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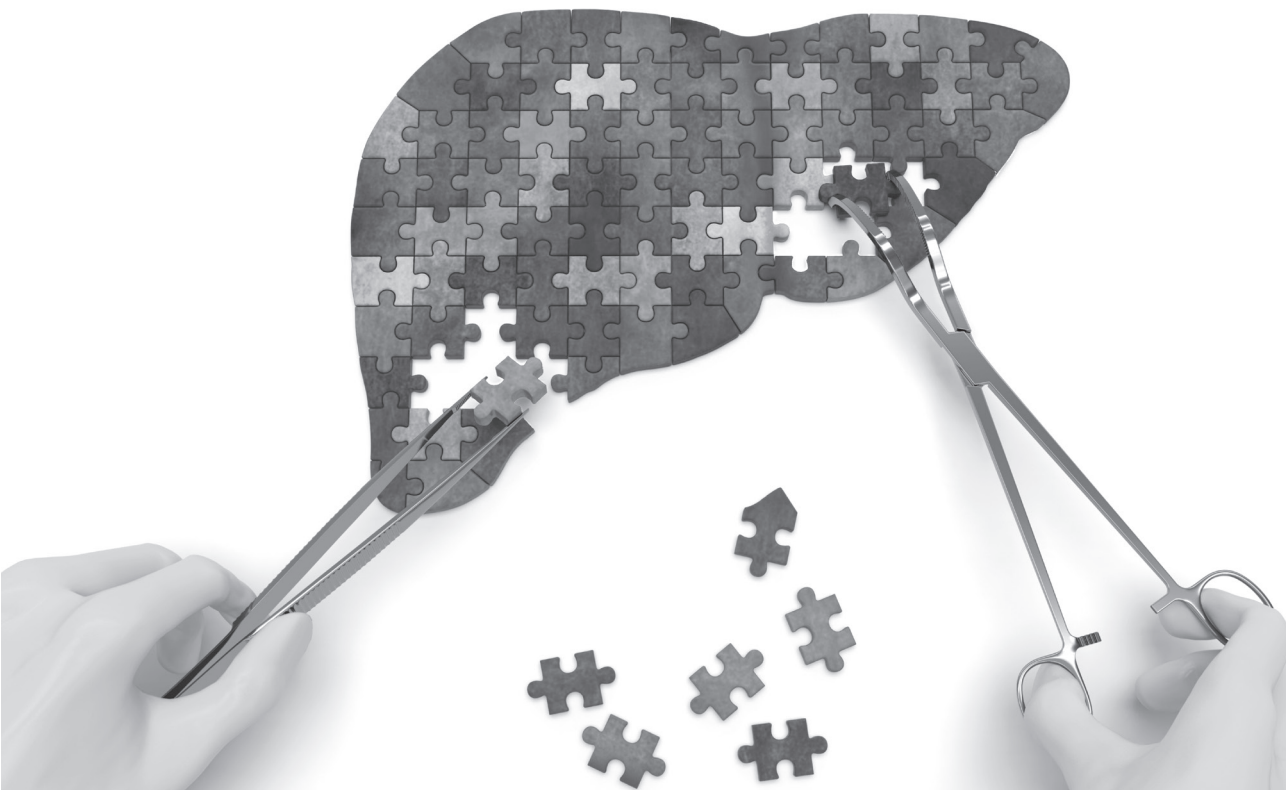
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CHAPTER 16

Hepatic parenchymal transection increases liver volume but not function after portal vein embolization in rabbits

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ABSTRACT

Background: Associating liver partition with portal vein ligation for staged hepatectomy (ALPPS) induces more extensive liver hypertrophy than ligation alone. However, the mechanisms underlying the accelerated liver regrowth and the functional quality of the hypertrophic liver are presently elusive. This study therefore investigated the effect of parenchymal transection on liver volume and function following portal vein embolization (PVE) in a standardized rabbit model.

Methods: Twelve rabbits were subjected to PVE of the cranial liver lobes and randomized between parenchymal transection of the left lateral liver lobe versus no transection (PVE-only). Liver volume of the non-embolized liver lobe was assessed using CT-volumetry and liver uptake function was determined by ^{99m}Tc -mebrofenin hepatobiliary scintigraphy before and 3 and 7 days after PVE.

Results: The increase in non-embolized liver volume 3 days after PVE was 2.7-fold greater in the transected group compared to the PVE-only group ($56\pm 16\%$ versus $21\pm 12\%$, respectively, $P<0.01$) and 1.7-fold greater 7 days after PVE ($113\pm 34\%$ versus $68\pm 24\%$, $P<0.01$). Liver uptake function did not differ between groups before PVE ($8.4\pm 3.7\%/min$ in the transection group versus $8.9\pm 1.6\%/min$), on day 3 ($33.2\pm 4.7\%$ after transection versus $30.3\pm 4.6\%/min$, respectively) and day 7 after PVE ($42.6\pm 8.4\%$ versus $39.1\pm 5.3\%/min$, respectively).

Conclusions: Parenchymal transection after PVE increases liver growth in terms of volume but not function. These results indicate that the rapid volume increase observed after ALPPS does not coincide with the clinically more relevant functional increase. Quantitative liver function tests might be essential in ALPPS to better assess the hypertrophy response and improve clinical decision-making.

INTRODUCTION

Portal vein embolization (PVE) is the gold standard procedure to preoperatively enhance the future liver remnant (FLR) in patients scheduled for major liver resection.^{1, 2} Recently, associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) was introduced, which induces rapid hypertrophy of the FLR, thereby allowing more extended liver resections in a shorter period of time.³ The hypertrophy induced by ALPPS is greater and faster compared to other conventional techniques such as PVE² or 'standard' two-stage hepatectomy.⁴

However, ALPPS is associated with substantial morbidity and mortality, which led to controversy in literature regarding its safety and its indications.^{5, 6} Although mortality has declined from the initially reported 12% to 9% in major series,^{7, 8} morbidity remains substantial and postoperative liver failure is the most common cause of death after resection despite the rapid hypertrophy.^{8, 9} Interestingly, the hypertrophied liver volume did not correlate to mortality in 320 patients collected from the ALPPS registry.⁴ This observation called into question the accuracy and relevance of liver volume assessment as the main parameter to time the second stage. Indeed, histological assessment of the hypertrophied FLR demonstrated that hepatocytes were smaller and more immature in patients after ALPPS compared to PVE.¹⁰ These preliminary results suggest that the observed liver volume increase after ALPPS might not reflect a proportional increase in liver function.

Several hypotheses have been postulated to explain the increased hypertrophy seen in ALPPS. These include the added inflammatory response of the parenchymal transection to the portal occlusion and the more complete portal occlusion by preventing collateral perfusion by the parenchymal division.¹¹ Several animal models have been developed to study the hypertrophy response induced by ALPPS, mostly in small rodents.¹²⁻¹⁶ However, these models likely lack translational value due to discrepancies in the observed response versus the response in humans. In rats, extensive necrosis of the ligated liver lobes is observed¹² and the increase in liver weight gain of ALPPS over PVL is less pronounced compared to humans.^{13, 15}

This study aimed to examine the effect of parenchymal transection on both liver volume and function following PVE in a standardized rabbit model, using ^{99m}Tc hepatobiliary scintigraphy for functional assessment. The main findings were that the addition of parenchymal transection to PVE indeed induced rapid hypertrophy. However, liver function was not increased by parenchymal transection and was similar to PVE alone. It was concluded that rapid hypertrophy leads to immature liver tissue that may not sustain patients after

resection despite the impressive volume increase. Due to the important clinical implications of the data, ALPPS should be used with caution and with functional monitoring of the FLR.

MATERIALS AND METHODS

Animals

Twelve New Zealand White rabbits (Female, mean \pm SD weight of $2,887 \pm 231$ kg) were obtained from Charles River (Saint-Germain-sur-l'Arbresle, France). Animals were housed individually with ad libitum access to water and standard chow in a temperature controlled room with a 12-h dark-light cycle. Rabbits were allowed to acclimatize for at least 7 days before inclusion in the experiments. All experimental protocols were approved by the Animal Ethics and Welfare committee of the Academic Medical Center (BEX35). Experiments were reported in accordance with the ARRIVE guidelines.

Experimental design

Rabbits were randomized in two groups of six animals. Six were planned for PVE of the cranial liver lobes (PVE group) and 6 rabbits underwent PVE of the cranial liver lobes combined with partial transection of the left lateral liver lobe (PVE with transection group). Primary outcome was regional hepatic mebrofenin uptake measured using ^{99m}Tc -mebrofenin hepatobiliary scintigraphy (HBS)¹⁷ and increase in liver volume measured on contrast enhanced CT images.¹⁸ Both scans were performed the day before PVE, as well as 3 and 7 days after PVE. All rabbits were sacrificed 7 days after PVE.

Portal vein embolization

PVE of the cranial liver lobes was performed as described previously.¹⁸⁻²⁰ In brief, rabbits were anesthetized by subcutaneous (s.c.) injection with ketamine (25 mg/kg, Nimatek, Eurovet, Bladel, the Netherlands) and medetomidine (0.2 mg/kg, Dexdomitor, Orian, Espoo, Finland) and maintained under anesthesia with isoflurane (2%, Forene, Abbott, Kent, United Kingdom). Analgesic care was given by s.c. injection of buprenorphine (0.03 mg/kg, Temgesic, Reckitt Benckiser, Hull, United Kingdom). Baytril (0.2mg/kg s.c., Bayer, Berlin, Germany) was administered daily; starting at PVE and continued for 3 days.

A branch of the inferior mesenteric vein was used to catheterize the portal system with an 18G catheter. Using a microcatheter (Renegade 3F, Boston Scientific, Natick, MA) and 0.36-mm diameter and 182-cm long guidewire (Transend-ex, Boston Scientific), the cranial lobes were embolized with polyvinyl alcohol particles (PVA 300-500 μm , Cook, Bloomington, IN) and platinum fibered coils (5 and 4 mm, Boston Scientific) under radiographic control. Complete embolization was confirmed by portography in each animal.

Parenchymal transection

In rabbits, the cranial liver lobes are almost completely separated from the caudal liver lobe. Therefore, collateral circulation between the caudal liver lobe and cranial liver lobe is unlikely, especially after PVE of the cranial liver lobes. In order to investigate the effects of combined PVE and parenchymal transection, the left lateral liver lobe was partially transected for 25 to 30 mm in the middle of the lobe using diathermic dissection (**Figure 1**). The approach was chosen to preserve arterial perfusion of the liver, which might be compromised with complete transection. Although the used set-up might not mimic all aspects of the ALPPS procedure, the complete deportalization of the cranial liver lobes with PVE compared to PVE combined with transection allows focused analysis of the added effect of the parenchymal transection on the hypertrophic response, and closely resembles the mini-ALPPS procedure recently developed by the group of de Santibanes et al.²¹

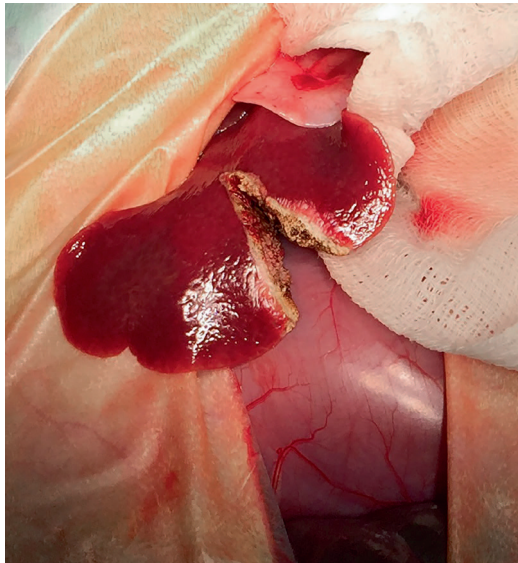


Figure 1: Parenchymal transection of the left lateral liver lobe after portal vein embolization using diathermic dissection.

Hepatobiliary scintigraphy and computed tomography

For HBS, rabbits were anesthetized as described above and a catheter was placed in the lateral ear vein. Rabbits were positioned supine on the imaging table with a 2-cm high foam block positioned under the thorax, in order to prevent overprojection of the cranial and caudal liver lobes on anterior and posterior views, which avoids the need for single photon emission computed tomography (SPECT) imaging. The position was optimized in pilot scans (data not shown). Rabbits were injected with 50mBq ^{99m}Tc-mebrofenin (Bridatec,

GE Healthcare, Little Chalfont, United Kingdom) and acquisition was started for 5min with a capture every 5s using a large field of view SPECT-CT camera (Siemens Symbia T16, Erlangen, Germany). The geometric mean of anterior and posterior camera capture was used for analysis and regions of interest were drawn around the left ventricle, entire liver, and caudal liver lobe. Hepatic ^{99m}Tc -mebrofenin uptake rate was calculated over 120s and corrected for perfusion. Total hepatic ^{99m}Tc -mebrofenin uptake rate was defined as total liver function (TLF) and expressed as %/min. The fractional uptake of the caudal liver lobe was calculated based on the segmental activity and defined as the caudal liver function (CLF). The same was done for the cranial liver lobe which was defined as cranial liver function (CrLF) and both CLF and CrLF were expressed as %/min.

After HBS, rabbits were subjected to contrast enhanced CT scanning. Following injection of 3mL contrast (Visipaque, GE Healthcare, Waukesha, WI) CT images from the diaphragm to the pelvis were captured with a delay of 18.2s for portal phase images. The CT protocol was optimized for rabbits based on pilot scans (data not shown). Total liver volume and caudal liver volume was calculated on 5-mm transversal reconstructions (MX VIEW, Philips, Eindhoven, the Netherlands). Increase in Caudal liver volume was calculated using the formula:

$$\text{Increase in CLV}_{day\ x} (\%) = \left(\frac{\text{CLV}_{day\ x} - \text{CLV}_{baseline}}{\text{CLV}_{baseline}} \right) \times 100\%$$

Clinical chemistry

Blood samples were obtained 7 and 1 day before PVE, 3h after, and 1, 3, and 7 days after PVE. Heparin anticoagulated samples were centrifuged at 3000 RPM for 10 min to obtain plasma, which was snap-frozen in liquid nitrogen and stored at -80 C until analysis. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed by the department of clinical chemistry.

Histology

In the PVE group, left lateral cranial liver lobe sections were obtained. For the PVE with transection group sections were obtained from the left lateral lobe adjacent and distant to the transection. In both group sections of the caudal liver lobe sections were obtained and all were fixed in % v/v buffered formalin solution. Sections were subsequently dehydrated and embedded in paraffin. Next, 0.2- μm sections were cut and stained with standard hematoxylin and eosin (H&E) or stained with for Ki67 as described previously.^{22, 23} H&E sections were scored in de semi-quantitative manner according to table S1 by an experienced hepatopathologist (JV) blinded to group allocation and Ki67 positive hepatocytes were counted on high power fields in the most proliferative region.

Cytokine determination

Plasma tumor necrosis factor alfa (TNF α) and interleukin-6 (IL-6) were determined using enzyme linked assay (ELISA, product numbers DY5670 and DY7984, RnD systems, Minneapolis, MN). Assays were performed according to manufacturer's guidelines on 100 μ L heparin-anticoagulated plasma and in addition in liver homogenates and corrected for protein content.

Total bile acids determination

Bile acids concentrations were quantified in heparin anticoagulated plasma samples using a Total Bile Acids Assay Kit (Diazyme, Poway, CA) according to the manufacturers' instructions.

Statistical analysis

All continuous variables that followed a normal distribution were displayed as mean with standard deviation (SD) and variables with a non-normal distribution as median with interquartile-range (IQR). Differences between continuous variables were analyzed using Mann-Whitney U-tests and differences over time were analyzed with Wilcoxon signed rank test or Friedman test. Differences in body weight and liver function were tested using two-way ANOVA. Correlations were tested using Spearman's correlation coefficient. All statistical analyses were performed using Graphpad (version 6, Graphpad, La Jolla, CA).

RESULTS**Transection accelerates liver regrowth in terms of volume but not function**

All rabbits tolerated the PVE or PVE-transection well, all animals finished the experiments, and no animals were excluded from the analyses.

Following PVE-transection the increase in CLV was 2.7-fold greater on day 3 compared to PVE alone (56 \pm 16% versus 21 \pm 12%, P <0.01) and remained 1.7-fold greater on day 7 after PVE (113 \pm 34% versus 68 \pm 24%, P <0.01, Figure 2A). CrLV decreased following PVE in both groups, however, the decrease was greater following PVE compared to PVE-transection 7 days after PVE (45 \pm 14% versus 27 \pm 4%, P =0.03, Figure 2B). Total liver volume decreased after PVE with 16 \pm 9% (P <0.01, compared to baseline) seven days after PVE, and remained stable following PVE with transection (Figure 2C). The weight of the caudal liver lobe at sacrifice was higher following PVE with transection compared to PVE alone (27.2 \pm 2.3g versus 21.3 \pm 1.4g, P <0.01, Figure 2D). Liver weight at sacrifice highly correlated with the measured CLV 7 days after PVE (Spearman's ρ =0.87, P <0.01), demonstrating the accuracy of liver volume measurements (Figure 2E). Body weight showed similar kinetics between

groups (Figure 2F). These measurements suggest the model adequately resembles the hypertrophic response seen in ALPPS.

HBS was performed in conjunction to liver volume to assess liver function. CLF increased after PVE in both groups, however no differences between groups could be detected on day 3 and day 7 after PVE (Figure 2G). CrLF decreased in both groups following PVE with no differences between groups (Figure 2H). TLF remained unchanged after PVE with transection (Figure 2I). In the PVE group, TLF was mildly elevated compared to baseline 3 days after PVE ($54.2 \pm 4.9\%/min$ at baseline compared to $63.4 \pm 3.7\%/min$ after 3 days, $P < 0.05$) and was similar to baseline 7 days after PVE (Figure 2I).

In order to examine the extent of hyperplasia, liver sections were stained for Ki67 to visualize hepatocytes with an active cell cycle. PVE alone appeared to induce more regional hyperplasia (Figure 2J,K) while PVE with transection resulted in more diffuse hyperplasia (Figure 2L,M). Quantification of Ki67-positive hepatocytes on a HPF in the most proliferative region revealed no differences between groups (Figure 2N)

Early increase in plasma transaminase levels following PVE with transection

In order to examine the extent of tissue damage, ALT and AST were determined before and after PVE. In both groups ALT and AST peaked one day after PVE and decreased towards baseline thereafter with no differences between groups. In the PVE with transection group, both ALT and AST were higher compared to PVE in blood samples obtained 3 hours after PVE.

Total bile acid concentration were measured in plasma of both groups, levels peaked 1 day after PVE and no differences were found between groups. A correlation between bile acids with liver hypertrophy (measured by liver volume)²⁴ was absent in the current experiments.

In order to analyze differences in the inflammatory response between groups, IL-6 and TNF α were quantified in plasma. TNF α was undetectable in all samples, as was IL-6 with the exception of the 3-hour and 1-day after PVE time points. No differences in IL-6 levels could be measured between groups (Figure 3D). Both IL-6 and TNF α were measured in liver homogenates of the cranial liver lobe after PVE obtained from remote and proximal biopsy sites relative to the parenchymal transection at sacrifice 7 days after PVE (Figure 3E, F). Both cytokines were detectable but no differences were found between groups. IL-6 and TNF levels were also similar between groups in caudal liver homogenates (Figure 3G, H).

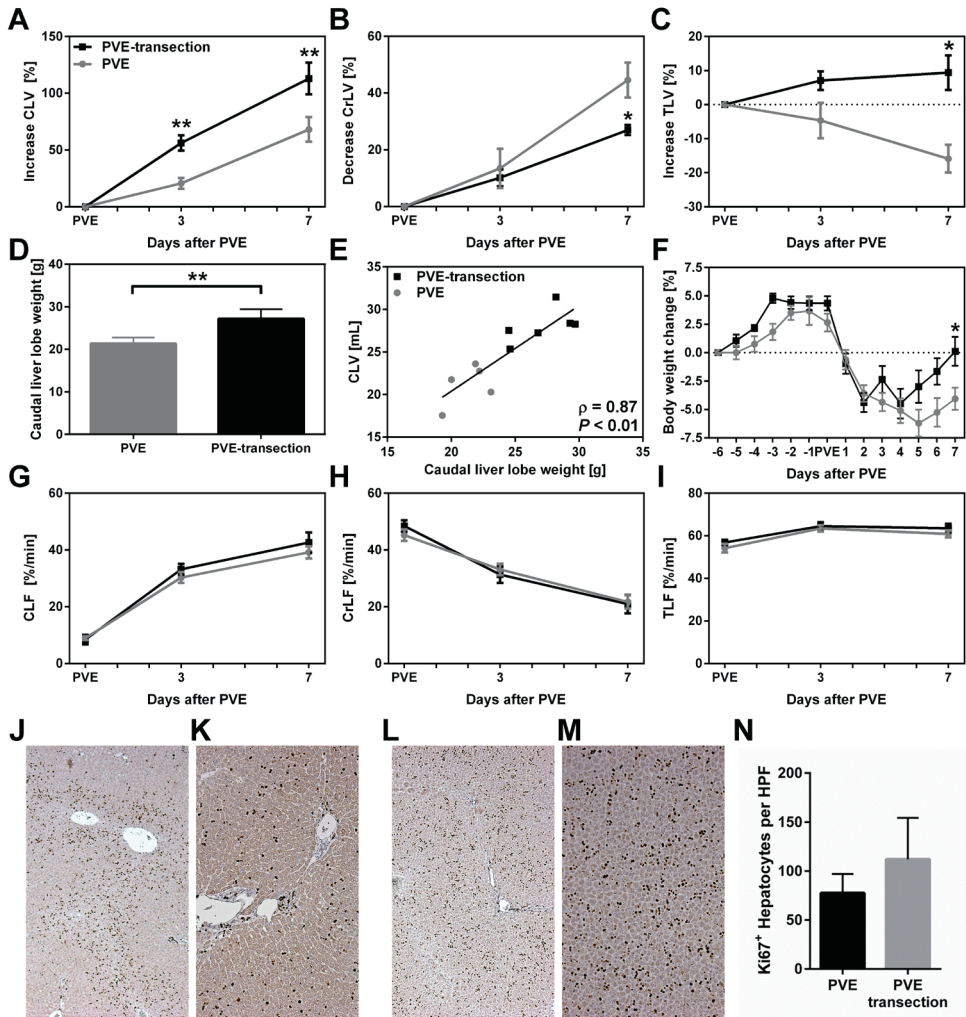


Figure 2: Caudal (A) and cranial (B) and total (C) liver volume increase compared to baseline measurements. Differences between groups were calculated by Mann-Whitney U-tests on area under the curve values. **D:** Caudal liver weight at sacrifice. Differences between groups were calculated by Mann-Whitney U-test. **E:** Correlation of CLV on day 7 with sacrifice liver weight. Correlations were tested using Spearman's rank correlation coefficient. **F:** Body weight change compared to baseline. Differences between groups were tested using two-way ANOVA. Caudal (G), cranial (H) and total (I) liver function at baseline and 3 and 7 days after PVE. Differences between groups were measured using two-way ANOVA. Representative Ki67-stained caudal liver lobe sections of the PVE group at 5x (J) and 10x (K) magnification and PVE transection group at 5x (L) and 10x (M) magnification. **N:** Ki67-positive hepatocytes per high power field in the most proliferative region. Differences between groups were tested using the Mann-Whitney U-test. All data are expressed as mean (SEM) for n=5-6 per group. * indicates $P < 0.05$ between groups and ** indicated $P < 0.01$ between groups.

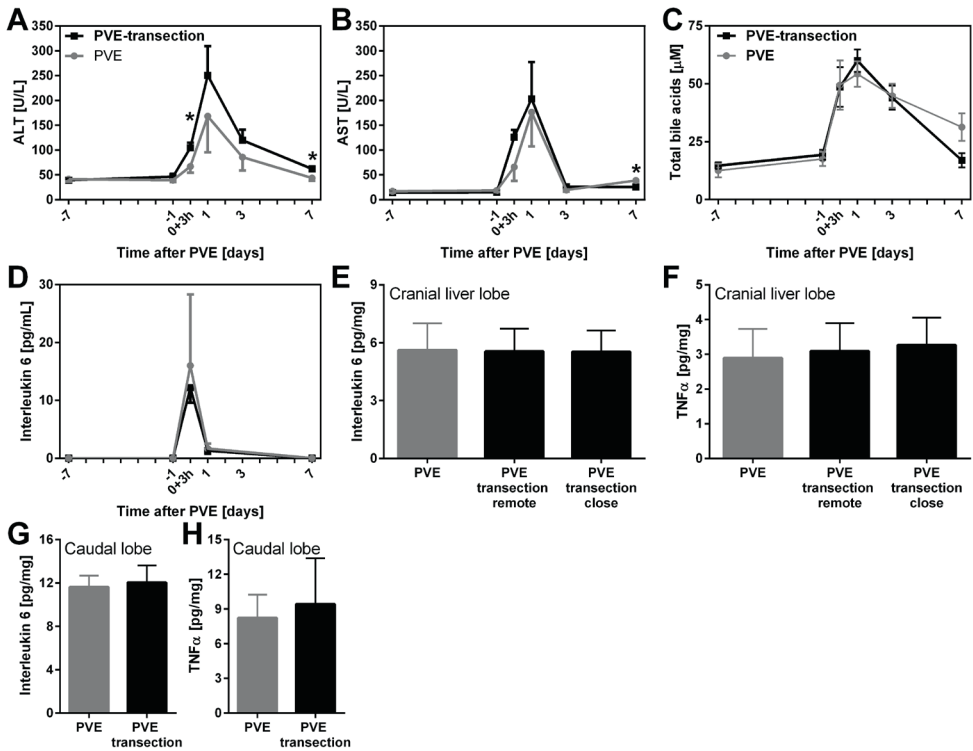


Figure 3: Plasma ALT (A) and AST (B) before and after PVE. Differences between groups were tested using two-way ANOVA. C: Plasma IL-6 before and after PVE. Differences between groups were tested using two-way ANOVA. Hepatic IL-6 (D) and TNF α (F) concentration in the caudal liver lobe on day 7 after PVE. E: Hepatic IL-6 (E) and TNF α (G) concentration in the cranial liver lobe after PVE and after PVE with transection remote and close to the parenchymal dissection on day 7. Differences in hepatic cytokine content were analyzed using the Mann-Whitney U test. All data represent mean (SEM) for n=6 per group. * indicates P<0.05 between groups.

Parenchymal transection does not alter liver histology

Non-embolized caudal liver lobe histology was examined in both groups and no differences were observed between the groups. Only minor portal and lobular inflammation was observed in both groups. Mild sinusoidal dilation was seen in all animals, although the clinical significance of this phenomenon is elusive. Moderate steatosis was observed in one animal in each group and mild steatosis in two animals in the PVE group, which is not uncommon during liver regeneration. The degree of steatosis was similar in both groups.

Cranial liver lobe histology was assessed after PVE (Figure 4C), and for the PVE transection group both in proximal (Figure 4D) and distant (Figure 4E) regions of the parenchymal transection plane. The parenchymal transection plane was flanked by a narrow rim of necrosis

(Figure 4E). In the distal segments, histology was comparable between groups (Figure 4E). Mild portal and lobular inflammation were similar between groups. The transection resulted in more severe sinusoidal dilatation compared to PVE. While necrosis was less than 5% in all PVE animals, transection resulted in up to 15% necrosis in the distant segments, albeit this effect was not statistically significant. Interestingly, embolization material was observed in all PVE animals but only in 2 out of 6 liver sections of the PVE transection animals.

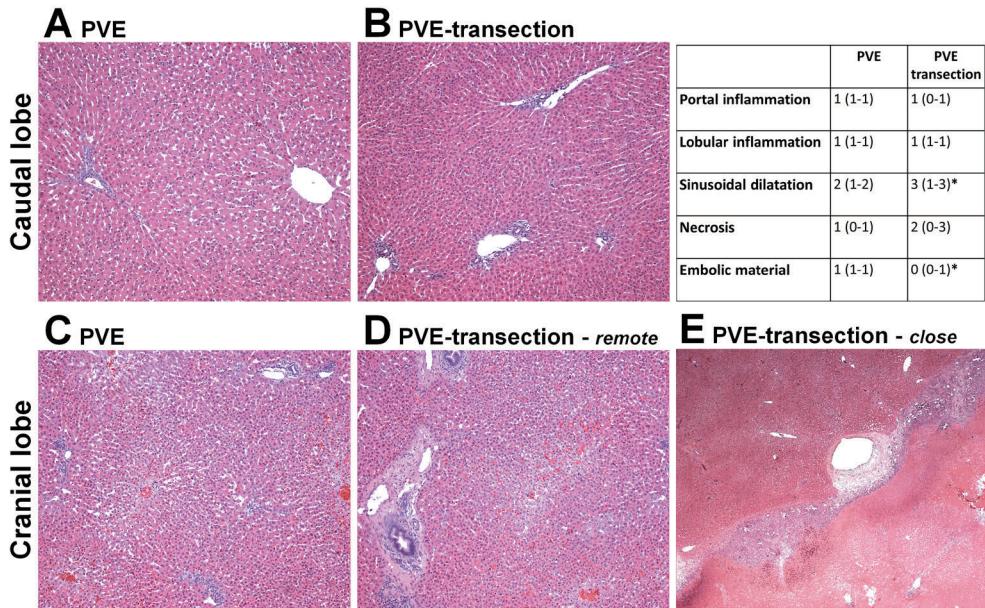


Figure 4: H&E-stained liver sections of the caudal lobe 7 days after PVE (A) or PVE with transection (B). H&E-stained liver section of the cranial lobe 7 days after PVE (C), after PVE with transection remote from the transection (D), or in the proximity of the transection (E). The table displays the quantitative scoring of cranial liver histology in the PVE group and PVE with transection group remote from the transection. Data represent median with range and differences between groups were analyzed using the chi-square test. * indicated $P < 0.05$ between groups.

DISCUSSION

This study demonstrated that parenchymal transection during PVE induced rapid hypertrophy in a rabbit model, but did not proportionally increase liver function compared to PVE alone, even after a week. These results give the first proof that rapid hypertrophy leads to dysfunctional parenchyma. Our findings explain the concerns regarding liver function in ALPPS voiced by many^{5, 6, 9} and support obligatory monitoring of remnant liver function after ALPPS.

Recently, ALPPS was introduced as a new technique to perform extended liver resections by inducing rapid liver hypertrophy. The initial report demonstrated a median 73% increase in FLR volume in just a median of 9 days.³ The reported hypertrophy was greater and faster compared to portal vein embolization, which are usually around a 38-39% increase in FLR volume in approximately 26 days.^{25, 26} Experienced centers might achieve rates of 62% FLR volume increase in selected patients with segment IV embolization. However, the response still takes a median of 34 days, which is considerably longer compared to ALPPS.²⁷ Despite the rapid hypertrophy seen in ALPPS, morbidity and mortality rates are substantial and liver failure accounts for the majority of deaths.^{8, 9} Accordingly, the low functional quality of the hypertrophied liver segment may lie at the basis of these unacceptable statistics.

The mechanisms of increased hypertrophy induced by ALPPS are currently elusive and several factors have been suggested to be involved. The mechanisms include more complete deportalization of the ligated liver by the prevention of collateral portal perfusion by the parenchymal transection.¹¹ However, partial or mini-ALPPS has been shown to have almost identical or similar hypertrophy rates,²¹ which suggests that the inflammatory response induced by the parenchymal transection may also play a role in the hypertrophy response. Several animal models of ALPPS have been developed to address these mechanisms.^{12, 13, 16} However, all have specific limitations that hamper translation of the obtained results.

Our rabbit model of PVE is a standardized model with reproducible results.¹⁸⁻²⁰ The rabbit liver anatomy with separated caudal and cranial liver lobes prevents collateral circulation following embolization of the cranial liver lobes. Therefore we hypothesized that the addition of (partial) parenchymal transection to a cranial liver lobe would closely resemble the (partial) ALPPS procedure and enable accurate investigation of the added effect of parenchymal transection to the hypertrophy response initiated by PVE. Although ALPPS is commonly applied with ligation of the portal vein, the recently introduced mini-ALPPS technique does use PVE yet yields similar results. In the current model, parenchymal transection does not separate the non-embolized segments from the embolized liver. Accordingly, collateral perfusion between the caudal and cranial liver is most likely not present in rabbits, and the model allows the study of the added effect of parenchymal transection over complete deportalization only. Although the embolic material was not observed in histology sections in all animals following PVE with transection, contrast-enhanced CT scans 7 days after PVE did not demonstrate portal perfusion in these animals, confirming patent portal occlusion.

In the current model we found that the increase in liver volume was indeed enhanced following PVE with transection compared to PVE alone. However, the increase in liver function was not affected and increased in a similar manner as PVE-subjected livers. These results are

of great interest and clinical relevance. ALPPS may suggest appropriate hypertrophy while the liver volumetry is in fact deceiving. The high incidence of post-hepatectomy liver failure and mortality of ALPPS may be a result of a dissociation of volume and function during rapid hypertrophy. This is the first study to demonstrate this dissociation using HBS for functional assessment of rabbit livers. The timing of the second stage of ALPPS remains an important issue in the discussion surrounding ALPPS. Because the observed liver volume following ALPPS does not readily reflect functional liver tissue, assessment of liver volume should not guide the timing of the second stage. Instead, functional assessment has to be performed. Several reports on hepatobiliary scintigraphy during ALPPS for timing of the second stage have been published.²⁸⁻³⁰ Perhaps functional liver assessment is key to reducing morbidity and mortality in ALPPS, especially when the current results can be translated to patients.

Other models of ALPPS have been developed and mostly involve rat models.^{12, 13} Small rodents have different portal perfusion compared to humans and most likely rabbits. Portal occlusion in rats often results in extensive necrosis in the deportalized liver, which is most likely not encountered in patients and rabbits, considering necrosis would induce a sterile inflammatory response and is usually not well-tolerated. All rodent models are partial ALPPS models because the vena cava runs inside of the liver parenchyma, and complete transection between two liver lobes cannot be performed. Additionally, in some rat models a part of the liver is resected. This reduction in mass could bias the obtained results. Similarly, ALPPS in a mouse model also involves a reduction in liver size.¹⁶ All of the published models use liver volume or mass as their primary outcome, and rapid hypertrophy induced by ALPPS compared to portal occlusion alone can be demonstrated. We suggest however, that assessment of liver function should be used routinely in this model, which could greatly influence the results and add major relevance to the models and provide insight into the true clinical relevance of rapid hypertrophy.

In conclusion, our current model suggests the induced rapid hypertrophy by ALPPS is associated with an increase in liver volume but not liver function. Liver volume assessment is not a good surrogate for function and therefore should not be used as a tool to determine the second stage. The ALPPS volume increase likely does not provide a clinical advantage prior to liver resection, and may be misleading in the worst case scenario. We suggest both experimental and clinical implementation of liver function assessment techniques in the context of ALPPS studies.

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SUPPORTIVE INFORMATION

Table S1: Semi-quantitative histologic scoring system

Parameter	Score			
	0	1	2	3
Portal inflammation	absent	mild	moderate	severe
Lobular inflammation	absent	< 2 foci	2-5 loci	>5 loci
Sinusoidal dilatation	absent	mild	moderate	severe
Confluent necrosis	absent	< 5%	5 – 10%	> 10 %
Embolic material	Absent	present		