# Vascular anatomy of the rat liver

# and its implications for extended hepatectomy

# and the determination of the minimal liver mass

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# 1. Introduction

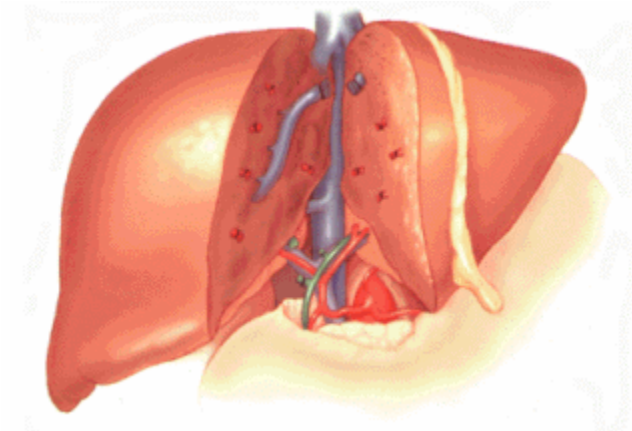
## *1.1. Extended hepatectomy in clinical surgery*

### 1.1.1. Rising need for extended hepatectomy

Surgical removal of the tumor remains the only realistic treatment offering the prospect of cure for hepatocellular cancer. This may be achieved, either by hepatic resection or complete hepatic removal with liver replacement, i.e. liver transplantation.

Lortat-Jacob's report of a true anatomical right hepatectomy *(Fig.1)* for cancer in 1952 ushered in the modern era of hepatic surgery (44;45). As a result, hepatic resection has evolved into the treatment of choice for selected patients with benign and malignant hepatobiliary disease (63). Also, with improvement in the safety of hepatic resection, indications for its use have broadened (23). Based on promising survival results and a perioperative mortality rate of below 5%, the frontiers of liver surgery are extending continuously towards more major liver resections leaving smaller fractions of residual liver (23;53;55;68).

**Figure 1. Illustration of right hepatectomy**



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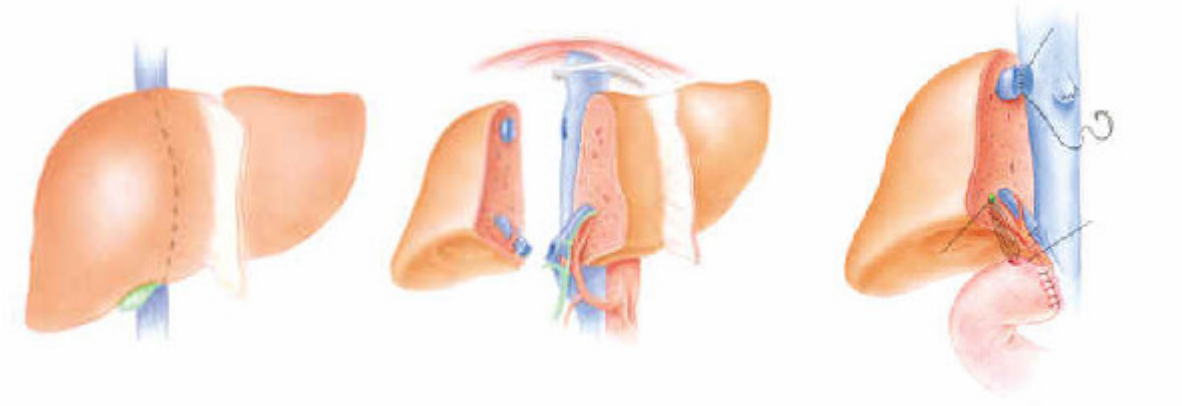
Although postoperative morbidity and mortality was significantly reduced over the last decades, no single factor is responsible for the marked improvement in perioperative outcome (3). A better understanding of hepatic anatomy and increasing application of anatomically based resections are perhaps the most important factors in this regard (23).

At the same time, the expansion of major liver surgery as a treatment option for large and inaccessible liver tumors has presented new challenges to surgeons and physicians in terms of the assessment and management of postoperative complications, particularly those involving hepatic insufficiency and susceptibility to infection (63). High rate of postoperative morbidity due to hepatic dysfunction and infectious complications following extended liver resection has been reported, even by very specialized centers (23;63).
The maximum extent of resection compatible with a safe postoperative outcome remains unknown, but the risk for perioperative complications is generally believed to increase when the remnant liver volume (RLV) is very small. Extending the amount of resected liver tissue beyond 70% increases the risk of facing such complications as major blood loss, coagulopathy, suppression of immune resistance against infection, liver function insufficiency or even failure. Surgical complications are attributed to portal hypertension, blood loss, outflow obstruction and ischemic zones in small remnant liver (46;70).

### 1.1.2. Extended hepatectomy in living liver donation

Since liver transplantation was established as a successful method for the treatment of end-stage liver diseases, results constantly improved due to the development of potent immunosuppressive drugs (65). This improvement led to an increase in the number of potential recipients waiting for liver transplantation which is by far exceeding the number of potential donors. Subsequently, organ shortage became a major problem in liver transplantation (64), especially in the pediatric population. Introduction of living donor transplantation for children in 1989 (4) contributed to overcome the problem of organ shortage for this group of patients. In 1998 cadaveric split liver transplantation (67) and shortly thereafter living liver donation (74) was introduced to clinical surgery to meet the rising need for donor organs for the adult population *(Fig. 2).*
As living donation requires the selection of an optimal healthy donor and allows selecting the optimal time point for transplantation, waiting time and pre-LTx mortality were reduced significantly.

**Figure 2. Right lobe LDLT**



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According to experience gained in hepatic surgery, an extended hepatectomy of up to 80% to 90% of the whole liver can be tolerated in noncirrhotic patients (68). This means that 10% to 20% of the liver may potentially support hepatic function. However, this cannot be translated directly into the lower limit of graft volume in LDLT, which involves periods of cold and warm ischemia and subsequent reperfusion injury of the graft (57).

Determination of the maximal amount of liver to be resected in a living donor and the minimal graft volume sufficient to meet the metabolic demand of the recipient remains the great challenge in LDLT(57).

Kiuchi et al (27) reported that the use of a graft with a graft-to-recipient weight ratio (GRWR) <1% can lead to lower graft survival. However, the reported minimal graft volume for a successful adult LDLT for fulminant hepatic failure is 0,6% of GRWR (43).

The use of small for size liver grafts is often associated with the so-called “small-for-size-syndrome”, characterized clinically by a combination of prolonged functional cholestasis, intractable ascites, and delayed recovery of both prothrombin time and encephalopathy (12). Development of the “small-for-size-syndrome” is attributed to enhanced parenchymal cell injury and reduced metabolic and synthetic capacity (27), presumably caused by both inflow and outflow compromise (46). Elevated portal venous pressure, decreased hepatic outflow, and decreased arterial flow frequently were frequently observed after small-for-size LTx, and associated with graft congestion, consecutive arterial thrombosis, and poor overall survival.

## *1.2. Extended hepatectomy in experimental surgery*

### 1.2.1. Controversial results after extended hepatectomy

Extended hepatectomy in rats has been used as a model for fulminant hepatic failure (FHF) in experimental medicine since 1964. Up to 1997 this model was regarded as lethal model leading to no or single survival after operation (1;5;6;9;11;15;16;26;29;30;32;34;40;56;76;77;79). Most of the animals died within the first 3 days after surgery due to acute hepatic failure.

The model was used to study liver function insufficiency, endotoxemia, hyperammonemia, bacterial translocation, cytokinemia as well as liver regeneration course after major hepatectomy (16;75;77). As reported, intraoperative bleeding, vena cava constriction and remnant liver necrosis were the main complications after such surgical procedures. Since 1997 reports appeared from one group (36) claiming that PH-90% in rats was a non-lethal model which allowed all animals to fully recover after the operation (8;25;36;54).

### 1.2.2. Surgical techniques

When analyzing the reasons for the strikingly different results despite the removal of a comparable liver mass, differences in the surgical techniques were identified. Low survival rates in these reports were associated with the use of a simple method of resection, performed by putting single ligations around the bases of the lobes (1;5;6;9;11;15;16;26;29;30;32;34;40;56;75;77;79). Mainly, the methods by Gaub and Iversen (16) or Weinbren and Woodward (75-77) were widely used in different modifications depending on selection of lobes to be resected for removal of 80% to 90% of liver mass.

High survival rates were only described from the group of Kubota et al (15). With his surgical technique 100% survival for 1 week was achieved. Isolation and dissection of portal vein and hepatic artery branches was followed by piercing sutures throughout the lobe to ligate separately the main hepatic veins.

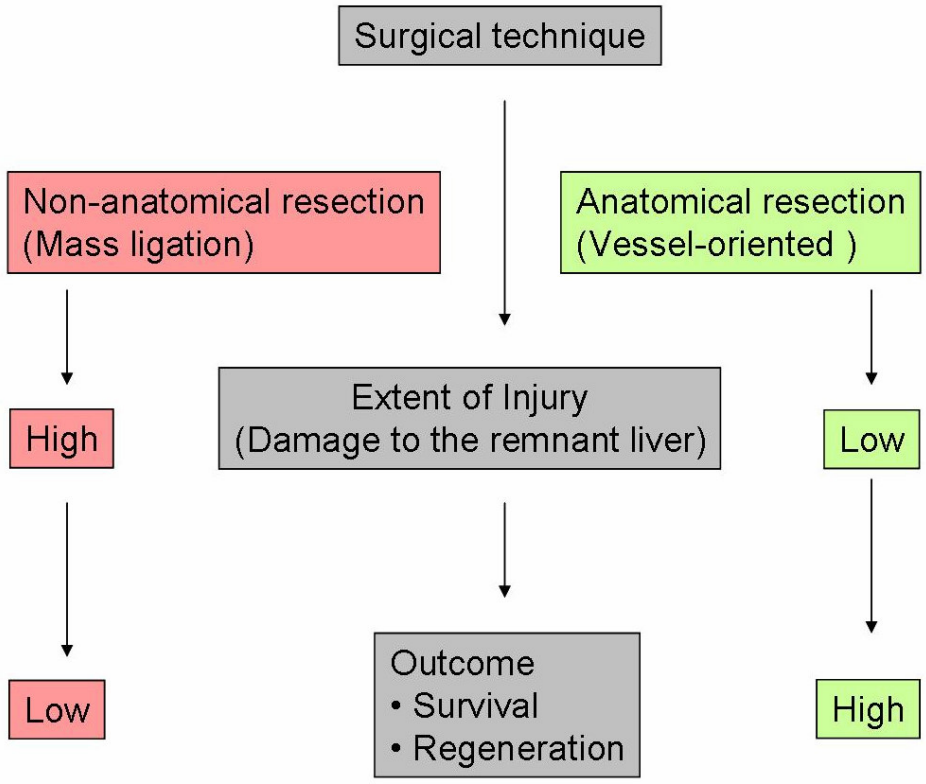
# 2. Hypothesis and aim

## *2.1. Hypothesis*

A careful analysis of all reported surgical techniques led to the hypothesis *(Fig.3)*, that the difference in outcome might be related to the extent of damage of the small remnant liver caused by the surgical procedure itself.

1. Non-anatomical mass ligation presumably caused more damage to the remnant liver compared to the anatomically oriented approach suggested by Kubota
2. Clinical outcome is related to the extent of local and systemic damage
3. Initiation and extent of regeneration is also dependent on the morphological and functional state of the remnant liver

**Figure 3. Hypothesis**



## *2.2. Aim*

In order to prove this hypothesis, the study was divided into 3 different parts:

### 1. Visualization of rat liver anatomy and development of an anatomically oriented parenchyma preserving surgical technique

Rat liver surgical anatomy from the view of extended hepatectomies is poorly described. Previous reports are limited to a description of gross anatomy and major hepatic vessels (9;16;31).

As detailed knowledge of the vascular and topographic anatomy is required for improving surgical technique, the first purpose of our study was to:

- *visualize rat liver anatomy using 3D anatomical models for developing an optimized method for extended hepatectomy*

### 2. Influence of surgical technique on outcome after 90% hepatectomy

The newly developed surgical technique was compared in a prospective study with two mass ligation techniques applied previously in our research group. Results obtained with all 3 techniques were analysed in a retrospective study for further confirmation.

The second purpose of our study was to:

- *investigate the effect of optimized parenchyma preserving and vessel oriented surgical technique on the outcome after 90% hepatectomy in comparison to mass ligation methods.*

### 3. Application of optimized technique for further reduction (95 and 97%) of liver mass

Since survival was dramatically improved, when using parenchyma preserving resection technique, the 3-rd purpose of the study was to:

- *determine the absolute minimal mass using the parenchyma preserving vessel oriented technique*

# 3. Material and methods

## *3.1. Experimental design*

### 3.1.1. Visualization of rat liver anatomy as a basis for optimizing the surgical technique

Rat liver anatomy was visualized by producing anatomical models from epoxy plastinates. Gross lobar anatomy of plastinates and explanted livers was documented by digital images from the ventral and dorsal side to allow comparison with intraoperative pictures *(Table 1)*. In order to confirm the volume of the separate liver lobes, n=6 animals were sacrificed and the weight of individual liver lobes was determined. Vascular anatomy was visualized by corrosion casts of the portal vein (n= 9), hepatic veins (n=30), hepatic artery (n=2) and bile duct (n=5). All models were subjected to digital imaging from different angles to analyze their structural relationship. The information was than transformed into schematic drawings.

**Table 1. Group distribution for visualizing rat liver anatomy**

| Object of study | Animal | Number | Method | Evaluation |
|---|---|---|---|---|
| Gross anatomy | Male Lewis rats | 7 | Intraoperative situs, explanted liver, plastinates | Detailed analysis of models with description |
| Portal vein | | 9 | Vascular corrosion casts | |
| Hepatic artery | | 2 | Vascular corrosion casts | |
| Vena cava and hepatic veins | | 30 | Vascular corrosion casts | |
| Bile duct tree | | 5 | Vascular corrosion casts | |

### 3.1.2. Influence of surgical technique on outcome after 90% hepatectomy

#### 3.1.2.1. Prospective study regarding influence of technique on outcome

A prospective study was designed to evaluate the effect of 3 different surgical techniques on the outcome after 90% hepatectomy in the early postoperative period. Planned groups consisted of 6 animals. Differences in surgical techniques are described in *Table 2.* 2 groups were operated according to "**mass ligation"** technique in

comparison to a "**parenchyma preserving vessel oriented"** technique. The following read-out parameters were analyzed:

1. Clinical outcome:
    - Clinical condition
    - Blood count
2. Liver damage
    - Blood biochemistry
    - Histological damage score
    - Three dimensional histomorphometry
3. Regeneration
    - Weight recovery of the remnant liver
    - Proliferative index (PI)

**Table 2. Group distribution in prospective study evaluating the effect of resection on outcome after 90% hepatectomy in a rat**

| Groups | Animals | Number | Left lateral lobe | Median lobe | Right superior lobe | Right inferior lobe |
|---|---|---|---|---|---|---|
| Technique 1 (T-1) | Male Lewis rats 250-320 g | 6 | Ligation of the pedicle | Running suture under the clamp | Running suture under clamp | Ligation of the pedicle |
| Technique 2 (T-2) | | 6 | Ligation of the pedicle | Extra mass ligation after running suture under clamp | Extra mass ligation after running suture under clamp | Ligation of the pedicle |
| Technique 3 (T-3) | | 6 | Ligation of the pedicle | Separate ligation of vessels within the parenchyma by piercing | Separate ligation of vessels within the parenchyma by piercing | Ligation of the pedicle |

### 3.1.2.2. Retrospective analysis of the effect of different surgical techniques on outcome

Since May 2002 to the end of 2003, totally 113 animals were subjected to 90% hepatectomy as control groups for different experiments. Since May 2002 till January 2003 mass ligation method involving running suture technique was used (T-1). In the period from January 2003 to August T-2 technique was applied *(Table 3).* From that time until now the anatomically based T-3 technique was employed. The same read-out

parameters as in prospective study were analyzed, except the assessment of necrosis volume in the remnant liver.

**Table 3. Group distribution in retrospective analysis**

| Groups/Time points | 24h | 48h | 72h | 1 week |
|---|---|---|---|---|
| Technique 1 (T-1) | 9 | not available | not available | 20 |
| Technique 2 (T-2) | 8 | 6 | 3 | 38 |
| Technique 3 (T-3) | 11 | 6 | 6 | 6 |
| **Total** | 28 | 12 | 9 | 64 |

### 3.1.3. Application of optimized technique for further reduction (95% and 97%) of liver mass

This experiment was designed to define the minimal amount of the remnant liver compatible with survival. The parenchyma preserving vessel oriented technique was used for 95% and 97% hepatectomy *(Table 4).*

**Table 4. Group distribution in the study of effect of "parenchyma preserving vessel oriented" technique on outcome after 90%PH, 95%PH and 97%PH**

| Groups | Animals | Number | Operation | Observation time | Read out parameters |
|---|---|---|---|---|---|
| 90% hepatectomy | Male Lewis rats 250-350g | 6 | 90% hepatectomy | 24h | SVR, liver function, morphological damage, liver regeneration |
| | | 6 | | 1 week and longer | |
| "mega"-extended | | 6 | 95% hepatectomy | 24h | |
| | | 6 | | 1 week | |
| "giga"-extended | | 6 | 97% hepatectomy | 24h | |
| | | 6 | | 1 week | |

The outcome was assessed using the same read-out parameters as in the retrospective study.

## *3.2. Animals*

*Male inbred Lewis* (LEW, RT[1]) rats (Charles River Wiga GmbH, Germany), were used in this study. The body weights of male rats were within 250~350g (10~14 weeks old). The animals were housed under standard animal care conditions (12 h light/dark cycle at 22 C) and were fed with rat chow *ad libitum* before and after the operation. All procedures and housing of the animals were carried out according to the German Animal Welfare Legislation.

## *3.3. 3-D Models*

Gross anatomy of the rat liver was studied by detailed observation of the intraoperative situs and explanted livers. Visualization of gross and vascular anatomy anatomy was achieved by producing plastinates and corrosion casts.

### 3.3.1. Plastination

For production of plastinates explanted livers were fixed in 4% buffered formalin for 24h. After fixation was finished the samples were subjected to impregnation using “5 min epoxy” glue (R&G GmbH, Waldenbuch, Germany) for 48h.

### 3.3.2. Corrosion cast

*Corrosion casts* were produced using Batson’s 17 anatomical corrosion kit (Polysciences Europe, Germany). The polymer mixture containing monomer base solution, catalyst and promoter was prepared as recommended by the manufacturer (Polysciences Europe, Germany) in a volume ratio of 200:20:1. Animals were subjected to isoflurane anesthesia (2,5 % of isoflurane, oxygen flow 0,5 L/min). Catheterization of infrahepatic vena cava, portal vein or hepatic artery was performed, followed by injection of 2-10 ml polymer mixture depending on the animal weight *(Table 5)* and vascular structure (portal vein, hepatic artery or vena cava) selected for casting. Explanted livers were left for polymerization for at least 2-3 hours at room temperature followed by maceration in 10% KOH solution for 48 hours at room temperature. After samples dried, small branches of the vascular cast were removed with a microsurgical forceps under 6x magnification using an Olympus stereomicroscope (Olympus, Japan).

**Table 5. Route of injection and volume of polymer**

| Vessel casted | Route of injection | Amount of polymer | Color |
|---|---|---|---|
| Portal vein | Proximal part of superior mesenteric vein | 3-6 ml | red |
| Hepatic vein | Infrarenal segment of inferior vena cava | 5-10ml | blue |
| Hepatic artery | Common hepatic artery | 2-4 ml | red |

## *3.4. Surgical procedures*

### 3.4.1. Anesthesia

For general anesthesia the semi closed circuit mask narcosis was used. Preoperative induction and intraoperative anesthesia were performed using Sigma Delta (UNO, Holland) isoflurane vaporizer with an isoflurane concentration of 1,5-3% and oxygen flow of 0,5 L/min. Before the operation the animal was put into the induction chamber for 4-5 min. After the animal was fully anesthetized, the abdomen was shaved, and the skin was disinfected with alcoholic disinfectant (Cutasept F, Bode Chemie, Hamburg, Germany) and the rat was transferred to the operation table.

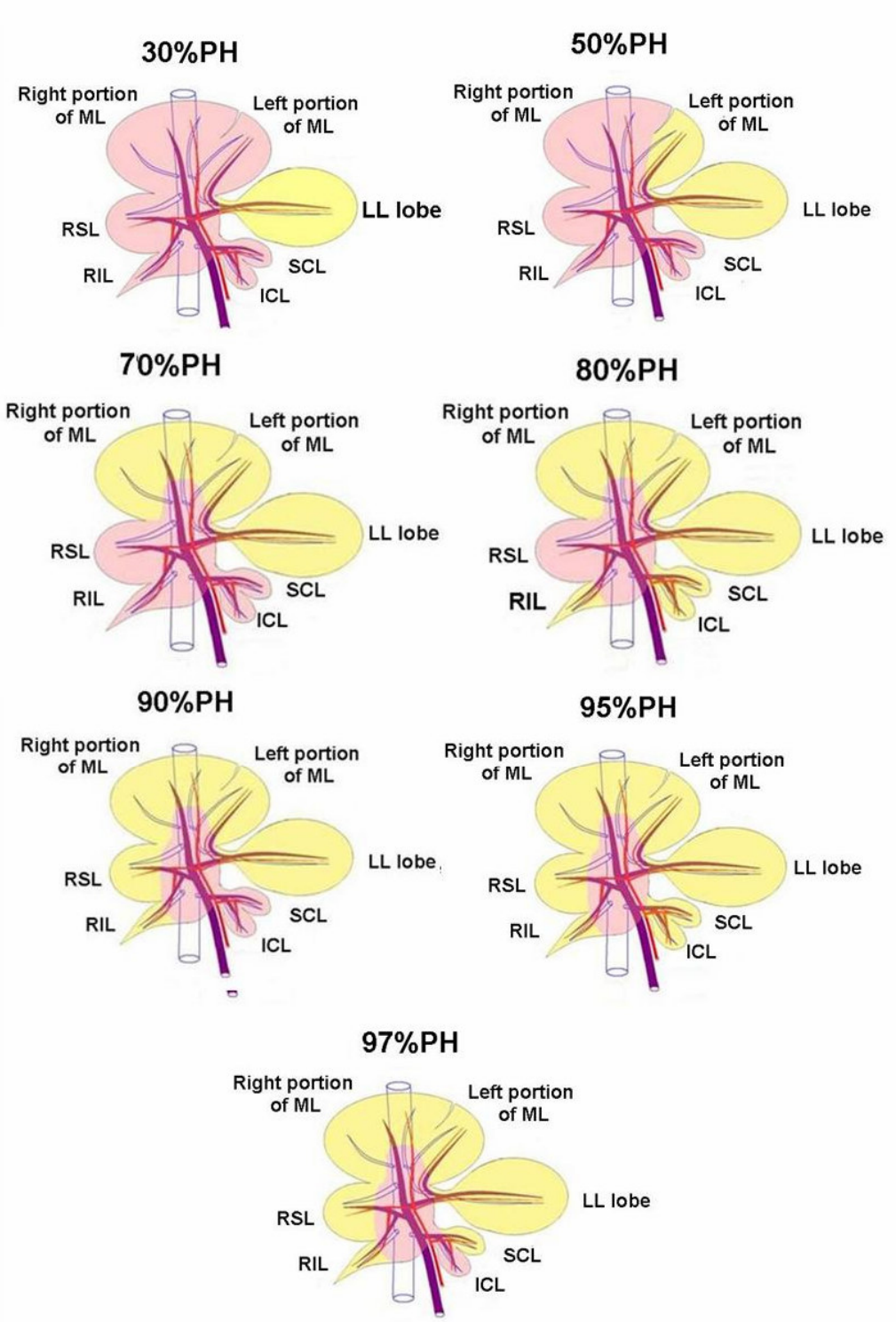
### 3.4.2. Extent, model and technique of liver resection

The extent of liver resections is determined according to the liver mass removed by resecting defined number of lobes as depicted in *Fig. 4* and *5*.

The model of hepatectomy is defined by the combination of lobes removed in given extent of resection.

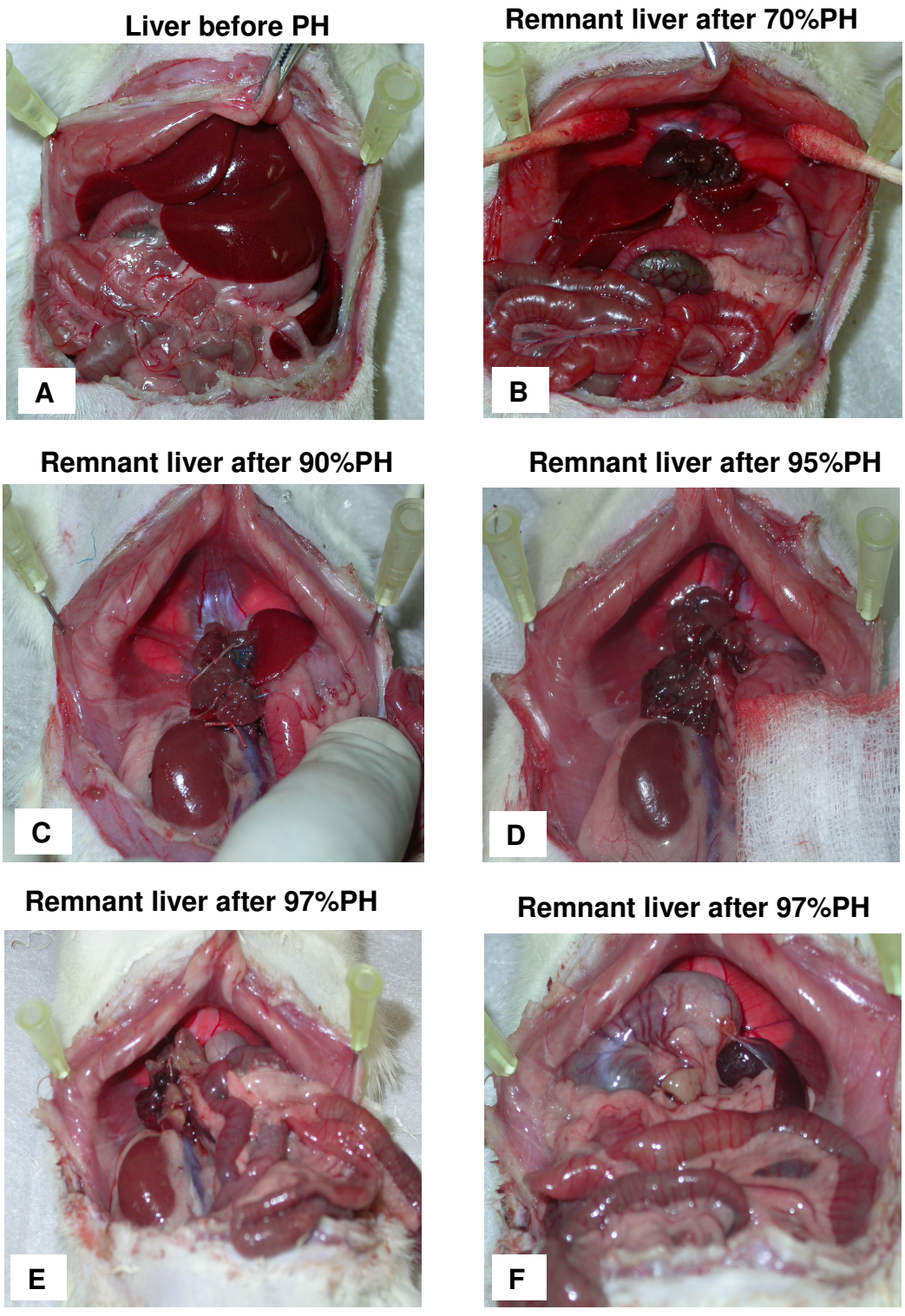
The technique refers to the surgical procedure based on the detailed description of all steps.

**Figure 4. Extent of hepatectomy in a rat (schematic illustrations)**




**ML** - median lobe
**LL** lobe – left lateral lobe
**RSL** - right superior lobe
**RIL** - right inferior lobe
**SCL** - superior caudate lobe
**ICL** - inferior caudate lobe

**Figure 5. Liver before and after hepatectomy**

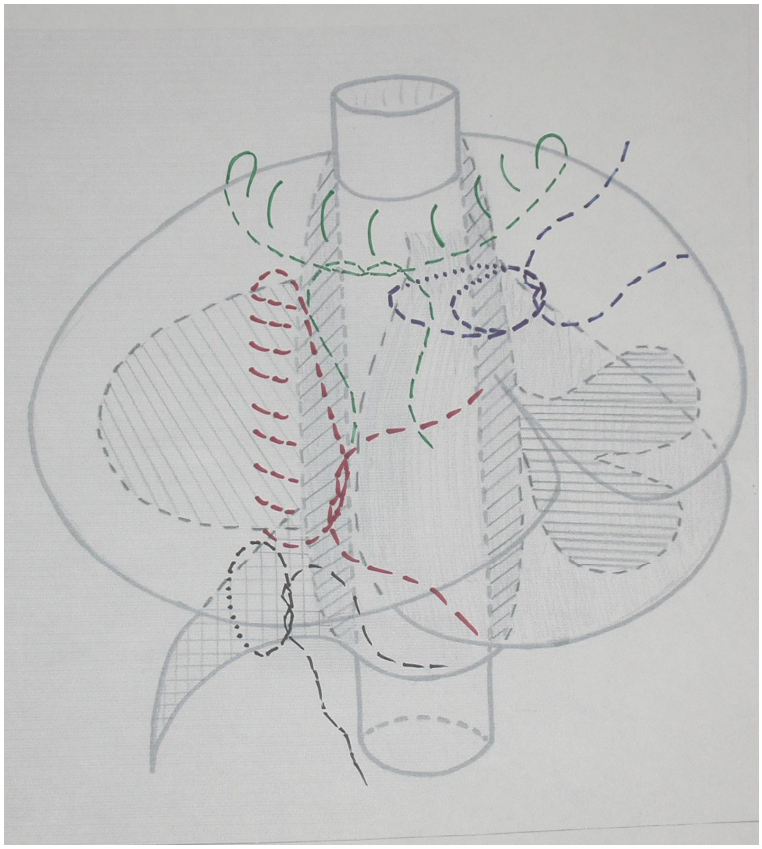


## Extended hepatectomy (90%PH)

After opening the abdomen aseptically with transversal incision, bowels were eventerated to the left and were wrapped with wet gauze. Interlobular ligaments were dissected. The left lateral lobe was removed after clamping and ligating the narrow pedicle with a 6-0 Prolene suture. Due to the wide base of the median lobe, resection was done in two clamping steps in all three different techniques.

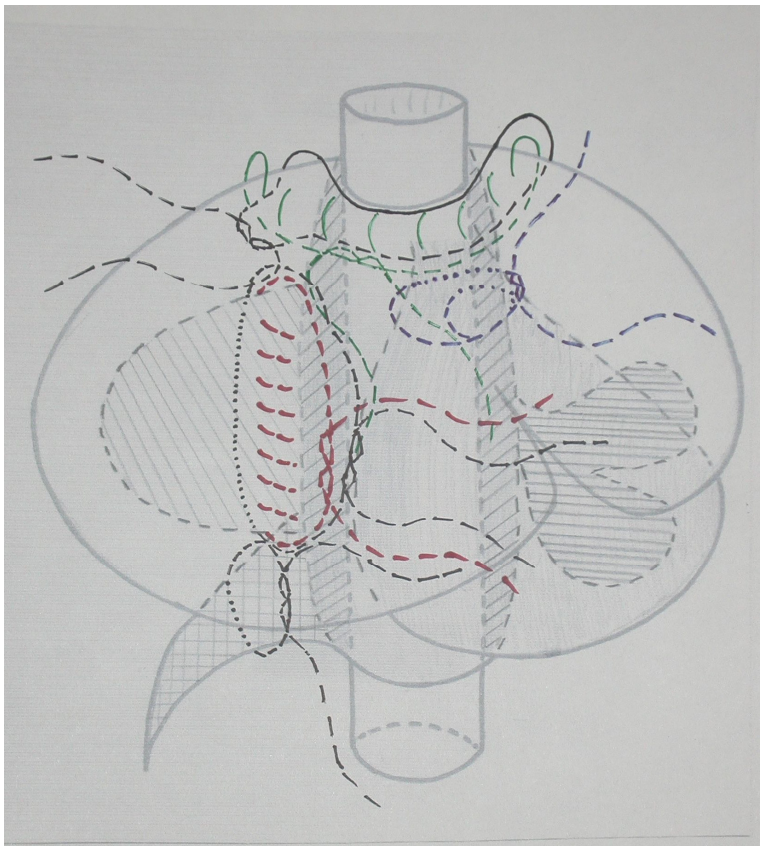
***Technique 1*** (T-1) - **"mass ligation"** - Median and right superior lobes were removed after clamping the lobe close to the base and placing a running polypropylene suture (Prolene 6-0, Resorba, Germany) over this clamp *(Fig.6)*. The clamp was than slowly released and the suture closed.

**Figure 6. Schematic illustration of *technique 1*** (T-1) - **"mass ligation" method**



***Technique 2*** (T-2) - **"extra mass ligation"** - The running suture at the base of the median and right superior lobes was further secured *(Fig.7)* by an additional ligation (resorbable Vicryl 3-0, Resorba, Germany).

**Figure 7. Schematic illustration of *technique 2*** (T-2) - **"extra mass ligation"**



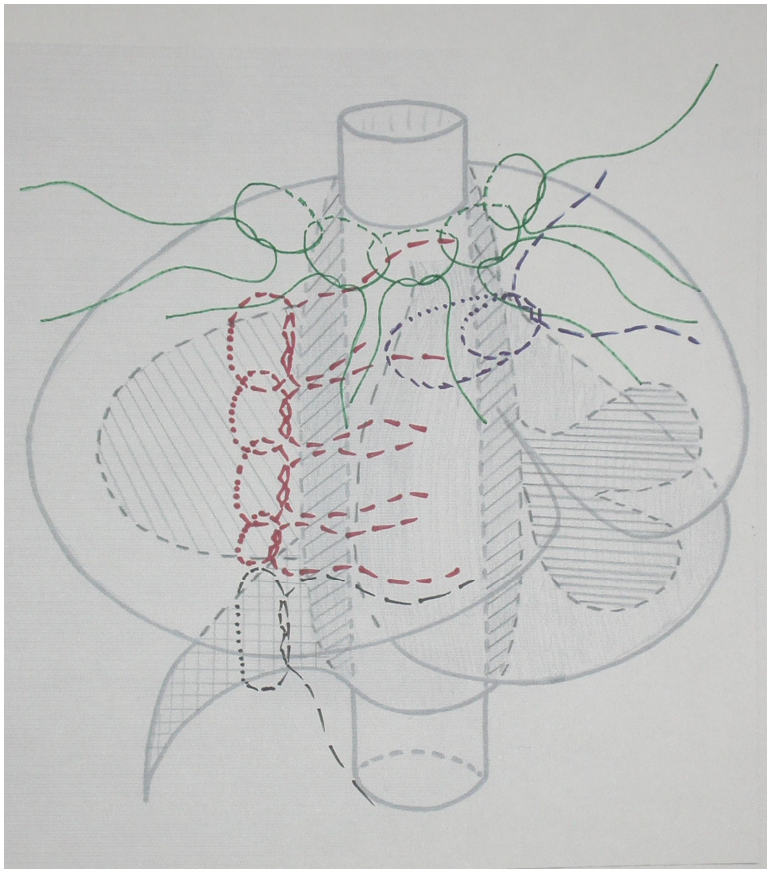
***Technique 3*** (T-3) - **"parenchyma preserving vessel oriented"** - The left lateral lobe was removed after clamping and ligating the narrow pedicle with a 6-0 Prolene suture. "Vessel oriented" resection of the median and right superior lobes was performed similarly in 4 steps. After placing the Mosquito clamp around the base of the respective lobe **(Step 1)**, the liver tissue was cut just above the instrument branches **(Step 2)**. Than, the piercing sutures (5 for median lobe, 4-5 for right superior lobe), penetrating the whole parenchyma, were placed below the clamp **(Step 3)** in order to ligate defined vessels separately *(Table 6).*

**Table 6. Structures ligated separately in vessel oriented piercing.**

| Lobe where vessel oriented piercing used | Piercing sutures | Portal vein | Hepatic vein | Hepatic artery | Bile duct |
|---|---|---|---|---|---|
| Median lobe | Suture 1. | right median | right median | Ligated together with PV | Ligated together with PV |
| | Suture 2. | middle median | | | |
| | Suture 3. | | middle median | | |
| | Suture 4. | left median | | | |
| | Suture 5. | | left median | | |
| Right superior lobe | Suture 1. | right superior | | | |
| | Suture 2. | | right superior | | |
| | Suture 3. | first branch of right superior | | | |
| | Suture 4. | | first branch of right superior | | |

The clamp was released **(Step 4)**. Thus, a plain cutting surface with only a thin layer of necrosis was achieved. The right inferior lobe was resected by using 2 sutures while the caudate lobes were removed by using a single ligation passing through the liver parenchyma of the pedicle *(Fig.8).*

**Figure 8. Schematic illustration of *technique 3*** (T-3) - **"parenchyma preserving vessel oriented"**



The abdominal incision was closed in two layers with running sutures using vicryl 3-0 (Resorba, Germany).

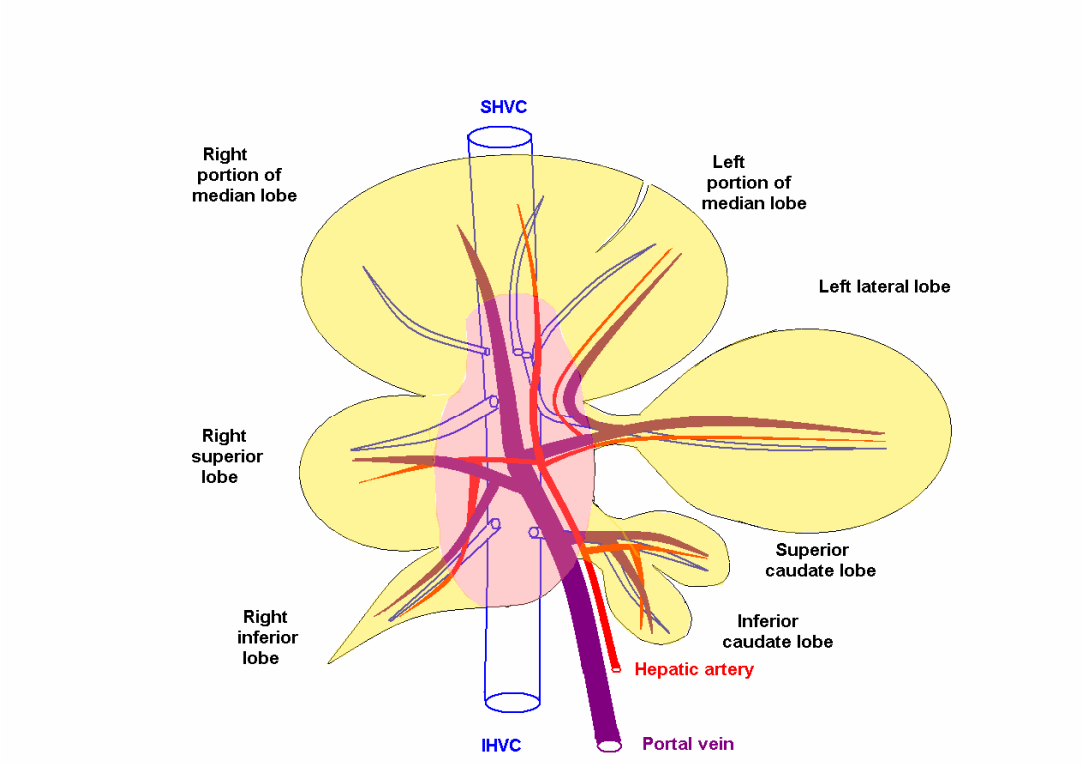
### "Mega"-extended hepatectomy (95%PH)

"Mega"-extended hepatectomy was performed according to the T-3 technique. In addition to the 90% hepatectomy the upper caudate lobe (5%) was removed by using a ligation around the pedicle, and leaving paracaval liver plus inferior caudate lobe.

### "Giga"-extended hepatectomy (97%PH)

In addition to the 95% hepatectomy the whole inferior caudate lobe was resected. Surgery was performed using "parenchyma preserving vessel oriented" technique *(Fig.9).*

**Figure 9. Model of 97% hepatectomy in a rat ("giga"-extended)**




This operation represented the maximal surgically achievable reduction of the liver mass *(Fig.10, 11).*

**Figure 10. Illustration of remnant liver after 97% hepatectomy using "parenchyma preserving vessel oriented" approach**

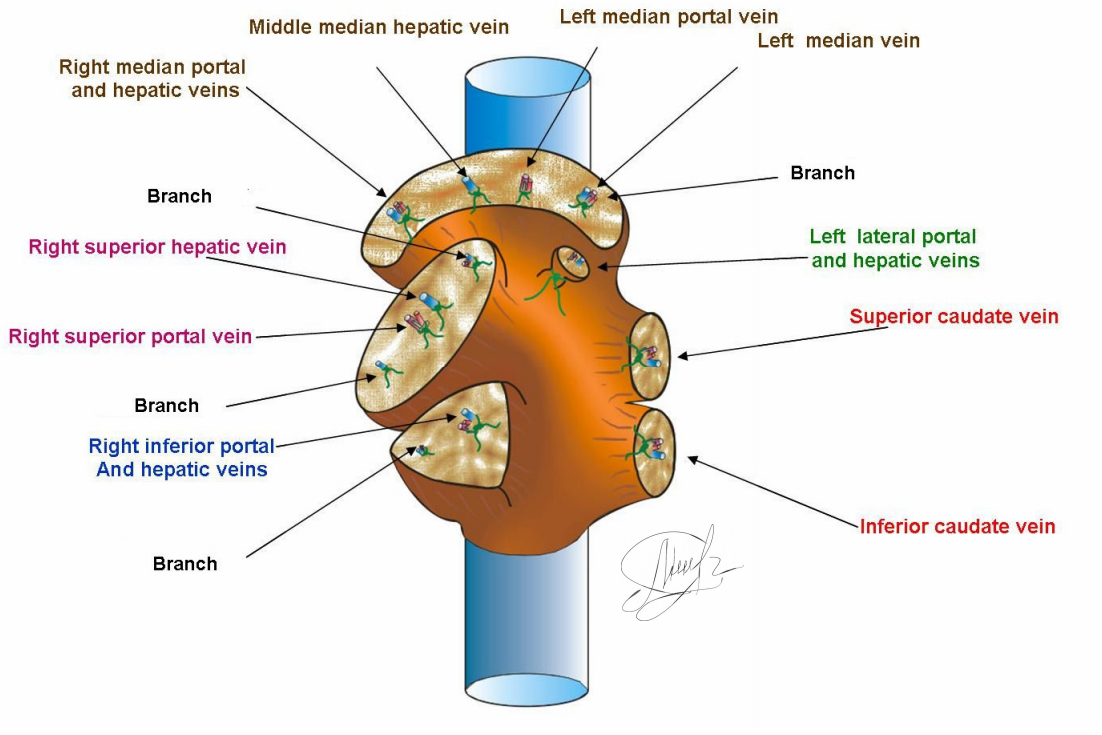
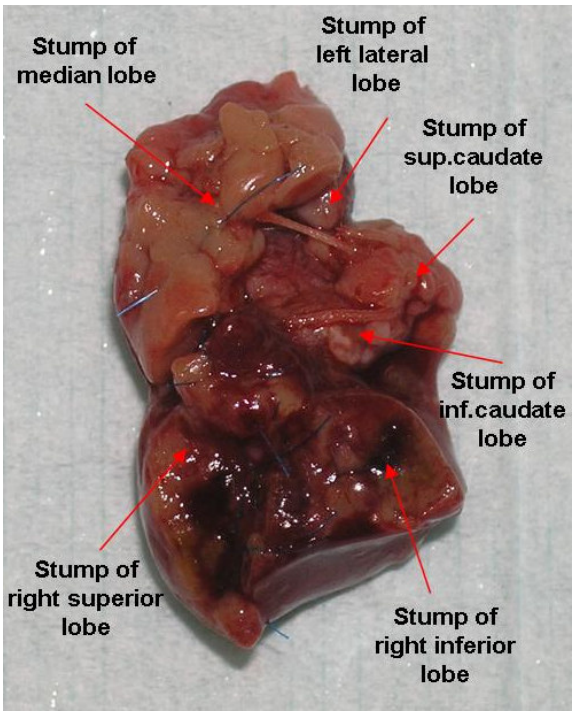



**Figure 11. Explanted remnant liver after 97% hepatectomy**



### 3.4.3. Postoperative management

All animals received 5 ml of 10% glucose subcutaneously and 0,3 ml penicillin (Baypen ™, Bayer GmbH, Germany) intramuscularily immediately after operation. Postoperative analgesia was performed by subcutaneous injection of buprenorphine (Temgesic ™, Essex Pharma GmbH, Germany) at a dose of 0,01 mg/kg body weight. In postoperative period animals had free access to 20% glucose solution for drinking and rat chow *ad libitum* for first 3 days after liver resection.

### 3.4.4. Euthanasia and sampling procedure

Administration of 5-bromo-2-deoxyuridine (BrdU, Sigma, USA) was performed 1 hour before sacrifice by intravenous injection (50mg/kg, dissolved in 1ml 0.9%NaCl). Sacrifice was performed under isoflurane anesthesia by exsanguination from the vena cava. In case of spontaneous death the autopsy was done within 12 h. Special gross findings were documented by a digital camera Nikon Coolpix 4500 (Nikon, Japan). The following samples were obtained:

**Samples taken during autopsy:**

a. For histology: Liver, kidney (left and right), lung, heart, small bowel, and bile duct

**Samples taken upon sacrifice:**

a. For biochemistry: serum (0.5 ml).
b. For blood counting: blood in EDTA tube (0.5 ml)
c. For histology and BrdU staining: Liver, kidney (right and left), lung, heart, small bowel, spleen, and bile duct.
d. For plastinates: explanted or remnant liver
e. For corrosion casts: explanted or remnant liver

## *3.5. Clinical outcome*

Survival time, body weight loss and clinical condition were used to assess clinical outcome.

### 3.5.1. Survival

Animals were observed daily, focusing on body weight loss and general condition: activity, jaundice and bleeding from eyes or nose. Survival time, body weight loss, and general condition were recorded for each animal throughout the observation period.

Survival assessment was based on daily observation of animals every 3-6 hours. Clinical condition was recorded. When clinical condition of animal was deteriorating and the condition score was reduced to (+), the animals were observed frequently at least every 1 h. In case an animal was found dead, the time interval between 2 last observations was recorded. For survival analysis the average time between two last observations was calculated.

### 3.5.2. Clinical condition

Clinical condition of the animals after operation was assessed using semiquantitative clinical condition score based divided in 5 grades:

**Active (+++)**

**Moderate active (+++/++)**

**Weak (++)**

**Sick (++/+)**

**Dying (+)**

Criteria for postoperative assessment included:

1. Activity
   - Movements
   - Response to grasping
   - Behavior
2. Body temperature
   - Skin temperature
   - Rectal temperature
3. External habitus
   - Position
   - Fur
   - Skin on paws and ears
4. Excrements
   - Stool (amount, rate, color and consistency)
   - Urination (amount, rate, color and crystallization)
5. Food and water consumption
6. Breathing
   - Type of breathing
   - Rate of breathing
7. Presence of abnormal secretions
   - Pus

- Hemorrhagic
- Ascites from wound

The definitive score for animals was given based on the surgeon's individual estimation.

### 3.5.3. Body weight loss

Body weight was recorded daily in the morning throughout the whole observation time. Accurat 2000 ™ (Werner Dorsch GmbH, Germany) scale with readability up to 0,1 g was used to check animal's weight.

## *3.6. Assessment of liver damage*

### 3.6.1. Liver function

Blood samples were kept at room temperature for 2 hours, and then centrifuged for 5 minutes at 35000 U/min (Hettich EBA 3s ™, Andreas Hettich GmbH & Co. KG, Germany). The serum was removed and stored at –20°C until use. The liver function, kidney function and electrolytes were measured by an Automated Chemical Analyzer (ADVIA 1650 ™, Bayer AG, Germany). To asses liver damage in terms of function the following parameters were included: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), Alkaline phosphatase (AP), Bilirubin-total, Bilirubin-direct, Albumin (Alb), Glucose (Glu).

### 3.6.2. Liver histology

#### Sample processing

Liver tissue was fixed in 4,5% buffered formalin for at least 24h and submitted for histological processing. Formalin fixed tissue was dehydrated through graded alcohols, and was then dipped into xylol prior to embedding into paraffin, by using a Tissue Processor TPC15 (Medite Inc., USA). Sections (4µm) were cut and stained with Hematoxylin-Eosin (HE). Also, the Elastica-von Giesson (EvG) staining was performed.

### Semiquantitiative scoring system

Histological evaluation of remnant liver was based on sections stained by the HE method and EvG method. The sections were submitted to an experienced pathologist and were analyzed according to a standard semi-quantitative histological scoring *(Table 7)*. Slides were revised for the presence of hepatocellular necrosis (single, confluent), vacuolar transformation (small, large), activation of Kupffer cells, eosinophilic globuli in the cytoplasm of the hepatocytes, sinusoidal dilatation and intrasinusoidal debris. Additionally, kidney, spleen, lung and heart lesions were assessed. The liver damage score was presented as a sum of the scores of all parameters, and the sinusoidal damage score was presented as a sum of sinusoidal dilatation and cell accumulation counted separately.

**Table 7. Histological damage score**

| Criteria for Damage Score | | | |
|---|---|---|---|
| **Criteria/Score** | **-** | **+** | **++** |
| Hepatocellular necrosis -single cell necrosis | No necrotic hepatocyte in 5 HPF (40X) | 1-10 in 5 HPF | >10 in 5 HPF |
| Confluent necrosis | No confluent necrosis | small in size and number | large size and /or large number |
| Small vacuolar transformation of the cytoplasm | No vacuolar transformation of the cytoplasm | 1-30% of all the hepatocytes | >30% of all the hepatocytes |
| Eosinophilic globuli | Absence of eosinophilic globuli | 1-5% of all hepatocytes | >5% of all hepatocytes |
| Activated Kupffer cells | negative | positive | n.a. |
| Alteration in sinusoids | no dilatation and cell accumulation | Dilatation of sinusoids or cell accumulation | dilatation and cell accumulation |
| Cholestasis | no cholestasis | hepatocellular or canalicular cholestasis | hepatocellular and canalicular cholestasis |

HPF= high power field = 400x

## 3.6.3. Mass necrosis

### Serial sections

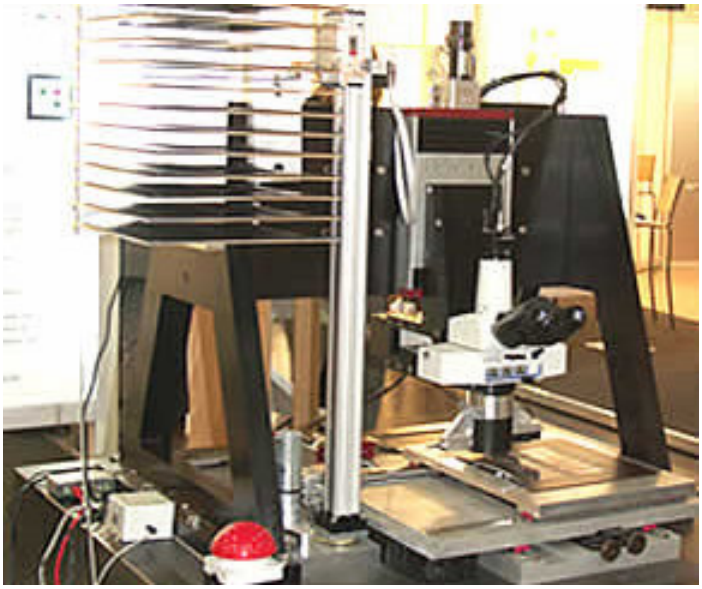
Remnant liver was fixed in 4.5% buffered formalin for 24h. In selected samples (n=1/group) both caudate lobes were removed. After standard processing the whole paracaval liver was embedded in paraffin blocks.

Serial sections were performed in interval of 50µm starting from cranial to caudal position following the axis of the vena cava. Sections were subjected to standard HE and EVG staining.

## Digitalization

All serial sections were digitalized using a High-Throughput Scanning-Microscope (MedXP-T-1 ™, MedXP GmbH, Germany) at 100x magnification *(Fig.12).* Up to 300 pictures/slide were aquired and stitched. Totally about 15000 pictures were obtained from one sample.

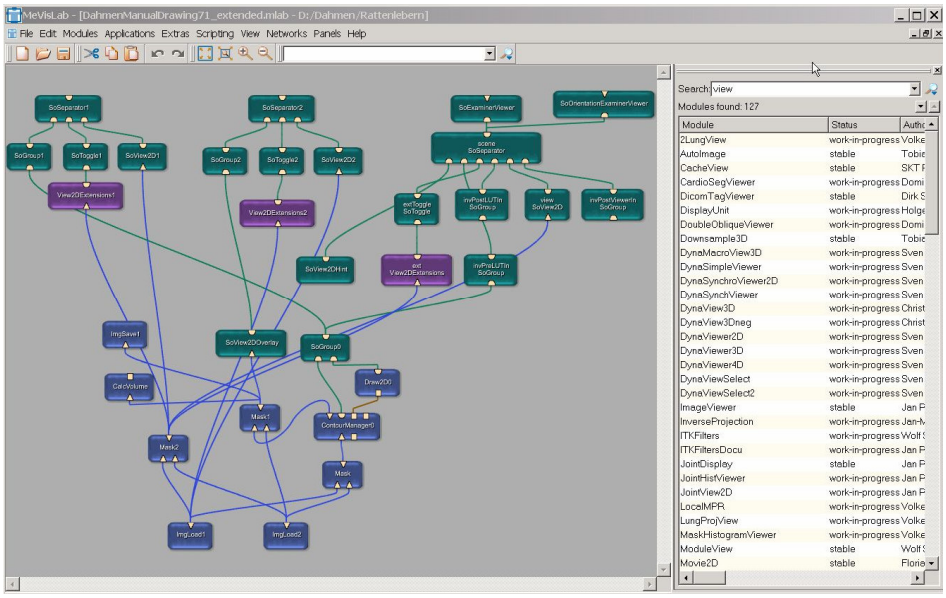
**Figure 12. High-Throughput Scanning-Microscope MedXP-T-1**



## 3D-Volumetry

Image processing for 3d reconstruction was done using MeVisLab software *(Fig.13)* with an adapted application provided by MeVis gGmbH, Bremen, Germany. The application was used for image processing and volumetry. Each stack of matched slices was imported into the software. Necrotic zone on the paracaval liver tissue was outlined manually for every slice. 3d volumetry was performed automatically. Results were presented in percentage of necrotic zone volume (microliters) of the whole remnant liver.

**Figure 13. MevisLab software and modules for volumetry (screenshot)**



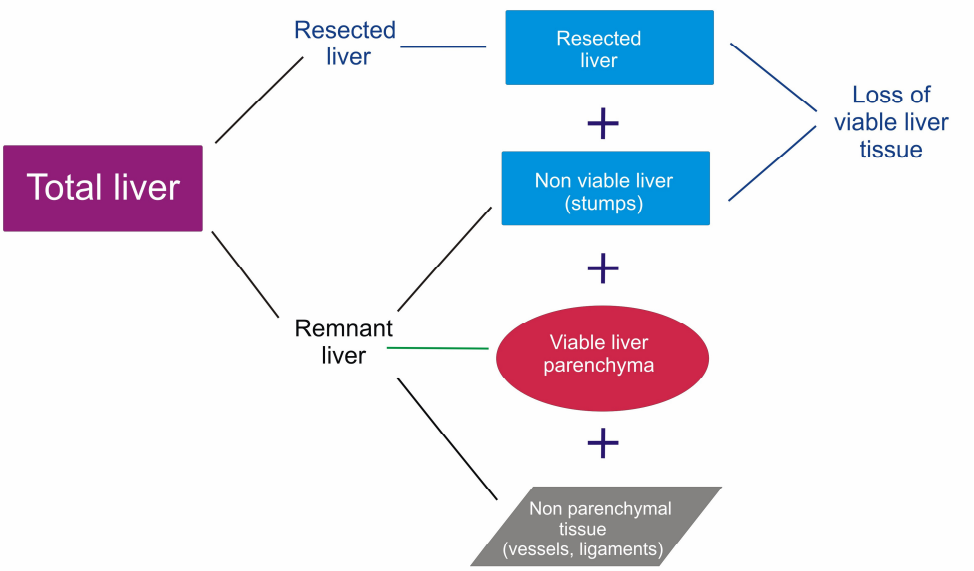

## *3.7. Assessment of liver regeneration*

### 3.7.1. Relative increase in liver weight

Resected and regenerated remnant liver was weighed using ACCULAB V-600 weight scale with capacity of 600 grams and 0.1 grams of readability.

In order to confirm the volume of the separate liver lobes, n=6 animals were sacrificed and the weight of individual liver lobes was determined.

**Figure 14. Structures of liver which should be considered for rat hepatectomy**



The following absolute values were obtained *(Fig.14)*:

1. *Weight of individual liver lobes (g)*
2. *Total explanted liver weight (g)*
3. *Resected liver weight (g)*
4. *Total regenerated remnant liver weight (g)*
5. *Weight of paracaval remnant liver including parenchymal and non-parenchymal tissue (stumps, ligaments, vessels) (g)*
6. *Weight of viable (parenchymal) liver tissue in paracaval remnant liver (g)*
7. *Weight of non parenchymal tissue in paracaval liver (stumps, vena cava and ligaments) (g)*

The following values were calculated:

1. *Calculated total LW= Resected liver weight (g) x 100% / Resected liver volume as indicated (%).*
2. *Relative weight of lobe= Weight of lobe (g)x 100% / Explanted liver weight (g)*
3. *Liver weight recovery (%)= Regenerated remnant liver (g) x 100%/Calculated original total liver weight (g)*
4. *Resected liver to body weight ratio (%)=Resected liver (g)*100%/Body weight (g)*
5. *Inferior caudate lobe to body weight ratio (%)= Weight of ICL (g)*100%/Body weight (g)*
6. *Regenerated liver weight to body weight ratio (Reg.Liv/BW) = Regenerated liver (g) x 100%/Body weight upon sacrifice (g)*
7. *Weight of inferior caudate lobe to body weight ratio (ICL/BW) = Inferior caudate lobe (g) x 100%/Body weight upon sacrifice (g)*

## 3.7.2. Proliferation index

**Immunohistochemical staining**

Incorporated BrdU was detected by immunohistochemical staining to allow calculation of the BrdU labelling index (LI). The staining procedure was based on a modified protocol of Sigma Inc., USA. After deparaffinization and rehydration, tissue sections were treated with prewarmed 0.1% trypsin solution (Sigma, Germany) at 37°C for 40 minutes, followed by denaturation of the DNA with 2N HCl (Merck, Germany) at 37°C for 30 minutes. In the next step sections were incubated with 1:50 monoclonal anti-BrdU antibody (Dako Corp., USA) at 37°C for 1 hour, then blocked with avidin (Dako Corp., USA) solution and subsequently with biotin (Dako Corp., USA) solution for 5 minutes,

respectively, followed by anti-mouse antibody (Dako Corp., USA) linked to alkaline phosphatase for 1 hour at room temperature, prior to the application of Neufuchsin (Chroma, Germany) solution for 20 minutes. The sections were counterstained with hematoxylin, and coverslipped using ImmuMount™ (Shandon Inc, USA).

**Quantitative Analysis**

BrdU LI was determined by analysing 10 digital pictures which were taken at 200x magnification. A computer-assisted image analysis system was used. After transferring the digital pictures to the computer, BrdU-labeled hepatocyte nuclei were counted using ImageTool 3.0, followed by the quantification of unlabeled hepatocyte nuclei using SigmaScan Pro 5.0 (SPSS Inc.,USA) with an adapted macro (28). In this way, more than 3000 hepatocytes were counted per slide. The LI was calculated as the percentage of BrdU-labeled nuclei of hepatocytes out of the total number of hepatocytes (labeled + unlabeled hepatocytes).

Labeling index (LI) = (number of BrdU-labeled nuclei in hepatocytes / number of counted hepatocytes) X 100%

## *3.8. Data analysis and statistics*

Data analysis was divided into following parts.

Clinical condition of rats was shown in individual scatter plots without statistical analysis

All survival rates are demonstrated by Kaplan-Meier charts. The comparison of survival rates in different groups is tested by *log* rank test.

Body weight development was shown in **Mean ± Standard Error of the Mean (SEM)** due to small sample size and high discrepancy presented in retrospective study. Liver weight recovery is demonstrated by weight to body weight ratios analyzed by Mann Whitney test.

All the biochemical results are issued as **Mean ± Standard Deviation (SD)**, the comparison of biochemical results in different groups is tested by *independent samples t*-test.

All damage scores are demonstrated by plot graphics, the comparison of damage scores in different groups is tested by *Mann Whitney* test.

Extent of necrosis in paracaval part is presented in % of volume in paracaval remnant liver as well as in microliters.

BrdU index (PI) is presented in % of positive cells to total number of hepatocytes

Statistical analysis was performed using SPSS v.11 statistical software, SPSS, Inc, USA. Graphs are produced by Sigma Plot v.8, Systat Software, Inc., USA.

# 4. Results

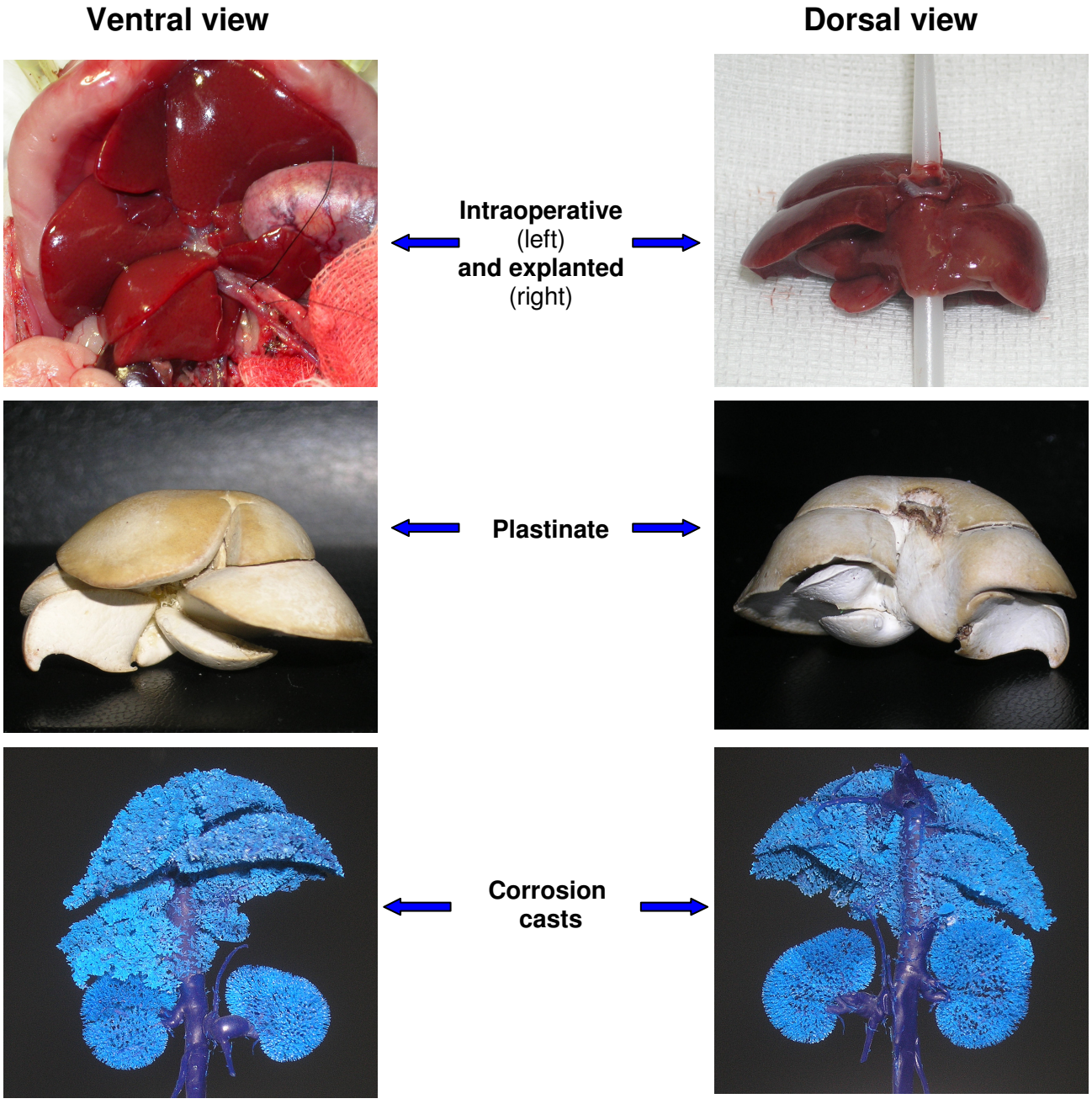
## *4.1. Visualization of rat liver anatomy and consequences for optimizing the vessel oriented resection technique*

### 4.1.1. Lobar anatomy

The rat liver consists of 4 distinct lobes of different size *(Fig.15, 16*). The *left lateral lobe* represents about 30-35% of the total liver weight and is located in the left lateral position dorsal to the median and cranio-ventral to caudate lobes and the stomach. It has a narrow pedicle containing portal vein, hepatic artery and bile duct. The pedicle is covered by the Glisson sheath and a narrow base, both located close to each other. The pedicle is attached to the infrahepatic caval vein and the base of the left portion of the median lobe. The base contains the left lateral liver vein. Interlobular ligaments connect the left lateral lobe with the upper caudate lobe.

The *median lobe* represents about 35-40% of total liver weight and consists of two portions. The left portion and the right portion are separated by a deep fissure. The median lobe is located under the diaphragm and is fixed with the falciform ligament, which spans from xyphoid and diaphragm to the liver beginning at the interlobular fissure. The left portion is smaller and represents about one third of the whole median lobe. The other two third are formed by the right portion. The median lobe has a wide base, surrounding almost half of the circumference of the vena cava.

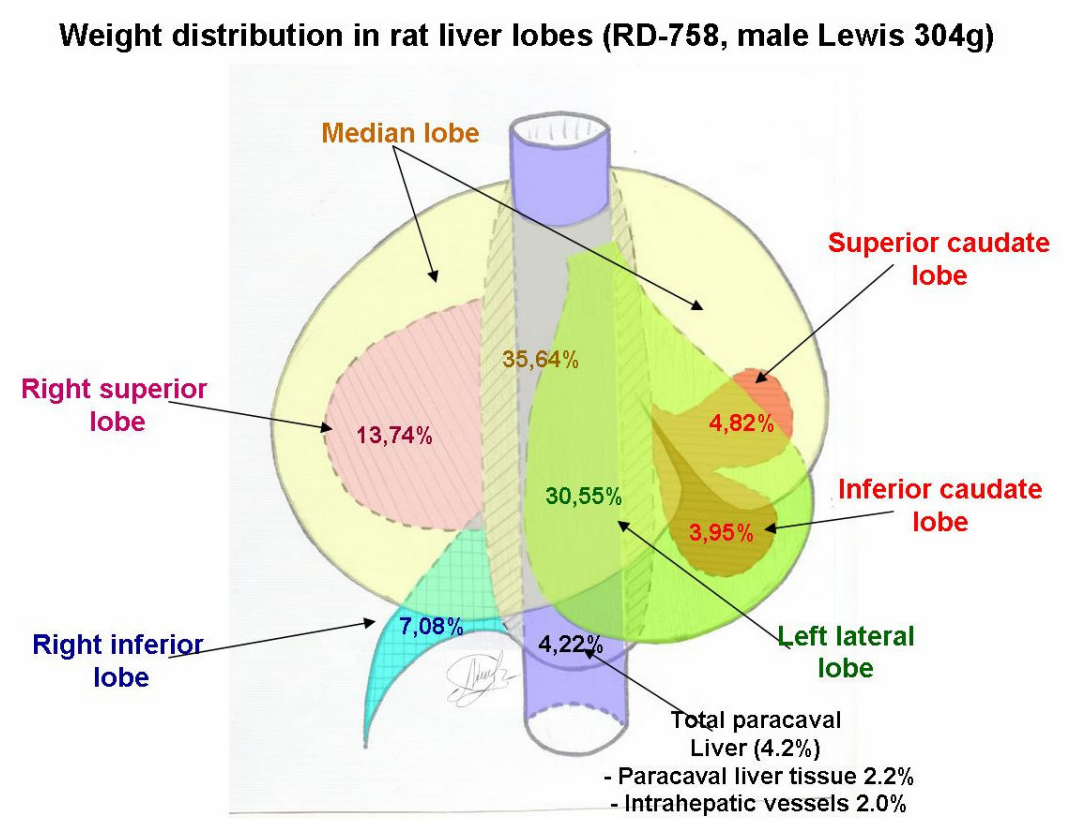
**Figure 15. Rat liver gross anatomy**



The *right liver lobe* is located on the right side of the vena cava and consists also of two distinct portions, the *right superior* and the *right inferior* lobes. Together they represent about 15% of the liver volume. The upper right lobe (10-15%) is shaped like an egg sitting on the intrahepatic cava with the wide base extending to the paracaval part of the liver. Dorsal fixation consists of a wide hepatodiaphragmal ligament. The pyramidal shaped right inferior lobe with the tip pointing to the vena cava comprises 5-7% of the total liver mass and is dorsally attached to the diaphragm and on the ventral side to the cava.

The *caudate lobe***,** also called Spiegel lobe, is located on the left side of the cava below the left lateral lobe and represents 8-10% of the total liver mass. The lobe is divided into two portions – superior or upper lobe and inferior or lower caudate lobe. Both are thin and flat and show an oval shape. The upper caudate lobe is connected to the left lateral lobe via a thin interlobular ligament and is fully covered by the minor omentum. The lower portion of the caudate lobe is located behind the stomach in close neighborhood to the pancreas and spleen and is also covered by a fibrous capsule, attached to the dorsal wall of minor omentum.

**Figure 16. Weight distribution in rat liver lobes**



In contrast to the other liver lobes, the so-called *paracaval liver* does not represent a distinct anatomic-functional unit. It is composed of the dorsal parts of the wide bases of right superior, right inferior and caudate lobes covering the dorsal wall of the intrahepatic cava and contributes to about 2-3% of the liver mass *(Table 8).* The paracaval liver tissue extends from the diaphragmal pedicles to the base of the right inferior lobe and is attached to the dorsal diaphragm by thin ligaments.

**Table 8. Relative contribution of individual liver lobes to the total liver weight and its meaning for definition of liver resection models (based on an individual rat (RD-758, male Lewis rat of 304gr body weight, LBWR 3.84%))**

| Liver lobes (LL) | Weight of ind. LL (gr) | Rel. contrib.(%) of ind. LL to TLW | Liver resection model* | Resected liver mass (%) | Remnant total liver mass (RLM) (%) (intrahepatic vessels included) | Rel% of intrahepatic vessels in RLM | Remnant liver tissue (%) (intrahepatic vessels excluded) | Resected LBWR (%) | Remnant tLBWR |
|---|---|---|---|---|---|---|---|---|---|
| Left lateral lobe | 3.56 | 30.55 | 30%PH | 30.55 | 69.45 | 2.89 | 68.83 | 1,18 | 2.66 |
| Median lobe | 4.16 | 35.64 | 70%PH | 66.19 | 33.81 | 5.94 | 32.45 | 2,55 | 1.49 |
| Right superior lobe | 1.60 | 13.74 | | | | | | | |
| Right inferior lobe | 0.83 | 7.08 | 90%PH | 87.01 | 12.99 | 15.47 | 11.20 | 3,34 | 0.50 |
| Superior caudate lobe | 0.56 | 4.82 | 95%PH | 91.83 | 8.17 | 24.60 | 6.28 | 3,53 | 0.31 |
| Inferior caudate lobe | 0.46 | 3.95 | 97%PH | 95.78 | 4.22 | 47.60 | 2.26 | 3,68 | 0.16 |
| Paracaval liver tissue | 0.26 | 2.21 | 100%PH** | 97.99 | 2.01 | 100.00 | 0.00 | 3,76 | 0.08 |
| Intrahepatic vessels*** (calc) | 0.23 | 2.01 | | 100.00 | 0.00 | | | | 0.00 |
| **Total liver weight (TLW)** | **11.66** | **100.00** | | | | | | | **3.84** |

- LL = Liver lobe
- TLW = Total liver weight including intrahepatic vessels
- PH = Partial hepatectomy
- RLM = Remnant liver mass
- LBWR = Liver-to-body weight ratio
- tLBWR = total liver-to-body weight ratio
- Rel. = relative
- Contrib. = contribution
- Calc. = calculated
- *
- ** only achieved by transplantion of vessel graft as suggested by Azoulay (4)
- ***includes vena cava, Glisson sheath and ligaments at the hilum attached to the paracaval liver

## 4.1.2. Portal vein

Based on the portal bifurcation of the portal vein and the hepatic artery the liver can be divided into a right and a left liver. Left lateral lobe, left portion of median lobe and caudate lobe belong to the left hemiliver, whereas the right hemiliver consists of the right lobe and the right portion of the median lobe *(Fig.17)*.

**Figure 17. Portal vein anatomy in rat liver (illustration)**

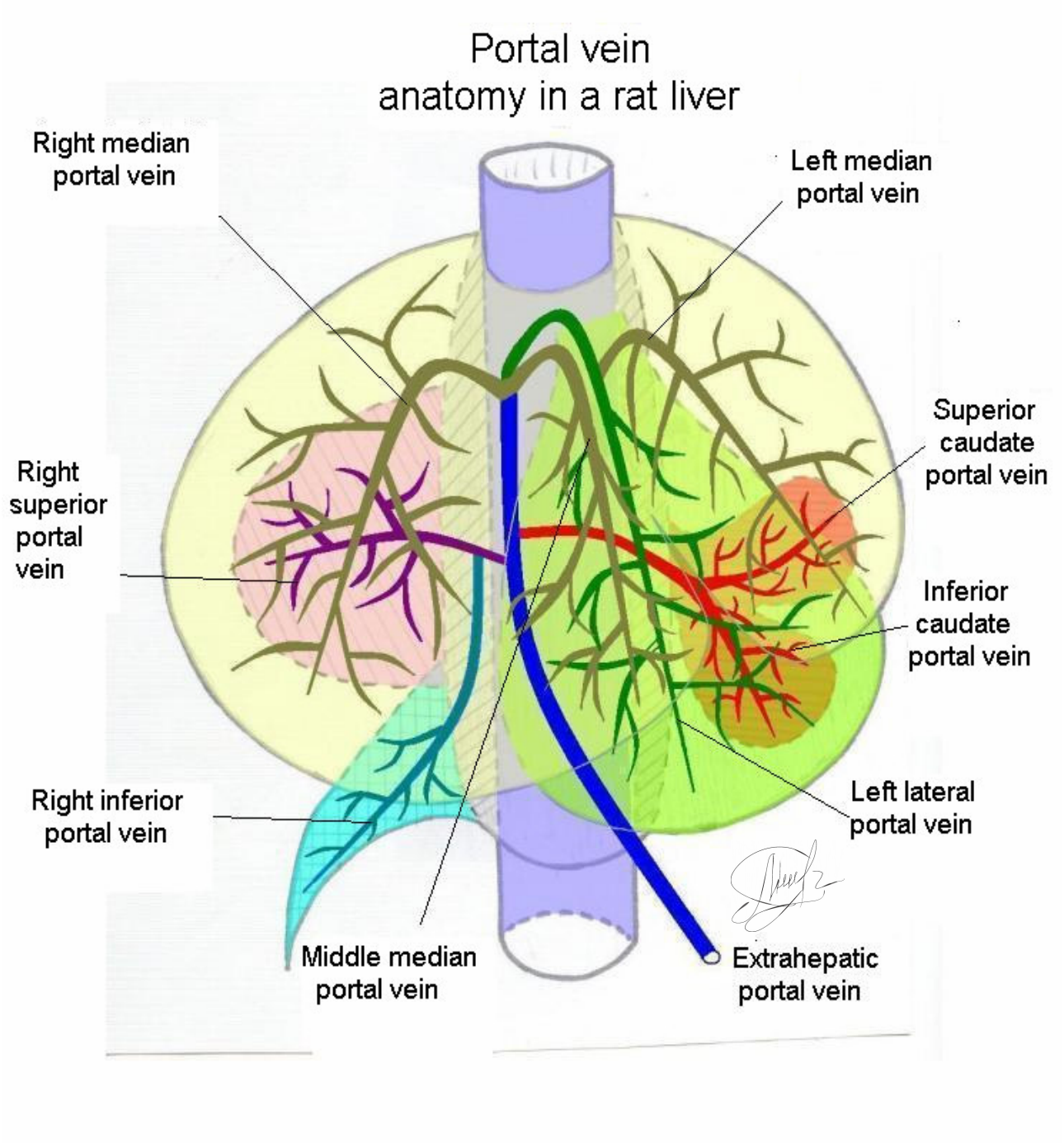


**Figure 18. Vascular cast of rat portal vein anatomy**

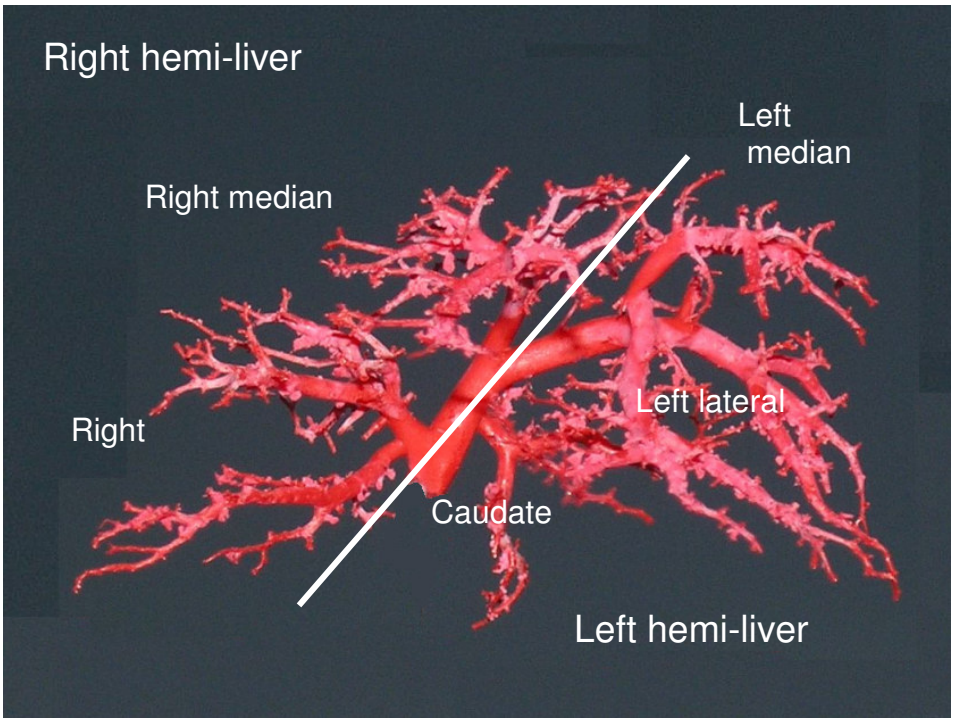
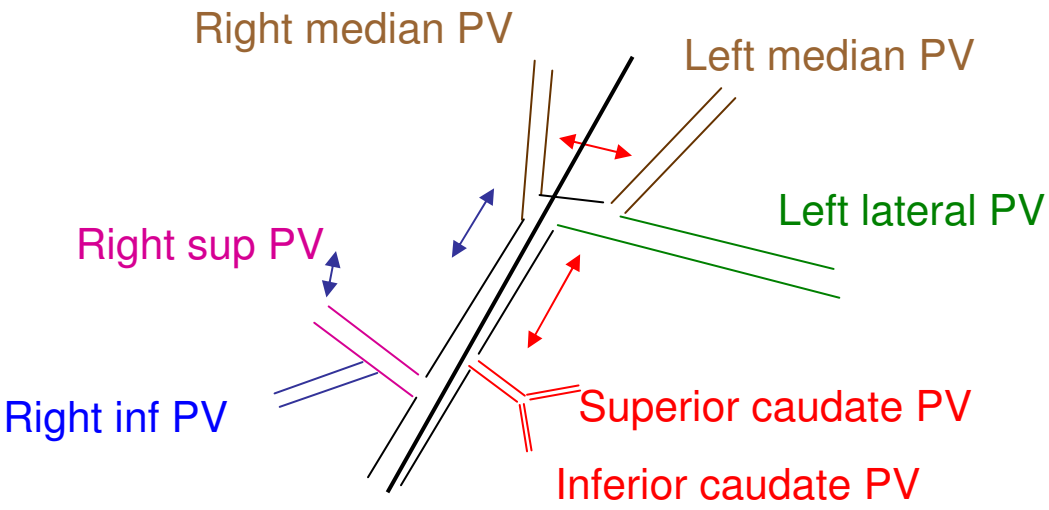



**Figure 19. Schematic drawing of rat portal vein anatomy**



The main bifurcation of the portal vein is the separation of the right median portal vein supplying the right portion of the median lobe and the left portal vein supplying the left portion of the median lobe and the left lateral lobe. The left median portal vein originates within the liver tissue distally to the pedicle of the left lateral lobe. Ligation of the pedicle therefore can result in portal hypoperfusion of the left portion of the median lobe.

The caudate portal vein originates directly from the left side of the main portal stem as the second branch. The right portal vein, which gives the portal supply to the right lobe, originates as the first branch directly from the stem of the portal vein below the bifurcation. Mainly one stem was observed, but in single cases the upper and lower right lobes were supplied by two vessels.

In contrast to the other lobes, which represent distinct anatomic functional units with separate individual portal venous supply and hepatic drainage, the paracaval liver mass receives its blood supply from three different origins. As anticipated by the shape of the paracaval tissue, the left portion is supplied by branches of the caudate portal vein, the right portion of the paracaval tissue receives its supply from the right portal vein, the intermediate and cranial tissue is supplied via separate veins originating from the back of the portal venous stem.

The branches of the hepatic artery and bile duct follow the branches of the portal vein *(Fig.18, 19).*

### 4.1.3. Hepatic veins

The main structure of the hepatic venous system is shown on *Figure 20, 21 and 22*. The left hemiliver is drained by the large left hepatic vein, collecting blood from the left lateral lobe and the left portion from the median lobe. According to the dual portal supply, the median liver lobe is divided into a right and a left portion. However, based on the triple venous drainage, three territories (left, large intermediate and small right territories) can be described. The left median vein receives the venous blood from the left territory of the median lobe.

**Figure 20. Rat hepatic vein anatomy**

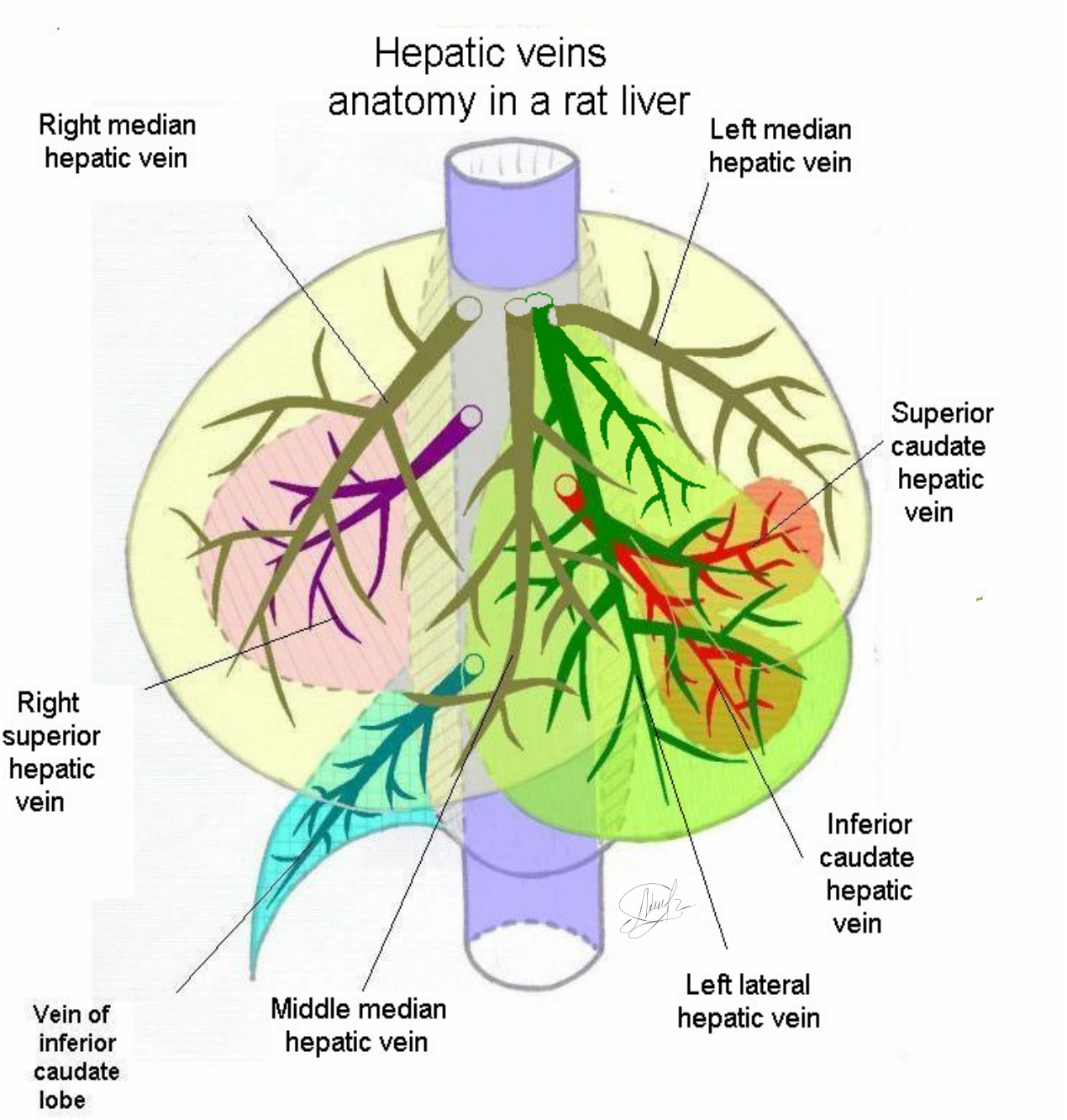


**Figure 21. Vascular cast of rat hepatic vein anatomy**

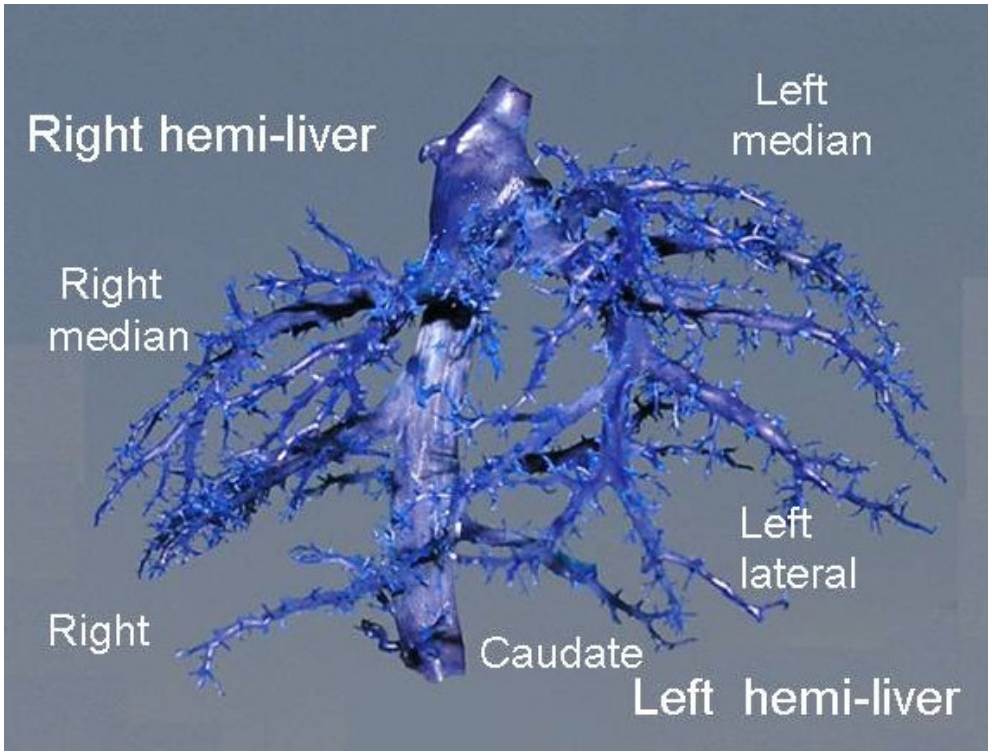
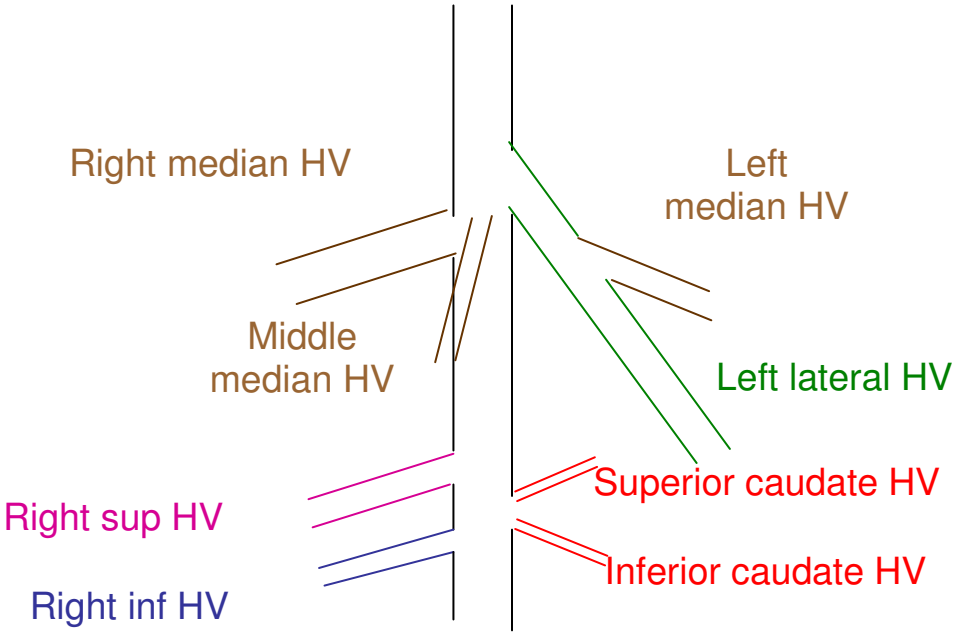



**Figure 22. Schematic drawing of rat hepatic vein anatomy**



The median middle hepatic vein drains part the intermediate median lobe, but flows also into the stem of the large left hepatic vein, sometimes even directly into the cava. The right median vein drains the small right territory of the median lobe. As it enters the cava on the right side, it belongs to the right liver.

The drainage of the right hemiliver consists of the right median hepatic vein entering the cava opposite to and below the left hepatic vein. Furthermore the superior and inferior right vein coming from the respective lobes are draining separately into the right ventral intrahepatic cava.

The caudate lobe is drained by a separate caudate vein, connecting to the left side of the intrahepatic cava.

The paracaval liver is drained by multiple small veins entering directly into the backside of the intrahepatic cava.

### 4.1.4. Technical considerations for resection of each lobe based on anatomical findings

As the liver lobes of the rat are of distinct shape and size, especially regarding the base and pedicle, resection of each lobe requires special attention based on topographic and vascular anatomy.

The *left lateral* lobe seems to be technically easily resectable, as it has a narrow pedicle containing portal as well as hepatic veins and is not connected to the paracaval liver. Simple clamping of the pedicle followed by ligation is appropriate to remove this lobe, but only if the median lobe is also resected within the same procedure.

If resection is limited to the removal of the left lateral lobe (30%PH), the left median portal vein together with the accompanying hepatic artery must be isolated deep in the left hilum and preserved prior to ligation of the pedicle. Simple ligation of the pedicle would severe this branch and lead to ischemia of the left portion of the median lobe.

As the *median lobe* has a wide base semisurrounding the suprahepatic cava, the removal of this lobe requires several (3-4) piercing sutures in a distance of 3mm to the cava for ligation of all vessels in the parenchyma. Removal by simply ligating the wide base of this lobe carries a high risk to cause constriction of the vena cava and an impairment of the liver tissue in the stump.

As the *right superior lobe* is sitting with a very wide base on the cava and is extending dorsally into the right paracaval liver tissue, it is the technically most difficult one to resect. Resection requires careful placement of the clamp in 3mm distance to the cava and 3-4 piercing sutures to ligate separately vessels in the parenchyma and to avoid damage of the stump and the paracaval liver tissue. As 70% of the paracaval tissue is supplied by a branch from the right superior portal vein, the potential damage is of major importance for the remnant liver.

The *right inferior lobe* is attached with a long base extending along the ventral side of the lower intrahepatic cava. Therefore resection is technically not complicated and requires the placement of the clamp close to the cava followed by one piercing suture ligating right inferior portal and heptic veins together.

Both *caudate lobes* have narrow pedicles which extend into the paracaval liver. Removal of theses lobes just requires the ligation of the pedicles prior to resection.

The *paracaval portion* is the only part of the liver, which cannot be surgically removed due to its tight connection to the vena cava. Any manipulation, damaging this part, will lead to penetration or constriction of the vena cava.

## *4.2. Influence of surgical technique on outcome after 90% hepatectomy*

### 4.2.1. Prospective study regarding influence of technique on outcome

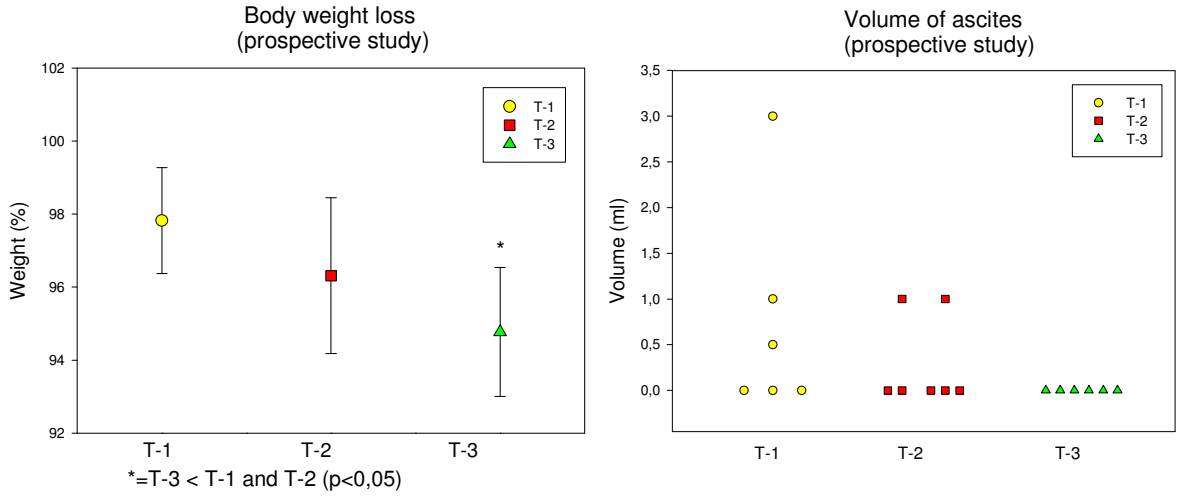
Totally 22 rats were operated according to the 3 different techniques. 4 animals were excluded from the evaluation. Criteria for exclusion were major intraoperative blood loss and necrosis of remnant liver under the line of sutures extending to paracaval part. This complication was caused by a technical error in placing sutures. All animals were sacrificed at 24 h after operation.

#### 4.2.1.1. Clinical outcome

Body weight loss

Within the observation time of 24h, animals lost maximally 15g of weight representing 7% of total body weight *(Fig.23).* Animals subjected to “mass ligation” procedure –T-1 and “extra mass ligation” procedure T-2 presented with 98,72+/-1,45% and 96,29+/-1,97% of original body weight respectively.

**Figure 23. Influence of surgical technique on body weight loss at 24 h after 90%PH**




Animals subjected to "parenchyma preserving vessel oriented" technique –T-3 experienced a significantly higher body weight loss of >5% ($p<0,005$).

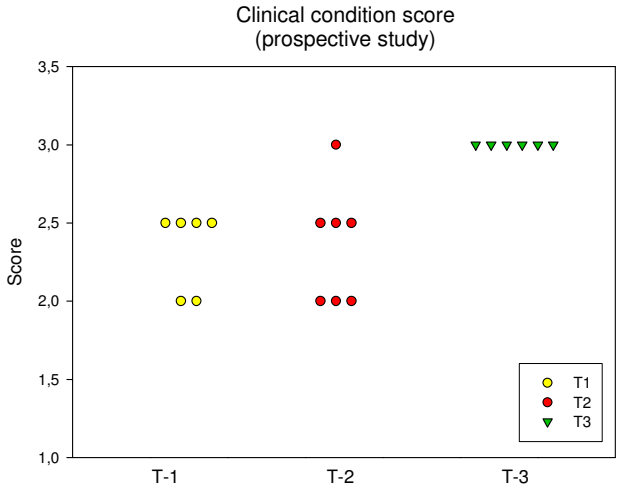
Mean body weight in this group was 94,7+/-1,63%. Interestingly, none of them developed ascites, whereas 2-3 animals operated according to both "mass ligation" techniques produced ascites up to 3 ml. Absence of ascites production partially explained the higher body weight loss in T-3 group.

Clinical condition

Animals operated according to mass ligation methods had a lower clinical condition score (++ in average) compared to the group operated according to vessel oriented technique *(Fig.24)*. All animals operated according to the vessel oriented anatomical resection were in excellent condition (+++). At the end of the first postoperative day they were active, food and glucose consumption was not affected. Animals, which underwent mass ligation, were weak but not dying at 24 h after operation.

Common signs of worsened clinical condition were "hunched back", hypodynamia, reduced consumption of 20% glucose, low resistance to grasping, moderate hypothermia (based on subjective feeling estimated as 35-36$^0$C of body temperature) and pale skin on paws and ears.

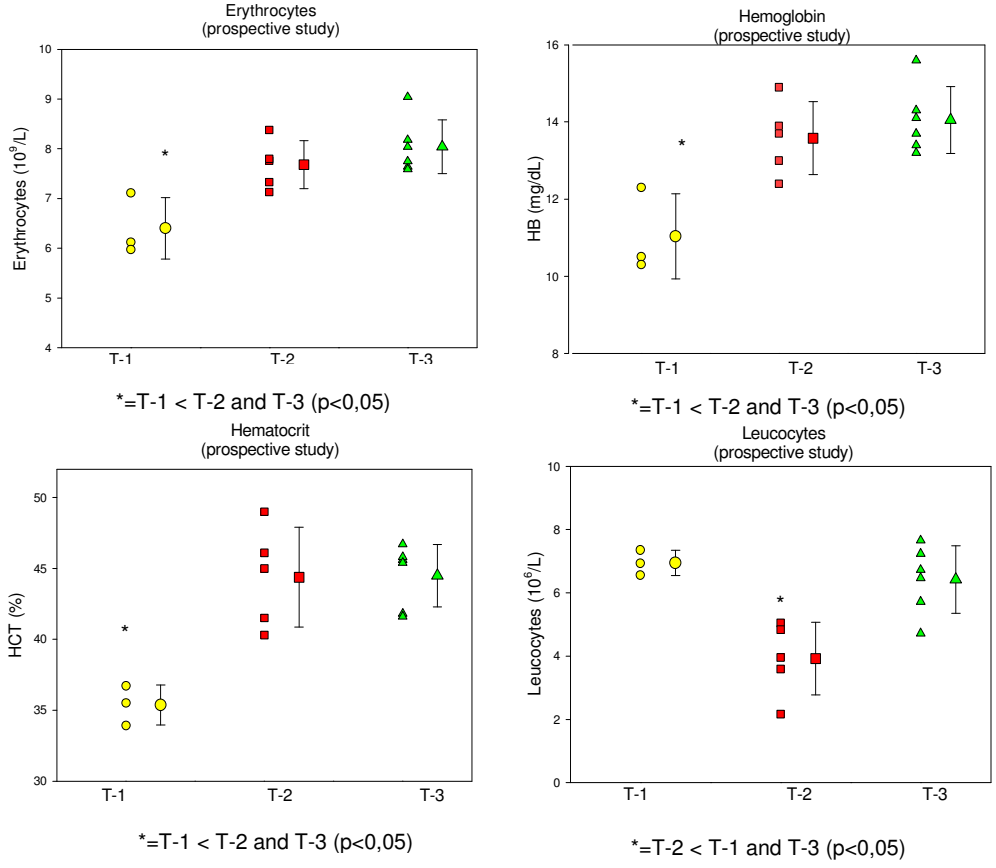
**Figure 24. Influence of surgical technique on clinical condition at 24 h after 90%PH**




Anemia

Animals operated according to T-1 technique showed obvious anemia with significantly lower levels of HB, HTC and Erythrocytes compared to other groups leading to the suspicion of a higher blood loss, when applying this technique *(Fig 25*). Red blood cells, HB and HTC values were in the range of 5,8-7,3 g/L, 10-12,5 x $10^6$/L and 34-37% respectively. In animals, operated according to T-2 and T-3 techniques, blood count values were within the normal range.

**Figure 25. Influence of the surgical technique on blood count at 24h after 90%PH**

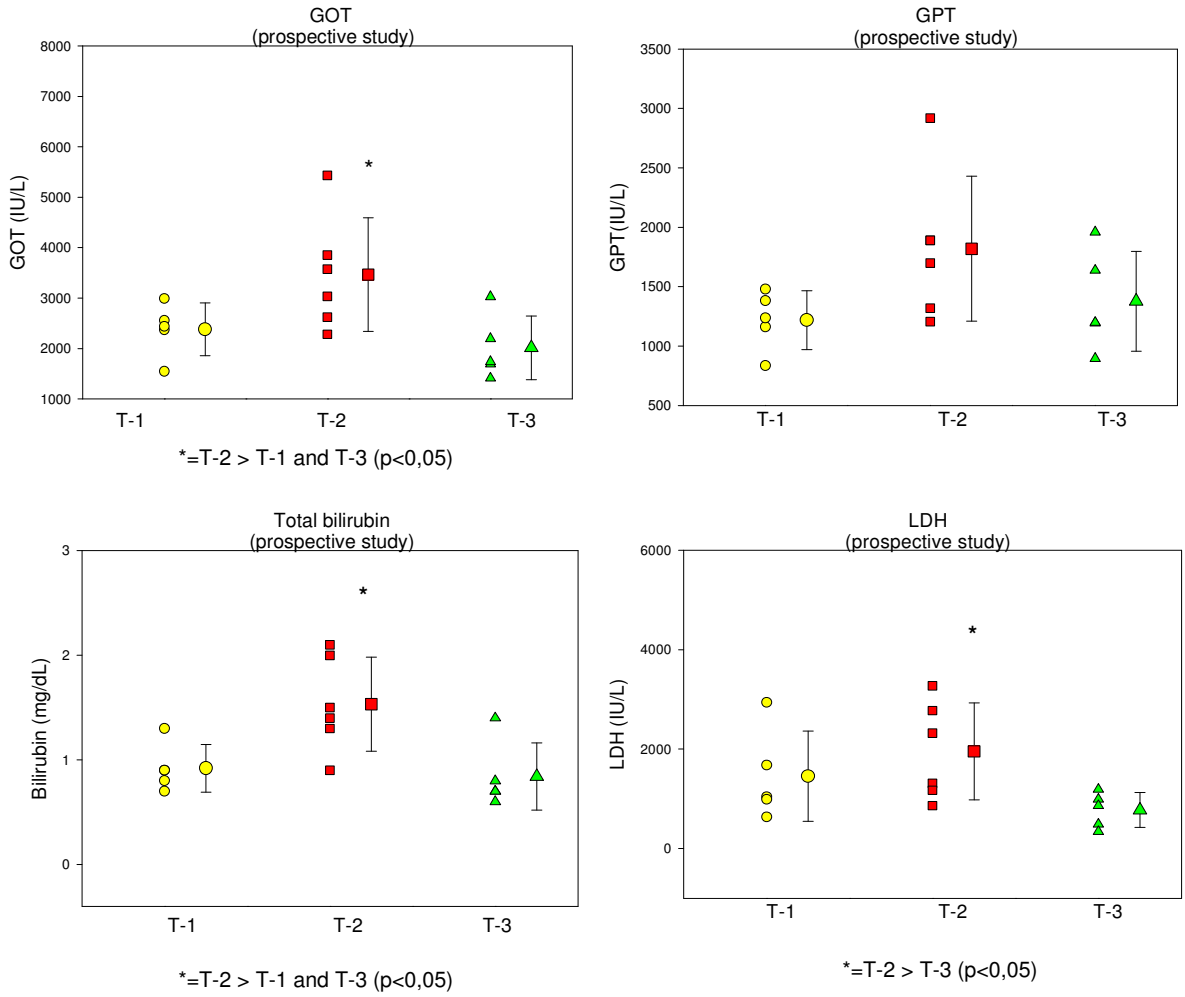


### 4.2.1.2. Liver damage

Liver function

Biochemical parameters indicating liver injury were highest in the group T-2, subjected to "extra mass ligation" procedure. Animals subjected to "mass ligation" only prevailed with slightly lower values, although no statistically significant difference was found in comparison with T-3 group. Nevertheless, lowest values were observed in the animals operated according to T-3 procedure. Difference between T-2 and T-3 was significant in terms of GOT, LDH and bilirubin values *(Fig.26).*

**Figure 26. Influence of the surgical technique on liver function at 24h after 90%PH**

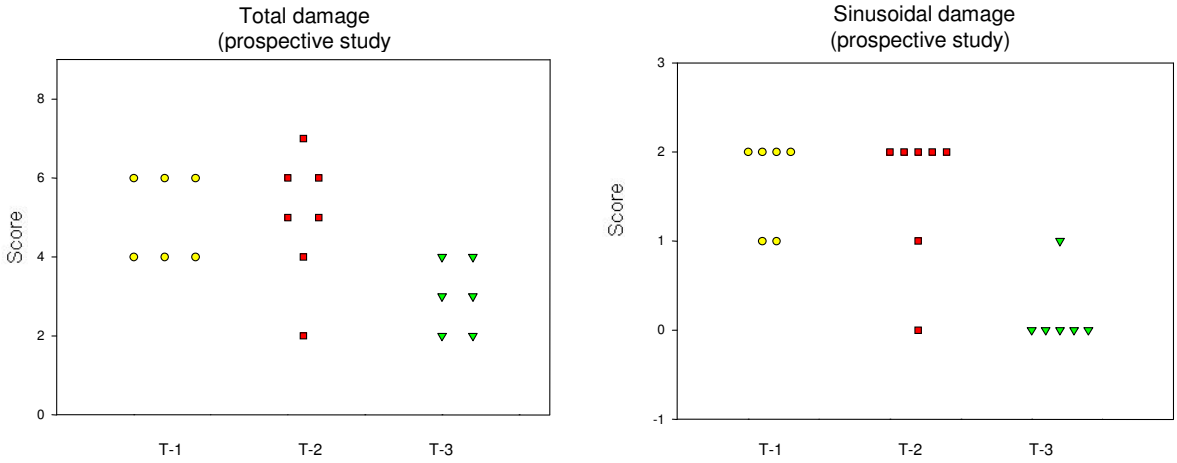



All 4 parameters suggested that the damage to the remnant liver was more severe when using an "extra mass ligation" (T-2) in comparison to the "parenchyma preserving vessel oriented" technique (T-3). *(Fig.26).*

Histological damage

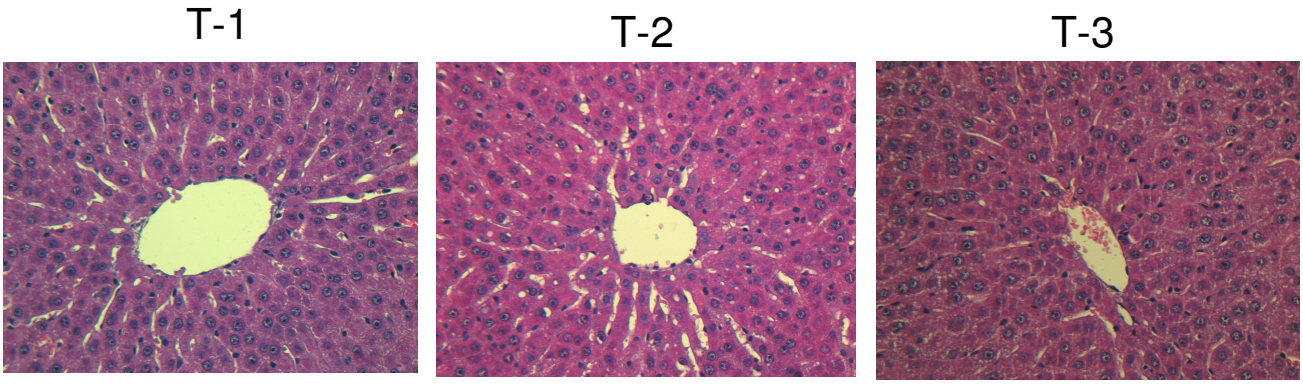
Histomorphologically, the total damage score was lower in the group operated according to T-3 procedure in comparison with the two groups and ranged from 2 to 4. Animals undergoing “mass ligation” and “extra mass ligation” methods presented with higher damage indicated by score ranging from 4 to 7 *(Fig.27).* The “mass ligation” techniques (T-1, T-2) showed a higher degree of sinusoidal dilatation compared to the “parenchyma preserving vessel oriented” technique T-3 *(Fig.28).*

**Figure 27. Influence of surgical technique on histomorphological damage at 24h after 90%PH (prospective study)**




In contrast, this finding was not pronounced in T-3 group. No debris was found in sinusoidal space, whereas in the other two groups these changes occurred frequently.

**Figure 28. Influence of surgical technique on liver histology (prospective study).**




REG-240 (200x,H&E) REG-234 (200x,H&E) REG-235 (200x,H&E)

Three dimensional histomorphometry

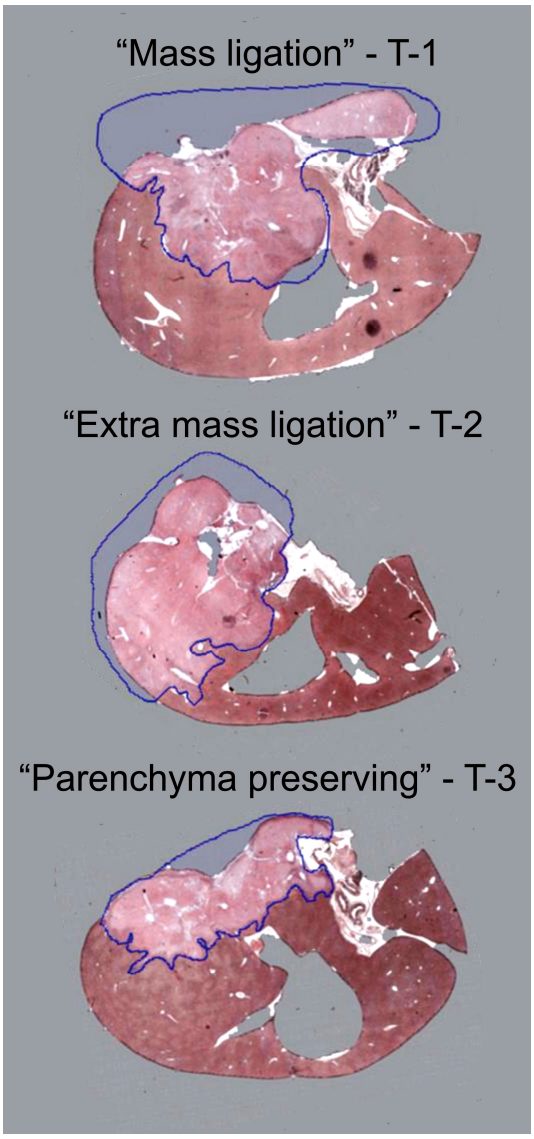
In microscopical 3D volumetry the extent of mass necrosis on paracaval liver, as measured on the serial sections after digitalization using MeVisLab, was lowest (27%) in animal subjected to T-3 technique. “Extra mass ligation” procedure caused the largest zone of necrosis in paracaval liver (57%), which was twice as large compared to T-3. Animal subjected to “mass ligation” had a necrotic zone occupying only 38% of the paracaval remnant liver (*Fig.29).*

**Figure 29. Influence of surgical technique on extent of necrosis in paracaval liver**

**Animal Liver Volume of necrosis**
**T04-72: 0.1620 ml 0.0614 ml (37.9%)**

**Animal Liver Volume of necrosis**
**T04-71: 0.1338 ml 0.0756 ml (56.5%)**

**Animal Liver Volume of necrosis**
**T04-70: 0.1890 ml 0.0508 ml (26.9%)**



### 4.2.1.3. Liver regeneration

Liver weight recovery

Although the resected liver mass was within the same range (**T-1**: 7,18+/-0,52, **T-2**: 7,43+/-0,72 and **T-3**: 7,18+/-0,61 g), the liver weight recovery was higher after "parenchyma preserving vessel oriented" resection *(Fig.30, 31)*. Liver weight recovery was significantly higher (p<0,05) in values of absolute liver weight (**T-1**: 2,25+/-0,15, **T-2**: 2,32+/-0,26 and **T-3**: 2,73+/-0,12 g) as well as in Reg.Liv/BW (**T-1**: 0,85+/-0,03, **T-2**: 0,86+/-0,08 and **T-3**: 1,07+/-0,04 %) *(Fig.32)*.

**Figure 30. Influence of surgical technique on liver weight recovery at 24h after 90%PH (prospective study)**

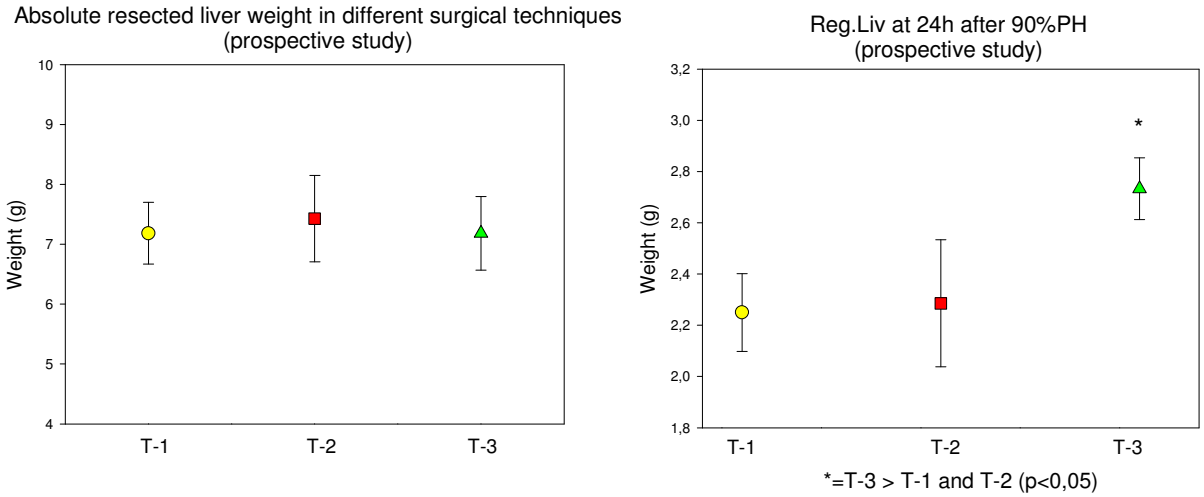



**Figure 31. Influence of surgical technique on liver weight recovery in relation to calculated original liver weight at 24h after 90%PH (prospective study)**

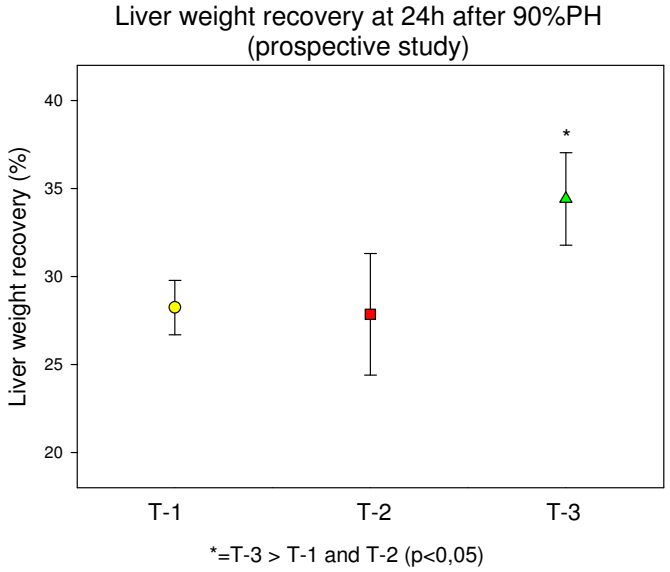


**Figure 32. Influence of surgical technique on Reg.Liv/BW at 24h after 90%PH (prospective study)**

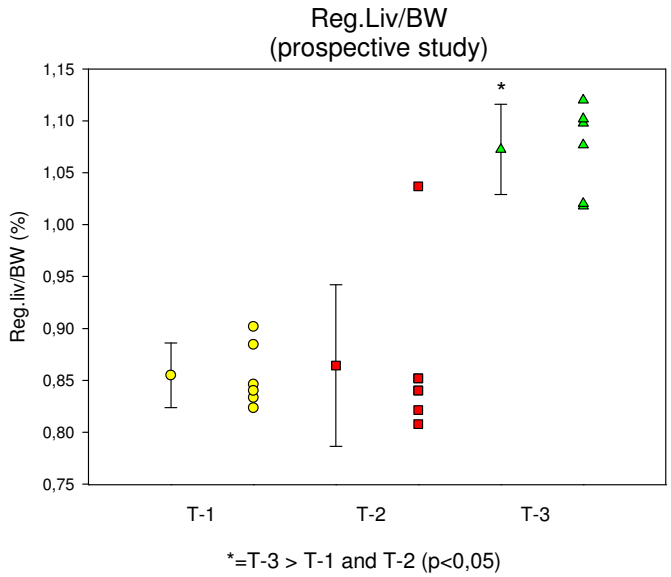
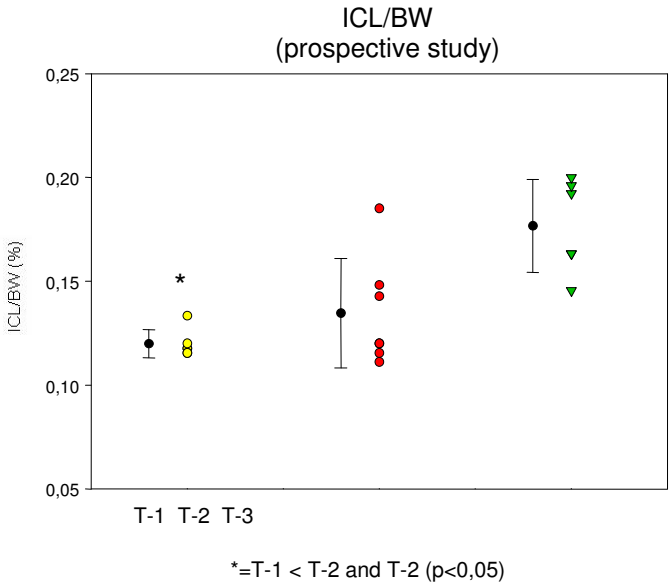



**Figure 33. Influence of surgical technique on ICL/BW at 24h after 90%PH (prospective study)**

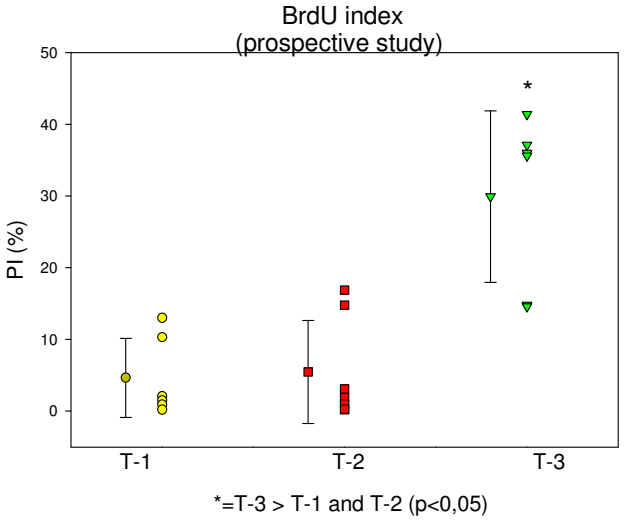



The calculated mean regenerated liver to body weight ratio also was significantly higher (Fig.32, 33) in animals from the group subjected to the T-3 technique in comparison to the two other groups ($p<0,05$).

Regeneration

The proliferative index (PI) derived from IHC staining of BrdU-labeling was also significantly higher in animals undergoing “parenchyma preserving vessel oriented” technique compared to both “mass ligation” procedures (29,92+/-11,97 % in T-3 group vs 4,67+/-5,5 of T-1 and 5,46 +/- 7,42 of T-2 group respectively).

**Figure 34. Influence of surgical technique on liver regeneration at 24h after 90%PH (prospective study)**




Analysis of the data distributions revealed that 4 out of 6 animals after “mass ligation” procedures showed no proliferative response at 24h (PI<1%), whereas in the “parenchyma preserving vessel oriented” group 5 out of 6 animals showed proliferation above 30%. All so called “escapers” prevailed with PI of about 10% *(Fig.34).* Further analysis of these individual animals allowed suspecting ineffective compression of the base of right superior lobe probably due to anatomical variation. Despite the fact, that single animals showed discrepant results, these data indicate clearly that liver regeneration was substantially enhanced after “parenchyma preserving vessel oriented” procedure.

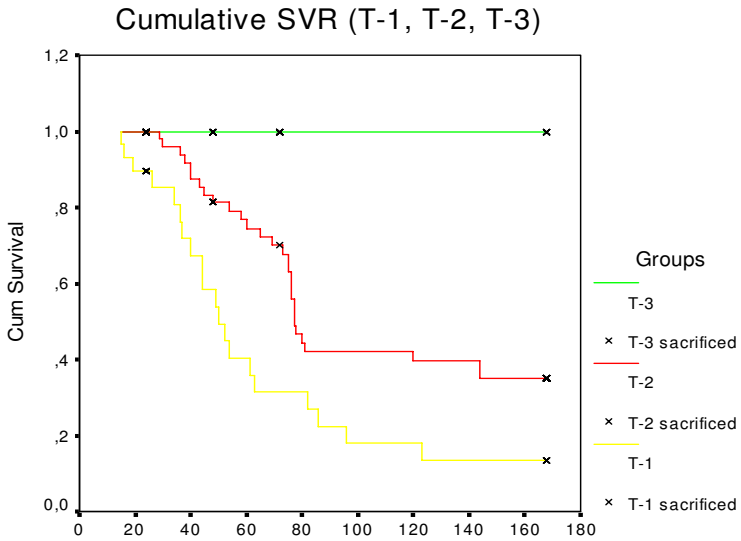
## 4.2.2. Confirmation of results in retrospective study

### 4.2.2.1. Clinical outcome

Survival

"Parenchyma preserving vessel oriented" technique resulted in survival of all animals throughout the observation period of 1 week *(Fig.35).* Both "mass ligation" techniques had 1 week survival rate below 50% (40% -T-2 and 20%-T-1). 60% of the animals died in the first 3 days after operation, the remaining animals on POD-4 and 5.

**Figure 35. Effect of surgical technique on survival after 90%PH (retrospective study)**




Clinical condition

Clinical condition score was highest (+++) in all animals operated according to vessel oriented anatomical resection technique whereas the score in animals of group T-1 and T-2 was ranging from (++) to (+++/++) throughout postoperative period. Dying of animals only occurred in groups subjected to mass ligation procedure. The clinical condition of dying animals substantially deteriorated while approaching death. The score of (+) was predictive of lethal outcome within the next few hours.

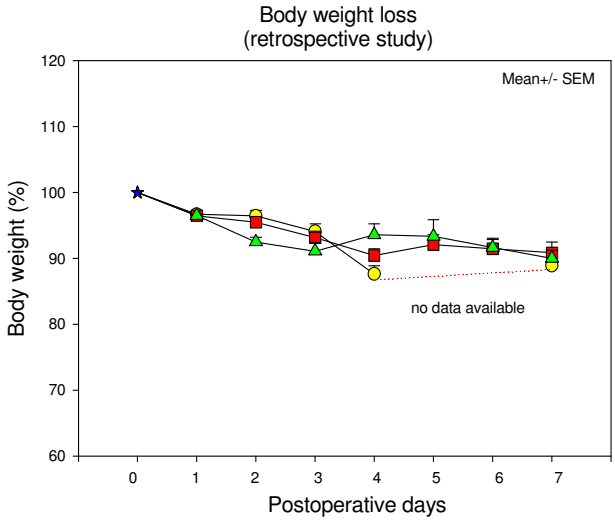
Body weight loss

In contrast to the prospective study the body weight loss at 24h was about 3-5% in all groups *(Fig.36).* Body weight ranged from 95-97% of original body weight. However, the

amount of ascites found in both “mass ligation” groups on POD-1 was 1-2 ml, therefore partially masking part of the body weight loss.

All animals had constant decrease in body weight within the observation time (1 week) without showing statistically significant difference between three groups. No drop below 85% of original body weight was observed.

**Figure 36. Influence of surgical technique on body weight loss after 90%PH (retrospective study)**



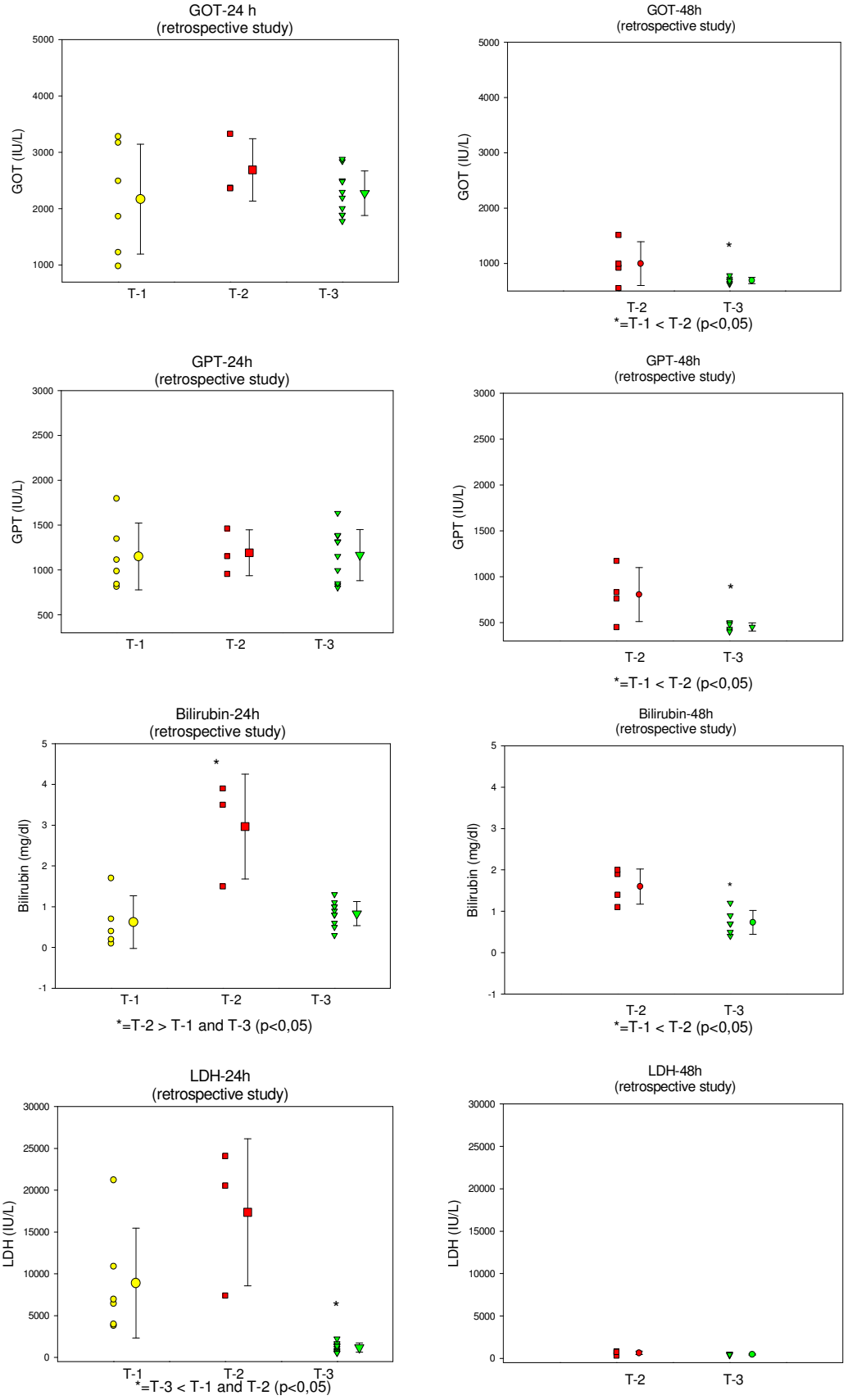

### 4.2.2.2. Liver damage

Liver function

Analysis of the liver function revealed that the average levels of all 4 parameters (GOT, GPT, Bili, LDH) were higher after “extra mass ligation” procedure. Animals operated according to T-3 technique had significantly lower bilirubin and LDH with little interindividual variations within the group.

By one week after operation, liver function of all sacrificed animals had almost fully recovered (GOT<100 IU/L; GPT<100 IU/L and bilirubin <0,3 mg/dL) *(Fig.37).* Recovery of the liver function seemed to take place later in animals from T-2 group, as all parameters were significantly higher compared to T-3 group at 48h after operation.

**Figure 37. Influence of surgical technique on liver function at 24h and 48h after 90%PH (retrospective study)**



Histomorphological damage to the remnant liver at 24h postoperatively, especially sinusoidal dilatation, was more pronounced when using either of the “mass ligation” methods *(Fig.39).* Mainly the total damage score in these groups ranged from 4-8 points. “Parenchyma preserving vessel oriented” resection resulted in moderate damage reflected in a scale of 2-6 (mainly 3-4) points. When looking at sinusoidal alterations separately, the difference between groups was more obvious: 12/17 from both “mass ligation” groups scored 2, whereas 10/11 from T-3 group scored 0 *(Fig.38).*

**Figure 38. Influence of surgical technique on histomorphological damage at 24 h after 90%PH (retrospective study)**

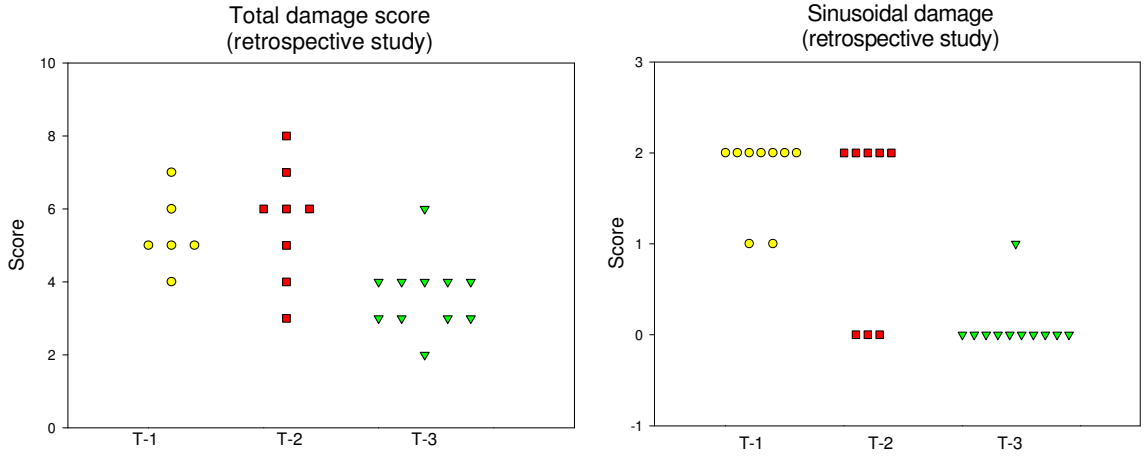
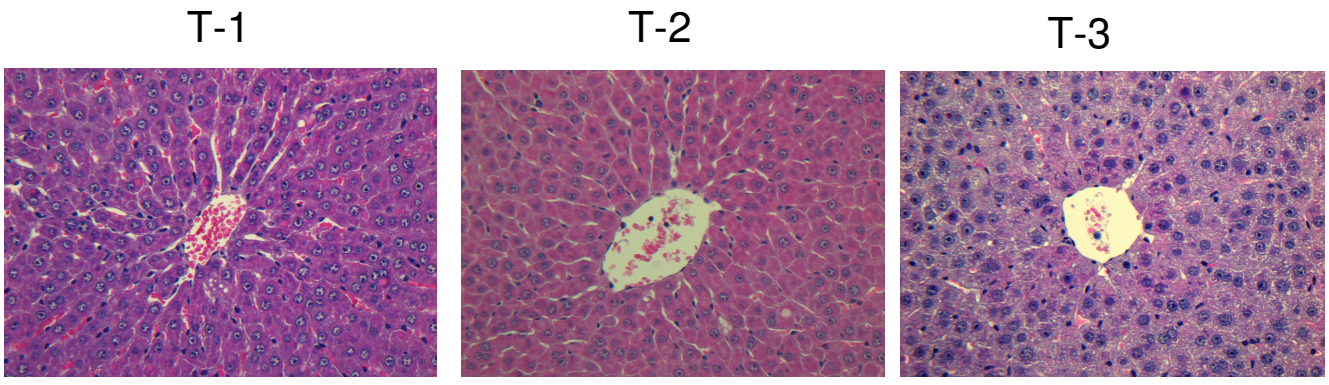



**Figure 39. Influence of surgical technique on liver histology (retrospective study).**




REG-129 (200x,H&E)

REG-162 (200x,H&E)

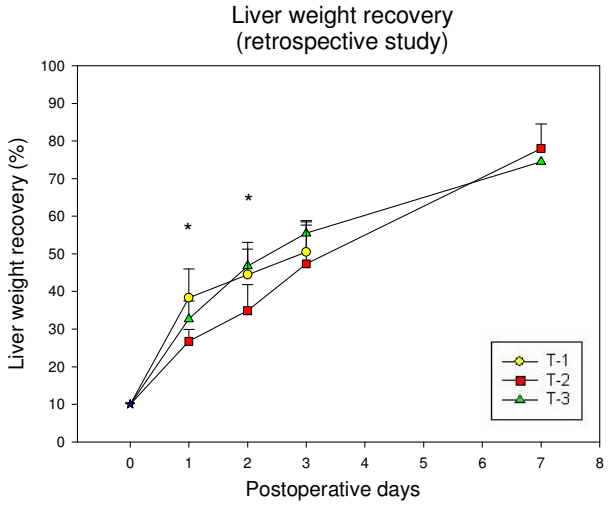
REG-216 (200x,H&E)

### 4.2.2.3. Liver regeneration

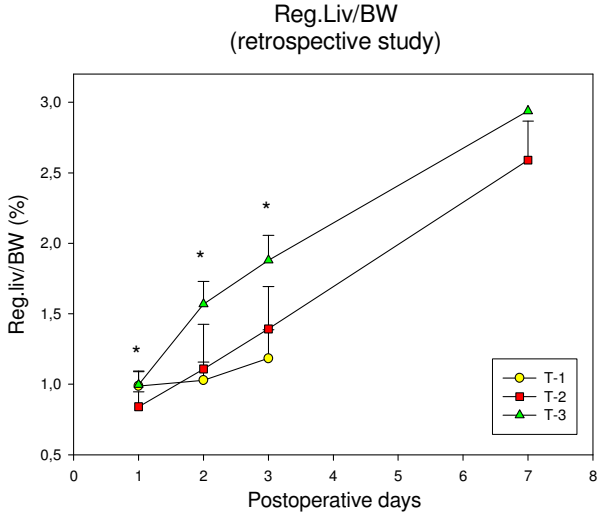
Liver weight recovery

Liver weight recovered up to 85% of the original calculated liver weight within the 7 days of observation in all 3 groups (Fig.41 and 42). However, the slope of recovery was significantly different, and relative increase in liver weight was higher at POD 1 and 2 in the T-3 group compared to T-2. When calculating the liver weight recovery in values of Reg.Liv/BW, similar results were found *(Fig.40).*

**Figure 40. Influence of surgical technique on liver weight recovery after 90%PH (retrospective study)**




**POD-1**
*=T-1 > T-2 and T-3 (p<0,05)
**POD-2**
*=T-2 < T-1 and T-3 (p<0,05)
T-3 (POD-2) >T-3 (POD-1) (p<0,05)




**POD-1**
*=T-3 > T-1 and T-2 (p<0,05)
**POD-2**
*=T-3 > T-1 and T-2 (p<0,05)
T-3 (POD-2) >T-3 (POD-1) (p<0,05)

**Figure 41. Situs at different time points after 90% hepatectomy**

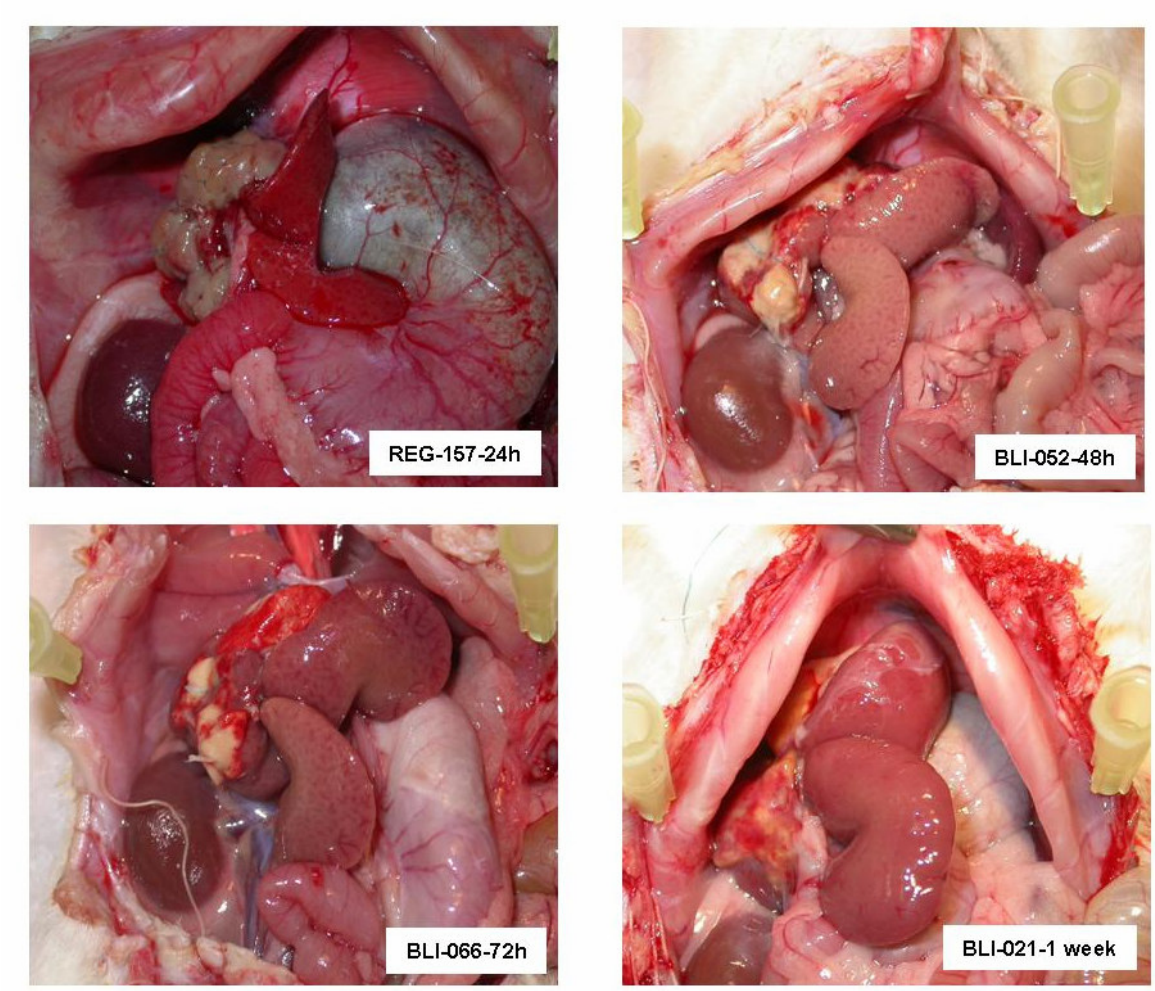
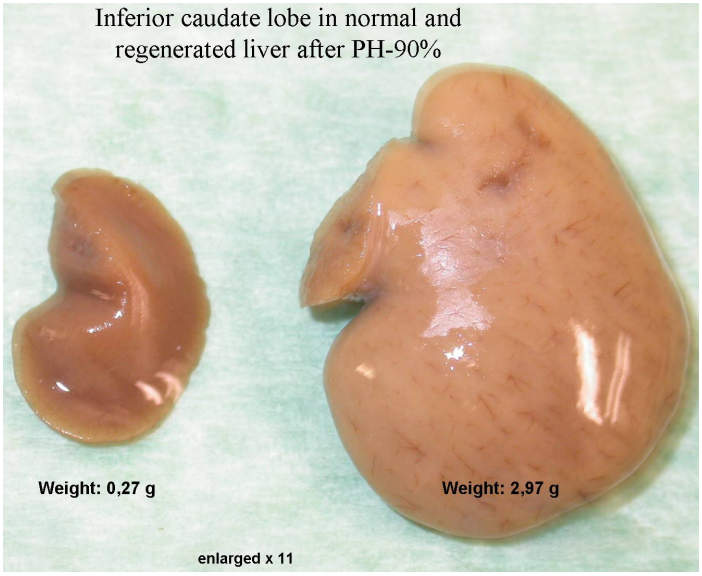


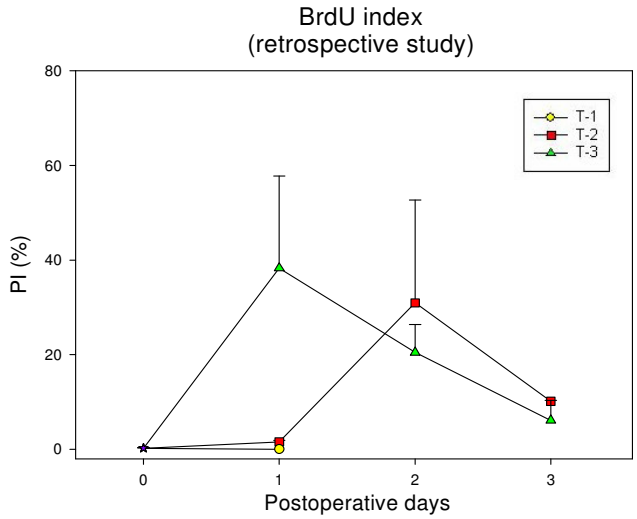

**Figure 42. Inferior caudate lobe in normal and regenerated liver after 90%PH**



Proliferation

Animals subjected to “both mass ligation” methods showed no signs of hepatocyte proliferation at the first postoperative day. Initiation of the regeneration was delayed to POD-2, with a PI of 10-30%. Animals subjected to “parenchyma preserving vessel oriented” resection showed completely different kinetics. As in the prospective study group, regeneration started at POD-1, with PI of up to 40% (mean 27,54 +/-8,8 %) and declined thereafter *(Fig.43).*

**Figure 43. Influence of surgical technique on kinetics of liver regeneration (PI) after 90%PH (retrospective study)**




## *4.3. Application of optimized technique for further reduction (95% and 97%) of liver mass*

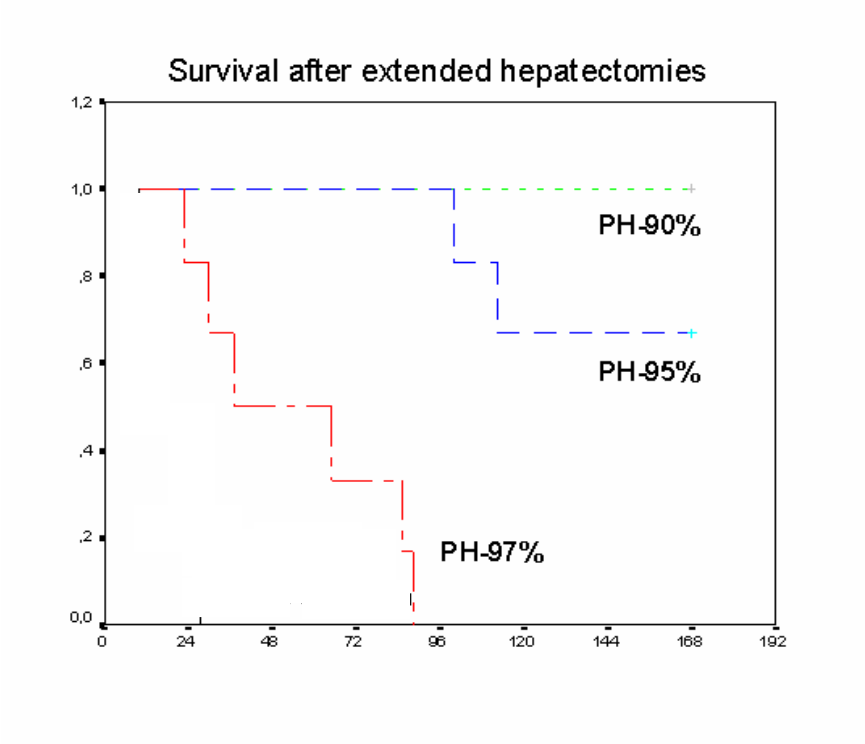
### 4.3.1. Clinical outcome

Survival Time

Extended 90% hepatectomy resulted in survival of all animals which were sacrificed one week postoperatively in excellent clinical condition. 66% of the animals reached one week after “mega”-extended hepatectomy. 2 out 4 animals died between POD 3 and 5. In contrast, “giga”-extended hepatectomy with removal of 97% of the liver mass was leading to the death of all animals within 4 days postoperatively. This was significantly

longer (log rank test) than the survival time reported after total hepatectomy by Azoulay *(Fig.44 and 62).*

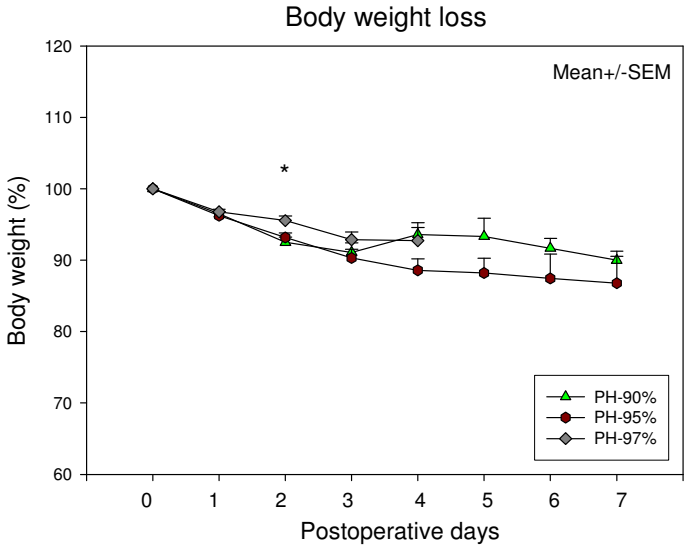
**Figure 44. Influence of "parenchyma preserving vessel oriented" technique on cumulative survival after 90%PH, 95%PH and 97%PH**




Body weight loss

Animals undergoing 90%PH and 95%PH experienced a continuous body weight loss of up to 5% in the first 3 days after operation *(Fig.45).* Thereafter the body weight stabilized. Total body weight loss at 1 week after operation ranged from 10-20% (mean 13,2+/-7,2 %). In contrast the animals subjected to 97%PH experienced a body weight loss of maximally 8% within the first 4 days. True body weight loss was masked partially by production of 3-5 ml of ascites in this group.

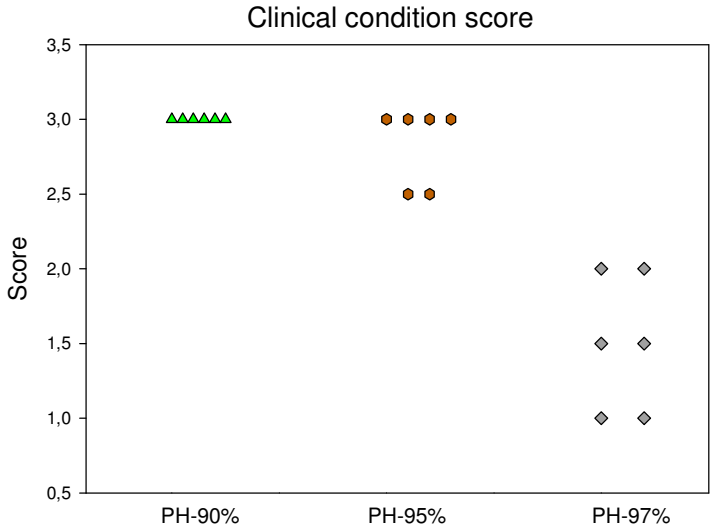
**Figure 45. Influence of "parenchyma preserving vessel oriented" technique on body weight loss after 90%PH, 95%PH and 97%PH**




Clinical condition

Clinical condition of rats undergoing 90% resection was excellent from the first day after operation *(Fig.46)* throughout the observation period of 7 days. 4/6 animals survived 95% resection and presented with a condition score of (+++) throughout the observation period. One of them deteriorated slightly towards the end of the POW-1.

**Figure 46. Influence of "parenchyma preserving vessel oriented" technique on clinical condition at 24h after 90%PH, 95%PH and 97%PH**



Postoperative outcome could be “somehow” predicted by the clinical condition score given given at POD-1. All animals after 90%PH presenting with a score of 3 points survived. The 3 animals after 95% and 97% liver resection receiving a lower score died within 5 days postoperatively.

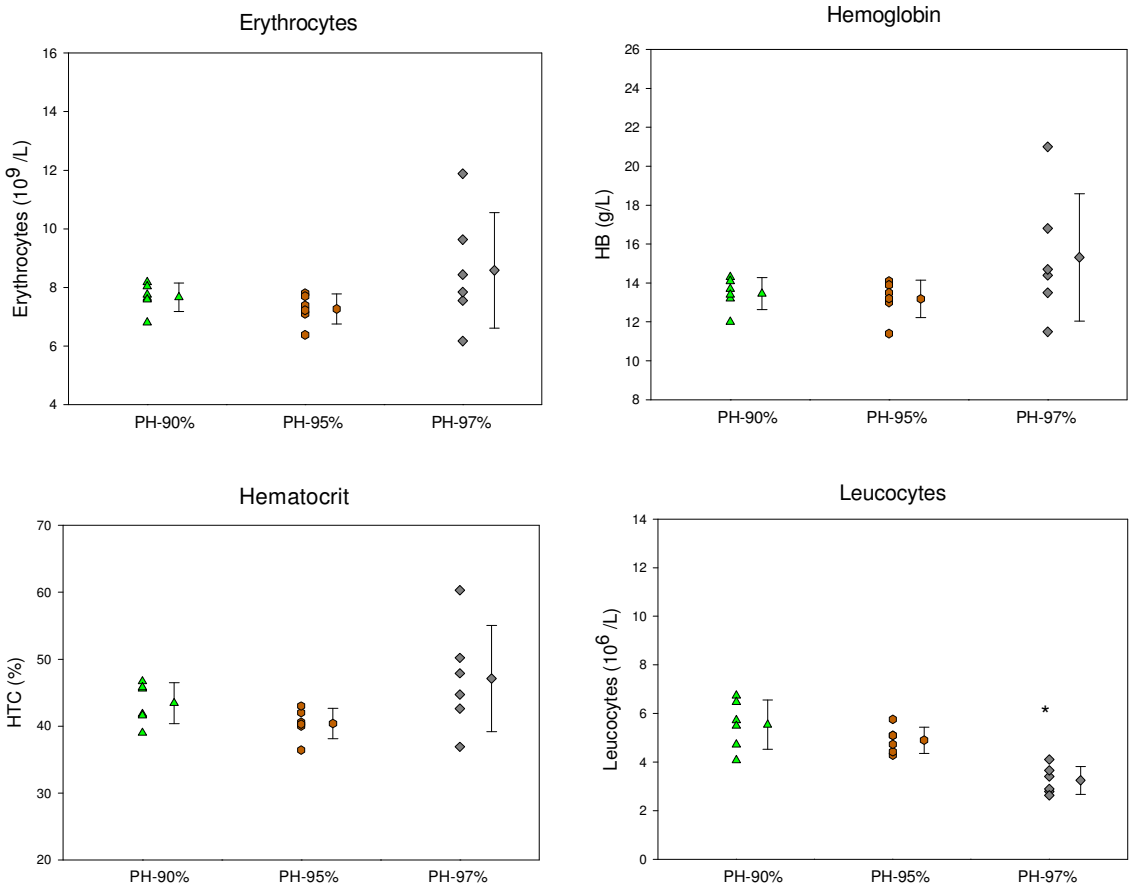
Animals undergoing 97%PH never recovered from the operation. All dying animals were hypothermic, lying in one location in the cage and did not display any resistance to grasping as well as any active reaction to visual or tactile stimuli. Pale skin on paws and ears could be observed. Food and glucose consumption was minimal, turgor of skin was reduced. Also, absence of urination and defecation could be observed. Hepatic encephalopathy was presented by reduced attention to external visual and tactile stimuli, disorientation, muscular tremor and somnolence suggesting the development of coma. At last phase of encephalopathy no response to pain stimuli was observed.

Anemia

Blood count values *(Fig.47)* were within the normal range in animals undergoing 90% and 95% liver resection (normal range: HB-13,5-15,5 g/L, erythrocytes-7-9 $10^9$/L ). In contrast 97% hepatectomy resulted in obvious anemia with high interindividual variability (HB-15,32+/-3,27 g/l and erythrocytes - 8,59+/-1,97 x$10^9$/L).

The relatively high values of HB together with HTC and clinical observation suggested exsiccosis of the animal (47,1+/-7,93 %; ranged from 36,9 to 60,3%). At the same time blood count revealed severe leucopenia of 3,25 +/- 0,57 x$10^6$/L (range 2,63-4,1 x$10^6$/L) in contrast to 90% and 95% liver resection *(Fig.47).* Pronounced leucopenia suggested severe alteration of the immune system.

**Figure 47. Influence of "parenchyma preserving vessel oriented" technique on blood values at 24h after 90%PH, 95%PH and 97%PH**




*=97%PH% < 90%PH and 95%PH on POD-1 (p<0,05)

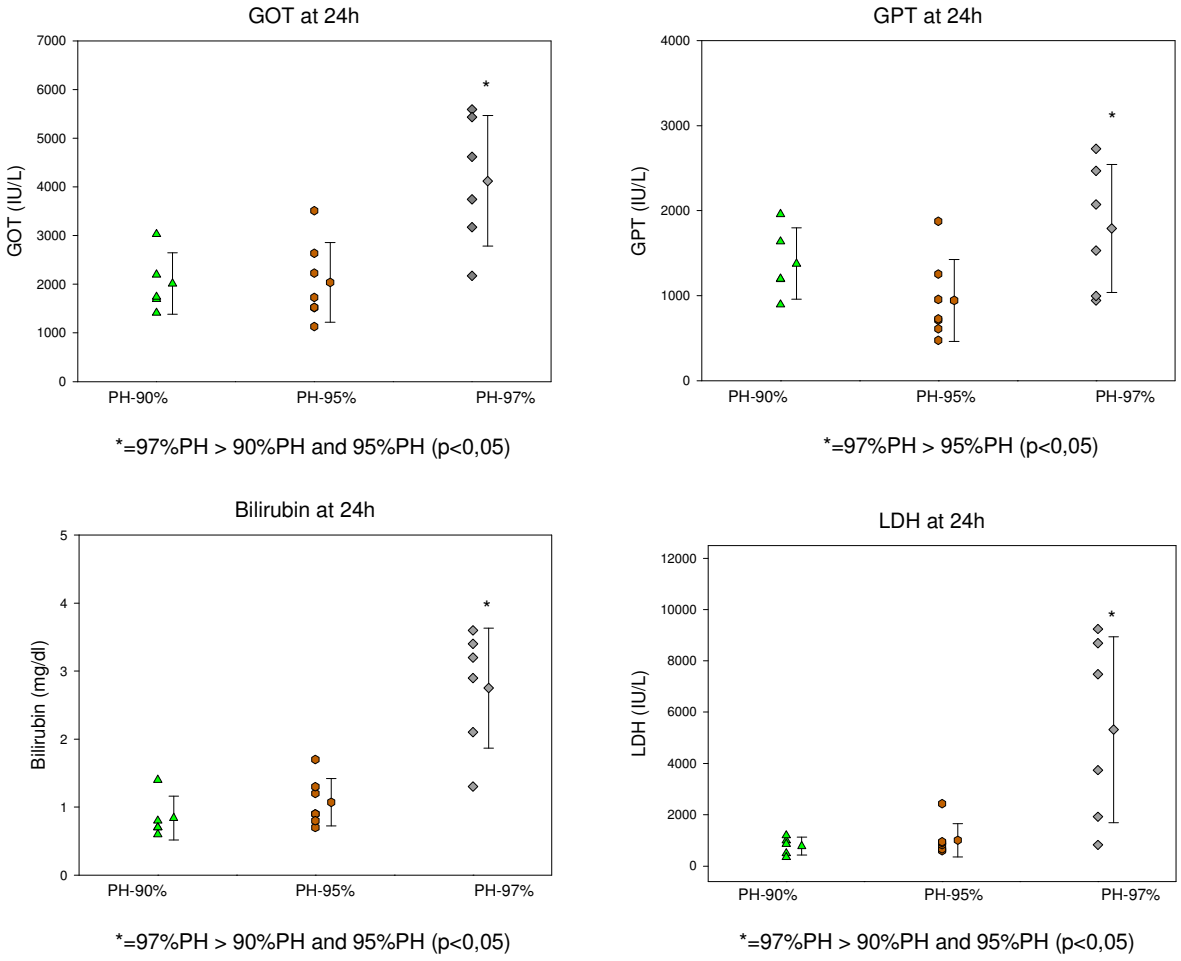
## 4.3.2. Liver damage

Liver function

Liver function parameters after 90% and 95% hepatectomy were elevated to a similar degree at 24h postoperatively *(Fig.48).* All values after 97%PH were substantially higher compared to the other two groups (p<0,05). GOT and GPT levels were twice as high and bilirubin levels were up to 3 times higher than after 90%PH and 95%PH.

Animals undergoing "giga"-extended hepatectomy suffered from a significantly more impaired liver function than the animals from the other two groups.

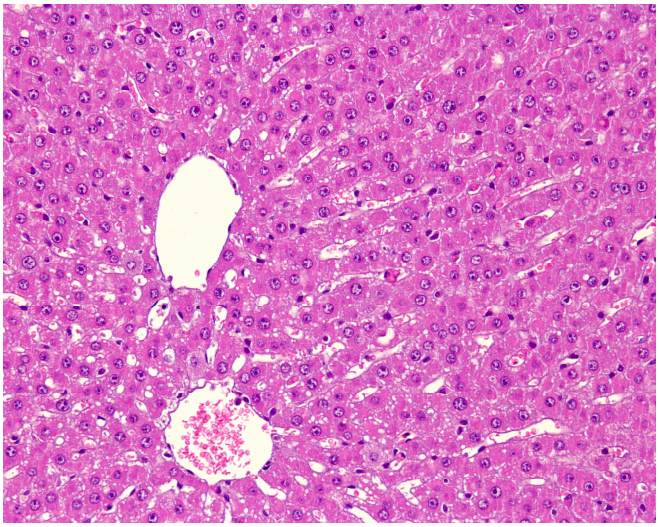
**Figure 48. Influence of "parenchyma preserving vessel oriented" technique on liver function at 24h after 90%PH, 95%PH and 97%PH**




Histological damage

24h after PH histologic damage score was not significantly higher in animals subjected to "mega"-extended hepatectomy compared to 90% PH. Histomorphological signs of damage were dominated by single cell necrosis fine vacuolar transformation of the cytoplasm of hepatocytes and sinusoidal dilatation *(Fig.49)*.

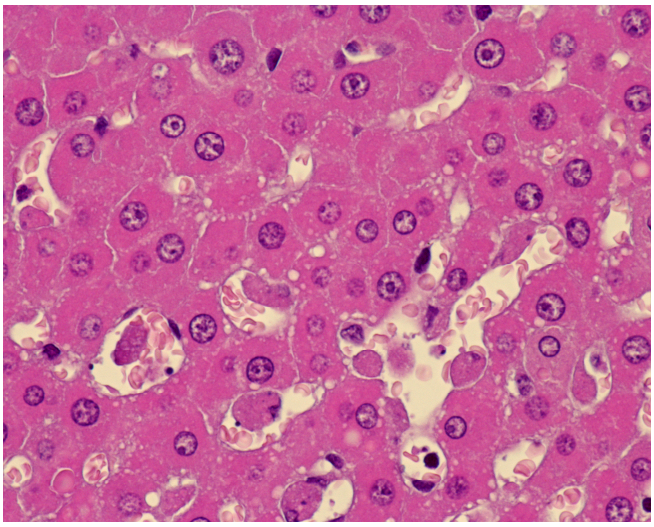
**Figure 49. Liver histology in 24h after 95%PH**



REG-257 (150x, H&E)

24 hours after “giga”-extended hepatectomy showed a higher amount of single cell necrosis and intrasinusoidal debri in dilated sinusoids *(Fig.50).*

**Figure 50. Liver histology in 24h after 97%PH**



REG-247 (400x,H&E)

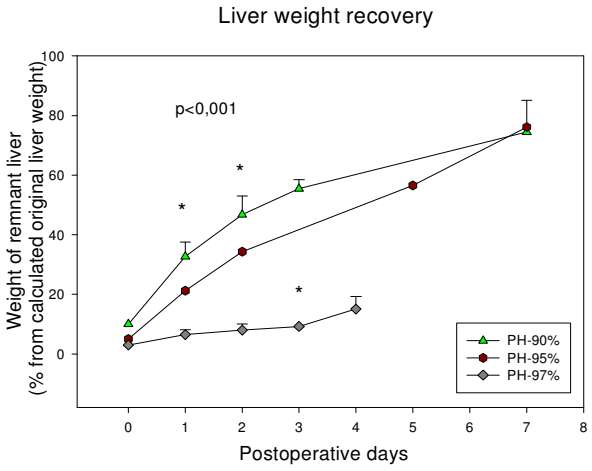
## 4.3.3. Liver regeneration

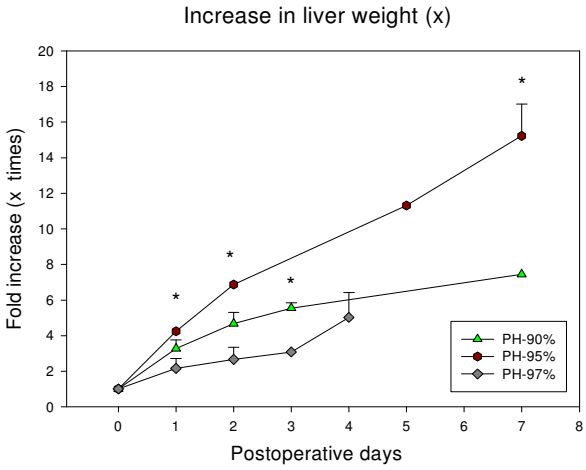
Liver weight recovery

Animals surviving extended (90%PH) and “mega” extended (95%PH) recovered about 75% (75,6+/-3,31%) of the original calculated total liver weight within 1 week

postoperatively. However, the course of liver weight recovery (based on animals sacrificed on POD-1 and POD-7 and spontaneously dying animals on day 2-6) followed a different kinetic *(Fig.51)*. On POD-2 and POD-3 liver weight after 90%PH reached 40-50% of the calculated original liver weight, whereas after 95%PH remnant liver mass reached 20-30% of the original calculated liver weight. The slope of absolute liver weight recovery was steeper after 90%PH than after 95%PH (p<0,001). When looking at the fold increase in the remnant liver weight over time, animals after 95%PH quadrupled their liver weight during the first 24h after operation and reached a 7 fold increase on the second day (p<0,001). At the end of observation time the remnant liver weight increased by 15-16 times. This slope was much flatter after 90%PH, a 3-fold increase on POD-1, a 4-fold increase on POD-2 and only 7-fold increase at POD-7. Apparently, the small remnant liver after 95%PH had a huge regenerative capacity, even higher than after 90%PH. In contrast regenerative capacity after 97%PH was impaired. Within the maximal observation time of 4 days, the liver weight increased from 3% to 15% of the calculated original liver mass, which represents only 5-fold increase. 5-fold increase in remnant liver weight was reached within 2 days after 90%PH and after 1,5 days after 95%PH respectively.

**Figure 51. Influence of "parenchyma preserving vessel oriented" technique on liver weight recovery after 90%PH, 95%PH and 97%PH**



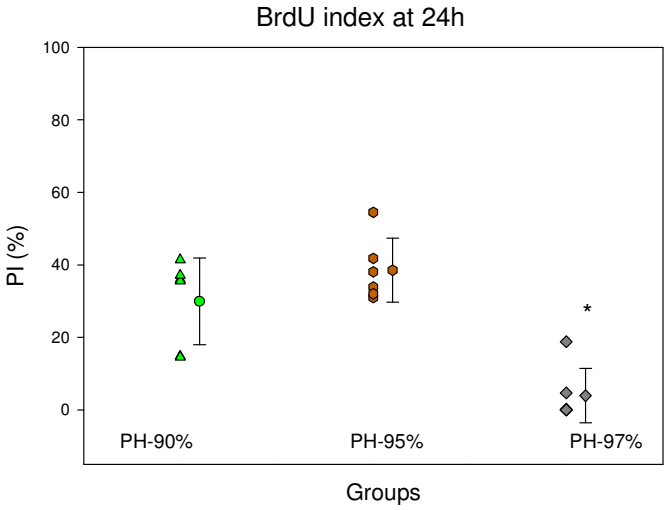

**POD-1**
*=90%PH > 95%PH and 97%PH (p<0,05)
**POD-2**
*=90%PH > 95%PH and 97%PH (p<0,05)
**POD-3**
*=97%PH < 90%PH and 95%PH (p<0,05)
90%PH (POD-2) >95%PH (POD-1) (p<0,05)

**POD-1**
*=95%PH > 90%PH and 97%PH (p<0,05)
**POD-2**
*=95%PH > 90%PH and 97%PH (p<0,05)
**POD-3**
*=97%PH < 90%PH (p<0,05)
95%PH (POD-2) >95%PH (POD-1) (p<0,05)

Proliferation

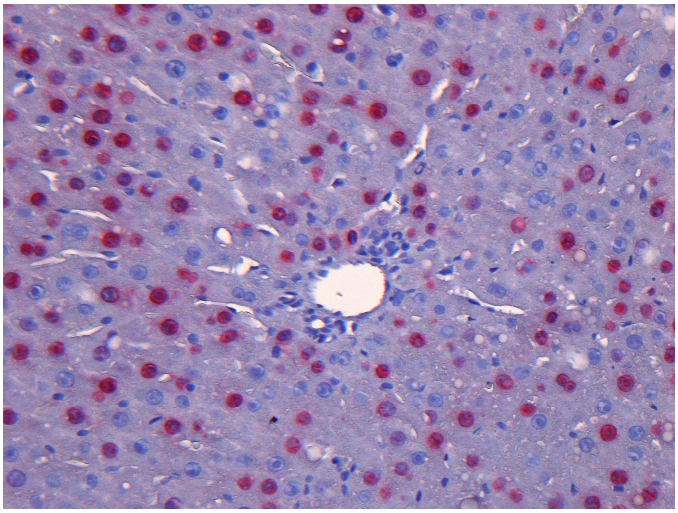
Regeneration was initiated at the first postoperative day after extended and “mega”-extended hepatectomy, whereas no signs of hepatocyte proliferation were detected in POD-1 after “giga”-extended resection *(Fig.52).* The high PI *(Fig.53)* after 90% and 95% resection (mean 29,92+/-11,97 % for 90%PH and 38,55+/-8,81 % for 95%PH respectively) supported, that the rise in liver weight was due to an increase in the number of hepatocytes

**Figure 52. Influence of “parenchyma preserving vessel oriented” technique on liver regeneration after 90%PH, 95%PH and 97%PH**



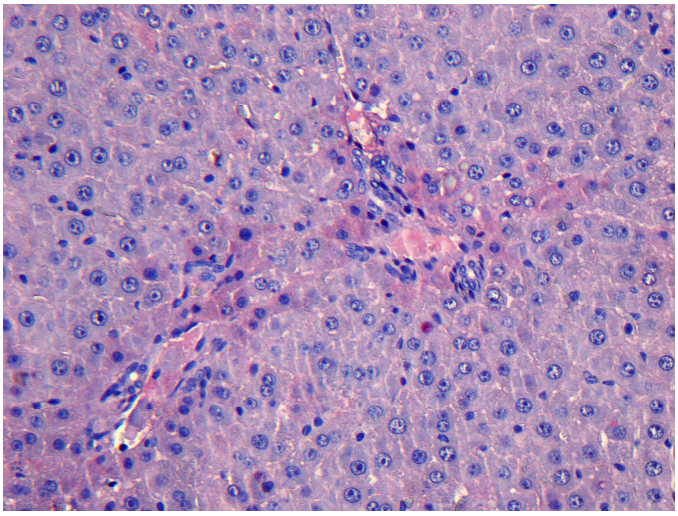

*=97%PH < 90%PH and 95%PH (p<0,05)

**Figure 53. BrdU staining at 24h after 95%PH**



REG-275 (200x,BrdU)

**Figure 54. BrdU staining at 24h after 97%PH**



REG-266 (200x, BrdU)

In contrast, PI at 24h after 97%PH was much lower ranging from 0 to 15% *(Fig.54)*. The moderate increase in liver weight observed after 97%PH in absence of hepatocyte proliferation might be also due to development of edema in remnant liver.

# 5. Discussion:

Born from the clinical and experimental need to define the absolute minimal liver mass, this study was designed and performed in 3 steps:

1. ***Analysis and hypothesis development***
2. ***Anatomically based development of surgical technique***
3. ***Evaluation of newly developed technique***

A thorough **meta-analysis** of the literature regarding the surgical technique was performed and disclosed highly contradictory results. The same 10% of remnant liver mass was insufficient to support the life of the animal when using mass ligation, but clearly sufficient when using a different surgical approach. This observation led to the hypothesis, that the outcome was related to the condition of the remnant liver, which itself was dependent on the surgical technique. In other words, improvement of the surgical technique should lead to a reduced damage of the remnant liver and therefore to an improved outcome.

The **experimental part** of the study was dedicated to confirm this hypothesis.

Three experiments were performed.

- Visualization of rat vascular anatomy of the liver as the basis for optimization of resection technique
- Comparison of the parenchyma preserving vascular oriented technique with mass ligation technique
- Application of the optimized technique to investigate the absolute minimal remnant liver mass

## *5.1. Analysis*

### 5.1.1. Meta-analysis of results after 90% PH

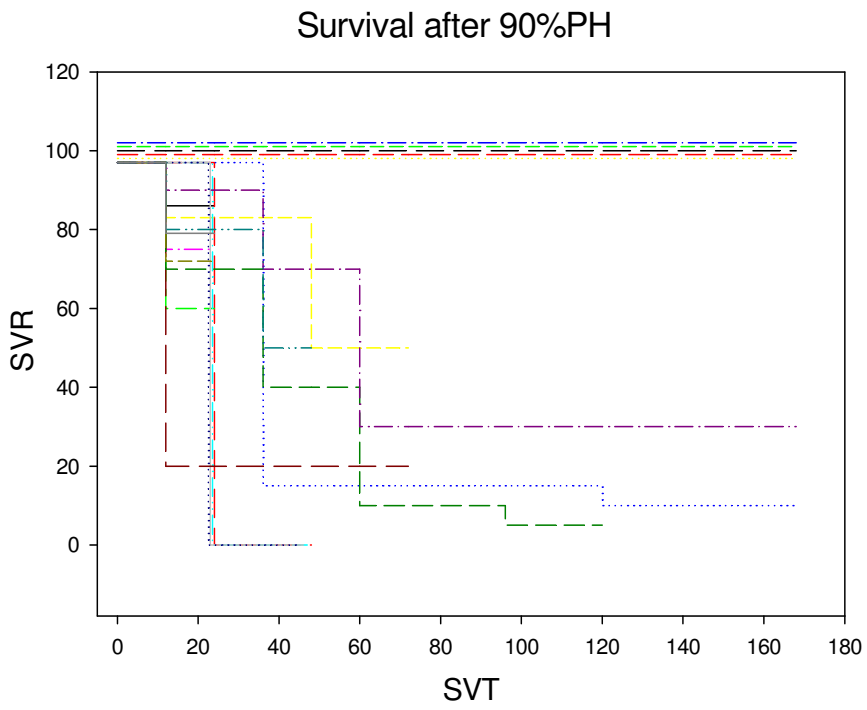
The meta-analysis of the existing body of literature regarding 90%PH has revealed a striking discrepancy in respect to the reported survival rates *(Table 9).* Starting from 1997, several reports, actually coming from one group at the Yokohama City University School of Medicine, Japan, demonstrated that rats can indeed tolerate the removal of 90% of their liver mass.

**Table 9. Survival rate after PH-90% (literature review)**

| Reference | Year | 24h | 48h | 72h | 1 week |
|---|---|---|---|---|---|
| **Gaub J, Iversen J. (16)** | 1984 | 86% | n.a. | n.a. | n.a. |
| **Caruana JA (6)** | 1986 | 75% | n.a. | n.a. | n.a. |
| **Emond J et al (11)** | 1989 | 60% | n.a. | n.a. | n.a. |
| **Sarac TP et al (61)** | 1994 | 80% | 50% | n.a. | n.a. |
| **Eguchi S et al (9)** | 1996 | n.a. | 0% | | |
| **Li B et al (41)** | 1997 | n.a. | 0% | | |
| **Kubota T et al (36)** | 1997 | 100% | 100% | 100% | 100% |
| **Kogure K et al (32)** | 1998 | 80% | n.a. | n.a. | n.a. |
| **Ando K et al (1)** | 2000 | 83,3 | n.a. | 50% | n.a. |
| **Cai SR et al (5)** | 2000 | n.a. | n.a. | 15% | 10% |
| **Kobayashi N et al (29;30)** | 2000 | n.a. | 0% | | |
| **Kim WH et al (26)** | 2000 | 70% | 40% | 10% | 5% |
| **Fukuchi T et al (15)** | 2000 | 90% | 70% | 30% | 30% |
| **Moser MJ et al (56)** | 2001 | 70% | n.a. | n.a. | n.a. |
| **Kamimukai N et al (25)** | 2001 | 100% | 100% | 100% | 100% |
| **Hamazaki K et al (17)** | 2002 | 20% | 20% | 20% | n.a. |
| **Mimuro A et al (52)** | 2002 | 100% | 100% | 100% | 100% |
| **Morita T et al (54)** | 2002 | 100% | 100% | 100% | 100% |
| **Chang TH et al (8)** | 2004 | 100% | 100% | 100% | 100% |

In 14/19 reports, 90%PH was described as a lethal model resulting in fulminant hepatic failure and leading to the death of the animals within 2-3 days *(Fig.55)*. Starting from 1997 it was demonstrated that rats can indeed tolerate the removal of 90% of their liver mass.

**Figure 55. Cumulative survival after 90%PH (literature review)**




When analyzing the potential reasons for the strikingly different results, it became obvious that the outcome was associated with the surgical technique *(Table 10)*. Poor survival was reported when a simple mass ligation procedure for liver resection was used. Animals survived 90%PH, when resection was performed using a vessel oriented resection technique.

**Table 10. Relationship between surgical technique and survival after 90%PH.**

| **Technique** | **Concept** | **Survival rate and time** |
|---|---|---|
| Mass ligation | Non-anatomical | High, > 1week |
| Vessel oriented | Anatomical | Low, < 1week |

### 5.1.2. Comparison of surgical techniques used for 90%PH

Liver resection in the rat model was introduced by Higgins and Anderson in 1931 (3).Totally; about 70% of the liver mass was removed. For resection of this amount of liver tissue the left lateral (20-30%) and median (40-50%) lobes were resected by putting ligations around the pedicles of the respective liver lobe. Nowadays, this surgical model is still widely used to study a variety of aspects in liver regeneration.

Liver resection models based on the removal of defined combinations of liver lobes are classified according to their relative resected liver mass as 30%, 50%, 70% and 90% PH model. Removal of 90% of the liver mass in a rat is achieved by two different selections of lobes to be resected. In the surgical model described by Weinbren et al (75) left lateral, median, right inferior and both caudate lobes are resected, whereas in the model of Gaub and Iversen (16) the left lateral, median and both right lobes are removed, leaving the two caudate lobes and the paracaval liver. The model established by Gaub and Iversen (16) is widely accepted and was frequently used to study fulminant hepatic failure.

Their resection technique consists of mass ligation of the liver lobes in three steps.

- First ligation is placed at the common base of left lateral and median lobe
- Second ligation is placed around the base of the pyramid-shaped right inferior lobe
- Third and last ligation is placed at the base of the pyramid-shaped right superior lobe.

Their major problem was the constriction of vena cava. Sign of this complication was the immediate "*darkening of the remnant liver*" after resection was finished. In order to avoid this problem they *"pulled to rat's right side upon ligating"*. This model, also used in the present study, resulted in the removal of about 85 to 90% of the total liver mass in adult male Lewis rats.

The technique described by Emond et al (11) in 1989, represents a modification of the mass ligation technique. He removed left lateral, median and right inferior lobes by separate ligations. The right superior lobe was treated differently. As he described *"the right superior lobe should be drawn anteriorly and to the left to liberate its posterior attachment to the diaphragm. The lobe is then removed using two separate ligatures to*

*clear the parenchyma posterior to the vena cava without causing caval compression*". Thus, the author separated the wide base into two parts and then ligated them. Besides, the author suggested that posterior parenchyma of right superior lobe should be completely removed.

In 1997, Kubota et al (36) described a new technique based on the vascular anatomy of the liver. Applying this vascular oriented technique allowed to reach 100% survival for 1 week. He reported that *"in the rat; the median and right inferior lobes, unlike the left lateral lobe, are attached to the inferior vena cava, and therefore ligatures at the bases of these lobes constrict the lumen of the inferior vena cava. If the ligature is made at a little distance from the vena, then constriction will not occur, but the danger of bleeding from the cut wedge of the liver parenchyma is increased, and it is difficult to achieve accurate resection".* To avoid this dilemma, they developed a new resection technique. They made a large midline incision and pushed out the liver after detaching it from the surrounding tissues; then ligated and divided hepatic arteries and the portal vein branches entering the lobes to be resected depending on the amount of liver to be removed. The pedicles of the lobes were then divided into two or three parts and "*ligated by piercing"* before the hepatic lobes were resected. With this method, *"the hepatic lobes can be precisely resected without constricting the inferior vena cava or causing any bleeding".*

Kim et al in 2000 (26) described a resection technique which represented a slight modification of Higgins and Anderson technique used for PH-70%. Instead of performing a mass ligation of both, the median and left lateral lobe, they ligated theses lobes separately. In contrast, instead of ligating the two right lobes separately, they performed a mass ligation of the large common base of these two lobes. The two caudate lobes remained. "*Special care was taken to fully mobilize the anterior liver lobes and to place ligature around their common pedicle high, so that there would be no interference with the arterial blood supply to the liver remnant or impairment of vena cava outflow."*

All techniques leading to low survival rate were modifications of the mass ligation technique. Only Kubota's technique taking the vascular anatomy of the liver into consideration was associated with survival of all animals *(Table 11).*

**Table 11. Technical differences in rat extended hepatectomy**

| Author | Year | Lobes resected | Resection technique | |
|---|---|---|---|---|
| | | | Parenchyma dissection | Vessel orientation |
| Weinbren and Woodward (75;77) | 1964 | Left lateral, median, right inferior and both caudate lobes | Mass-ligation of all lobes | |
| Gaub and Iversen (16) | 1984 | Left lateral, median, both right lobes | Mass-ligation of all lobes | |
| Emond et al (11) | 1989 | | Mass-ligation of left lateral, median and right inferior lobes | Resection of right superior lobe by placing piercing sutures in two steps |
| Kubota et al (36) | 1997 | | | Preliminary dissection of artery and portal vein branches and then resection by piercing |
| Kim et al (26) | 2000 | | Mass-ligation of all lobes | |
| Optimized vessel-oriented parenchyma preserving technique | 2005 | | Ligation of pedicle of left lateral lobe | Clamping of liver lobe and positioning of piercing sutures proximal to clamp in number and location according to topographical vascular anatomy of the individual liver lobe |

## *5.2. Experimental approach*

### 5.2.1. Anatomical considerations

#### 5.2.1.1. Visualization of liver anatomy by corrosion cast and 3-D imaging as a prerequisite for surgical development

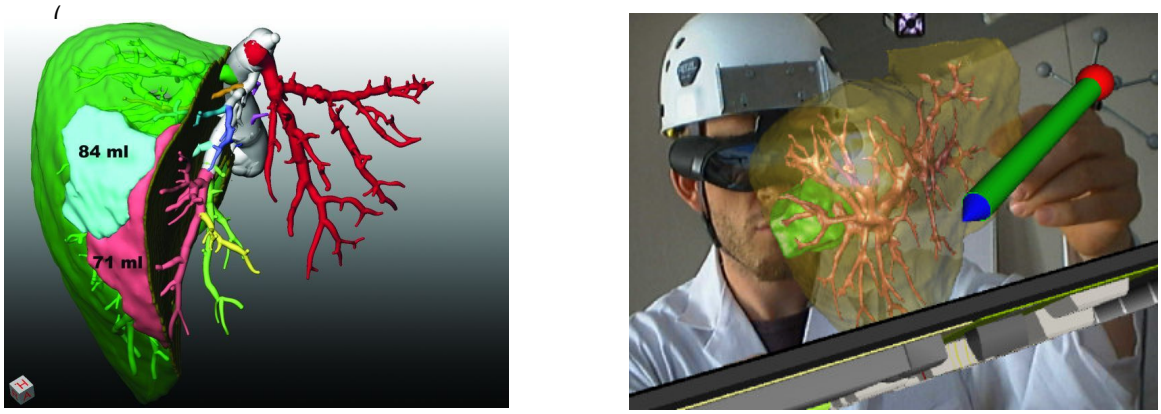
Profound topographical anatomical knowledge is the basis for devising a surgical technique, which aims for the maximal preservation of the remnant organ and its adjacent structures. Development of clinical hepatobiliary surgery especially of living liver donation was profoundly enhanced by modern tools such as 3D-visualization of the individual hepatic vascular tree (38;50;51;59;71). Three-dimensional information is provided by 3D reconstruction of individual vascular anatomy based on CT-imaging (38;50). Three-dimensional (3D) visualization improves anatomic assessment, allows for interactive surgical planning, and acts as an intraoperative guide (18;39;69). Virtual liver surgery planning *(Fig.56)* based on individual 3d anatomy facilitates the optimal

preservation of the small remnant liver by reducing the surgical injury to the vascular, biliary and parenchymatous structures. Nowadays, these methods used more and more frequently prior to complex resection procedures *(Table 12).*

**Table 12. Reason to use 3D-Imaging**

| Criteria | Clinical | Experimental |
|---|---|---|
| Object of study | 3D computer image | Physical model |
| Purpose/strategy | Individual op planning | Development of surgical strategy |

**Figure 56. Virtual liver surgery planning**




Copied from : **http://www.mevis.de** (left) and **http://liverplanner.icg.tu-graz.ac.at** (right))

Modern experimental surgery in rodents has to follow the same principles. Selection of investigative tools must be appropriate for the animal model. Application of imaging technologies such as computer tomography to depict individual liver vascular anatomy in the rat prior to the operation requires extreme radiation doses (5 Gy, which equals the $LD_{50}$) for highest quality images with an isotropic voxel size of 100 microm (14) and is therefore not compatible with the life of the animal. Furthermore, demonstration of the individual anatomy prior to resection is not even necessary, as inbred animals are used, which do not show the same high amount of vascular variations as encountered in clinical surgery. This was the reason to use a different tool e.g. corrosion casts, for understanding rat vascular anatomy *(Table 13).* Complete casts of the vasculature of organs and tissues are obtained by infusing low viscosity resins into the vasculature and

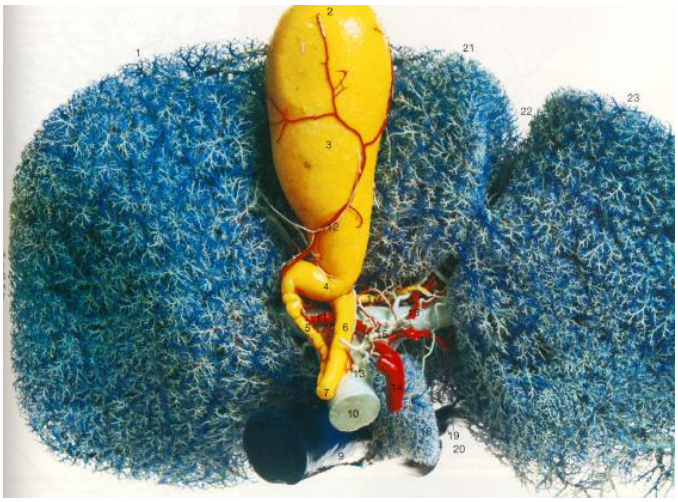
allowing the resin to polymerize. Maceration of the surrounding tissue with alkali leaves a model of the intricate, three-dimensional distribution of vessels in that tissue, which is not easily obtainable by any other means *(Fig.57).*

**Table 13. Comparison between 3D-Imaging and corrosion casting**

| Criteria | Computed tomography | Corrosion casts |
|---|---|---|
| Application | Individual anatomical variant | Deduction on species anatomy |
| Accuracy | Dependent on CT-quality<br>Dependent on segmentation algorithm | Dependent on injection quality |
| Disadvantage | Lethal, as extreme radiation dose necessary for required resolution | Lethal, as model is generated |

Well prepared casts appear to faithfully replicate the true vascular anatomy of organs including the dimensions of vessels and details of imprints of the endothelial cells lining their lumens (35).

**Figure 57. Corrosion cast of all hepatic vessels from human liver**



(Copied from : **www.esg.montana.edu,** original from from **R. M. H. McMinn, et al., 1993. Color Atlas of Human Anatomy, Third Edition. Mosby-Wolfe, London. pg. 225**.)

Production of 3D-models in sufficient quantities confirmed the presence and frequency of anatomical variations and enabled the deduction on the characteristics of the species-specific anatomy. The corrosion casts were very helpful to generate a stereoscopic image regarding the topography of the vessels in relation to the liver lobes. By revising

the cast it became possible to draw virtually the lines for the resection plane as well as for the vessels to be ligated at specific zones.

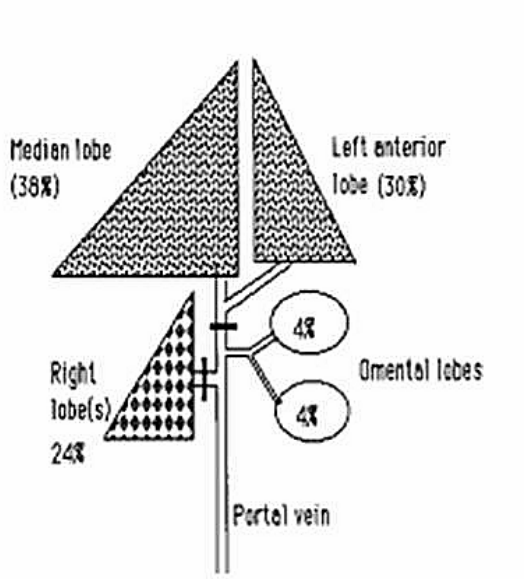
It was shown that the paracaval liver has 3 sources of blood supply and a drainage system consisting of multiple veins having a diameter much less than 1 mm. This observation could not have been obtained using CT or MRI scanning due to the limits in maximal resolution and doses required. However, this information was very important for the development of the surgical technique.

### 5.2.1.2. Rat hepatic vascular anatomy

**State of the art**

In 1996 Eguchi et al. (9) described the basic vascular anatomy of the rat liver. Only major lobar vessels are roughly depicted *(Fig.58).* Neither the dual portal supply of the median lobe is described nor the existence of the paracaval portion of the liver.

**Figure 58. Distribution of liver mass in rat liver ( Eguchi(9))**

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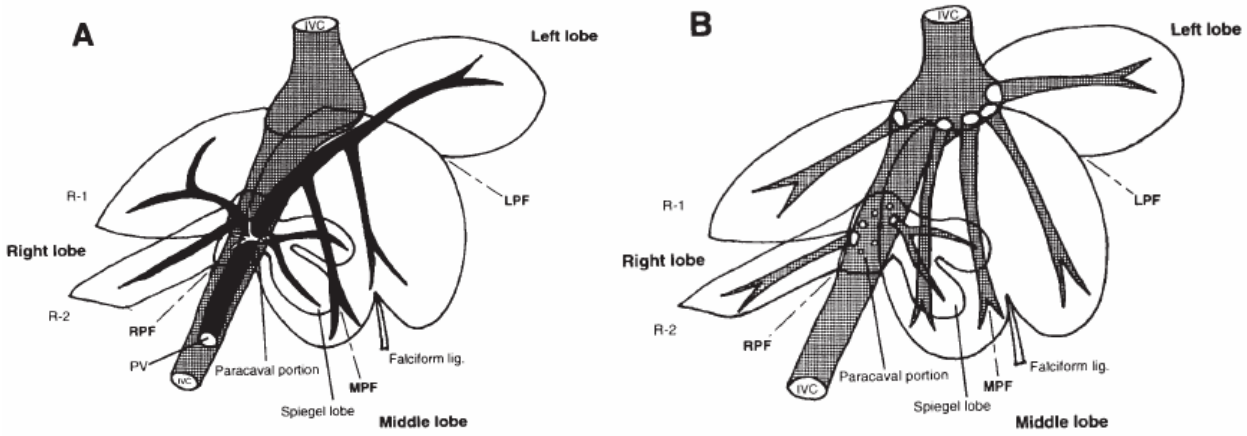
Kogure et al. (31) presented a more detailed description of the vascular anatomy of the rat liver *(Fig.59).*

**Figure 59. Hepatic vascular anatomy of the rat according to Kogure (31). A) Portal vein anatomy and B) Venous outflow**

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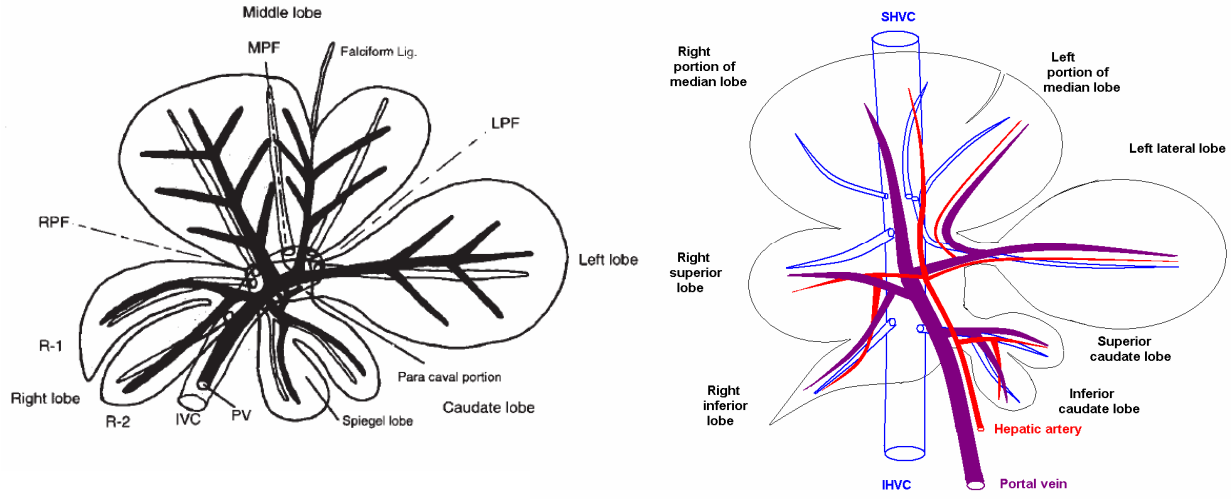
Compared to our anatomical analysis there are a few differences *(Fig.60).* Regarding *gross anatomy* of the rat liver, the left and right portion of the median lobe are depicted as being of equal size. In our experience the left portion of the median lobe is considerably smaller that the right portion. Furthermore, the paracaval part of the liver is not shown in its full extension.

**Figure 60. Comparison of schematic anatomic drawing of Kogure (31) (left) and own modification (right)**



Vascular anatomy of branches of the *portal vein* does not depict that the left portion of the median lobe is supplied by a branch of the left portal vein stem supplying the left lateral lobe, a fact, which is important to know in case the left lateral lobe representing about 30% of the liver mass is to be resected. The schematic drawings of Kogure were informative regarding the main lobar veins, but did not disclose information regarding the topography of the vessels. When planning an extended resection topographical knowledge regarding the localization of the vessels in respect to the liver lobes proved to be very helpful. Analysis of the corrosion casts of the hepatic veins revealed that the right superior hepatic vein drains into the intrahepatic vena cava just below and slightly lateral to the right median hepatic vein. The middle median hepatic vein drains either into the stem of the left hepatic veins or directly into the cava, whereas the left median hepatic vein drains into the left hepatic vein distally and laterally to the middle median hepatic vein.

#### 5.2.1.3. Paracaval liver and its contribution to the remnant liver after 90% resection

Identification of the multiple portal blood supply and drainage system of the paracaval liver allowed its perception as a separate functional unit. Determination of the weight of the paracaval liver revealed that it contributes up to 4% of the liver mass, half of it being parenchymal tissue, half nonparenchymal tissue. Each of the caudate lobes contributes about 4-5% of liver mass.

In the anatomical description of Eguchi (9) the paracaval portion is not even mentioned as a part of the liver parenchyma. He described only the relative volume contribution of each of the other liver lobes to the total liver volume but not of this structure.

The paracaval liver is mentioned in other papers (11;16;36) as a structure to remain together with both caudate lobes as remnant liver after 90% resection. Kogure et al regarded the so-called “paracaval liver” as a part of caudate or Spiegel lobe (31). He called this part a “fundamental structure” of the liver, which was observed in all 20 examined animals. However, he described that this part of the liver was not of sufficient size for a detailed macroscopic assessment.

Discovering the paracaval liver as unique structure having its own multiple blood supply and drainage system helped us to extend the level of our resection beyond 95% hepatectomy. By using the vessel oriented approach for the resection of both right liver

lobes the paracaval portion remained undisturbed in terms of devascularization and outflow obstruction. Necrosis in this part of the liver was widely avoided which improved the outcome in extended hepatectomy also. The small remnant paracaval liver consisted of viable parenchyma, irrespectively of the extents of liver resection.

#### 5.2.1.4 Surgical consequences of the enhanced understanding of rat vascular anatomy

Partial hepatectomy starts with defining the plane of resection by placing a clamp on the respective liver lobe in about 4mm distance to the cava. The key point of the newly developed resection procedure consists of placing the piercing sutures proximal to the clamp (2-3mm distance to cava) in number and location according to the topographical vascular anatomy of the individual liver lobe. This step requires the intuitive knowledge of topographical liver vascular anatomy for optimal positioning of piercing sutures to include both portal vein and hepatic vein. If the sutures are placed correctly, there is no or only a minimal risk of bleeding from the stump unless small branches of liver veins are not included in piercing sutures. Portal ischemia of the remnant liver, especially the paracaval portion, is completely avoided. In contrast to mass ligation procedures, the risk of outflow obstruction is negligible, as piercing suture are placed in 2-3mm distance to cava.

In contrast *(Table 14),* Kubota's resection procedure started with ligation of the portal vein and the hepatic artery in the liver hilum close to the respective lobe. This step was followed by placing 3 piercing sutures in equal distances from each other in the pedicle of median and right superior lobes. As this step is not necessarily reducing the risk of venous bleeding, Kubota proposed to isolate and ligate the portal vessels prior to placing the piercing sutures. However, cutting off the portal blood supply to the right part of the liver leads to development of ischemic zones in the stumps and the paracaval liver which represents a major part of the remnant liver after extended hepatectomy. In addition, due to the extra step, the total operation time seems to take longer.

Defined piercing sutures ligating all inflowing and outflowing vessels and bile ducts is a decisive step in parenchyma preserving vessel oriented resection technique. Omitting the ligation of the portal vein and hepatic artery prior to this procedure reduces the operation time and does not subject the remnant stumps to the loss of portovenous and

arterial blood supply. This is especially important for the resection of the right superior lobe because the basal parts of this lobe extend into the paracaval part of the liver, which should not be subjected to a loss of vascular supply.

**Table 14. Difference in surgical technique of Kubota and own procedure**

| Criteria | Vessel-oriented technique, Kubota (1997) | Vessel-oriented, parenchyma-preserving technique |
|---|---|---|
| **Principle** | • PV and HA ligation prior to resection of lobe<br>• Piercing suture through liver parenchyma at the base of individual LL with the aim to ligate 3 hepatic veins within the lobe<br>• Used for median and right superior lobes | • Positioning of piercing sutures proximal to clamp in number and location according to topographical vascular anatomy of the individual liver<br>• No ligation or clamping of vessels in liver hilum prior to resection thus avoiding ischemic damage to small remnant liver |
| **Technical difficulties** | • Special skills for microsurgical isolation of portal vessels in liver hilum<br>• Special skills to place piercing sutures to include HV | • Intuitive knowledge of topographical liver vascular anatomy required for optimal positioning of piercing sutures to include PV and HV |
| **Operation time** | • Additional time for separate ligation of portal vessels in liver hilum | • Limited to piercing time only |
| Risk of bleeding | Risk of bleeding from portal vessels during isolation procedure | No risk of bleeding from main vessels |
| | No risk of bleeding from stump unless small branches of liver veins are not included in piercing sutures | |
| **Risk of ischemic damage to remnant liver** | Risk of ischemic damage to right paracaval liver tissue due to ligation of portal vessels in liver hilum | Minor risk of ischemic damage as portal vessels are not ligated in liver hilum |
| **Risk of vena cava constriction** | Negligible, as piercing suture is placed in 2-3mm distance to cava | |
| **Risk of bile leakage** | Small, as not all capillary bile ducts ligated with piercing suture | |

## 5.2.2. Comparison of surgical techniques

90% PH was performed in our research group for a long time using a mass ligation technique *(Table 15).* Meta-analysis of the literature plus extensive anatomical studies of the rat liver enabled us to develop a parenchyma preserving vessel oriented surgical technique. The effect of this surgical technique was compared to the mass ligation techniques in a retrospective analysis of 90% PHs performed previously. In addition, a prospective study was designed to evaluate the T-3 in a systematic fashion.

**Table 15. Evolution of the surgical technique of 90% PH in our group**

| Technique applied | Timepoint developed | Comments |
|---|---|---|
| **T-1:** Mass ligation | 1999-December 2002 | Introduced as a simple and fast procedure |
| **T-2:** Extra mass ligation | January 2002-August 2003 | Modification of T-1 by additional ligation to secure stumps in order to prevent bleeding |
| **T-3:** parenchyma preserving vessel oriented surgical technique | August 2003-up to present | Developed as a result of meta-analysis of the literature |

### 5.2.2.1. Improved outcome and reduced damage in case of "parenchyma preserving vessel oriented" resection: Implication of technique on damage

In contrast to previous studies *(Table 3)* the influence of the surgical technique on the overall outcome and the damage to the remnant liver, but also on regeneration, was studied in detail.

Application of the parenchyma preserving vessel oriented technique reduced the extent of injury to the remnant liver. Mass ligation and extra mass ligation was associated with a considerable damage of the remnant liver, as indicated by the high release of liver enzymes, high serum bilirubin, high total and sinusoidal liver damage score, but also the enhanced local necrosis surrounding the resection margins. The parenchyma preserving vessel oriented technique was associated with a significantly lower damage to the remnant liver with lower release of liver enzymes, lower bilirubinemia and a significantly lower amount of local necrosis.

**Removal of liver mass** represents a reduction of the hepatic vascular bed, leading to severe injury of the small remnant liver via an elevation of the portal pressure, the portal perfusion and the intrahepatic vascular resistance. Furthermore, the **surgical procedure** itself is causing damage to the remnant liver. Therefore, it is even more important to control and reduce the potential sequalae directly related to the surgical procedure.

Reduction of injury to the liver was reached by reducing the perioperative blood loss, by avoiding portal ischemia and presumably by avoiding outflow obstruction, all achieved by optimal placing of the piercing sutures.

Perioperative blood loss was minimized by placing the sutures “intuitively” to ligate the hepatic veins respectively portal veins of the lobe to be resected, which requires the visualization of the 3-dimensional vascular tree of both venous systems.

In contrast to Kubota’s approach, portal vein ischemia was completely avoided. Kubota tried to reduce blood loss by ligating the portal vessels of the lobe to be resected close to the hilum, hereby causing ischemic damage to and potentially necrosis of the remaining stump of the resected lobe and of the paracaval liver. Reduction of the viable remnant liver mass is the necessary consequence.

Temporary occlusion of the portal vein respectively the hepatoduodenal ligament is used more frequently in both, experimental and clinical surgery. However, there are number of clinical and experimental reports describing unwanted effects of Pringle maneuver on outcome after major hepatectomies (13;24;37;66). Ito et all reported that Pringle maneuver significantly affected outcome even after 70% liver resection in rats (22). Kukita et al (37) observed an increased injury of remnant liver after applying Pringle maneuver in pigs. Normothermic Pringle maneuver of 30 min duration in study of Filos et al (13) resulted in immediate and delayed gut barrier failure by significantly increasing bacterial translocation and endotoxinemia which was attributed to portal stasis leading to intestinal congestion as well as temporary liver ischemia. Scatton et al (62) reported improved outcome after major hepatectomy done without clamping. The author suggested that good preservation of remnant liver could be achieved by avoiding warm ischemia zones.

Outflow obstruction was suspected to occur after mass ligation. In these animals severe sinusoidal damage was observed. Constriction of hepatic veins draining into the suprahepatic vena cava leads to venous congestion in the small remnant liver experiencing portal hypertension. In contrast, no or little damage was observed in animals subjected to the newly developed technique. The presumed outflow obstruction was avoided by placing the piercing sutures at 2-3mm distance to the cava.

Outflow obstruction was recently identified to be one of the major problems in clinical surgery after extended hepatectomy and in living donor related liver transplantations (7;21;46). Transection of hepatic veins is leaving insufficiently drained hepatic segments behind. Incomplete drainage of the remnant liver or parts thereof is further compromising the condition residual liver parenchyma. Liver dysfunction, respectively liver failure and death of the patient are the ultimate consequences. Malago et al reported (46;48) that graft dysfunction can correspond to the impaired function of some insufficiently drained

segments of the graft. By optimizing hepatic vein reconstruction the author significantly improved graft survival and function.

### 5.2.2.2. Enhanced liver regeneration in case of "parenchyma preserving technique": Role of nonspecific damage of the remnant liver on regeneration

Animals operated according to the parenchyma preserving vessel oriented technique showed a higher PI after 24 h and a faster recovery of the liver weight than those in the two other groups. Most animals operated according to the two mass ligation techniques showed no proliferation at 24 hours after PH.

This observation is in line with most of the reports related to 90% PH resection in the rat, which state that liver regeneration is delayed by 1 day compared to 70% hepatectomy (1;5;9;16;32;52;60). Little or no proliferation is observed on the first postoperative day. Onset of regeneration was only observed at 48h after operation. In 6 of these reports 90% liver resection was performed using mass ligation technique, mostly according to the technique of Gaub and Iversen, one author used the vessel oriented technique of Kubota. Delay of liver regeneration (79) in control animals was accompanied by decreased protein synthesis (36), impaired hepatic metabolism of the remnant liver by endogenous endotoxin (1), decreased reticuloendotelial system function and, decreased glycogenolysis (16), but was not related to the extent of damage of the remnant liver. Onset of regeneration at 24h postoperatively was only observed in animals treated with a variety of drugs (testosterone (73), ethanolamine (52), prostaglandin (1) and follistatin (32)).

However, there are a few reports *(Table 16)* demonstrating the onset of liver regeneration at 24 h after 90% hepatectomy in untreated animals (6;8;75). The analysis of these papers revealed, that regeneration was initiated, but the proliferation index was much lower compared to the proliferative activity at 24 hours after a standard 70% hepatectomy.

**Table 16. Controversial reports regarding onset of liver regeneration after 90% PH in untreated animals.**

| Author | Resection technique | Proliferation at 24h | Proliferation at 48h | Method to assess proliferation |
|---|---|---|---|---|
| Eguchi et al (9) | 70% by Higgins and Anderson (20) + other right lobes with one ligation | 0,1% | 0,05% | BrdU-incorporation |
| Cai et al (5) | Emond (11) | Not detected | 14% | BrdU-incorporation |
| Gaub and Iverson (16) | Gaub and Iverson (16) | 5-7% | 40% | incorporation of [3H]-thymidine |
| Ando et al(1) | Gaub and Iverson (16) | 1000 dpm per $10^3$x A600 | 1600 dpm per $10^3$xA600 | incorporation of [3H]-thymidine |
| Kogure et al (32) | Gaub and Iverson (16) | 2% | 25% | BrdU-incorporation |
| Sakaguchi et al (60) | Gaub and Iverson (16) | 5% | 45% | Ki-67 |
| Minuro et al (52) | Kubota (36) | <2% | No performed 5% at 72h | BrdU-incorporation |
| Weinbren et al (75) | Weinbren and Woodward (75) | 4,7mitoses per 1000 hepatocytes compared to 14 after 70%PH | Not performed | Mitotic figures |
| Chang (8) | Combination of methods by Gaub and Iverson (16) and Kubota (36) in own modification | 35% compared to 50% after 70%PH | Not detected 60% at 36h and 70% at 72h | PCNA labeling |
| | | | | |
| Li et al (41) | Ligation of PV 2w prior to 90%PH acc to Gaub and Iverson (16) | 550 DPM/micgDNA vs 10 DPM/micgDNA of control standard | 500 DPM /micgDNA | Incorporation of [3H]-thymidine |
| | | | | |
| Own observation | Modifcation of Kubota without ligation of portal vessels | >30% | nd | BrDU |
| | Mass ligation | <5% | nd | BrdU-incorporation |

Weinbren et al (75) using also a mass ligation technique described that enhanced feeding with glucose via gastric tube after extended hepatectomy (80%PH to 90%PH) was associated with an elevated mitotic index observed at 24h after operation. However, the mitotic index was only one third of that observed after 70% hepatectomy.

In the study of Chang et al (8) PCNA labeling index reached 35% in non treated animals after 90% PH, which was lower than the PI observed after 70% hepatectomy (PI of 50%) in the same study. Furthermore, the peak of regeneration did not occur at 24 hours as known after 70%PH, but occurred at a later time point, resulting in an impaired course of regeneration. The technique he employed was described as his own modification of the technique of Gaub and Iversen (16) and Kubota (36), but he did not disclose any details of the procedure.

The work of Li et al (41) is actually not comparable to the results obtained after standard 90%PH. He described the onset of regeneration at 24 h after 90% hepatectomy but performed a ligation of portal vein branches 2 weeks prior to resection. Increase in DNA synthesis was 40 times higher compared to control animals without ligation.

In none of the cited reports, the potential importance of the surgical technique for the initiation of regeneration was not identified. The present study is the first systematic experimental study investigating the relationship between the surgical procedure, the damage caused to the remnant liver and its influence on the initiation and course of liver regeneration.

Recent papers regarding liver regeneration after LDLT suggest that regulation of the liver proliferation is dependent on liver perfusion (10;58;72;78). Acute elevation of portal pressure, reflecting wall shear stress of sinusoidal endothelial cells, is supposed to trigger liver regeneration after partial hepatectomy, whereas excessive portal hypertension induces liver failure (58). However, liver perfusion may be impaired by outflow obstruction in the small remnant liver (19;33;42;47;58), respectively the small partial graft. Marcos et al (49) proposed a triangular relationship theory between the graft-to-recipient body weight ratio (GRBWR), portal hypertension (inflow), and outflow capacity. They suggested that the interrelationship between these variables determined the fate of the graft.

## 5.2.3. Extending the extended hepatectomy

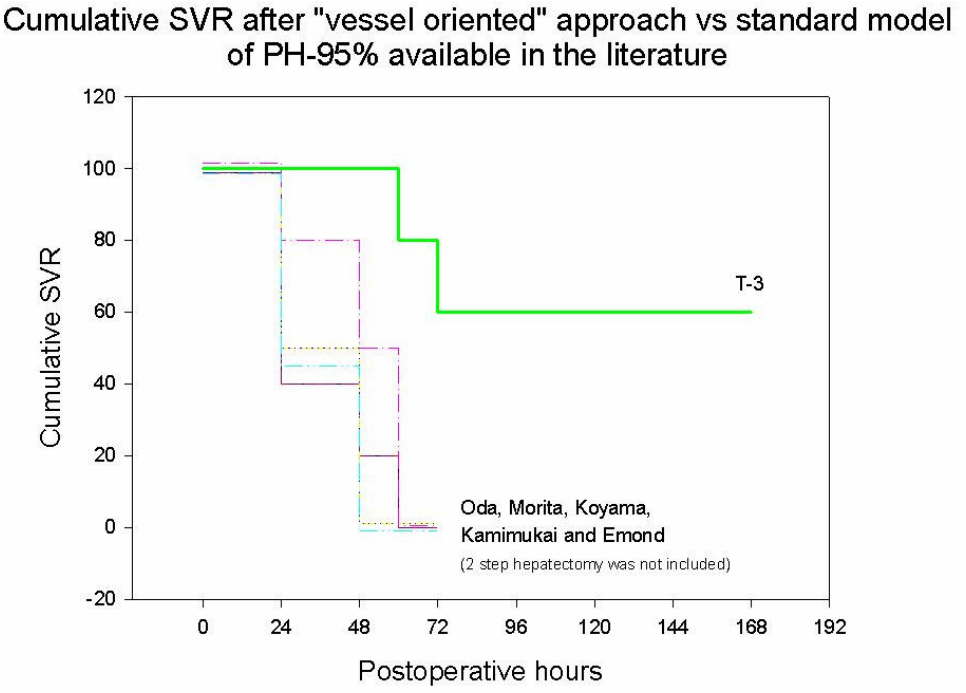
### 5.2.3.1. Determination of the minimal liver mass

Determination of the minimal liver mass is important for clinical as well as for experimental liver surgery. Up to now, the maximal reduction of liver mass in the rat compatible with survival of the host was the removal of 90% of the liver. Previous reports

dealing with 95% resection reported a maximal survival time of up to 3 days. However, the authors did use mass ligation techniques, but not a vessel oriented technique.

Contrary to the reported results we observed a one week survival rate of 66% after 95% resection *(Fig.61)*. The reduction of the liver mass by 95% model leaving about 5-6% of liver tissue behind represented the "absolute" minimal liver mass after resection compatible with survival of at least more than half of the animals.

**Figure 61. Cumulative survival after 95% hepatectomy**




This absolute minimal liver mass in rats observed in this study is the lowest reported up to now and much lower than the reported clinical maximal removal of liver mass. Single case reports can be retrieved, even as early as 1975, where a successful outcome after a 85-90% resection is described (68).

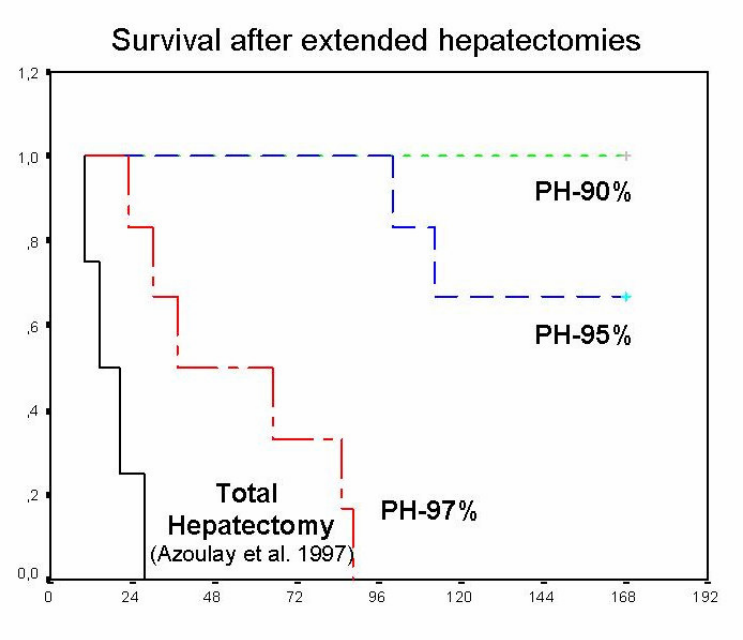
In transplantation surgery, a minimal graft to body weight ratio of 0,8 is regarded to be safe in humans, but survival of the recipient was also achieved when GBWR of 0,6 was used. This is obviously much higher than the size of the remnant liver and respectively the corresponding remnant liver to body weight ratio observed here.

### 5.2.3.2. Generation of 3 clearly defined different surgical models with different outcome

The investigation of the minimal liver mass by expanding the extent of resection up to surgically maximal achievable reduction of the liver mass led not only to the determination of the absolute limit, but also to the establishment of three clearly defined surgical models *(Fig.62)*.

Extended liver reesction (90%PH) in a rat done using "parenchyma preserving" technique is a survival model of extended hepatectomy with compensated portal hypertension, transient liver dysfunction and a timely onset of regeneration.

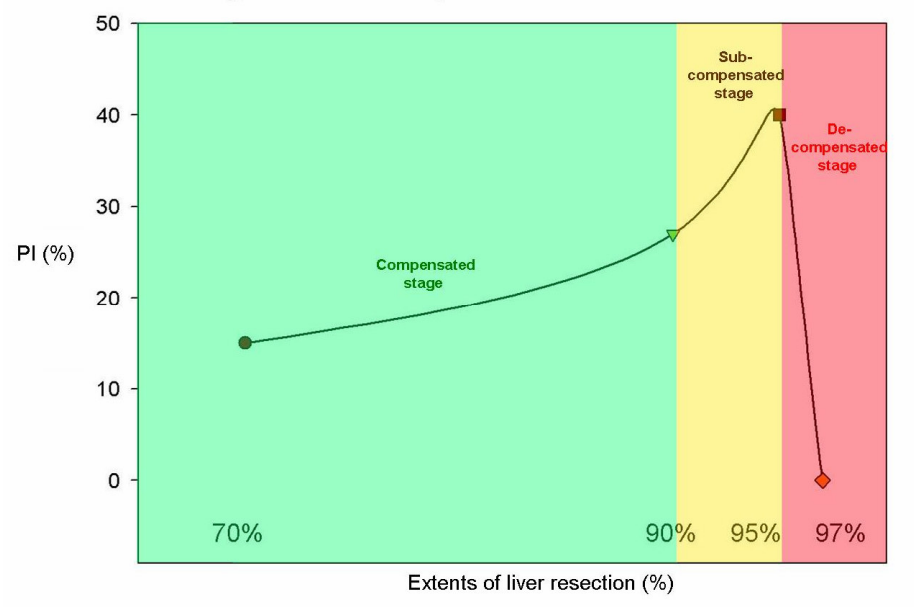
**Figure 62. Cumulative survival after different extents of hepatectomy performed using PPVO technique in comparison with total hepatectomy by Azoulay.**




In contrast to 90%PH the "mega"-extended hepatectomy (PH-95%) turned out to represent a critical but subcompensated model *(Fig.62)*. Massive reduction of the hepatic vascular bed, presumably causing severe portal hypertension, was tolerated by half of the animals, but caused severe liver dysfunction and eventually the death of the remaining animals. Regeneration was initiated timely, though the proliferation index was higher than after 90% PH, as expected when leaving smaller liver mass behind *(Fig. 63)*.

Extending the liver resection to 97% removal of liver mass resulted in the generation of a reproducibly lethal model representing decompensated liver failure. However, survival time after “giga”-extended resection using 97% hepatectomy model was twice as long compared to a survival time of 20 +/- 5 hours in total hepatectomies performed by Azoulay (2). This result suggests that the minimal remnant liver tissue of about 2% is at least partially functional.

**Figure 63. Proliferative response versus extent of anatomically based resection**



# 6. Summary:

Based on a detailed study of the hepatic vascular anatomy a parenchyma preserving vessel-oriented technique for 90% extended liver resection in the rat was developed. The paracaval liver was identified as a separate “liver lobe” with its own multiple portal blood supply and hepatic drainage contributing to about 4% of the total liver mass.

Damage of the remnant liver was clearly dependent on the surgical approach. Mass ligation procedures caused severe damage to the remnant liver, whereas the newly developed technique was preserving the remnant liver parenchyma. Timely initiation of regeneration was related to the extent of morphological damage in the small remnant liver, which was dependent on technique used.

Investigation of the absolute minimal liver mass revealed that a remnant liver of 10% of the calculated original liver mass is sufficient for survival. Reduction of the liver mass to 5% allowed 1 week survival of more than 50% of the rats. Removal of all resectable liver lobes leaving 3% of liver mass behind was causing the death of all rats within 4 days.

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# 8. Abbreviation:

| | |
|---|---|
| **ALT** | Alanine aminotransferase |
| **AP** | Alkaline phosphatase |
| **AST** | Aspartate aminotransferase |
| **BrdU** | bromodeoxyuridine |
| **CT** | Computed tomography |
| **Eryth.** | Erythrocytes |
| **EvG** | Elastic von Gieson |
| **FHF** | Fulminant hepatic failure |
| **Fig.** | figure |
| **GBWR** | Graft-to-body-weight ratio |
| **Glu** | Glucose |
| **GOT** | Glutamat-Oxalacetat-Transaminase (AST) |
| **GPT** | Glutamat-Pyruvat-Transaminase (ALT) |
| **GRWR** | Graft-to-recipient weight ratio |
| **HA** | Hepatic artery |
| **HB** | Hemoglobin |
| **HE** | Hematoxylin-Eosine |
| **HPF** | High power field |
| **Ht** | Hematokrit |
| **HV** | Hepatic vein |
| **ICL** | Inferior caudate lobe |
| **ICL/BW** | Inferior caudate lobe to body weight ratio |
| **IHVC** | Infrahepatic vena cava |
| **IU** | International Units |
| **LBWR** | Liver-to-body-weight ratio |
| **LDH** | Lactate dehydrogenase |
| **LDLT** | living donor liver transplantation |
| **Lew** | Lewis (breed) |
| **LI** | Labelling index (i.e. PI) |
| **LL lobe** | Left lateral lobe |
| **LTx** | Liver transplantation |
| **ML** | Median lobe |
| **MRI** | Magnetic Resonance Imaging |
| **PCL** | Paracaval liver |
| **PH** | Partial hepatectomy |
| **PI** | Proliferative index |
| **POD** | Postoperative day(s) |
| **POW** | Post operative week(s) |

| | |
|---|---|
| **PV** | portal vein |
| **Reg.Liv/BW** | Regenerated liver to body weight ratio |
| **RIL** | Right inferior lobe |
| **RLBW(R)** | Resected liver-to-body weight(ratio) |
| **RLM** | Remnant liver mass |
| **RSL** | Right superior lobe |
| **SCL** | Superior caudate lobe |
| **SD** | Standard deviation |
| **SEM** | Standard error of the mean |
| **SHVC** | Suprahepatic vena cava |
| **SVR** | Survival rate |
| **SVT** | Survival time |
| **T-1** | Technique 1 |
| **T-2** | Technique 2 |
| **T-3** | Technique 3 |
| **Tab.** | Table |
| **TLW** | Total liver weight |
| **3-d** | Three dimensional |

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