential, resulting in a larger tumor load at the end when the tumor is also larger at the beginning of the analysis. Tumor sizes were possibly different between groups after initial injection of the tumor fragments. We therefore used TGR as marker for tumor growth, since these outcomes were compared to baseline values, and therefore, were more reliable.

Regarding the pathology results, no major differences were found in terms of steatosis, portal and lobular inflammation, focal lytic necrosis, portal edema, the extent of sinusoidal dilation or confluent necrosis, the latter being absent or mild in both lobes in both groups. The groups might have been too small however, to detect statistically significant differences, although the findings are in agreement with the transient, minimal increase in liver damage parameters found after PVE. In the embolized lobes, a giant cell reaction was found in association with embolization material, but this could not be demonstrated in one animal, probably due to a sampling error since effective embolization of the cranial lobe was evident at imaging. No differences were observed in cytokines or growth factors in the liver parenchyma between the PVE- and control-group at sacrifice. This is probably due to the cytokine responses which peak earlier in rabbits and therefore, were missed in this study. Along the same lines, growth factors released early after PVE, were possibly depleted after 14 days, showing no increase at that time anymore.

In conclusion, TGR was significantly increased over time in the PVE rabbit tumor model, supporting the notion that PVE potentially enhances tumor growth along with regeneration of the non-embolized liver lobe. Therefore, new interventions should be directed to preventing tumor growth after PVE.
Acknowledgement

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References

Chapter 12
Tumor progression after preoperative portal vein embolization

L.T. Hoekstra
K.P. van Lienden
A. Doets
O.R.C. Busch
D.J. Gouma
T.M. van Gulik

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Abstract

Objective: To evaluate tumor growth in a series of patients undergoing liver resection after portal vein embolization (PVE).

Background: The regenerative response after PVE leading to compensatory hypertrophy of the non-embolized liver segments, potentially enhances tumor growth.

Methods: PVE was performed in 28 patients diagnosed with colorectal metastases (CRM) between 2004 and 2011. Tumor volume (TV) was measured by CT volumetry before and after PVE. Tumor growth rate (TGR) was measured by CT volumetry and compared with a non-PVE control group with CRM of whom 30 had two CT-scans preoperatively. Also, newly diagnosed tumors in the future remnant liver (FRL) after PVE and after resection were analyzed.

Results: The median TGR of PVE patients was 0.53 mL/day (IQR 0.02; 1.88) vs 0.09 mL/day (IQR -0.04; 0.40; p=0.03) in non-PVE patients. TGR was 0.15 (IQR -0.52; 0.66) mL/day before PVE, and 0.85 (IQR -0.10; 1.62) mL/day after PVE in the same patients (p=0.03). Seven (25%) patients showed new tumor lesions in the FRL after PVE, of whom three patients (11%) were not resectable. Patients after PVE also showed a higher rate (8/19; 42%) of recurrent metastases in the remnant liver at follow-up compared to non-PVE (1/28; 4%). Survival was significantly better for non-PVE patients with a 3-year survival rate of 77% versus 26% in patients undergoing PVE.

Conclusions: PVE is associated with increased TGR and new tumor in the FRL and recurrent tumor after resection. Short intervals as well as interval chemotherapy between PVE and resection are therefore advised.
Introduction
The only curative treatment for malignant liver tumors is (partial) liver resection. Not all tumors are resectable, in many cases because the future remnant liver (FRL) is too small with a high risk of postoperative liver failure which is the major cause of mortality after extended liver resections, especially in patients with compromised liver, such as cirrhosis, steatosis, cholestasis, fibrosis, or after extensive chemotherapy. Portal vein embolization (PVE) is an accepted method worldwide, to increase the resectability rate of patients with liver tumors by inducing hypertrophy of the non-embolized FRL. Several studies describe the possibility of enhanced tumor growth after PVE as a result of cytokines, growth factors and an increased arterial blood supply, but the exact mechanisms of this phenomenon are still unknown. Growth of tumor may be accelerated, while micrometastases in the non-embolized remnant liver may also develop or progress. The potential boost of tumor proliferation, therefore, creates a dilemma in terms of optimal waiting time until resection. The aim of this study was to examine the consequences of preoperative PVE for tumor growth in a series of patients prepared for resection in our department.

Methods
Study-characteristics
The results of patients with colorectal liver metastases (CRM; n=28) undergoing preoperative PVE (PVE group) from 2004 until 2011 were compared with a series of patients with CRM (n=30) who underwent liver resection without PVE (non-PVE-group), in whom two sequential CT-scans were performed before liver resection. The median follow-up was 6 (IQR 0; 27) months in the PVE-group, and 40 (IQR 26; 52) months in the non-PVE group.

Management policy
The standard diagnostic work-up included a multiphase CT-scan, MR imaging, or dynamic ultrasound of the liver as required. A multidisciplinary team evaluated the imaging studies and came up with a proposal for treatment of patients with CRM. Of all PVE-patients, CT-scans were performed in the portal phase. The volumes of total liver (TLV), tumor (TV) in the embolized liver lobe, and future remnant liver (FRLV) were determined by CT-volumetry in the prePVE and postPVE scans. The percentage of FRL was calculated according to the following formula: FRLV x100/(TLV-TV). Tumor progression was also recorded if presenting in the future remnant liver after PVE. 28 patients with CRM were analyzed in the PVE-group. In all PVE-patients, CT-scans were made before PVE and three weeks later. However, two sequential scans were performed prior to PVE in ten patients. These scans were made to assess tumor response to chemotherapy or to check for new, extra-hepatic disease during therapy. Another reason for an extra CT scan was to perform the CT-volumetry calculations, which can only be determined on our own work-station, indicating that scanning techniques and images were comparable. In these patients, tumor volumes and growth could be
determined before PVE in the same patient group. Follow-up CT scans were made after liver resection to detect recurrent tumor.

In 30 patients of the non-PVE group, CT volumetric data (TLV, TV and FRLV) were assessed in two sequential CT-scans performed before liver resection. Firstly, the volumes were determined, after which the calculations were performed. The results of the volumes measured by CT-volumetry were determined by two independent, experienced investigators, showing no major variations and resulting in reproducible assessments. Calculations were made using established formulas.

To assess tumor volume changes, tumor volumes were determined and the linear tumor growth rate (TGR) per day was calculated by the following formulas:

- For PVE patients: \( \frac{(TV_{\text{after PVE}} - TV_{\text{before PVE}})}{\text{days between scans before surgery}} \) if only one scan was available before PVE, and \( \frac{(TV_{\text{second scan before PVE}} - TV_{\text{first scan before PVE}})}{\text{days between scans before PVE}} \) for patients in whom two sequential scans were performed prior to PVE (n=10)

- For non-PVE patients: \( \frac{(TV_{\text{second scan before surgery}} - TV_{\text{first scan before surgery}})}{\text{days between scans before surgery}} \)

Whereas the abovementioned formulas to calculate tumor growth rate implies a linear growth, tumor growth of CRM is likely exponential. Therefore, we also calculated the exponential TGR (ETGR) for characterization of an exponentially growing tumor, by using the formula \( \text{ETGR} = \ln\left(\frac{TV_2}{TV_1}\right)/(t_2-t_1) \) in which \( TV \)=tumor volume, and \( t \)=time, described as the “specific growth rate” by Mehrara.10

New tumor lesions in the FRL after resection were also reported. Follow-up time was recorded as the period between resection date and the last date of follow-up. Survival was analyzed according to the date of liver resection until the date of death.

**Chemotherapy**

The administered chemotherapy regimens (number of cycles) varied among patients and groups. In most patients, the combination of Oxaliplatin and/or Capecitabine with or without Bevacuzimab was given. Some patients received Capecitabine, Irinotecan, Panitumumab, or Oxaliplatin with 5-Fluorouracil/leucovorin. In view of the large variation, we only took into account the mere fact that patients received chemotherapy or not.

**Statistical analysis**

The data were analyzed by statistical software (SPSS for Windows 18.0; SPSS, Chicago, Illinois, USA) and GraphPad Prism (GraphPad Software, San Diego, CA). The non-parametric Mann Whitney \( U \) test was used for comparing unpaired data that was not normally distributed between the PVE-group and non-PVE group. For parametric, paired data the paired T-test was used. Normally distributed data was described as mean±SEM. The Wilcoxon signed rank test was used for comparisons between pre-PVE and post-PVE in the same patients undergoing PVE (n=10), for paired data that was not normally distributed. The chi-square
test was used for comparing binary data for comparisons across the PVE-group and non-PVE group (unpaired). The Spearman correlation coefficient was calculated for the correlation between tumor growth rate or tumor volume increase and increased FRL, and between number of cyclus of chemotherapy and tumor growth rate or tumor size changes. Survival curves were generated by the Kaplan-Meier method. A p-value of <0.05 was considered statistically significant.

Results

PVE was successfully performed in all patients of the present series, without PVE-related complications. Following PVE, liver resection was carried out in the great majority of patients. Characteristics of patients with and without PVE are shown in table 1.

Tumor volume (TV)

In 28 PVE-patients, a mean TV of 131.4±44.3mL pre-PVE versus 180.0±55.2mL after PVE was seen following an overall time-interval of 51.4±5.4days (p=0.011). In this group, an increase of tumor volume after PVE was found in 23 patients, of whom 13 patients (57%) had received chemotherapy before PVE, whereas five patients showed a decrease in tumor size after embolization, in whom chemotherapy was administered in 4 patients (80%). There was a time-interval of 29.1±5.4 days between the first CT scan and PVE, compared to 22.2±0.7 days between PVE and the second scan (three weeks after PVE). In a subgroup of 10 patients two sequential CT scans were made prior to PVE with a time-interval of 44.2±12.1days between scans. These patients showed a stable TV from 176.3±87.3mL to 179.4±87.2mL (p=0.758) before PVE.

In the non-PVE group (n=30), a mean TV of 153.2±54.9mL was seen on the first scan versus 118.2±36.5mL on the second scan. An increase in tumor volume was found in 19 patients (63%), with a mean time-interval of 107.85±19.15 days between the two scans performed prior to liver resection for all patients (n=30). The decrease in tumor size in 11 patients is probably related to the use of chemotherapy. In patients who received chemotherapy preoperatively in the non-PVE group (n=14), a decrease in tumor volume was seen from 267.5±108.8mL to 158.7±68.1mL (p=0.245). Conversely, patients who had no chemotherapy in the non-PVE group (n=16) showed an increase in tumor volume between the initial and second scans (from 53.3±22.9mL to 82.7±33.8mL), although not significantly different (p=0.099).

Tumor growth rate (TGR)

The TGR of the patients (n=28) who underwent PVE was significantly greater before surgery than that of the non-PVE patients, showing median TGR 0.53 (IQR 0.02; 1.88)mL/day and 0.09 (IQR -0.04; 0.40)mL/day, respectively (p=0.03). No significant differences were seen in patients in whom chemotherapy was administered preoperatively (table 1).
Table 1. Patient characteristics of PVE patients and non-PVE patients with CRM. Values are shown in means±SEM or in n(%). PVE = portal vein embolization

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<td>Resection type</td>
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<td>Right hemihepatectomy</td>
<td>14</td>
<td>6</td>
<td></td>
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<tr>
<td>Left hemihepatectomy</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Right extended hemihepatectomy</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Left extended hemihepatectomy</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Metastectomy</td>
<td>3</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Unresectable</td>
<td>3</td>
<td>0</td>
<td></td>
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<tr>
<td>Tumor volume pre-PVE/resection (mL)</td>
<td>131±44</td>
<td>153±55</td>
<td>ns</td>
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</table>

The median TGR in the ten PVE patients in whom two scans were performed before PVE was 0.15 (IQR -0.52; 0.66)mL/day, which increased to 0.85 (IQR -0.10; 1.62)mL/day after PVE in the same patients (p=0.03). Figure 1 summarizes the results of TGR.

Exponential tumor growth rate (ETGR)

In the PVE-patients, a median ETGR of 0.0061 (IQR 0.0023; 0.0244)ln(ml)/day was found, compared to a median of 0.0040 (IQR -0.0039; 0.0099)ln/ml/day in the non-PVE
group (p=0.269). In the patients undergoing PVE (n=10), the median ETGR was 0.0004 (IQR -0.0073; 0.0143)ln(ml)/day before PVE, and 0.0054 (IQR -0.0090.0186)ln(ml)/day following PVE, showing enhanced tumor proliferation after PVE compared to pre-PVE (p=0.139). These results did not reach statistical significance but are numerically in line with the outcomes of the linear tumor growth rates, showing tumor progression after PVE.

Future remnant liver

The PVE-group (n=28) showed a significant increase in FRL volume after PVE. The mean FRL volume pre-PVE was 480.7±31.9mL versus 716.1±48.7mL post-PVE (p<0.001), which corresponds with a 28.5% FRL prior to PVE and 42.1% after PVE. No significant correlations between tumor volume increase and increased FRL were found (p=0.423).

Seven of 28 PVE-patients (25%) showed new tumor lesions in the FRL three weeks after PVE. Three of these patients (11%) were not deemed resectable after PVE for this reason. When examining the first follow-up imaging after resection, PVE-patients showed a higher proportion (8/19; 42%) of recurrent metastases in the remnant liver as compared to the non-PVE patients (1/28; 4%). The median time-interval between resection and first follow-up imaging was 82 (range 6-297) days in the PVE group and 102 (range 5-762) days in the non-PVE group (p=0.011).

Chemotherapy

Chemotherapy before PVE was administered in 17 out of 28 patients (61%), of whom one patient also received chemotherapy after PVE, before resection. Another patient received chemotherapy only in the time period between PVE and surgery (three cycles). No significant correlations were found between the changes in tumor volume after PVE in patients who received chemotherapy or not preceding PVE (r=0.262, p=0.178). Also no significant correlations were found between TGR and the number of cycles of chemotherapy (p=-0.075, p=0.703). In the non-PVE group (n=30), 14 patients received chemotherapy before PVE. 

Figure 1. Tumor growth rate (TGR) was 0.15 (range -3.79–1.00)ml before portal vein embolization (PVE), and 0.85 (range -1.46–4.67)ml/day after PVE in the same patients (n=10, p=0.08). The median overall TGR of PVE patients (n=28) was 0.53 mL/day (range -4.24–8.00) vs 0.09mL/day (range -5.01–8.74) in non-PVE patients (n=30, p=0.03).
surgery. Again, no significant correlations were seen between tumor size changes or TGR and (cycles of) chemotherapy ($\rho=-0.081$, $p=0.782$ and $\rho=-0.024$, $p=0.935$ respectively).

Survival

We demonstrate a 3-year survival rate of 26% in our series of PVE-patients (figure 2). These patients had otherwise not been resected on the basis of the initial results of CT volumetry. The three patients with CRM (11%) who proved unresectable after PVE survived 5, 10 and 20 months respectively, while palliative chemotherapy was administered. These patients were considered unresectable, due to disease progression. Survival was better for non-PVE patients with a 3-year survival rate of 77% versus 26% in patients undergoing PVE.

Discussion

A schematic overview of the literature results pertinent to PVE and tumor growth is shown in table 2. The literature review suggests that PVE potentially induces tumor proliferation after PVE but there are no solid data to corroborate this notion. An important point is the natural history of tumor growth over time. It has been reported that the mean doubling time of CRM found by the surgeon at laparotomy is $155\pm34$ days, in comparison to $86\pm12$ days for CRM detected by the CT scan post-operatively.$^{11}$ We assessed the outcomes of PVE in our department using a large sample size, with the main focus on tumor volume and growth changes after PVE. Furthermore, we paid special attention to potential tumor development in the future remnant liver after PVE, and the effects of chemotherapy. We showed a significant increase in mean tumor volume after PVE, although this increase cannot be ascribed to PVE alone. A control group was therefore included in this study to compare the outcomes with patients who did not undergo PVE. This is the first study comparing patients with and without PVE in which tumor growth before and after PVE are reported, allowing us to compare clinical tumor progression before and after PVE.
The time period between the scans were different within and between groups, therefore, we calculated the TGR and ETGR per day which are better indicators of tumor proliferation. We found a higher TGR after PVE compared to pre-PVE (0.85 vs 0.15mL/day) in the same patients in our series, which is consistent with the results of Hayashi et al.\textsuperscript{6} Furthermore, our results show that PVE is associated with larger TGR in comparison to patients that do not require preoperative PVE, a similar finding as found in the study of Pamecha et al.\textsuperscript{9}

The effects of PVE on tumor progression are not always clinically relevant since the tumor is commonly located in the part of the liver that will be resected. However, when the tumor is located near the intended resection plane or liver hilum, increase of tumor may become troublesome. Besides that, if PVE also increases tumorigenesis, new tumors may develop in

<table>
<thead>
<tr>
<th>Authors</th>
<th>Patients (n)</th>
<th>Diagnosis (n)</th>
<th>Conclusion</th>
<th>Decrease/Increase</th>
</tr>
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<tr>
<td>Elias et al Br J Surg 1999</td>
<td>PVE (5)</td>
<td>CRM (5)</td>
<td>Increase TV, NEL (n=4), 60-970% Slightly decrease TV, NEL (n=1), -30%</td>
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<td>Azoulay et al Ann Surg 2000</td>
<td>PVE (30) Non-PVE (88)</td>
<td>CRM</td>
<td>10 patients (33%) no resection after PVE because of tumoral extension</td>
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<td>Kokudo et al Hepatology 2001</td>
<td>PVE (18) Non-PVE (29)</td>
<td>CRM</td>
<td>PVE: TV increase EL (n=15): 20.8% NEL+EL (n=3): NEL: 9.7 (0.5-42.1)% EL: 2.8 (2.5-6.3)% TV unchanged EL (n=3, carcinoid)</td>
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<td>Barbaro et al Acta Radiol 2003</td>
<td>PVE (9) Carcinoid (3)</td>
<td>CRM</td>
<td>TV increase EL (n=6, CRM): 84.4 (62.4-562)% TV unchanged EL (n=3)</td>
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<td>Hayashi et al Acta Radiol 2007</td>
<td>PVE (8) HCC (6) CCC (2)</td>
<td>CRM</td>
<td>TGR increase EL: 0.59 (0.22-6.01) to 2.37 (0.29-13.97)cm(^3)/day</td>
<td>Increase TGR EL, HCC</td>
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<td>Ribero et al Br J Surg 2007</td>
<td>PVE (112)</td>
<td>CRM (50) HCC (24) CCC (14) Gallbladder carcinoma (6) Other (18)</td>
<td>TV change (n=80): 5.3 (2.2-12.8) to 5.4 (1.9-15.2) cm 10 patients (8.9%) no resection after PVE because of tumoral extension</td>
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<tr>
<td>Pamecha et al Br J Cancer 2009</td>
<td>PVE (22) Non-PVE (20)</td>
<td>CRM</td>
<td>TGR increase: PVE: mean 0.36±0.7mL/day Non-PVE: mean 0.05±0.3mL/day</td>
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<tr>
<td>Mailey et al J Surg Oncol 2009</td>
<td>PVE (20)</td>
<td>CRA (9) HCC (4) CCC (4) Other (3)</td>
<td>Change in max diameter: Unresected: mean 45±63% Resected: mean -6±27% 8 patients (40%) no resection after PVE because of tumoral extension</td>
<td>Increase TV</td>
</tr>
<tr>
<td>Treska et al Rozhl Chir 2010</td>
<td>PVE (40)</td>
<td>CRM (35) Breast metast (2) Ovarian metast (1) HCC (2)</td>
<td>11 patients (28%) tumoral extension</td>
<td>Unclear</td>
</tr>
</tbody>
</table>

PVE = portal vein embolization; CRM = colorectal metastases; TV = tumor volume; NEL = non-embolized liver lobe; EL = embolized liver lobe; HCC = hepatocellular carcinoma; CCC = cholangiocarcinoma; TGR = tumor growth rate; CRA = colorectal adenocarcinoma.

Table 2. Summary of literature. Numbers are expressed as median values with range, unless otherwise stated.

The time period between the scans were different within and between groups, therefore, we calculated the TGR and ETGR per day which are better indicators of tumor proliferation. We found a higher TGR after PVE compared to pre-PVE (0.85 vs 0.15mL/day) in the same patients in our series, which is consistent with the results of Hayashi et al.\textsuperscript{6} Furthermore, our results show that PVE is associated with larger TGR in comparison to patients that do not require preoperative PVE, a similar finding as found in the study of Pamecha et al.\textsuperscript{9}

The effects of PVE on tumor progression are not always clinically relevant since the tumor is commonly located in the part of the liver that will be resected. However, when the tumor is located near the intended resection plane or liver hilum, increase of tumor may become troublesome. Besides that, if PVE also increases tumorigenesis, new tumors may develop in
the FRL endangering resectability of the patient. We assume that PVE can lead to activation of dormant micrometastases in the FRL, while the presence of microtumors is not detectable by imaging studies prior to PVE or to liver resection. These micrometastases are stimulated to grow by the process of liver regeneration triggered by PVE, comprising both cytokines and growth factors. It remains uncertain whether new tumors in the remnant liver are true new tumors or microtumors that were present but not detectable by imaging studies prior to PVE. Either way, PVE does provide a biological test to identify undetectable lesions before undertaking resection, which otherwise would obviously become apparent in the follow-up after resection (under the influence of post-resectional regeneration). In this regard, these new findings are helpful in that they may prevent a futile liver resection.

In literature, survival outcomes have been reported of patients resected after PVE compared to non-PVE patients. In the study of Wicherts et al, non-PVE-patients had a significantly better survival rate compared to PVE patients, with a 3-year survival rate of 61% and 44%, respectively, outcomes which compare reasonably well with the 3-year survival rates reported in our series, i.e. 77% and 26%, respectively. Remarkably, of the 99 patients who received PVE in the study of Wicherts et al, 32 (32%) patients were not resectable following PVE since tumor had spread (n=27), or an insufficient hypertrophy response had occurred. Of the latter patients, 10 patients survived for one year, 8 patients died within two years and finally, no patients survived longer than 3 years after PVE. In our study, only three patients (10.7%) were not able to undergo resection after PVE, and these patients showed a survival of 10.3±8.4 (range 5-20) months. One of the remaining questions is whether there are other reasons why survival should be different between patients that underwent PVE and non-PVE-patients. Survival could be influenced by chemotherapy, the size of the biggest lesion, the number of lesions, and synchronous or metachronous tumors (table 1). Ideally, independent predictors of poor long-term outcomes are determined by multivariate analysis; however, this was not possible in our study comprising 58 patients in total.

Our study has some limitations. Firstly, a PVE-group was compared with a non-PVE group, although the tumor burden in patients requiring PVE is usually higher and prognosis worse, leading to a bias in selection. Furthermore, the volumes of liver metastases were different between both groups at presentation (i.e., non-PVE: 153.3±54.9, PVE: 131.4±44.3), although not statistically significant (p=0.472). However, this is the first report of a series with a large sample size, primarily focusing on tumor changes in patients after PVE and development of new tumor in the future remnant liver.

Many patients undergo both PVE and chemotherapy. The latter, because of its anti-proliferative effect, may hamper regeneration and influence postoperative complications. In most patients of this study, chemotherapy was administered before PVE. Some studies showed excellent results of the combination of chemotherapy and PVE in relation to the liver hypertrophy response after PVE. Chemotherapy pre-PVE did not impair liver regeneration in response to PVE. Also, survival, morbidity and mortality rates were similar for patients undergoing a two-stage hepatectomy (chemotherapy first, then minor hepatectomy, followed by portal vein ligation or PVE if indicated, and finally major hepatectomy), compared
to a single stage hepatectomy. Several studies favor stopping chemoembolization 6 to 8 weeks before any intervention, such as PVE or liver resection. Key questions are whether chemotherapy administered post-PVE inhibits tumor progression, and which time interval should be observed between cessation of chemotherapy and resection after PVE. These are important issues to be studied in future research. Recently, de Graaf et al compared the increase in FRL function after PVE as measured by dynamic $^{99m}$Tcmebrofenin hepatobiliary scintigraphy, with the increase in FRL volume as measured by CT volumetry. They showed that 23±4.9 days following PVE, the increase in FRL function exceeded the increase in FRL volume. These findings suggest that the recommended waiting time until operation may be shorter than usually indicated by volumetric parameters. Therefore, we assume that a waiting-time of two to three weeks is sufficient between PVE and resection. Furthermore, there seems to be a place for chemotherapy in the waiting time after PVE to control tumor growth. Goéré et al compared 10 patients treated by chemotherapy in the interval between PVE and hepatectomy with 10 patients without chemotherapy. They reported that chemotherapy can be safely continued until liver surgery when the portal vein is embolized without impairment of the hypertrophy of the future remnant volume or the postoperative course after liver resection. In contrast, Beal et al showed that chemotherapy administered in the interval between PVE and liver resection impaired liver hypertrophy. However, the latter authors observed that patients without chemotherapy were more likely to have tumor progression between embolization and liver resection. Concluding from their study, chemotherapy between PVE and hepatectomy did not prevent, but did reduce liver hypertrophy after PVE. Transarterial chemoembolization (TACE) has also been used to prevent tumor progression. The combination of TACE and PVE has a strong anticancer effect, and therefore, has a strong potential to suppress tumor growth after PVE.

Although our results support the evidence from literature that PVE increases tumor growth, further research is required to confirm these findings. Ideally, in a clinical trial, patients would be randomized to undergo liver resection for similar tumor burden to receive preoperative PVE or not. Only one prospective clinical trial has been published in which patients were randomized to undergo PVE or not. The authors concluded that in patients with normal livers, there was no benefit of liver regeneration induced by PVE on postoperative outcomes. A criticism on the latter study design is that only standard right hepatectomies were performed, leaving out the extended right liver resections which are the resections prone to insufficient FRL. A randomized controlled trial is unethical to perform in our opinion, since most patients that require preoperative PVE are unresectable without PVE.

In conclusion, there is evidence that PVE increases tumor growth in both the embolized and non-embolized side of the liver. The beneficial effects of preoperative PVE on FRL volume must therefore be weighed against potential enhancement of tumor growth in the tumor bearing lobe, and induction of new tumor in the FRL after PVE, or recurrent tumor after PVE and resection. We therefore advise short intervals (i.e. 2-3 weeks) between PVE and resection as well as interval chemotherapy.
References


5. Elias D, de BT, Roche A et al. During liver regeneration following right portal embolization the growth rate of liver metastases is more rapid than that of the liver parenchyma. Br J Surg 1999; 86:784-788.


Chapter 13

The outcomes of hepatic artery embolization in a rabbit liver VX2 tumor model

K.P. van Lienden
L.T. Hoekstra
J.D. van Trigt
J. J.T.M. Roelofs
O.M. van Delden
T.M. van Gulik

Abstract

**Background:** The aim of this study is to determine the effects of trans arterial embolization (TAE) on tumor volume and liver regeneration in a rabbit VX2 tumor model.

**Methods:** Twenty-three rabbits underwent subcapsular tumor implantation with a VX2 tumor. Two weeks after implantation, eighteen rabbits were used for TAE experiments, five rabbits were used as a sham group. Tumor response and liver regeneration response of the embolized cranial and non-embolized caudal liver lobes were assessed by CT volumetry, liver to body weight index, and the amount of proliferating hepatocytes.

**Results:** All super selective arterial tumor embolization procedures were performed successfully. Despite embolization, the tumor volume increased after an initial steady state. The tumor volume after embolization, was smaller, compared to the sham group, but this difference was not significant. Massive necrosis of the tumor however, was seen after embolization, without damage of the surrounding liver parenchyma. There was a significant atrophy response of the tumor bearing cranial lobe after super-selective arterial embolization of the tumor with a concomitant hypertrophy response of the non-embolized, caudal lobe. This regeneration response was confirmed histologically by a significantly higher number of proliferating hepatocytes on the Ki-67 stained slides.

**Conclusion:** Super selective, bland arterial coil embolization causes massive necrosis of the tumor, despite increase of volume on CT-volumetry. Atrophy of the tumor bearing liver lobe is seen after arterial embolization of the tumor with a concomitant hypertrophy response of the non-embolized lobe, despite absence of histological damage of the tumor-surrounding liver parenchyma.
Introduction

For patients with primary or secondary hepatic tumors, the treatment deemed most effective is liver resection. However, for this intervention to be considered, a minimum volume of remnant liver is required for sufficient liver function and volume after resection. If the future remnant liver (FRL) is too small, patients are considered to be unresectable. Unfortunately, in patients with malignant liver tumors, less than 15 to 20% are eligible for surgical resection.

Portal vein embolization (PVE), in which either the right or left branch of the portal vein is occluded, is an option to increase the volume of FRL by inducing atrophy of the embolized liver lobe, and consequently, hypertrophy of the contralateral, non-occluded liver lobe.[1] However, there is evidence that after PVE the regenerating liver releases growth factors and cytokines, which also enhances tumor growth.[1-8] In addition, PVE results in a compensatory hyperperfusion of the ipsilateral hepatic artery, which will boost the growth of tumors mainly fed by the hepatic artery, such as hepatocellular carcinomas (HCC) and colorectal liver metastases (CRLM).

Embolizing the hepatic arteries supplying the tumor-bearing liver segments is a conceivable treatment option, alone or in combination with PVE. Transcatheter hepatic artery embolization (TAE), with or without additional chemotherapy, may be offered to patients with primary liver tumors or liver metastases who are unsuitable for resection. Embolizing the tumor-supplying artery will decrease arterial perfusion of the tumor, resulting in selective ischemia of the tumor and possibly, reduction of tumor size.

We explored the effects of an arterial embolization strategy as described above in an animal model in which tumor growth rate can be assessed in relation with TAE. The VX2 liver tumor model in rabbits is used, enabling evaluation of liver regeneration response and tumor changes after TAE. The aim of this study was therefore, to determine the effects of TAE on tumor volume and hypertrophy response in the VX2 liver tumor model in rabbits.

Methods

The Institutional Animal Ethics Committee of the Academic Medical Center of the University of Amsterdam approved this study protocol.

Animals

Twenty-three female New Zealand White rabbits (Harlan, Charles River, France) were acclimatized for 2 weeks under standardized laboratory conditions. This included a temperature-controlled room with a 12-hour light/dark cycle and access to standard chow and water ad libitum. The subjects had a mean weight of 3253±288 grams.
Tumor model

After anesthetization of the rabbit, four tumor fragments of 0.5x0.5 mm each were injected superficially in the subcapsular area of the left-medial liver lobe using a 16-gauge angiocatheter. After the angiocatheter was removed, the liver capsule was manually compressed, and the abdomen was closed in two layers. Five rabbits were used as a control group, 18 rabbits were used for the embolization experiments. Two weeks after implantation, the injected VX2 carcinoma had acquired sufficient mass in the rabbit model to be used for the TAE experiments. Two weeks after TAE, and thus four weeks after tumor implantation, the rabbits were sacrificed.

Anaesthesia and TAE conditions

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, Pharmacemie, Haarlem, the Netherlands). After subcutaneous injection of 0.03mg/ kg buprenorphine (Temgesic, Reckitt Benckiser Healthcare Limited, Hull, Great-Britain) and 0.2mg/kg Enrofloxacin (Baytril, Bayer Healthcare, Berlin, Germany) the rabbit was placed in a supine position. 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.

Transcatheter Hepatic Artery Embolization

Hepatic artery embolizations were performed by an interventional radiologist (KPvL) with over 10 years of experience. The left central auricular artery, located in the center of the left ear, was punctured and canulated with an 18-gauge (18G) catheter (Hospira Venisystems, Lake Forest, IL). A 3fr Renegade microcatheter (Boston Scientific, Place Natick, MA) with a 0.014 inch Transend-ex wire (Boston Scientific, Place Natick, MA) was subsequently introduced into the 18G catheter and under fluoroscopic guidance using a mobile C arm (Oldelft Benelux, Veenendaal, The Netherlands), advanced in a retrograde direction through the external carotid artery and the common carotid artery into the aortic arch and the descending aorta. An aortogram was performed, and the origin of the celiac trunk was located. After canulating the celiac trunk and passing the gastric and gastro-duodenal branches, the common hepatic artery was catheterized and the feeding branches of the tumour were visualized. A super-selective embolization was performed using a single 2 mm straight platinum coil (Boston Scientific, Place Natick, MA) as close to the tumor as possible. Finally, control angiography was performed to assure complete embolization of the target vessels, and to verify flow in the spared right cranial and the caudal hepatic arterial branches. After removing the catheter, the puncture site was manually compressed to avoid bleeding.
Angiography

Using a mobile C-arm Exposcop 8000 (Ziehm Imaging, Nürnberg, Germany), angiography was performed at different time points during the experiments. Preceding TAE, the first angiograph is performed to visualize hepatic arterial anatomy and tumor vascularisation. Immediately after TAE a control angiogram is performed to check the result of complete tumor embolization. Finally, on day 14 after TAE the third angiography was executed to confirm complete occlusion of the hepatic artery.

CT volumetry

Tumor growth was determined by means of CT-volumetry. Using a 64-slice CT scan (Brilliance 64-channel, Philips, Eindhoven, The Netherlands), contrast-enhanced multiphasic CT-scans were made for the arterial (15s), portal (30s), and venous phase (45s) after injection of contrast solution (3 mL Visipaque, GE Healthcare, Waukesha, WI) in an ear vein, which was subsequently flushed with 4 mL sterile physiological saline. On each section of the CT scan, total liver, caudal liver lobe and the tumor(s) were outlined manually after which total liver volume (TLV), caudal liver lobe volume (CLV) and tumor volume (TV) were calculated, respectively. CT-scans were made 14 days before embolization (on the day of tumor implantation), directly following TAE, and on day 3, 7, 10 and 14 after TAE. On day 14 after TAE, after the last CT-volumetry, the rabbits were sacrificed. During CT-scans, the rabbits were anesthetized and positioned in supine position.

Liver / tumor volumes

Tumor growth rate (TGR) after TAE was calculated by means of dividing the calculated tumor volume on x days after TAE (dx) by the calculated tumor volume two weeks after tumor implantation (d0), giving us the following formula: TGR = TV_{dx}/TV_{d0}. The volume ratio of the caudal, non-embolized liver lobe to the total liver volume (%CLV) was calculated by dividing the caudal liver lobe volume by the TLV minus the TV, using the following formula: %CLV = CLV/(TLV-TV) x 100%. In a similar way as %CLV, atrophy of the cranial liver lobe (%CRLV) was calculated with the formula: %CRLV = (TLV-CLV-TV)/TLV x 100%.

Liver to body weight index

After sacrifice, the entire liver and the caudal liver lobes were weighed using a precision scale (Sartorius, Göttingen, Germany). Body weight can influence total liver weight; this is why the caudal liver lobe weight was divided by the body weight.

Biochemical assessments

Liver function and liver damage parameters were assessed in blood samples by routine clinical chemistry. These samples were obtained before tumor implantation, after two weeks of tumor growth, and before TAE. Following TAE, blood samples were taken three hours after the procedure, and on post-TAE day 1, 3, 7, 10 and 14. AST (aspartate aminotransferase), ALT (alanine aminotransferase), AP (alkaline phosphatase), $\gamma$GT
(gamma-glutamyltranspeptidase), and bilirubin were determined to serve as liver damage parameters. Liver synthesis function was determined by measuring plasma prothrombin time and albumin.

Histological examination

Biopsies from the embolized liver lobes and the non-embolized liver lobes were taken at sacrifice. Tissue samples were routinely fixed in 4% formalin (48 hours) and then processed to paraffin tissue blocks. 4μm sections of these blocks were cut and stained with haematoxylin and eosin (H&E). The H&E slides were blindly evaluated by an experienced liver pathologist.

Portal inflammation was indiscriminately graded as follows: 0 (absent), 1 (mild), 2 (moderate), or 3 (severe). Sinusoidal dilation was graded as: 0 (absent), 1 (mild; involving ≤ one-third of the (centro-) lobular area), 2 (moderate; involvement ≤ 2/3 of the parenchyma), or 3 (severe; involving ≥ 2/3 of the liver parenchyma). Portal oedema was scored by determining the percentage of portal tracts involved: 0 (not present), 1 (<25%), 2 (25-50%), 3 (50-75%), and 4 (>75%). The presence of areas with merging necrosis of liver parenchyma was scored as: 0 (absent), 1 (affecting <25% of the parenchyma), 2 (affecting 25-50% of the parenchyma), 3 (affecting 50-75% of the parenchyma), and 4 (affecting >75% of the parenchyma).

For the evaluation of hepatocyte proliferation in normal, non-tumorous liver parenchyma of the embolized cranial liver lobes and the non-embolized caudal liver lobe, staining with the proliferation marker Ki67 was performed (monoclonal mouse anti-rat Ki-67 antigen, clone MIB-5, Dako Cytomation, Glostrup, Denmark). The immunostained sections were counterstained with haematoxylin. The immunostained sections were quantified in 10 fields of view per section (40x magnification) using a Leica CMLB microscope (Leica Microsystems, Wetzlar, GmbH, Rijswijk, the Netherlands) 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 18.0), and GraphPad Prism (GraphPad Software, San Diego, CA). Data are tested for normal distribution, and equal variances. Values are expressed as means ± SEM, unless otherwise stated. Continuous, non-parametric data were compared (TAE versus sham) by the Mann-Whitney U test. The Wilcoxon signed rank test was used for non-parametric continuous data for different time points within groups. Correlation between variables (total/caudal liver volume as measured by CT volumetry and actual liver weight determined at sacrifice) was tested using the Pearson’s r correlation coefficient. Histological specimens were evaluated using the Fisher’s Exact test and Chi-square test where appropriate. Since most of the histology scores are ordinal (there is a ranking in the categories), the linear by linear association test was also used, which is identical to the Fisher’s Exact test. The median of differences between cranial and caudal hepatocyte proliferation, as determined by Ki67
staining, were evaluated using the Wilcoxon signed rank test. Statistical significance was accepted when \( p<0.05 \).

**Results**

**Angiography**

Selective angiography and subsequent embolization of the hepatic artery using a minimally invasive route, succeeded in all procedures. All initial angiographies, performed before the embolization procedure, showed a normal blood supply by the hepatic artery and an arterial hypervascularization of the tumor in the cranial liver lobes. The control angiography, following super selective embolization, showed no flow in the embolized hepatic artery, and normal flow in the caudal artery and the gastro-duodenal artery. Angiograms performed after 14 days, before sacrifice, again showed normal blood flow in the hepatic artery but no flow in the arterial branches feeding the tumor. The tumor was clearly visible on angiography, as a hypo-vascular mass within the normal enhancing parenchyma of the cranial liver lobe.

**Liver regeneration response**

Using CT volumetry, absolute total volume measurements were calculated. The absolute data and % CLV rates are presented in table 1 and 2, respectively. The % CLV before TAE, calculated 14 days after tumor implantation, was 22.7\( \pm \)0.7%. The % CLV increased to 24.4\( \pm \)1.1 \( (p=0.172) \), 25.3\( \pm \)1.3\% \( (p=0.081) \), 25.0\( \pm \)0.8\% \( (p=0.032) \), and 26.6\( \pm \)1.0\% \( (p=0.004) \) on day 3, 7, 10 and 14, respectively. In the sham-group, there were no significant differences over time. When the time points were analyzed one-by-one comparing both groups, differences were observed from day 7 till 14, post-TAE (table 2 and figure 1A). The volume percentage of the cranial liver lobe significantly decreased from 76.6\( \pm \)0.7\% on day 0, to 70.8\( \pm \)1.7\% on day 3, 69.9\( \pm \)2.1\% on day 7, 68.4\( \pm \)2.1\% on day 10, and 64.9\( \pm \)2.5\% on day 14 (figure 1B). The total liver volumes as measured by means of CT volumetry correlated well with liver weight at sacrifice \( (r=0.965, p<0.001) \) (figure 2A). This was also the case for the caudal liver lobe calculated by CT volumetry, which correlated strongly with the actual weight of the caudal liver lobe at sacrifice \( (r=0.905, p<0.001) \) (figure 2B).

<table>
<thead>
<tr>
<th>Measurement (mean±SD)</th>
<th>Pre-tumor</th>
<th>Pre-TAE (day 0)</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume [mL]</td>
<td>84.6±19.9</td>
<td>79.7±13.6</td>
<td>72.4±16.9</td>
<td>79.7±16.0</td>
<td>90.2±20.5</td>
<td>95.7±28.9</td>
</tr>
<tr>
<td>Caudal volume [mL]</td>
<td>16.7±5.0</td>
<td>17.4±3.7</td>
<td>16.1±4.3</td>
<td>17.5±3.9</td>
<td>19.2±4.5</td>
<td>20.8±6.1</td>
</tr>
<tr>
<td>Cranial volume [mL]</td>
<td>67.9±16.4</td>
<td>62.4±11.7</td>
<td>56.4±14.2</td>
<td>62.3±14.8</td>
<td>71.0±17.6</td>
<td>75.0±24.2</td>
</tr>
<tr>
<td>Tumor volume [mL]</td>
<td>0</td>
<td>0.74±0.6</td>
<td>4.66±4.9</td>
<td>4.77±4.7</td>
<td>7.61±6.7</td>
<td>11.30±11.2</td>
</tr>
</tbody>
</table>

**Table 1.** Absolute measurements of CT volumetry data of total, caudal, cranial and tumor volumes before and after TAE.
Tumor response

Tumor volume increased over time in both groups (figure 3A). Considering the separate time points post-embolization, tumor volumes remained stable between day 3 and 7, after which re-growth was seen. The mean volume of the tumor mass after TAE was smaller compared to the sham group, but there was no statistical difference between the groups after 14 days. TGR increased over time in both groups, with a lower trend seen in the TAE-group, although no significant differences were observed between both groups (figure 3B).

Table 2. %CLV (caudal liver volume), %CRLV (cranial liver volume), and TGR (tumor growth rate) before and after TAE

<table>
<thead>
<tr>
<th>CT volumetry</th>
<th>Sham</th>
<th>TAE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 (pre-TAE)</td>
<td>%CLV 18.4±2.0</td>
<td>22.7±0.7</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>%CRLV 79.1±2.0</td>
<td>76.6±0.7</td>
<td>0.380</td>
</tr>
<tr>
<td></td>
<td>TGR 1.00±0.0</td>
<td>1.00±0.0</td>
<td>1.000</td>
</tr>
<tr>
<td>Day 3</td>
<td>%CLV 20.5±2.1</td>
<td>24.4±1.1</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td>%CRLV 71.7±2.1</td>
<td>70.8±1.7</td>
<td>0.941</td>
</tr>
<tr>
<td></td>
<td>TGR 3.4±0.5</td>
<td>7.6±2.7</td>
<td>0.411</td>
</tr>
<tr>
<td>Day 7</td>
<td>%CLV 18.8±2.3</td>
<td>25.3±1.3</td>
<td>0.038*</td>
</tr>
<tr>
<td></td>
<td>%CRLV 64.3±2.2</td>
<td>69.9±2.1</td>
<td>0.092</td>
</tr>
<tr>
<td></td>
<td>TGR 9.1±2.4</td>
<td>7.1±2.1</td>
<td>0.186</td>
</tr>
<tr>
<td>Day 10</td>
<td>%CLV 19.8±1.7</td>
<td>25.0±0.8</td>
<td>0.016*</td>
</tr>
<tr>
<td></td>
<td>%CRLV 56.9±1.9</td>
<td>68.4±2.1</td>
<td>0.009*</td>
</tr>
<tr>
<td></td>
<td>TGR 14.4±3.5</td>
<td>12.0±4.9</td>
<td>0.085</td>
</tr>
<tr>
<td>Day 14</td>
<td>%CLV 20.5±1.8</td>
<td>26.6±1.0</td>
<td>0.016*</td>
</tr>
<tr>
<td></td>
<td>%CRLV 48.8±2.4</td>
<td>64.9±2.5</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>TGR 24.1±7.2</td>
<td>18.3±7.7</td>
<td>0.055</td>
</tr>
</tbody>
</table>

Figure 1. %CLV data for the sham- and TAE-group. Analyzing the separate time-points, TAE showed significant higher CLV-values on day 7 (p=0.038), 10 (p=0.016), and 14 (p=0.016) compared to the sham-group (panel A). Panel B shows a decrease in %cranial liver lobes in the TAE-group.
Biochemistry

There was a transient elevation of plasma AST/ALT levels after TAE (figure 4). Also, GGT, LDH and albumin were shortly significantly increased, with peak concentrations on day 1 and 3, returning to normal within 7 days (data not shown).

Histology

Histopathological evaluation of sections of the tumor demonstrated no signs of inflammation. The tumor showed confluencing areas of necrosis and apoptosis. A capsule of fibrotic tissue was seen around the tumor. Although 60-70% of tumor was necrotic, approximately 30-40% of the tumor still contained viable tumor cells, whereas in the sham group no tumor necrosis was apparent. Sections of parenchyma of the embolized cranial liver lobe

Figure 2. There was a strong and significant correlation between total liver volume (TLV) as measured by CT volumetry and actual liver weight determined at sacrifice ($r=0.965$, $p<0.001$). Additionally, caudal liver volume (CLV) as measured by CT volumetry also correlated well with actual caudal liver lobe weight measured after sacrifice ($r=0.905$, $p<0.001$).

Figure 3. Tumor% (panel A) increased significantly after TAE (*$p<0.05$). %TGR data plotted as a function of time in panel B. TGR increased over time for both groups, without significant differences between groups.
demonstrated normal liver structure without tissue necrosis nor apoptosis, but with minimal portal inflammation. Also, mild sinusoidal dilatation was observed in the cranial liver lobe.

In accordance with the volumetric results as mentioned above, the caudal liver lobe contained a significantly higher number of proliferating hepatocytes in the Ki-67 stained slides, compared to the cranial liver lobe. The median number of proliferating hepatocytes per field of view in the cranial liver lobe was 18 (range 9-106), compared to 24 (range 11-77) in the caudal lobe (p=0.004).

Discussion

For patients with primary or metastatic liver tumor who are unresectable due to insufficient FRL-volume, preoperative PVE is a successful technique to induce hypertrophy of the FRL, and to minimize the risk of extensive liver resection. [9,10,11] In recent years, however, an increasing number of articles have been published showing induction of tumor growth as side effect of PVE. [12,13,14] Therefore, TAE alone or sequentially in combination with PVE has been suggested to induce hypertrophy of the FRL, in the meantime preventing tumor growth. [15,16,17] A similar hypertrophy response has also been described after hepatic arterial radioembolization using yttrium-90. [18] Besides destructing the tumor, the radiation treatment causes intrahepatic parenchymal damage, fibrosis and portal hypertension, generating a regeneration response of the untreated, contralateral lobe.

Our study was limited to assessment of the effects of bland hepatic artery embolization on tumor growth and liver regeneration. The technique of TAE, using a transauricular approach, appeared feasible. Only minor spasm, caused by the microcatheter and guidewire, was seen in the small target vessels, but this did not impede the embolization process. In previous experiments, using rabbits, spill of Polyvinyl Alcohol (PVA) particles was seen in non-targeted arterial branches, causing ischemia of the stomach and duodenum (data not shown). Therefore, the present experiments were conducted using a single 2 mm microcoil, resulting in complete occlusion of the tumor feeding arterial branches without damaging the surrounding liver tissue. Although arterial embolization did cause atrophy of

Figure 4. There was a transient increase in AST/ALT-levels following TAE. Asterisks represent p<0.05.
the tumor bearing cranial liver lobes, normal liver parenchymal structure with minimal portal inflammation and mild sinusoidal dilatation in the cranial lobes was seen on the histological investigation.

Despite arterial embolization, increase of tumor volume was seen on CT volumetry, after an initial steady state. Tumor volume was smaller compared to the sham group but this difference was not significant. On histopathological examination however, massive necrosis and apoptosis of the tumor was seen, whereas tumors in the sham group did not show any necrosis or apoptosis. The percentage of necrosis was probably underestimated, as during sacrifice of the rabbits, necrotic parts of the tumor were not included in the histological sections.

Although histologically undamaged parenchyma was seen in the cranial lobe after embolization of the tumor, a significant hypertrophy response was apparent in the caudal, non-embolized lobe. These observations indicate that arterial embolization of only the hepatic arterial branches supplying the tumor, causes significant tumor necrosis and in addition a hypertrophy response of the non-embolized caudal lobe, in the absence of histological damage of the embolized lobe. The findings at CT-volumetry were confirmed by the increased regeneration response observed using Ki-67 staining of histological sections. This is line with the publication of Vogl et al.[15] who already in 1998 described a 10% volume decrease of the embolized lobe after embolization of the right hepatic artery in patients with an initially unresectable cholangiocarcinoma, and a hypertrophy response of the unaffected left lobe, with a mean increase of 37%. In 2011 Denecke et al. [19] compared TAE with PVE in patients with cholangiocarcinoma and also described a hypertrophy response after arterial embolization. The increase of the FRL-volume, however, was less than that after PVE.

It is believed that the regeneration response after portal vein embolization is not only caused by the hemodynamic changes after occlusion of part of the portal system, but is also set off by upregulation of several humoral mediators (HGF, TNF-α, TGF-α, IL-6, insulin) released after hepatocellular damage, causing a regeneration response that leads to hypertrophy of the non-embolized lobe.[19] Although the exact mechanism is not specifically investigated, it is assumed that the hypertrophy response after arterial embolization is mediated by the same pathways.[20] The fact that PVE has a superior hypertrophy response compared to arterial embolization can be explained by its greater effect on overall hepatic blood perfusion. The question remains however, whether the regeneration response of the caudal lobe is caused only by the damage incurred in the tumor after embolization. On basis of the results of this study, this seems highly suggestive.

Conclusion

The transauricular approach of hepatic artery embolization is a feasible technique in a rabbit VX2 tumor model. Super selective, bland arterial coil embolization causes massive necrosis of the tumor, despite increase of volume on CT-volumetry. Atrophy of the tumor bearing
liver lobe is seen after arterial embolization of the tumor with a concomitant hypertrophy response of the non-embolized lobe, despite absence of histological damage of the tumor-surrounding liver parenchyma.
References

Chapter 14

Summary, conclusions and future perspectives

K.P. van Lienden
Summary and conclusions

This thesis deals with liver regeneration after portal vein embolization (PVE) or portal vein ligation (PVL). Several aspects of these portal vein occlusion techniques have been evaluated in clinical and experimental studies. In addition, the role of dynamic liver function tests and CT-volumetry in risk assessment before major liver resection has been evaluated in several clinical studies.

After a general introduction in chapter 1, a systematic review was presented of literature over the last 20 years (1990-2011) in chapter 2, describing the clinical use of portal vein embolization (PVE). After critical evaluation, 44 publications were finally included for meta-analysis, consisting of 1791 patients with a mean age of 61 years.

Overall technical success rate was 99.3%. The mean hypertrophy rate of the FRL after PVE was 37.9 ± 0.1%. In 70 patients (3.9%), surgery was not performed because of failure of PVE (clinical success rate 96.1%). In 51 patients (2.8%) the hypertrophy response was insufficient to perform liver resection. In the other 19 cases, 12 were not technically successful (0.7%) and in 7, a complication led to unresectability (0.4%). In 6.1% of patients, resection was cancelled because of local tumor progression after PVE. Major complications were seen in 2.5% and the mortality rate was 0.1%. A meta-analysis of the subgroups could not be performed because of the small numbers of articles or the inhomogeneity of the data. A head-to-head comparison showed a negative effect of liver cirrhosis on hypertrophy response. N-butyl cyanoacrylate seemed to result in a greater hypertrophy response compared to other embolization materials used. No difference in regeneration response was seen in patients with a predamaged liver caused by steatosis, cholestasis or chemotherapy.

Chapter 3 described the outcomes of PVE and extensive resection in patients with a predamaged liver. Between January 2005 and July 2011, 56 consecutive patients underwent successful PVE by percutaneous ipsilateral approach. The mean increase of the FRL was 51% (0-305%). Insufficient hypertrophy response precluding surgical resection was seen in only one patient (1.7%). There were no significant differences in hypertrophy response of FRL after PVE between patients with or without chemotherapy (p=0.51), fibrosis/steatosis (p=0.43) and patients with or without cholestasis (p=0.58). There were no significant differences in regeneration three months after liver resection. It was concluded that PVE is a safe and efficient technique to increase the FRL, also in patients with a predamaged liver.

Assessment of the difference in hypertrophy response between portal vein embolization (PVE) and portal vein ligation (PVL) was described in chapter 4 using a standardized rabbit model. The increase of the unaffected caudal lobe was greater after PVE than after PVL (P = .001), with a mean degree of hypertrophy of 20% ± 2%, and 15% ± 4%, respectively. This was confirmed by Ki-67 staining, which showed a significantly greater number of proliferating hepatocytes after PVE (P = .016). This study proved the superiority of PVE over PVL in a standardized rabbit model.

In chapter 5 intrahepatic vascular changes were studied in patients undergoing PVL or PVE. Between December 2008 and October 2011, 7 patients underwent right PVL and
14 patients PVE. CT-volumetry and $^{99m}$Tc-mebrofinin-HBS was performed in all patients before and 3 weeks after portal occlusion. In 18 patients an intra-operative portography was performed to assess perfusion through the occluded portal branches. In all patients after initially successful PVL, reperfused portal veins were seen on CT scan three weeks after portal vein occlusion. This finding was confirmed in all cases using intra-operative portography.

Intrahepatic porto-portal collaterals were identified in all patients in the PVL group and in one patient of the PVE group. Porto-portal collateral flow was seen from segment 4 to segment 5 or 8. The median increase of FRL volume after PVE was 41.6% (range 10-305), and after PVL only 8.1% (range 0-102) (p=0.179). Therefore we concluded that PVE and PVL are both useful methods to induce hypertrophy of the FRL before major liver resection. PVL, however, is less efficient in inducing hypertrophy, compared to PVE. This is most likely caused by the formation of intrahepatic porto-portal neo-collateral vessels through which the ligated portal branches are reperfused despite adequate ligation procedure.

Chapter 6 discussed the influence of permanent versus absorbable embolization materials on hypertrophy response after PVE in a rabbit model. The use of Polidocanol was discontinued because of toxic reactions in 3 rabbits. Gelatin sponge was the only material that was absorbed within 7 days resulting in less hypertrophy of the non-embolized lobe compared to the permanent occluding materials (fibrin glue, polyvinyl alcohol particles with coils (PVAc), n-butylcyanoacrylate (nBCA)). No other mechanism was found to explain the differences in liver regeneration.

For patients who clinically fail PVE because of insufficient hypertrophy response, alternative methods to optimize the hypertrophy response after portal vein embolization (PVE) are desired. In chapter 7 the effect of hepatic vein embolization (HVE) in addition to PVE on the liver hypertrophy response in a standardized rabbit model was assessed. Although histological and additional regenerative changes were seen, HVE in addition to PVE, caused no greater or earlier hypertrophy response than PVE alone. The combination of HVE and PVE may, therefore, have little use in a clinical setting.

The possibility of performing an extended liver resection is dependent on the volume of the remnant liver. Therefore, preoperative risk assessment is very important. In Chapter 8, future remnant liver (FRL)-function assessed by $^{99m}$Tc-mebrofinin hepatobiliary scintigraphy (HBS) was compared with FRL-volume assessed by CT-volumetry, in the prediction of liver failure after major liver resection.

CT-volumetry and $^{99m}$Tc-mebrofinin-HBS were performed prior to major resection in 55 high-risk patients, including 30 patients with parenchymal liver disease. Liver volume was expressed as percentage of total liver volume or as standardized future remnant liver volume. Receiver operating characteristic (ROC) curve analysis was performed to identify a cut-off value for future remnant liver function in predicting postoperative liver failure. Postoperative liver failure occurred in nine patients. A liver function cut-off value of 2.69%/min/m$^2$ was calculated by ROC-curve analysis.

$^{99m}$Tc-mebrofinin-HBS demonstrated better sensitivity, specificity, and positive and negative predictive value compared to FRL-volume. Therefore, the technique is valuable to
estimate the risk of postoperative liver failure, especially in patients with uncertain quality of the liver parenchyma. $^{99m}$Tc-mebrofinin-HBS proved of more value than CT volumetry.

In **chapter 9** the additional value of dynamic $^{99m}$Tc-mebrofinin-HBS single photon emission computed tomography (SPECT) for assessment of the 3D segmental liver function and liver functional volume was evaluated. Preoperative CT volumetry and $^{99m}$Tc-mebrofenin HBS with SPECT were performed in 36 patients undergoing liver resection. In 18 patients postoperative scans were performed within 3 days after operation. Dual-head dynamic acquisitions were used to calculate FRL function using anterior and posterior geometric mean (G-mean) datasets. Total and FRL functional liver volumes were measured by SPECT. Because of the anatomical position of the liver, the anterior projection resulted in an underestimation of FRL function in patients undergoing left hemihepatectomy. In patients with normal liver parenchyma, total functional liver volume was comparable to total liver volume measured by CT volumetry, indicating that $^{99m}$Tc-mebrofinin SPECT is an accurate method to measure hepatic volume. In compromised livers, compared with normal livers, FRL function per cm$^3$ of liver volume was significantly less, and liver function was not distributed homogeneously, with the segments to be resected being relatively more affected. FRL function, measured by a combination of SPECT and dynamic HBS, was able to accurately predict actual postoperative remnant liver function. Therefore the G-mean dataset is recommended for the assessment of hepatic function by dynamic planar $^{99m}$Tc-mebrofenin HBS. The combination of SPECT data with the dynamic uptake function measured by planar HBS provides valuable visible and quantitative information regarding segmental liver function and is an accurate measure for FRL function.

The $^{99m}$Tc-mebrofenin SPECT and CT-volumetry was further evaluated in patients who underwent PVE prior to liver resection in chapter 10. In 24 patients scans were performed before and 3–4 weeks after PVE to measure FRL volume, standardized FRL and FRL function. A hypothetical model was used to assess safe resectability after PVE. The limit for safe resection for FRL function was set at an uptake of 2.69 %/min/m$^2$. For FRL volume and standardized FRL, 25 or 40 per cent of total liver volume was used, depending on the presence of underlying liver disease. After PVE, FRL-function increased significantly more than FRL-volume. The correlation between the increase in FRL volume and FRL function was poor. Using the hypothetical model, 7 patients did not achieve a sufficient increase in FRL-function to allow safe resection 3–4 weeks after PVE, compared with 12 and nine patients based on FRL volume and standardized FRL respectively. In conclusion, the increase in FRL-function after PVE is more pronounced than the increase in FRL-volume, suggesting that the necessary waiting time until resection may be shorter than indicated by volumetric parameters.

An increasing number of publications report on progression of tumorgrowth after PVE, causing unresectability. However, the exact mechanisms are still unknown. **Chapter 11** described an experimental study using a rabbit hepatic VX2-tumor model. Two weeks after subcapsular implantation of a VX2 carcinoma in the cranial liver lobe, New Zealand White rabbits were allocated to a control group or PVE group (n=5/group). Tumor growth rate (TGR) was increased in both groups, with a significantly larger increase in the PVE-group over
time (day 14: mean 34.4±4.3mL/day vs. control: 24.1±7.2mL/day). This was confirmed by a significantly higher hypertrophy response and proliferation rate in the non-embolized liver lobes of the PVE group. This finding supports the notion that PVE potentially enhances tumor growth, along with regeneration of the non-embolized liver lobe.

This same issue has been evaluated clinically in chapter 12, where 28 patients with colorectal liver metastases (CRLM) who underwent PVE were compared to a non-PVE control group. The tumor growth rate (TGR) was higher after PVE and seven patients (25%) showed new tumor lesions in the FRL after PVE. Patients after PVE also showed a higher rate (42%) of recurrent metastases after resection in the remnant liver at follow-up. The survival was significantly better for non-PVE patients with a 3 and 5 year survival rate of 77% and 60%, respectively, versus 26% and 22% in patients undergoing PVE (p< 0.001). Therefore we concluded that PVE is associated with increased TGR, new tumor in the FRL and recurrent tumor after resection. This corresponds with the outcomes of the experimental study described in chapter 11. Short intervals as well as interval chemotherapy between PVE and resection are therefore advised.

As an alternative treatment option, to prevent tumor growth, the effect of hepatic arterial embolization was studied in chapter 13 in a rabbit VX2-tumor model. Super selective, arterial bland coil embolization caused massive necrosis of the tumor, despite increase of tumor volume on CT-volumetry. Atrophy of the tumor bearing liver lobe was seen after arterial embolization of the tumor with a concomitant hypertrophy response of the non-embolized lobe, despite absence of histological damage of the tumor-surrounding liver parenchyma. Although the exact mechanism was not specifically investigated, it is assumed that the hypertrophy response after arterial embolization is mediated by the same pathways as after PVE. The fact that PVE has a superior hypertrophy response compared to arterial embolization can be explained by its greater effect on overall hepatic blood perfusion. The question remains however, whether the regeneration response of the caudal lobe is caused only by the damage incurred in the tumor after embolization. On basis of the results of this study, this seems highly suggestive.
Future perspectives

Despite the fact that gradually more and more between portal vein embolization and liver regeneration has been unravelled, there are still many issues that need to be further explored, such as the underlying mechanisms of the regeneration response following portal vein occlusion, the influence of pre-existing liver disease on induced liver regeneration, the effect of single arterial embolization on the atrophy-hypertrophy response and the use of absorbable embolization materials in PVE.

The latter issue is not only important in oncological liver surgery in patients with uncertain resectability but could also play a role in living related liver transplantation in case of small-for-size left liver lobes. Further experimental and clinical research on this topic is required.

Acceleration of tumor growth after PVE is a major concern. For this reason, optimizing treatment strategies is of extreme importance. Accurate monitoring of FRL-function is important to shorten the waiting time until resection. $^{99m}$Tc-mebrofenin hepatobiliary scintigraphy with SPECT for the assessment of hepatic function and liver functional volume has proven useful for this purpose.

Also, there is room in existing liver augmenting techniques to be modified to induce a greater hypertrophy response in shorter time. On the other hand, more effective techniques must be developed to prevent increased tumor growth. Single hepatic artery embolization (HAE) appears promising in suppressing tumor growth, however, has limited effect on the hypertrophy response. Only limited experiences have been reported on sequential application of HAE and PVE in patients. The optimal time interval between the two procedures is also unknown. Additional clinical and experimental research, therefore, is required to further exploit this combined treatment technique.

Recent reports demonstrate a marked volumetric response after Yttrium-90 radioembolization of the hemi-liver. This heralds a very promising strategy as this treatment combines local radiation therapy of the tumor with induction of a hypertrophy response of the untreated liver. Further investigation, however, is needed to determine the factors that contribute to this effect.
Chapter 15

Samenvatting en conclusie

K.P. van Lienden
Samenvatting en conclusie

In dit proefschrift is het effect beschreven van vena porta embolisatie (VPE) en vena porta ligatie (VPL) op regeneratie van de lever. Verschillende aspecten van de portale occlusie technieken zijn geëvalueerd aan de hand van klinische en experimentele studies. Daarnaast werd de waarde van $^{99m}$Tc-mebrofinine hepatobiliaire scintigrafie in aanvulling op conventionele CT-volumetrie onderzocht bij risico-analyse voor uitgebreide lever resectie. Tevens werd onderzocht wat de waarde van deze techniek was bij de beoordeling van de functionele restlever na VPE, voor lever resectie.

Hoofdstuk 1 is een algemene inleiding en beschrijft tevens het doel van dit proefschrift. De doelstellingen van de afzonderlijke hoofdstukken werden hierin toegelicht.

In hoofdstuk 2 werd een samenvatting gegeven van de literatuur, welke is verschenen in de laatste 20 jaar (1990-2011) over het klinisch gebruik en de resultaten van vena porta embolisatie. Na kritische selectie werden uiteindelijk 44 artikelen gebruikt voor meta-analyse. Het betrof 1791 patiënten met een gemiddelde leeftijd van 61 jaar. Het overall succespercentage bedroeg 99.3%. Het percentage groei van de toekomstige restlever bedroeg gemiddeld 37.9 +/- 0.1 %. Bij 70 (3.9%) patiënten werd de geplande leverresectie niet uitgevoerd vanwege het falen van de VPE. Hiermee komt het klinisch succespercentage op 96.1%. Bij 51 patiënten (2.8%) uit deze groep was er onvoldoende hypertrofie respons ondanks een technisch succesvolle embolisatie en bij 7 patiënten (0.4%) ontstond er een complicatie die tot irresectabiliteit leidde. Bij 6.1% van de patiënten werd de uiteindelijke leverresectie niet verricht vanwege progressie van de tumor.

Een meta-analyse van verschillende subgroepen (pre-existente cirrhose / steatose / cholestase, status na chemotherapie, het type embolisatie-materiaal) bleek niet mogelijk vanwege de inhomogeniciteit van de artikelen in de subgroepen. Een head-to-head vergelijking kon echter wel worden gemaakt.

Pre-existent lever schade door cholestase of chemotherapie, leek geen invloed te hebben op de hypertrofie respons. Cirrhose bleek een negatieve invloed te hebben op de lever regeneratie. Tenslotte leek het gebruik van n-butyl cyanoacrylaat te leiden tot een grotere regeneratie respons dan bij de andere embolisatie materialen. Concluderend bleek dat VPE een effectieve methode is om de toekomstige rest lever te vergroten, met een hoog technisch en klinisch succespercentage.

In hoofdstuk 3 zijn de resultaten beschreven van een retrospectieve studie naar de resultaten van PVE en leverresectie bij patiënten met pre-existent lever schade. In de periode van januari 2005 tot en met juli 2011, zijn 56 achtereenvolgende patiënten succesvol behandeld met PVE via de percutane ipsilaterale methode. De gemiddelde groei van de toekomstige restlever bedroeg 51% (0-305%). Bij één patiënt was er geen groei van de restlever, waardoor leverresectie uiteindelijk niet mogelijk was.

Er werden geen significante verschillen gezien in hypertrofie respons tussen patiënten met en zonder chemotherapie ($p=51$), fibrose / steatose ($p=43$) en patiënten met en zonder
cholestase (p=0.58). Ook werd er drie maanden na leverresectie geen verschil in leverregeneratie gezien tussen de verschillende groepen.

Aan de hand van deze resultaten kon worden geconcludeerd dat VPE een veilige en zeer succesvolle behandeling is, ook bij patiënten met pre-existentie leverafwijkingen.

Het verschil in hypertrofie respons tussen VPE en VPL in een gestandaardiseerd konijnenmodel is beschreven in hoofdstuk 4. Dit experimentele konijnenmodel is in eerdere publicaties reeds uitgebreid beschreven en leent zich uitstekend voor het onderzoeken van hypertrofie respons na portale oclusie technieken. De groei van de caudale, niet-geëmboliseerde, leverkwab was groter na VPE dan na VPL (p=0.001). Dit werd bij histologisch onderzoek bevestigd. De Ki-67 gekleurde preparaten toonde een significant groter aantal prolifererende hepatocyten na VPE (p=0.016). Met deze studie is de superioriteit aangetoond van VPE in het konijnenmodel.

Naar aanleiding van deze resultaten hebben we in hoofdstuk 5 de verschillen tussen PVE en VPL in patiënten onderzocht.

In de periode december 2008 en oktober 2011 hebben 7 patiënten een PVL en 14 patiënten een PVE ondergaan. CT-volumetrie en $^{99m}$Tc-mebrofinine-hepatobiliaire scintigrafie (HBS) zijn verricht, direct voor en drie weken na oclusie van de vena portae. Bij 18 patiënten is ook intra-operatief een portogram gemaakt om informatie te krijgen over eventuele reperfusie van het afgesloten portale systeem.

Bij alle ligatie-patiënten werd op de CT-scan, ondanks succesvolle PVL, reperfusie gezien van het centraal afgesloten rechter portale systeem. Dit werd bevestigd door de intra-operatief verrichte portogrammen. De reperfusie werd veroorzaakt door intrahepatische porto-portale collateralen van segment 4 naar segment 5 of 8. De volume toename van de toekomstige restlever (TRL) was na VPE gemiddeld 41.6% (10-305%) en na VPL gemiddeld 8.1% (0-102%) (p=0.179). Geconcludeerd kon worden dat zowel VPE als VPL hypertrofie van de restlever indiceren, maar dat VPE significant effectiever is. Reperfusie van het afgesloten portale systeem door porto-portale collateralen na VPL, is waarschijnlijk een van de belangrijke oorzaken van de verminderde hypertrofie respons.

Het gebruik van verschillende oplosbare en permanente embolisatiematerialen voor VPE werd vergeleken in een konijnen model in hoofdstuk 6. Het gebruik van Polidocanol werd vroegtijdig gestopt in verband met een toxische reactie bij 3 van de 5 konijnen. Gelfoam was het enige materiaal dat binnen 7 dagen werd geresorbeerd. Dit leidde tot een mindere hypertrofie respons van de niet-geëmboliseerde leverlobben, in vergelijking met de permanente embolisatiematerialen zoals fibrineliem, polyvinylalcohol partikels met coils en n-butyl cyanoacrylaat. Een andere oorzaak voor de verschillen kon niet worden gevonden.

Bij patiënten die ondanks adequate VPE toch te weinig groei van de toekomstige restlever (TRL) hebben moet worden gezocht naar additionele behandelingen, die de hypertrofie verder stimuleren. In hoofdstuk 7 werd het effect van embolisatie van de levervenen (HVE), in combinatie met PVE, geëvalueerd in een gestandaardiseerd konijnenmodel. Dertig konijnen werden onderverdeeld in drie groepen: PVE, HVE en combinatie van beide technieken. HVE alleen veroorzaakte helemaal geen hypertrofie respons.
De combinatie van VPE met HVE resulteerde niet in een grotere hypertrofie respons, dan na VPE alleen. Er is dus geen bewijs, dat deze behandeling bij patiënten nut zal hebben.

In hoofdstuk 8 werd de betrouwbaarheid van de $^{99m}$Tc-mebrofinine hepato-biliaire scintigraphy (HBS) onderzocht bij het voorspellen van leverfalen na leverresectie.

CT-volumetrie en $^{99m}$Tc-mebrofinine HBS werd verricht bij 55 patiënten, waaronder 30 patiënten met een pre-existent lever afwijking. Door middel van ROC curve analyse kon een afkapwaarde worden berekend voor de restlever functie als voorspellende waarde voor postoperatief leverfalen. Er werd een afkapwaarde gevonden van 2.69%/min/m$^2$. $^{99m}$Tc-mebrofinine HBS bleek een betere sensitiviteit, specificiteit en positieve en negatieve voorspellende waarde te hebben in vergelijking met CT volumetrie. Geconcludeerd kon worden dat $^{99m}$Tc-mebrofinine HBS een waardevolle methode is bij het evalueren van de restlever functie en een belangrijke bijdrage levert aan de preoperatieve risico analyse bij patiënten waarvan de kwaliteit van het leverparenchym voor de operatie onbekend is.

In hoofdstuk 9 werd de toegevoegde waarde van $^{99m}$Tc-mebrofinine SPECT (Single Photon Emission Computed Tomography) beschreven voor het bepalen van segmentele leverfunctie en het meten van het functionele levervolume. Dit is mogelijk door een combinatie van de functionele informatie van de HBS te combineren met de additionele 3-dimensionale informatie van de CT scan. Dubbelkops gamma camera’s met twee detectoren kunnen gelijktijdig data verzamelen van de anterieure en posterieure projectie van de lever.

Geometrische gemiddelden (Gmean) kunnen worden berekend zodat over- of onderschatting van de leverfunctie, door verzwakking van straling door weefsel, vermindert.

$^{99m}$Tc-mebrofinine SPECT werd uitgevoerd bij 36 patiënten voorafgaande aan leverresectie en bij 18 patiënten, 3 dagen postoperatief.

Aanbevolen wordt om de Gmean data set te gebruiken bij de dynamische $^{99m}$Tc-mebrofinine HBS voor het bepalen van het functionele volume van de toekomstige restlever. Bij patiënten met pre-existent leverafwijkingen bleek er per milliliter TRL-volume minder functie dan bij patiënten met een normale lever. Tevens bleek de voorspelde TRL-functie goed overeen te komen met de postoperatieve gemeten restlever functie.

$^{99m}$Tc-mebrofinine SPECT is daarmee een waardevolle, betrouwbare methode om volumetrische data te combineren met functionele data en daarmee de functie van de TRL te bepalen.

In hoofdstuk 10 werd toename van TRL-volume bij CT volumetrie vergeleken met de TRL-functie bij $^{99m}$Tc-mebrofinine SPECT, geëvalueerd bij 24 patiënten, voor en 3 weken na VPE. Er werd een hypothetisch model opgesteld waarin criteria voor een veilige leverresectie werden gedefinieerd. Als grens werd een minimale uptake van 2.69 %/min/m$^2$ aangehouden bij functionele TRL en 25 of 40% van het totale lever volume bij volumetrische TRL, afhankelijk van de aan- of afwezigheid van pre-existent leverparenchymafwijkingen.

Na VPE werd een significant grotere toename gezien van functionele TRL ten opzichte van het TRL-volume. Er werd echter geen correlatie gevonden tussen toename van TRL-functie en TRL-volume. Gebruik makend van het hypothetisch model, bleek 3 weken na VPE, bij 7 patiënten onvoldoende TRL-functie en bij 12 patiënten onvoldoende TRL-volume, om
een veilige resectie mogelijk te maken. Dit toont aan dat toename van de TRL-functie meer uitgesproken is dan toename van TRL-volume. Deze uitkomsten suggereren dat op basis van de grotere toename van de TRL-functie ten opzichte van TRL-volume, de wachttijd tussen VPE en leverresectie mogelijk zou kunnen worden verkort.

In een toenemend aantal publicaties wordt toename van tumor groei na VPE beschreven, welke uiteindelijk kan leiden tot irresectabiliteit. De literatuur is echter niet eenduidig over dit probleem. In hoofdstuk 11 werd de invloed van VPE op tumor groei onderzocht in een levertumor model in konijnen. Hiervoor werd in 10 New Zealand White konijnen tumorcellen geïmplanteerd, subcapsulair in de craniale leverlob. Twee weken na implantatie werden de konijnen onderverdeeld in een controle groep (geen VPE) en een VPE groep. Alle VPE procedures waren succesvol uitgevoerd, waarbij ook bij controle na 14 dagen geen flow in geëmboliseerde segmenten werd gezien.

In beide groepen werd evident toename van het tumorvolume, maar de Tumor Growth Rate (TGR) was significant groter in de VPE groep (op dag 14 bij de VPE groep : gemiddeld 34.4 ± 4.3 ml/dag t.o.v. de controle groep : 24.1 ± 7.2 ml/dag). Deze uitkomst ondersteunt de hypothese dat VPE tumorgroei induceert.

In aansluiting hierop werd in hoofdstuk 12 de tumorgroei geëvalueerd bij patiënten met colorectale levermetastasen (CRLM) na VPE. In de periode 2004 - 2011 werd bij 28 patiënten een VPE verricht. Deze patiënten werden vergeleken met een controle groep van 30 patiënten met CRLM, in dezelfde periode, zonder VPE.

Er was een significante toename van de tumorgroei na VPE. Ook de gemiddelde TGR was significant groter na VPE (0.53 ml/dag) in vergelijking met de controle groep (0.09 ml/dag).

Bij zeven patiënten (25%) werden nieuwe metastasen gevonden, waardoor drie patiënten (10.7%) irresectabel werden na VPE. De overleving na resectie was significant beter voor de niet-VPE patiënten met een 3- en 5-jaars overleving van respectievelijk 77% en 60% versus 26% en 22% voor VPE patiënten (p<0.001).

Geconcludeerd kan worden dat VPE niet alleen leverregeneratie stimuleert, maar ook de groei van mogelijk nieuwe tumor in de TRL, en recidieven na resectie. Daarom wordt verkorting van de wachttijd tussen VPE en resectie en het continueren van chemotherapie gedurende de wachttijd geadviseerd.

Om eventuele tumorgroei tegen te gaan, is in hoofdstuk 13 het effect van embolisatie van de arteria hepatica in het eerder beschreven VX2-tumormodel in konijnen geëvalueerd. Hiervoor werd bij 18 konijnen een superselectieve coil embolisatie uitgevoerd van tumor voedende arterie takken in de craniale leverkwab. De uitkomsten werden vergeleken met die van een groep van 5 controle konijnen zonder embolisatie. Ondanks groei van het tumorvolume op CT-scan, werd na embolisatie massale necrose van de tumor gezien. Dit werd ook histologisch bevestigd. Daarnaast werd significante atrofie gezien van de geëmboliseerde craniale leverlob ondanks afwezigheid van histologische schade van het leverparenchym, in combinatie met een hypertrofie respons van de caudale niet-geëmboliseerde leverlob.
Mogelijk wordt de atrofie / hypertrofie respons via het zelfde mechanisme geïnduceerd als bij VPE. Het is zeer opmerkelijk dat het erop lijkt dat schade toegebracht aan alleen het tumorweefsel ook in staat is een hypertrofie response te veroorzaken in de niet-geëmboliseerde leverkwab.

Toekomstig onderzoek

De afgelopen jaren is veel energie gestoken in het ontrafelen van de onderliggende leverregeneratie mechanismen na portale occlusie technieken. Er zijn echter nog een aantal belangrijke aandachtspunten, binnen deze behandelingstechniek, die verder onderzoek behoeven.

Het gebruik van oplosbare embolisatiematerialen kan van essentieel belang zijn bij patienten met een hilair cholangiocarcinoom. Deze groep patienten heeft meestal een uitgebreide lever resectie nodig om gecureerd te worden, maar resectabiliteit is preoperatief vaak onzeker. Bij permanente portale occlusie en uiteindelijk gebleken irresectabiliteit, kan de combinatie met intrahepatische galstuwing leiden tot complicaties zoals abcesvorming en levernecrose. Uit de huidige literatuur blijkt vooral een inferieure hypertrofie respons en een onvoorspelbare resorptie termijn. Verdere ontwikkeling van resorbeerbare embolisatiematerialen, die wel voldoende leverregeneratie genereren en na enkele weken volledig zijn opgelost zouden dergelijke complicaties bij deze specifieke patiëntengroep kunnen voorkomen. Tevens zou deze modificatie op de bestaande VPE-techniek een rol kunnen spelen bij living-related levertransplantaties in geval van een relatief kleine linker leverkwab bij de donor.

Daarnaast is de door VPE geïnduceerde tumorgroei een grote zorg. Bij het beperken van de tumorgroei is het optimaliseren van de behandelingstrategieën van groot belang.

Allereerst is het nauwkeurig monitoren van de functionele restlever na VPE van belang bij het verkorten van de wachtijd voor resectie en daarmee het verkleinen van de kans op irresectabiliteit door tumorgroei. 99mTc-mebrofenine hepatobiliaire scintigrafie met SPECT kan daar een belangrijke rol bij spelen.

Daarnaast zal meer onderzoek moet gedaan worden naar de ontwikkeling van modificaties van de porta embolisatie techniek, zodat in kortere tijd meer hypertrofie kan worden bewerkstelligd. Dit in combinatie met technieken waarin tumor groei wordt geremd. Selectieve embolisatie van de tumorvoedende arteria hepatica lijkt veel belovend, maar geeft op zich zelf te weinig hypertrofie respons. Een oplossing hiervoor zou sequentiële embolisatie van de arteria hepatica en vena portae kunnen zijn. Met deze combinatie behandeling is nog maar zeer beperkte ervaring zodat langere termijn resultaten ontbreken.

Kennis omtrent de volgorde van embolisatie en het optimale tijdsinterval tussen de twee procedures ontbreekt eveneens. Uitgebreid experimenteel en klinisch onderzoek is dus nodig om deze combinatie techniek verder te ontwikkelen.

Tenslotte wordt in recente publicaties gewezen op het volumetrische hypertrofie respons na Yttrium-90 radio-embolisatie van rechter lever. Locale radiotherapie zou niet
alleen de tumor behandelen maar ook een hypertrofie respons veroorzaken van de niet behandelde leverhelft. Ook hier zal verder experimenteel en klinisch onderzoek nodig zijn om onderliggende mechanismen en toepasbaarheid verder uit te zoeken.
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List of publications

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Dankwoord
Dankwoord

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Curriculum Vitae
Curriculum Vitae


Vanaf 2006 is hij intensief betrokken geraakt bij wetenschappelijk onderzoek op de afdeling experimentele heelkunde van het AMC, o.l.v. prof. dr. T.M. van Gulik. Met name bij het ontwikkelen van een konijnen proefdiermodel voor studies op gebied van portale embolisatie en de effecten hiervan op leverregeneratie. Zijn ervaring met vena portae embolisatie bij patiënten en expertise in het gebruik van microcatheters en embolisatiematerialen waren van belang bij deze onderzoekslijn. Na een bijdrage te hebben geleverd aan de promotie van drie voorgangers ontstond de mogelijkheid en de wens om zelf ook op dit onderwerp te promoveren, hetgeen uiteindelijk heeft geleid tot dit proefschrift.