# Radiological assesment of the postoperative liver

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# Radiological assessment of the postoperative liver

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## Contents

# General introduction

The liver is the largest organ in the human body. It weighs approximately 1-2.5 kg, depending on the individual body size[1]. The liver has a very rich blood supply which consists of a dual system: (1) the portal system, which carries blood from the spleen and intestines and accounts for approximately 80% of the livers blood supply and (2) the hepatic arterial system, which arises indirectly from the aorta and delivers the remaining 20%. Additionally, the biliary system runs through the liver with its numerous branches. The liver is involved in many metabolic, synthetic and detoxifying processes. Besides these vitally important functions, the liver is unique due to its remarkable capacity to regenerate after injury. This regenerative ability has already been described in the ancient Greek mythology. Prometheus reminds us that the liver is the only organ that can regenerate. According to Greek mythology, Zeus was furious with the titan Prometheus because he gave fire to the mortals. In return, Zeus chained Prometheus to a rock in the Caucasus and sent his giant eagle to eat his liver during the day, only to have it regenerate by night. Although this is an exaggeration, the principles are correct that after partial hepatectomy, the remnant liver will hypertrophy over weeks to months to regain most of its original volume. It is interesting to note that the ancient Greeks seem to have been aware of this fact, because the Greek word for liver, "hepar", is derived from the verb "hepaomai", which means to mend or to repair. Hence "hepar" can be roughly translated as repairable. The regenerative capacity of the liver is important in liver transplantation and after liver surgery

## 1.1 Liver Surgery.

### 1.1.1 Historical perspective.

Liver surgery has been traditionally difficult. Partial hepatectomy carries the risk of massive bleeding because of the rich blood supply of the liver. Sepsis is favoured by bile leak due to the extensive biliary system. Additionally, there is a risk of liver failure when the volume and/or quality of the remaining parenchyma does not ensure sufficient metabolic, synthetic and detoxifying functions. There are scattered reports of liver surgery for battlefield injuries in the past, but the first recorded elective hepatic resection was performed in 1887 in Germany by Langenbuch[2]. Other reports of liver resection followed soon. The main problem in the early history of liver surgery was the potential for massive bleeding during liver surgery. Because of this, very little progress in surgical techniques was recorded for the next half-century[2]. Thereafter, major advances have been made in hepatic surgery, which have been greatly aided by improved understanding of the intrahepatic anatomy. Externally, the liver *appears* to consist of a large right lobe and a smaller left lobe, separated by the falciform ligament. However, this division does not correspond to external and internal vascular and biliary territories. It was not until the 1950s that the anatomy of the liver was well described. In 1957, Claude Couinaud saw a regular segmentation pattern that was constant from one liver to another and recognized the surgical importance of these observations[3]. He showed that the distribution of the feeding arteries and the portal system firstly divide the liver into two units. These are the right and left lobe, that

each can be subdivided into subunits or segments. There are eight segments in total. These segments are enumerated clockwise, starting with the caudate lobe as segment I. The right lobe of the liver consists of four segments (segments V-VIII). The left lobe of the liver comprises three segments (II-IV). Segment one, the caudate lobe, has a bilateral (both right-sided and left-sided) portal, arterial and biliary supply and is regarded as a separate structure (Figure 1). When partial hepatectomy meets these anatomical areas - a so called anatomical resection - the remaining parenchyma is kept well vascularized and drained. Additionally, when these anatomical borders are respected, it will lead to a better removal of tumors and their vascular territories, which is very important in surgical oncology. Another widely used classification is the Bismuth classification, which is mostly used in the United States. The Bismuth classification was introduced in 1982 and resembles the Couinaud classification, except some small differences. It is debated which classification provides the most detailed description of hepatic anatomy. In parallel with understanding the anatomy, progress in liver surgery was advanced by medical and surgical technological developments for control of hemorrhage and bloodless dissection[3].

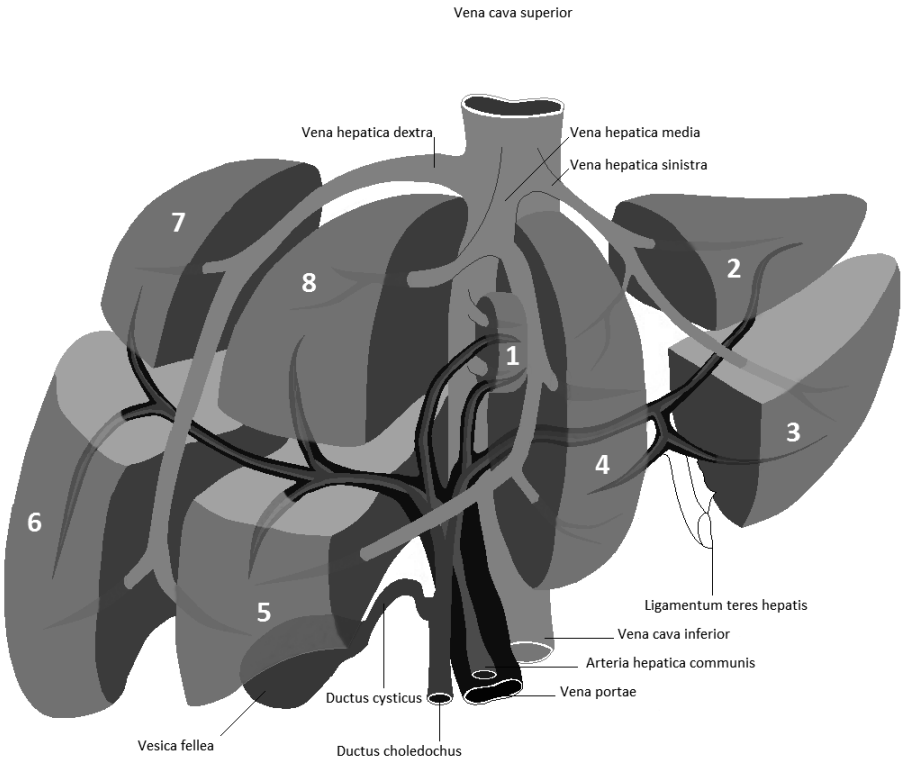


**Figure 1.** Liver anatomy according to Couinaud.

### 1.1.2. Indications for liver surgery.

There are four main indications for partial hepatectomy: (1) neoplasms, (2) living donor liver transplants, (3) infections and (4) traumatic injuries to the liver. The majority of liver resections is performed for malignant tumors, most commonly liver metastases, with colorectal cancer as the primary source. Hepatocellular carcinoma and cholangiocarcinoma are primary liver tumors and encountered less frequently. Benign neoplasms, including hepatic adenomas, focal nodular hyperplasia and hemangiomas are discovered incidentally and do not require resection in the absence of symptoms. However, adenomas are associated with a risk of spontaneous bleeding, causing intra-abdominal hemorrhage. Additionally, there is a risk of malignant degeneration in large adenomas and in these cases resection is sometimes required. Living donor liver transplantation has evolved in response to the severe shortage of organ donors. Since the 1980s, donation of liver lobes from adults into pediatric recipients has been widely practiced. These procedures typically involve resection and transplantation of the left lateral segment. Adult-to-adult liver transplantation is more challenging because of the requirement for a greater hepatic mass. Right-sided hepatectomy is usually necessary to provide sufficient volume. Infection is a rare indication for hepatic resection, but particular abscesses from bacterial or fungal origin and hydatid cysts can be best treated with partial hepatectomy.

### 1.1.3. Morbidity, mortality and survival.

Nowadays, mortality after partial hepatectomy has decreased from nearly 100% in the first reports to 0-6.6% in the modern era[4]. Postoperative morbidity ranges between 22-45%[5]. The 5-year survival rates for partial hepatectomy in patients with colorectal liver metastases, the most common indication for surgery, are 12-67%, depending on the patient selection criteria.

### 1.1.4. Malignant tumors of the liver.

Malignant hepatic tumors, especially colorectal liver metastases, are the main indication for partial hepatectomy. Surgical resection is the therapy of first choice in these patients. Unfortunately, only 10-20% of the patients are candidates for partial hepatectomy[6-9]. Contraindications for liver resections in oncological patients are a high number of tumors in the liver, bilobar liver tumors, unfavourable localization of the tumor(s) in the liver, patient comorbidity and the presence of extrahepatic metastases. Although the resectability criteria for patients with liver tumors are changing - mainly because of improvement in chemotherapy, surgical techniques and postoperative care - the majority of patients still remain ineligible for partial hepatectomy. For these patients, a series of minimally invasive tumor ablation techniques have been developed. These techniques kill the tumor in situ by localized injection of chemicals – for example ethanol and acetic acid – or by intratumoral delivery of lethal energies to freeze or heat the tumor, as in cryotherapy, thermotherapy, radioembolization, chemoembolization, stereotactic radiotherapy and brachytherapy[10].

## 1.2 Thermal ablation.

Hippocrates: "Those diseases which medicines do not cure, iron cures; those which iron cannot cure, fire cures; and those which fire cannot cure, are to be reckoned wholly incurable."

### 1.2.1 Principles.

Radiofrequency ablation (RFA) is a viable and effective therapeutic option in the treatment of solid tumors in the skeleton, liver, spleen, kidneys, adrenal glands and lungs. It can be performed either percutaneously or by an open approach (Figure 2). The basic concept of RFA is a localized and contained heat generation, which induces focal coagulative necrosis and cell death. An RF system consists of a very high frequency alternating current generator (200 to 1200 kHz), an RF needle, a grounding pad which serves as a large dispersive electrode and the patient. All must be connected in series. In this circuit, electric current enters through both the electrodes with the patient as resistor. As the electric current alternates in directions at high frequency, tissue ions that are attempting to follow the direction of the current get agitated. Due to natural high resistivity in the living tissue, ionic agitation produces frictional heat at the immediate vicinity of the electrodes. Because the grounding pad has a very large surface area, the electrical resistance is low. Hence, the production of frictional heat is concentrated at the needle electrode. Thus, deposition of the energy from the electric current produces thermal injury. The extent and nature of this injury are dependent on two important factors which are (1) the temperature and (2) the duration of the RF application. To produce irreversible cell damage, it takes several hours at 45 C°, but it takes only 4-5 minutes at 50 to 55 C°. At temperatures between 60 and 100 C°, there is immediate tissue coagulation because of irreversible damage to mitochondrial and cytosolic enzymes by heat induced denaturation of proteins. Above 100 C°, tissue simply vaporizes. Therefore, temperatures between 50 and 100 C° are ideal for RFA to induce cytotoxicity[11, 12].

*1.2.2 Historical perspective.*

The effect of radiofrequency waves was first reported by Jaques d'Arsonval in 1891[13]. He discovered that RF waves could pass through living tissue without causing neuromuscular excitation, i.e. an electric shock. This led to the development of medical diathermy by the German physician Carl Franz Nagelschmidt in 1897 and surgical electrocautery in the early to mid 1900s. In the 1990s, two independent groups of investigators used modified RF equipments for percutaneously created focal thermal injuries in the liver. The first needles used were simple in design and consisted of standard stock needles insulated to the distal tip. These needles created a well-defined concentric region of coagulation necrosis – the ablation zone - around the exposed needle tip. However, the size of the ablation zone was small due to superficial charring around the needle tip. Subsequent research revealed several factors which increase the volume of coagulated tissue, such as a minimally required local temperature for effective tissue coagulation, which should be above

50 C°, a slow increase in generator power, prolonged RF application and an increase in the exposed surface area of RF needle electrodes. RFA for the management of solid malignancies necessitates a more dispersed distribution of relatively mild RF energy to cause a more extensive sphere of tissue destruction. This has been realized thanks to advances in imaging diagnosis with ultrasound (US), computer tomography (CT) and magnetic resonance imaging (MRI) and improved guidance in monitoring of interventional procedures with implementation of dedicated imaging modalities and endoscopic equipment. The efficacy of RFA has been improved significantly by upgrading RF generators to yield more appropriate power (pulsed current depositions), controllable tip temperature, impedance adaptation, optimizing RFA electrode configuration to improve heat generation and distribution and by decreasing local heat loss through vascular occlusion and induced hypotension. This enables ablation of larger lesions in the order of several centimeters compared with only several millimeters previously. Thus, as an alternative to standard surgical resection for the treatment of malignant tumors, RFA has rapidly evolved into one of the most popular minimally invasive therapies[12].

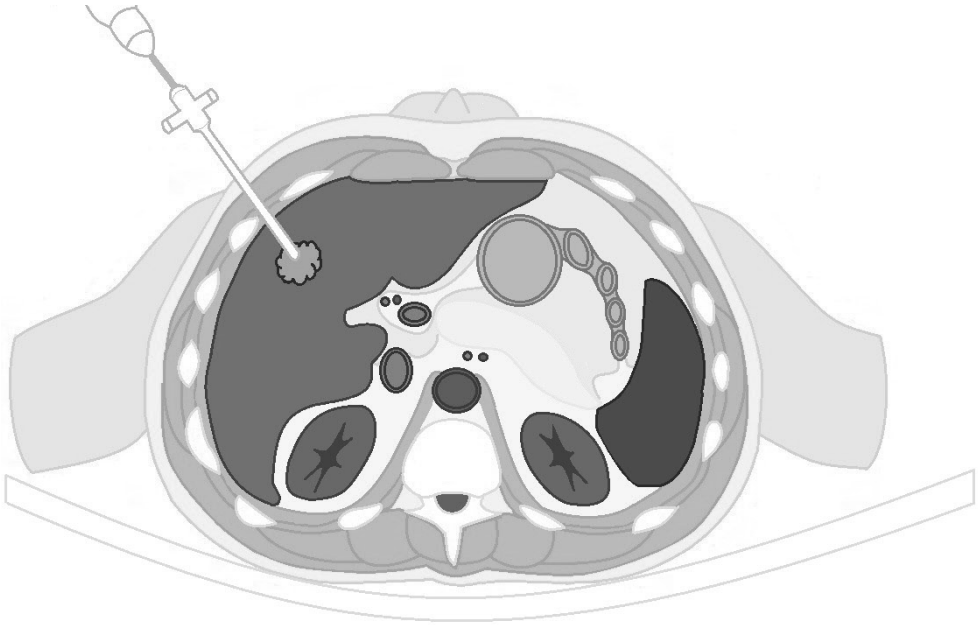

**Figure 2.** Schematic representation of radiofrequency ablation.

### 1.2.3. Indications for RFA.

RFA has been used to treat tumors of the skin, liver, bladder, gastrointestinal tract, lungs and brain. Meanwhile, the "electric scalpel", an RF-instrument, has widened the therapeutic scope of all surgical specialities by allowing the safe division of tissue and by coagulating bleeding vessels. In addition, RFA is used for the destruction of peripheral sensory nerves for control of constant pain and as transcatheter ablation for the treatment of cardiac arrhythmia caused by abnormal cardiac conducting pathways.

### 1.2.4. Morbidity, mortality and survival after RFA for colorectal liver metastases.

Since part one of the thesis focuses on RFA as a treatment for colorectal liver metastases, the following morbidity, mortality and survival rates concern on this issue. The overall complication rate of RFA is below 10% with a mortality of less than 1%. RFA-associated morbidity and mortality are related to the age of the patient, RFA technique (open versus percutaneous), number of RFA sessions, type and size of the treated lesions, the presence of liver disease and concomitant liver surgery. The 5-year survival rate after RFA ranges between 17-55% with a median survival of 24-52 months[14]. These rates appear to rival with the 5-year survival following partial hepatectomy, which ranges between 12-67%[5]. Unfortunately, long-term data are sparse and randomized controlled trials failed in recruitment, thus resection remains the therapy of first choice in patients with colorectal liver metastases.

### 1.2.5. Ablation site recurrences after RFA for colorectal liver metastases.

Ablation site recurrences, also known as "local recurrences" or "local tumor progression" are the key determinant of the technical success of RFA and result from incomplete ablation. We prefer the term "ablation site recurrence" over "local recurrence" because the term "local" generally reflects a recurrence at the site of the primary tumor, which may be in the pelvis in the case of a colorectal carcinoma. Several factors are associated with a lower risk of ablation site recurrences, such as a small index tumor size (< 3 cm), a low number of treated tumors, a safety margin of at least 10 mm coagulation necrosis around the tumor, open RFA approach versus percutaneous CT-targeted approach and tumor location away from large vessels and biliary structures (heat sink phenomenon)[15, 167, 8, 17]. Reported rates of ASR range between 2-55%[7, 8, 10, 16, 18, 19], mainly due to different data collection times.



There are different other treatment modalities in thermal ablative procedures. The most commonly used techniques are microwave ablation (MWA), high-intensity focused ultrasound (HIFU), laser ablation and cryoablation.

### 1.2.6. Microwave ablation.

As with radiofrequency ablation, heat is used in MWA to destroy cells. In MWA, antennas are inserted in the tumor. These antennas create electromagnetic fields which cause ionic agitation to induce frictional heat that destroys tissue. An important difference with RFA is that these electromagnetic fields do not require an intact electrical circuit or physical contact (no grounding pads). This leads theoretically to a higher heating effectiveness in tissues with high impedance (lung and bone). Additionally, there is a fast heat generation over a larger volume of active heating. MWA can penetrate tissues which are localized deeper and it produces higher temperatures than RFA without needle charring. Finally, MWA is reported to be less sensitive to the heat sink effect, which allows the technique to be applied in tumors in the proximity of large vascular structures. Clinical applications of MWA are tumors of the liver, lung, kidney and bones. MWA is widely used in Asian countries, but is not widespread in Europe and the United States[20, 21].

### 1.2.7. Cryoablation.

Cryoablation uses extremely cold temperatures to destroy tumor tissue. Freezing is achieved by circulating liquid nitrogen through the cryoprobe or by the rapid expansion of argon gas in the tip of the probe placed in the tumor. Cell death is induced by two mechanisms. The subzero temperatures cause both intracellular and extracellular ice crystal formation. Intracellular ice crystal formation damages the cell membrane, intracellular proteins and organelles. Extracellular ice crystals cause a transmembrane osmotic gradient, which draws water from the cells, thereby dehydrating them. This leads ultimately to cell death. Cryoablation has been used in the treatment of tumors in the liver, kidney, lung, prostate, breast and skin. A main advantage of cryoablation is that it is independent from electrical current application, as freezing purely is a thermal process. Thus, it can be used in tissues with high impedance, such as lung and bone. Furthermore, the iceball created with the procedure can be monitored intraprocedurally with US, CT and MRI, since cryoablation does not interfere with these imaging techniques as the other ablation techniques do. However, there are several disadvantages of cryoablation. The main problem is that excessive torque or displacement of the probes in the tissue can lead to organ fracture and significant bleeding since frozen tissues are more brittle than heated tissues. Additionally, the probe tract cannot be cauterized, theoretically leading to an increased risk of tumor seeding. Furthermore, procedure times are longer than with other ablative techniques and the costs of cryoablation are higher, mainly because purchase and storage of sufficient quantities of argon and nitrogen gas can be costly. These are the main reasons that RFA and increasingly MWA have replaced cryoablation as ablative therapy[20-22].

### 1.2.8. High-intensity Focused Ultrasound.

In HIFU, high-power, highly focused ultrasound beams are used, which are targeted to converge on a specific point within the body. These ultrasound beams cause tissue vibration, which creates the heat to destroy tissue. It can be compared to the focusing of sun rays through a magnifying glass to start a fire. The main indications for HIFU are malignant tumors of the prostate, breast, liver and benign tumors such as uterine fibroids[20]. HIFU is a new technique on which data are scarce and its long-term effectiveness is yet not well established.

## 1.3. Radiology: Imaging of the liver.

### 1.3.1 Historical perspective.

The history of radiology goes back to 1895 when the x-ray was discovered by Wilhelm Conrad Röntgen. The x-ray was a previously unknown, extremely penetrating form of radiation. Medicine was revolutionalized by his discovery. The new diagnostic tool allowed the human body to be opened without dissection, so that anatomy and physiology could be studied on living patients. The first X-ray techniques were used to examine fractures, foreign bodies and pathological skeletal conditions. The wider physiological application of the technology had to wait for the development of appropriate apparatus and methods. Improvements were made step by step. Greater radiation intensities were achieved while the exposure time to x-rays was reduced. The introduction of ultrasound in the 1960s, the advent of the digital computer – making the invention of computed tomography (CT) possible in 1972 by Godfrey Hounsfield in England - and the introduction of magnetic resonance imaging (MRI) (1978) – have combined to create an explosion of diagnostic imaging techniques in the past 30 years.

For several decades, imaging of the liver was limited to plain radiography of the abdomen on which the shadow of the liver was scanned for calcifications, intestinal air or air in the biliary tract. Oral and intravenous cholecystography was used for examination of the bile ducts. Angiography of the liver was used for the detection of (hypervascular) tumors. With the introduction of ultrasound in the 1960s and CT and MRI from the 1970s, hepatic imaging underwent a revolution[23].

### 1.3.2. Ultrasonography.

Ultrasound as used in medicine is a noninvasive imaging technique to visualize internal organs and structures. This technique uses high-frequency sound waves greater than 2 megahertz. These ultrasound waves are emitted and then received by a transducer. The transducer is placed against the skin of the patient. A thin layer of coupling gel is applied to the skin of the patient to displace the air that would otherwise reflect virtually the entire ultrasound beam. As the ultrasound waves travel into the patient, wave fronts spread out and diminish the overall beam intensity. Partial tissue absorption with associated heat conversion also contributes to beam attenuation. The beam is partially reflected and transmitted at tissue interfaces. The reflected sound waves

- or echoes - travel back to the transducer and are converted into electric signals, which are subsequently amplified. The amplitude of the returning wave partially depends on the degree of beam absorption and a shade of gray is then assigned to each of these amplitudes. Strong echoes are being assigned a shade near the white end of the spectrum. Weak echoes are assigned a shade near the black end of the spectrum. Additionally, the depth of the reflecting tissue can be calculated from the known total beam travel time and the average sound velocity in human tissue, which is 1.54 m/s. Limitations of ultrasound are primarily operator-dependent in nature. An additional limitation is the variable visualization of midline abdominal organs (pancreas) and vasculature when these structures are obscured by overlying bowels or bone, since ultrasound waves are unable to penetrate gas or bone.

Applications of ultrasonography are imaging of the abdomen, pelvis, fetus, vascular system, testicles, breasts, pediatric brain and chest. Furthermore, ultrasound guided interventions are routinely used to facilitate biopsies, drainages and RFA.

Another application of ultrasound in medicine is the Doppler ultrasound. This ultrasound technique utilizes the Doppler effect, which occurs when a sound emitter or reflector is moving relative to the stationary receiver of sound. An object which moves toward the detector appears to have a higher frequency and shorter wavelength. In contrast, an object which moves away form the detector has a lower frequency and longer wavelength. If the ultrasound beam strikes a reflector (the object) which moves toward it, the reflected sound will have a higher frequency than the original beam. Alternatively, if the ultrasound beam strikes a reflector which moves away from it, the reflected sound will have a lower frequency than the original beam. The Doppler shift is the frequency difference between the original beam frequency and the reflected beam frequency. Frequency differences are used to calculate the corresponding flow frequencies from which a Doppler waveform or tracing can be generated. This tracing describes the relationship between velocity and time and is unique to the flow pattern within the vessel. Color flow Doppler assigns colors (blue and red) to structures according to their motion toward or away from transducers. This information can be superimposed on a gray-scale image.

### 1.3.3. Computed tomography.

Computed tomography is an imaging technique in which cross-sectional images are created with the use of x-rays and computerized image processing. A large series of two-dimensional x-ray images are taken perpendicularly to a single axis of rotation as the patient passes through a gantry. This gantry contains one or more x-ray tubes on one side and an array of detectors on the other side. As the gantry rotates around the patient, information obtained from the detectors is analyzed by a computer and displayed as an image. The image information can be manipulated by the computer to reflect a greater spectrum of densities than possible with conventional x-ray films. Images can be manipulated - "windowed" - to display various tissue densities based on their x-ray blocking properties and to optimize the appearance of the image. Selecting a window

width determines the range of CT numbers that will be represented on a specific image. The computer assigns different shades of gray to CT numbers that fall within the selected range. CT numbers that are above the chosen range will appear white, and numbers below the chosen range will appear black. By increasing the window width, a greater range of CT numbers is assigned to a shade of gray. This technique is used when it is desirable to view a variety of tissues that vary greatly in density (e.g., lung). The disadvantage to wider windows (400 to 2000 HU) is that subtle differences in density will not be visualized. CT images were initially limited to the axial plane, but modern scanners allow reconstruction of the pictures into coronal and sagittal planes. Additionally, three-dimensional (3D) reconstructions of the structures can be created, which is a useful tool in volumetric measurements of organs and lesions.
Contrast agents are often used to improve the sensitivity and specificity of CT imaging. Contrast materials for abdominal CT examinations can be administered by mouth, intravenous or rectal routes. Oral contrast agents are used for many abdominal CT examinations in an attempt to improve contrast opacification and distention of the bowel. Iodine, which has an atomic weight of 127, is used as a radiopaque intravenous contrast agent for improved demarcation of vascular structures.

### 1.3.4. Radiation risks.

With the increase in the diagnostic capability of CT and its availability, CT has become an indispensable tool in the medical diagnostic armamentarium. However, the widespread use of CT has led to increasing concerns about radiation exposure. Additionally, the widespread use of MDCT has raised concern because the dose of radiation delivered by MDCT is 27% greater than for single-detector CT and the dose increases with decreasing slice thickness.

The radiation risk from CT is modified by patient factors – for example body habitus - but is also affected by numerous potentially controllable factors, such as the used scan protocol.

### 1.3.5. Magnetic resonance imaging.

Felix Bloch and Edward Purcell were awarded the Nobel Prize in 1952 for their independent discovery of the phenomenon of magnetic resonance in 1946. Between 1950 and 1970, nuclear magnetic resonance (NMR) was developed and used for chemical and physical molecular analysis. Raymond Damadian demonstrated in 1971 the utility of NMR in the diagnosis of cancer. This was based on prolonged relaxation times in pathologic lesions compared to the relaxation times in normal tissue. The first 2D proton NMR image of a water sample was generated in 1972 by Paul Lauterbur who used a back-projection technique, similar to that used in CT. In 1975, Richard Ernst used phase and frequency encoding, as well as Fourier transform analysis, to form the basis of current magnetic resonance imaging (MRI) techniques.

### 1.3.6. Principles of MRI.

MRI is based on the principle of nuclear magnetic resonance, in which energy emissions from nuclei in the presence of an applied, external magnetic field are detected after the nuclei have been stimulated by radiofrequency pulses with the same, or resonant, frequency as the nuclei themselves. The key atom in MRI is hydrogen, which is abundant within the body with more than $10^{20}$ nuclei per cubic centimeter of tissue. Since hydrogen nuclei do not contain any neutrons, they are often referred to simply as protons. When placed in a strong magnetic field, nuclei such as hydrogen, resonate and emit radio signals when pulsed with radio waves. A defined sequence of magnetic pulses and interval pauses produces measured changes in the magnetic vectors of the tissue. This results in an MR image. Proton relaxation is characterized by two time parameters, T1 and T2. T1, or longitudinal relaxation time, is the measurement of magnetic vector changes in the *z*-axis during the relaxation pause. T2, or transverse relaxation time, is the magnetic vector change in the *x*-axis and the y-axis.

Each tissue, whether normal or pathologic, has its own unique T1 and T2 for a given MRI field strength. The inherent tissue differences between various T1 and T2 values lead to the visual contrast seen between tissues on the MR image. An image is T1-weighted if it depends on the differences in T1 measurements for visual contrast and T2-weighted if it depends on differences in T2 measurements. T1-weighted images are often thought of as "anatomy" scans because they clearly delineate the boundaries between different tissue planes. Fluid is very dark, solid organs and muscles are gray, and fat (including fatty bone marrow) is very bright. T1-weighting is also chosen for contrast-enhanced scans because gadolinium, the most common MRI contrast agent, appears bright, owing to its T1 shortening effect. On T2-weighted images, fluid is very bright, while most other tissues are gray or dark. Such images are often thought of as "pathology" scans because abnormal fluid collections and edematous tissue are bright against the darker background of normal tissue. With contrast-enhanced and T2-weighted sequences, abnormal tissue is made even more conspicuous by suppressing the background fat signal using either chemically selective fat saturation pulses or (in combination with T2-weighting) inversion recovery sequences. A recently developed sequence of interest is diffusion weighted imaging, which is thought to be capable of differentiation between benign and malignant tissue. Another emerging sequence is MR spectroscopy, which detects metabolic changes in tissues and tumors. MR images are obtained in the transverse, sagittal, oblique, and coronal views.

Gadolinium (gadopentetic dimeglumine) is an ionic contrast agent that acts as a paramagnetic agent and enhances vessels and lesions of abnormal vascularity.

A major advantage of MRI compared to CT is that no ionizing radiation is involved. Additionally, it provides a better display of vascular anatomy without contrast, linear structures (spine and spinal cord, aorta, vena cava), the posterior fossa and other difficult-to-see CT areas are better visualized. Lastly, MRI provides high-contrast soft-tissue images.

A major drawback of MRI is the long scanning time. Additionally, MRI is more expensive compared to CT. Another disadvantage of MRI is the presence of claustrophobia. Open bore MRI systems may help, but these are often not readily available. Lastly, the presence of metallic foreign bodies such as pacemakers, vascular clips, metallic eye fragments, and cochlear implants are contraindications for undergoing MRI examination.

### 1.3.7. Integration of radiology and surgery.

There is no doubt that the improvement in the results of surgical endeavors in the treatment of liver disease has been in part related to the developments in imaging. Imaging leads to a better knowledge of the liver anatomy and the opportunity to make a well pre-operative planning. Intra-operative use of ultrasound from the early 1980s changed the surgical effectiveness dramatically, since it leads to better identification of vessels, intrahepatic structures and their relationship with tumors. The role of diagnostic imaging in liver surgery has gained increasing importance over the past decades. Whereas 30 years ago the thorough history taking, clinical judgement and laboratory analyses were the most important and often only tools to establish a diagnosis, nowadays a multitude of imaging methods provide complementary information. Frequently, the surgical decision making regarding the indication for partial hepatectomy relies heavily on imaging methods. Ultrasound, computed tomography, magnetic resonance imaging and positron emission tomography are the most often used imaging modalities in hepatic imaging. Tumors of the liver – both benign and malignant - are the main indication for liver imaging. Surgical removal with resection of negative margins remains the optimal therapy for primary and secondary liver tumors. Therefore, pre-operative knowledge regarding the number and size of liver lesions as well as their location regarding major liver vessels and the biliary system is crucial for successful surgical removal. Unfortunately, many patients who undergo curative liver surgery for primary or secondary malignancies will relapse due to undiagnosed small malignant lesions. Therefore, the goal of imaging methods is not only the characterization of the liver lesions and their anatomical localizations, but also the detection of small tumor burden.



## 1.4. Aims and outline of the thesis.



### 1.4.1. Part one: Imaging after thermal ablation.

Colorectal cancer is the third most common malignancy after lung, prostate and breast cancer. It is the second most common cause of cancer related death. Approximately 50% of the patients with colorectal cancer will develop liver metastases, but unfortunately only 10-20% of the patients are eligible for partial hepatectomy. As mentioned in the introduction, radiofrequency ablation has emerged as a widely accepted alternative for these patients[6-9].

Incomplete ablation is one of the major problems with RFA, leading to ablation site recurrences. In contrast with partial hepatectomy, treated tissue is left in situ after an RFA procedure. Since

histopathology does not play a role after RFA, the evaluation of the effectiveness of the RFA procedure fully relies on imaging. Additionally, post-RFA imaging is performed to detect ablation site recurrences as early as possible to have the opportunity for repeating the RFA procedure. Although it is still unclear which imaging modality is preferable for post-RFA follow-up, most institutions use multiphase contrast-enhanced computed-tomography (CT) or magnetic resonance imaging (MRI) with or without fluoride-radiolabeled-deoxy-glucose positron emission tomography (FDG-PET)[24, 25]. Post-RFA evaluation can be performed by measuring ablative margins or by fusing the pre-RFA and post-RFA scans to see whether the ablative margin is overlapping the index tumour. A disadvantage of these techniques is the variation that can occur in the position of the liver between the pre-RFA scan and post-RFA scan, which results in inaccurate measurements of the ablative margin. This may give false reassurance to the adequacy of the treatment and thus increase the probability of an ASR, since insufficient ablative margins remain unnoticed. Another strategy for the evaluation of the RFA-procedure is to focus on the presence or absence of contrast-enhancement in the ablation zone. Successfully and insufficiently ablated metastases have specific characteristics on contrast-enhanced post-RFA CT-scans and MRI-scans. However, ASR of hypovascular metastases – for example colorectal liver metastases - may present atypically. Furthermore, a benign pattern of contrast-enhancement can be observed shortly after an RFA procedure, due to inflammatory changes around the ablation zone. Although this benign periablational enhancement can be distinguished from contrast-enhancement due to residual tumor origin, differentiation between these two entities may be difficult[26, 27]. In addition, detection of subtle changes in tumor shape and size can be challenging with two-dimensional cross-sectional-imaging[28]. Three-dimensional assessment, such as volumetric quantification of ablation zones could theoretically be more accurate than two-dimensional measurements. It has been reported previously that ablation zones gradually become smaller because of shrinkage of the metastases and resolution of post-RFA-inflammation. Theoretically, ablation zones with viable metastatic cells should have a smaller decrease in volume between two subsequent time points of measurement. Residual tumor cells will continue to multiply and inhibit or slow down the volume-decrease of the ablation zone. Therefore, the first aim of part one of the thesis was to assess the impact of the variation in the position of the liver between pre-RFA scan and post-RFA scan on the evaluation of the success of the RFA procedure. These results are presented in **Chapter 2**. The second aim was to study the relationship between volume changes of ablation zones on successive post-RFA CT-scans and the development of ablation site recurrences. This is discussed in **Chapter 3**. Thirdly, we studied whether immediate post-procedural contrast enhanced scanning provides reliable quantitative assessment of the effectiveness of the ablational procedure in patients who underwent microwave ablation for liver tumors. The results are presented in **Chapter 4**.

### 1.4.2. Part two: Liver regeneration after partial hepatectomy.

Among all organs, the liver has a remarkable capacity of regeneration after surgery. The liver can tolerate resections as much as 75-80% with the prerequisite that there is no underlying parenchymal disease[29, 29-31]. Microscopically, liver regeneration is the result of cell replication which leads to macroscopic liver regeneration, thus volume increase. This increase in volume can be assessed with post-processing software for CT and MRI-scans. Liver regeneration depends on different factors, such as the presence of an underlying liver diseases, age and sex. There is a relationship between the resected volume and the amount of liver tissue that needs to regenerate, which implies that the resected volume might also be a trigger in the regeneration process after surgery[32-34]. However, the relationship between resected volume and liver regeneration has been studied less well. Studies which have evaluated liver regeneration have mainly based their results on the type of partial hepatectomy rather than on the exact amount of resected volume. Reported rates of liver regeneration range between 28-64% with lower regeneration rates after smaller resections, for example a bisegmentectomy. However, regeneration rates between patients with the same type of partial hepatectomy vary also substantially. This discordance might be explained by the fact that there is considerable interpatient variability in the size of the different segments of the liver. This means that the resected volume after a particular type of partial hepatectomy may differ considerably between patients[35]. Thus, liver regeneration should be seen in perspective of the resected volume rather than the type of partial hepatectomy.

Parenchymal diseases of the liver are well known factors which have negative influence on liver regeneration. The most well known disease of the liver parenchyma is hepatic cirrhosis and it has been shown that cirrhotic livers do not regenerate well[31]. Hepatic steatosis, characterized by lipid accumulation within hepatocytes is a far more commonly encountered condition of the liver parenchyma. It is estimated to affect up to 30% of the Western population[36, 37]. It is reasonable to assume that its prevalence will further increase due to the current obesity epidemic. Although formerly regarded as a benign condition, hepatic steatosis can lead to non-alcoholic steatohepatitis, cirrhosis and development of hepatocellular carcinoma. In addition, steatosis is thought to be a risk factor for post-operative complications, although reports are contradicting[38, 39]. Clinically significant steatosis is defined as steatosis of 30% or more. The regenerative capacity of steatotic livers is less well established. Studies which have evaluated liver regeneration in the presence of steatosis report that regeneration is not impaired by the presence of steatosis[38, 40]. However, these studies concern only living donors and may therefore not be comparable with patients undergoing elective liver surgery, since steatosis is not a major exclusion criterion in the latter group as it is in living donors.

The main indications for partial hepatectomy are currently primary and secondary malignant liver tumours. Unfortunately, the majority of these patients are no candidates for liver resection because of relevant comorbidities and, even more important, unresectable disease. Chemotherapy is used increasingly to downstage patients who are initially regarded as having non-resectable

disease[41]. A major side effect of chemotherapy are its considerable hepatotoxic effects[31, 41]. It has been reported that postoperative morbidity and mortality is increased in patients who received pre-operative chemotherapy[31]. Additionally, chemotherapeutic agents can induce different types of injury to liver cells, which theoretically may lead to impaired liver regeneration after partial hepatectomy[31, 41], but the impact of pre-operative chemotherapy on liver regeneration remains unknown.

On the molecular level, hepatic regeneration is an orchestrated interplay of signaling events, consisting of growth factors, cytokines and transcription factors[42]. Platelets, which contain multiple growth factors, have recently gained interest in their possible role in liver regeneration. Platelets are thought to be directly involved in liver regeneration, as demonstrated in several studies in rodents[43-45]. Thrombocytopenia resulted in a markedly reduced proliferative activity in the liver. On the other hand, trombocytosis was associated with accelerated liver regeneration. In humans, it has been shown that a low postoperative platelet count (PC) was associated with delayed functional recovery of the liver and trombocytopenic patients have increased markers of liver injury, function and mortality[46]. It has been reported that PC decreases after PH and that this decrease is related to the extent of resection[47, 48]. This could be due to the suggested role of platelets in liver regeneration. However, the extent of decrease in PC has never been related to posthepatectomy liver regeneration.

Part two of this thesis focuses on liver regeneration. Firstly, we aimed to study the rate of liver regeneration in patients who underwent partial hepatectomy and whether the rate of liver regeneration is proportional to the amount of resected volume. This is discussed in **Chapter 5**. Secondly, the impact of steatosis on the rate of liver regeneration was evaluated, which is evaluated in **Chapter 6**. Thirdly, since pre-operative and postoperative platelet counts were available in the patient database, the decrease in postoperative platelet count in relationship to the regeneration response was evaluated. These results are presented in **Chapter 7**. Fourthly, the impact of pre-operative chemotherapy on early liver regeneration in patients undergoing right hemihepatectomy was studied. This is discussed in **Chapter 8**.

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# PART 1

# 2

# Lack of anatomical concordance between pre-ablation and post-ablation CT-images: a risk factor related to ablation site recurrence

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## Abstract

**Objective**:
Variation in the position of the liver between pre-ablation and post-ablation CT-images hampers assessment of treatment of colorectal-liver-metastasis (CRLM). The aim of this study was to test the hypothesis that discordant pre-ablation and post-ablation imaging is associated with more ablation-site-recurrences (ASR).

**Methods**:
Patients with CRLM were included. Index-tumour-size, location, number, RFA-approach and ablative-margins were obtained on CT-scans. Pre-ablation and post-ablation CT-images were assigned a "Similarity-of-Position-Score" (SiPS). A suitable cut-off was determined. Images were classified as identical (SiPS-id) or non-identical (SiPS-diff). ASR was identified prospectively on follow-up-imaging.

**Results**:
Forty-seven patients with 97 tumours underwent 64 RFA-procedures (39 patients/63 tumours open-RFA, 25 patients/34 tumours CT-targeted-RFA, 12 patients underwent >1 RFA). Images of 52 (54%) ablation-sites were classified as SiPS-id, 45 (46%) as SiPS-diff. Index-tumour size, tumour location and number, concomitant partial hepatectomy and RFA-approach did not influence the SiPS. ASR developed in 11/47 (23%) patients and 20/97 (21%) tumours. ASR occurred less frequently after open-RFA than after CT-targeted-RFA ($P<0.001$). ASR was associated with larger index-tumour-size (18.9 versus 12.8mm, $P=0.011$). Cox-proportional-hazards-model confirmed SiPS-diff, index-tumour-size >20mm and CT-targeted-RFA as independent risk-factors for ASR.

**Conclusion**:
Variation in anatomical concordance between pre-ablation and post-ablation-images, index-tumour-size and a CT-targeted approach are risk factors for ASR in CRLM.

## Introduction

Liver metastases develop in approximately 50% of patients with colorectal carcinoma. Partial hepatectomy is a potentially curative treatment, but only 10-20% of the patients are eligible for partial hepatectomy. Radiofrequency ablation (RFA) is an alternative for patients with unresectable tumours and is often used as an adjunct to partial heptaectomy[1-4]. By using an image-guided approach, electrodes are positioned in the tumour either percutaneously or by an open approach[5-8]. One of the major problems with RFA is incomplete ablation, leading to ablation site recurrences (ASR)[9]. Factors associated with low ASR rates are small index-tumour size[10], low number of treated tumours[11], at least 10 mm margins of coagulation around the tumour[1, 12], open surgical approach (versus percutaneous CT-targeted approach)[2, 3] and tumour location distant from large vessels[2, 3]. The purpose of post-RFA imaging is the early detection of ASR, providing the opportunity to repeat the RFA procedure. Strategies used for post-RFA evaluation include measuring ablative margins or focusing on contrast-enhancement in the ablation zone. A disadvantage of these techniques is the variation that can occur in the position of the liver between the pre-RFA scan and post-RFA scan, resulting in an inaccurate quantitative assessment of the ablative margin. This may give false reassurance to the adequacy of the treatment and might thus be an indirect risk factor for development of ASR. In this study, we test the hypothesis that variation in the position of the liver between pre-RFA scan and post-RFA scan makes the assessment of completeness of ablation of colorectal liver metastases difficult, and as an indirect risk factor is associated with the development of future ablation site recurrences (ASR).

## Patients and methods

### Patients

The study was approved by our institutional review board. Between July 2000 and July 2008, 142 RFA procedures were performed for primary (benign and malignant) and secondary liver tumours in our center. Sixty-five percent of these procedures were done with an open approach, 35% was performed percutaneously under CT-targeting. Open procedures were performed in patients who also underwent a partial hepatectomy or if the tumour could not be safely reached using the percutaneous route. Laparoscopic procedures were not performed. All procedures were performed by one of the authors, an experienced hepatobiliary surgeon in collaboration with dedicated radiologists. CT-targeted RFA was performed in collaboration with a radiologist. Fifty-two patients (37%) underwent RFA for colorectal liver metastases. Five patients were excluded - missing pre-RFA images (n=2), multiple and widespread liver metastases shortly after RFA making assessment of ASR impossible (n=2) and lost to follow-up (n=1). Thus, 47 patients who underwent 64 RFA procedures for 97 liver metastases were included in the study. Partial

hepatectomy was performed as described previously and is considered the gold standard[13]. It is standard praxis to fix the liver remnant after partial hepatectomy with the aim to keep the liver remnant in the same position in order to prevent rotation of the remaining liver lobe. Rotation can lead to torsion of the draining hepatic vein and congestion of the liver lobe. RFA was only performed if partial hepatectomy was not able to render the liver tumour-free.

**RFA procedure**

RFA was performed by one staff HPB surgeon (KPdJ) in collaboration with a staff radiologist (EJvdJ) for the CT-guided procedures. Ablation procedures are performed in our hospital since 1995 with about 20 procedures per year for colorectal liver metastases. We used the RF 3000 TM Radio Frequency Ablation System (Boston Scientific, Boston, MA, USA). A LeVeen-electrode of 2, 3.5, 4 or 5 cm diameter was used, depending on tumour diameter. The RFA-electrode was positioned using ultrasonography in open and CT-guided in CT-targeted RFA. RFA was applied according to the protocol of the manufacturer. RFA was continued until the generation of radiofrequency waves was blocked by the rise in tissue impedance. Large tumours were treated by several overlapping positions of the deployed RFA-electrode. Terminology used in this paper is in accordance to the guidelines by Goldberg *et al*[14].

**CT-protocol**

Patients underwent triphasic CT-scanning before the RFA procedure, one week after the RFA procedure, then at three-monthly intervals during the first two years and every six months thereafter. CT was performed on a 16- or 64-slice multidetector CT scanner (Somatom Sensation 64, Siemens, Erlangen, Germany). Intravenous contrast was used, 120 ml iodixanol 320 mg I/ml (Visipaque 320, GE Healthcare, Chalfont St Giles, UK), with a flow rate of 4.0 ml/sec. All subjects were scanned in craniocaudal direction during inspiratory breath-hold. CT-images were acquired in a supine position using a 16 x 1.5 (16-slice) or 24 x 1.2 (64-slice) collimation, tube potential 120 kV, tube current time product 130 mAs, pitch 1, slice thickness of 2 mm, reconstruction Kernel B30f and reconstruction increment 1.5.

**Follow- up**

Follow-up of the ablated tumours consisted of CT-imaging or [F-18]-fluorodeoxyglucose-positron emission tomography (FDG-PET) when CT-imaging was inconclusive. Patients were considered to have recurrences when there was a typical pattern of contrast-enhancement on CT-imaging and/or pathological glucose uptake on PET-scanning.

**Post-RFA evaluation**

Radiological evaluation of the tumours before and one week after the RFA procedure was performed on an Aquarius Workstation (version 1.8.3.6, TeraRecon Inc., San Mateo, CA, USA).

Images in axial and reconstructed coronal planes were used for three-dimensional measurements and comparison of the pre-RFA scan and the post-RFA scan (explanation in Figure 1). This was done by two of the authors (PGK, EJvdJ). Reliable comparison was only possible when the position of the liver was identical or almost identical on the pre-RFA scan and post-RFA scan. Therefore, a dichotomous "Similarity of Positioning Score" (SiPS) was developed in which post-RFA scans were compared to pre-RFA scans. Post-RFA scans were centrally and blindly classified as SiPS-identical (SiPS-id, i.e. comparable to the pre-RFA scans, Figure 2) or SiPS-different (SiPS-diff, i.e. not comparable to the pre-RFA scan, Figure 3). A post-RFA scan was considered SiPS-id when the vascular configuration (especially hepatic and portal veins) was identical or nearly identical to that on the pre-RFA scan. In addition, the projection of the abdominal organs, bony structures (vertebrae and ribs) and the position of previously placed surgical clips had to be identical or nearly identical. When these criteria were not met, a scan was regarded as SiPS-diff. For validation of SiPS, one of the authors (PK) classified all tumours twice for intra-observer agreement. Another radiologist with two years CT-experience performed the same classifications to obtain the interobserver agreement. Ablative margins > 10 mm were considered sufficient. The smallest margin in one of the six directions was considered the most imperfect one. Therefore, tumours with an ablative margin <10 mm in only one of the six directions were regarded as having an insufficient ablative margin.

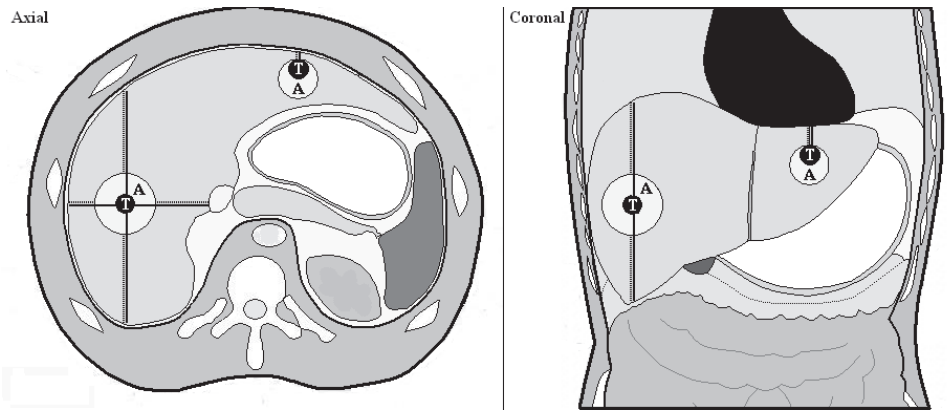


**Figure 1.** Explanation of the method of measurement

Schematic representation of the method of measurement of the ablation zone.
Figure 1 representing the axial and coronal view respectively of the tumour (black circle with white T) and the ablation zone (white circle with black A).
Ablative margins were calculated as follows. The distance from the edge of the tumour to the surface of the liver was measured in all six directions on the pre-RFA scan (continuous line). The same measurements were performed for the post-RFA scan from the edge of the ablation zone scan to the surface of the liver (dotted line). The ablative margins are the difference between both distances.
The tumour in the left liver lobe is considered to be incompletely ablated because in one of the six directions the difference between both distances is zero.

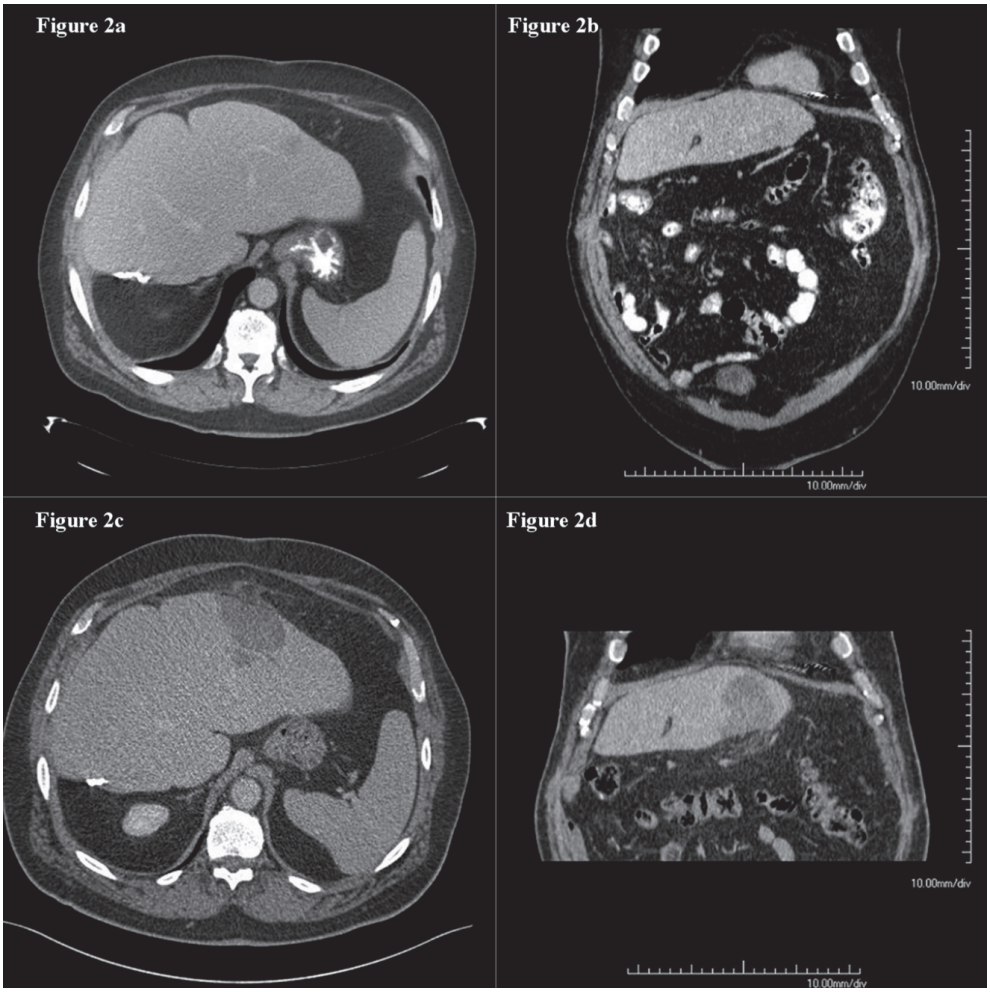


**Figure 2.** Similarity of Positioning Score-identical (SiPS-id)

Example of identical (concordant) pre-RFA CT-images and post-RFA CT-images, classified as Similarity of Positioning Score-identical (SiPS-id). The pre-RFA CT-scan (A axial, B coronal) and post-RFA CT-scan (C axial, D coronal) are well comparable.

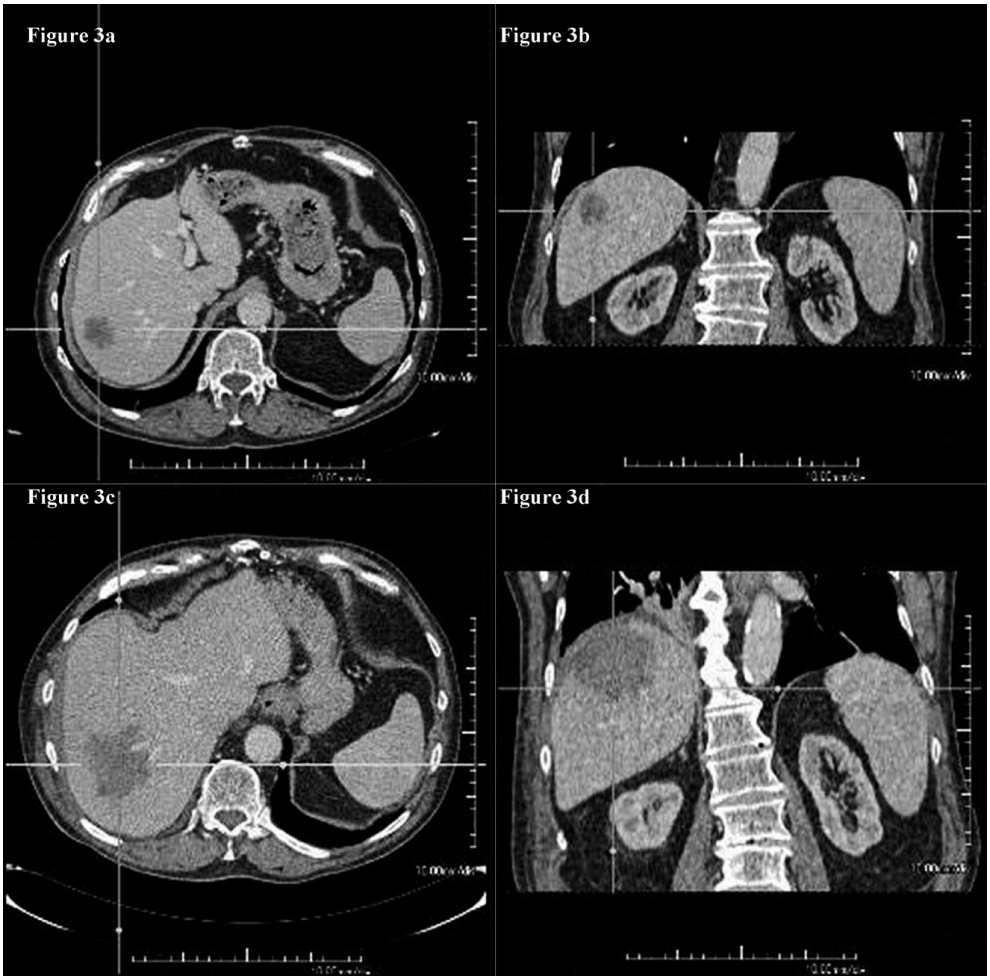


**Figure 3.** Similarity of Positioning Score-different (SiPS-diff)

Example of non-identical (discordant) pre-RFA CT-images and post-RFA CT-images, classified as Similarity of Positioning Score-different (SiPS-diff). The pre-RFA CT-scan (A axial, B coronal) and post-RFA CT-scan (C axial, D coronal) are not comparable.





### Definition of ablation site recurrence

Progression at the site of a previously RFA-treated tumour was considered ASR when it met both of the following criteria: (1) growth of a contrast-enhancing lesion within or directly adjacent to the ablation zone and (2) the largest diameter of the lesion was in direct contact with the ablation zone. The latter prerequisite is to exclude outgrowth of satellite lesions in the vicinity of the ablated tumour (Figure 4).

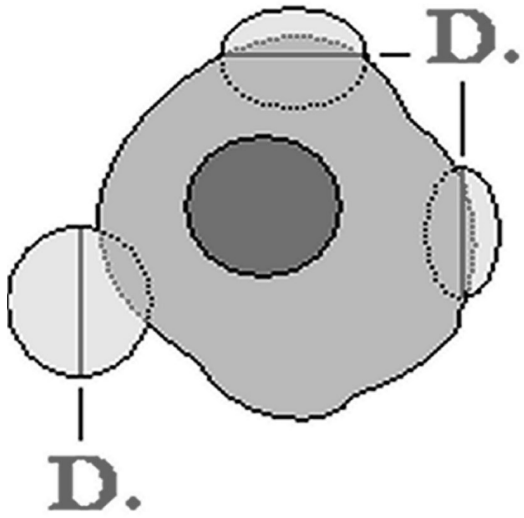


**Figure 4.** Definition of ablation site recurrence

Examples of ablation site recurrences (ASR). The largest diameter of both lesions on the right is in direct contact with the ablation zone. The lesion on the left is not an ASR, because the center of the line representing the largest diameter is not in direct contact with the ablation zone. It is more probably a satellite metastasis which was already present at the time of the ablation. Outgrowth of this lesion took place after the RFA procedure. This prerequisite is necessary to prevent erroneously identified outgrowing satellite lesions in close vicinity of the ablated tumour as ASR.

**Statistical analysis**

Chi-square and Fisher's exact test were applied to assess the relationship between categorical variables SiPS (identical versus different), RFA approach (open versus CT-targeted), partial hepatectomy in the history (yes versus no), number of tumours ablated (<3 versus ≥3), localization of tumours (subcapsular, i.e. <10 mm under the liver capsule, versus central), ablative margins (>10 mm versus <10 mm and >5 mm versus <5 mm) and ASR (yes versus no). For comparing the continuous variable index-tumour size between tumours with and without ASR, Student's t-test was used after correction for non-normal distribution (log-transformation). Survival was assessed with Kaplan-Meier analysis. Variables possibly contributing to ASR were analyzed by using log-rank test. Cox proportional hazard model was used to identify independent risk factors for ASR. Kappa statistics were calculated to test the intra-observer and interobserver agreement of SiPS. Agreement was rated as poor (kappa 0-0.2), fair (kappa 0.21-0.40), moderate (kappa 0.41-0.60), substantial (kappa 0.61-0.80) or excellent (kappa 0.81-1.0)[15]. The significance level was set at a $P < 0.05$ for all tests. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences version 16.0 Inc., Chicago, IL, USA).

**Table 1.** Patient and tumour characteristics

| | Patients | Tumours |
|---|---|---|
| Number | 47 | 97 |
| Sex ♂/♀ | 30/17 (64 %/ 36 %) | - |
| Age (mean, range) | 61.8 years (39-81) | - |
| Deceased | 12/47 (26 %) | - |
| Partial hepatectomy | 34 (72 %) | 73 (75 %) |
| - Before RFA[a] | 5 (15 %) | 20 (27 %) |
| - During RFA | 25 (73 %) | 43 (59 %) |
| - After RFA | 4 (12 %) | 10 (14 %) |
| Type of partial hepatectomy (n=34) | | |
| - Right-sided hemihepatectomy | 12 (35 %) | - |
| - Left-sided hemihepatectomy | 8 (24 %) | - |
| - Segment 2 & 3 resection | 12 (35 %) | - |
| - Other | 2 (6 %) | - |
| Synchronous/ metachronous disease | 26/21 (55 %/ 45 %) | 57/40 (59 %/ 41 %) |
| Indication RFA | | |
| - Bilobar disease | 29 (62 %) | - |
| - Recurrence after partial hepatectomy | 8 (17 %) | - |
| - Major comorbidity | 7 (15 %) | - |
| - Minimal residual disease | 2 (4 %) | - |
| - Severe steatosis | 1 (2 %) | - |
| RFA procedures | | |
| - 1 RFA | 35 (75 %) | - |
| - 2 RFAs | 9 (19 %) | - |
| - 3 RFAs | 1 (2 %) | - |
| - 4 RFAs | 2 (4 %) | - |
| RFA approach (64 procedures) | | |
| - Open | 39 (61 %) | 63 (65 %) |
| - CT-targeted[b] | 25 (39 %) | 34 (35 %) |
| No. of tumours ablated (64 procedures) | | |
| - 1 tumour | 44 (69 %) | - |
| - 2 tumours | 13 (20 %) | - |
| - ≥3 tumours | 7 (11 %) | - |
| Recurrence | 33 (70 %) | 74 (76 %) |
| ASR[c] | 11 (23 %) | 20 (21 %) |
| Repeat RFA for ASR | | |
| - Yes | 5 (11 %) | 10 (10 %)[d] |
| - No | 42 (89 %) | 87 (90 %) |
| Partial hepatectomy for ASR | | |
| - Yes | 1 (2 %) | 2 (2 %) |
| - No | 46 (98 %) | 95 (98 %) |

[a]RFA: radiofrequency ablation.
[b]CT-targeted: computer tomography-targeted.
[c]ASR: ablation site recurrence.
[d]CT-targeted RFA was performed initially in all tumours which underwent repeat RFA for ASR.

## Results

### General characteristics and recurrence patterns

In 47 patients with 97 colorectal liver metastases, 64 RFA procedures were performed. An open approach was used in 39 patients with 63 metastases. Percutaneous CT-targeted RFA was performed in 25 patients with 34 metastases. There were 12 patients who underwent one or more further RFA procedures, of which 5 patients had repeat RFA for ASR. ASR was seen in 11 patients (23%) with 20 metastases (21%). There were 2 patients (4%) with 3 metastases (3%) who showed ASR without recurrences elsewhere. Recurrent disease elsewhere occurred in 33 patients (70%) with 74 ablated liver metastases (76%) and was concomitant with ASR in 9 patients (19%) with 17 metastases (18%). Recurrence without ASR was seen in 24 patients (51%) with 57 tumours (59%) (Table 1). Mean index-tumour size before RFA was 13.9 mm (SD 1.8, range 3.9-78.0 mm).

### Similarity of Positioning Score (SiPS)

After CT-targeted RFA, 15 of the 34 tumours (44%) were classified as SiPS-id, the remaining 56% as SiPS-diff. After open RFA, 37 of the 63 tumours (59%) were classified as SiPS-id, the remaining 41% as SiPS-diff. After open RFA with concomitant partial hepatectomy, 24 tumours were classified as SiPS-id (56%), the remaining 44% as SiPS-diff. Kappa statistics for intra-observer agreement were excellent (kappa 0.834, $p<0.001$) and substantial for interobserver agreement (kappa 0.752, $p<0.001$). Index-tumour size, RFA approach, concomitant partial hepatectomy, number of ablated tumours and tumour localization were not different in the SiPS-diff group versus the SiPS-id groups (Table 2).

**Table 2.** Effect of different factors on the Similarity of Positioning Score (SiPS)

| | SiPS-id[a] (n=52) | SiPS-diff[a] (n=45) | *P* value |
|---|---|---|---|
| Index-tumour size (mean ±SD)[b] | 14.1 mm (1.9) | 13.6 mm (1.8) | 0.773 |
| RFA approach[c] | | | |
| - Open RFA (n=63) | 37 (59 %) | 26 (41 %) | 0.203 |
| - CT-targeted RFA (n=34) | 15 (44 %) | 19 (56 %) | |
| Partial hepatectomy during RFA[c] | | | |
| - Yes (n=43) | 24 (56 %) | 19 (44 %) | 0.838 |
| - No (n=54) | 28 (52 %) | 26 (48 %) | |
| No. tumours ablated[c] | | | |
| - 1-2 (n=70) | 40 (57 %) | 30 (43 %) | 0.364 |
| - ≥ 3 (n=27) | 12 (44 %) | 15 (56 %) | |
| Localization[c] | | | |
| - Subcapsular (n=76) | 44 (58 %) | 32 (42 %) | 0.140 |
| - Central (n=21) | 8 (38 %) | 13 (62 %) | |

[a]SiPS: Similarity of Positioning Score, identical (SiPS-id) or different (SiPS-diff).
[b]Student's t-test.
[c]Chi-square or Fisher's exact test.

**ASR**

ASR occurred in 20 of 97 metastases (21%). Tumours with ASR were larger than tumours without ASR (18.9 mm versus 12.8 mm, $P$=0.011). ASR was seen in 17 (50%) tumours treated with CT-targeted RFA and 3 (5%) tumours with open RFA ($P$<0.001). ASR was seen in 6 (12%) tumours classified as SiPS-id and 14 (31%) tumours classified as SiPS-diff ($P$=0.017). ASR was not different in tumours with ablative margins <5 mm ($P$=0.464).

Univariate analysis showed more ASR in tumours treated with CT-targeted RFA ($P$<0.001), in tumours classified as SiPS-diff ($P$=0.023) and in tumours with an index-tumour size >20 mm ($P$=0.009). Tumour localization (subcapsular versus central) and ablative margins were not associated with ASR ($P$=0.483 and $P$=0.576, respectively). Cox proportional hazard model identified RFA approach, SiPS and index-tumour size as independent predictors of ASR. CT-targeted RFA was associated with the highest risk for developing ASR, followed by SiPS-diff and an index-tumour size >20 mm (Table 3).

**Table 3.** Cox proportional hazard model showing the relative risk for development of ablation site recurrence compared to the reference standard (1.0).

| | Relative risk (95%-CI) | $P$ value |
|---|---|---|
| RFA approach | | |
| - Open RFA | 1.0 | 0.001 |
| - CT-targeted RFA | 9.5 (2.6-34.0) | |
| Similarity of Positioning Score (SiPS) | | |
| - SiPS-identical | 1.0 | 0.019 |
| - SiPS-different | 3.9 (1.2-12.3) | |
| Index-tumour size | | |
| - < 20 mm | 1.0 | 0.010 |
| - ≥ 20 mm | 3.6 (1.4-9.4) | |

**Survival**

Median time of follow-up was 36 months (interquartile range 25-49). Median overall survival in the open RFA group was 40.7 months (95%-CI 23.3 - 58.2) and was not statistically different for the CT-targeted RFA group ($P$=0.23). As the proportion of disease-free patients in the latter group was more than 50% at the end of the study, the median survival could not be estimated. After open RFA, median disease-free survival was 35.2 months (95%-CI 29.7 - 40.7) and 32.6 months (95%-CI 15.8 - 49.5) after CT-targeted RFA ($P$=0.50).

## Discussion

RFA is increasingly used in patients with malignant liver tumours in whom partial hepatectomy is not able to render the liver tumour-free. RFA seems to be a highly attractive treatment modality since it is associated with lower morbidity and mortality compared to partial hepatectomy. However, a major concern is the reported high incidence of ablation site recurrences (ASR). Early evaluation of the completeness of RFA - followed by immediate repeat RFA in case of an incomplete procedure – is essential to reduce the high incidence of ASR. A prerequisite for evaluation of the completeness of RFA is the anatomical concordance or comparability of the pre-RFA scan with the post-RFA scan. In the present study, we hypothesized that incomparability of the pre-RFA scan and post-RFA scan may result in an increased number of future ASR, since completeness of ablation cannot be evaluated reliably. Indeed we found that this incomparability is a risk factor associated with ASR. Other risk factors were CT-targeted RFA approach (as opposed to open RFA) and an index-tumour size >20mm.

The reason for using Similarity of Positioning Score (SiPS) in this study was to evaluate the problem and consequences of incomparable pre-RFA imaging and post-RFA imaging. Fifty-four percent of the post-RFA scans were classified as anatomically concordant or SiPS-identical (SiPS-id), the remaining 46% as anatomically discordant or SiPS-different (SiPS-diff). Open RFA and CT-targeted RFA were equally represented, suggesting that SiPS is not influenced by RFA approach and concomitant partial hepatectomy. Although intuitively it seems reasonable to expect that partial hepatectomy is associated with a change in position and configuration of the liver – and thus influences SiPS - we did not encounter this. A probable explanation is that the liver remnant is fixed in position at the end of the operation. This means that SiPS is determined by other factors, for example changes in the position of the liver as a result of longitudinal or rotational movements of the liver related to variations in diaphragm position. These factors could result in substantial organ position differences. These issues are well-known in the field of radiotherapy and nuclear medicine. In radiotherapy, this problem is improved by using implanted markers which optimize accurate tumour targeting and advanced scanning techniques such as four-dimensional CT-planning[16, 17]. In nuclear medicine, movements – particularly respiratory movements - can result in mismatch between PET and CT-images. Respiratory-motion tracking systems, mathematical correction models or scanning correction models and post-processional motion-correction methods are used to minimize this problem[18, 19]. These techniques could be useful in reducing organ position differences between subsequent scans in the post-RFA follow-up.

Radiological evaluation of RFA procedures can be performed by different strategies. Firstly, ablative margins can be estimated by fusing pre-RFA images and post-RFA images. Unfortunately, this method is often hindered by incomparable pre-RFA images and post-RFA images. Secondly, comparing surfaces, volumes or diameters of the index-tumour and post-RFA ablation zone is

often not reliable because of geometrical constraints or incomplete overlap of the index-tumour and ablation zone[7, 20]. Thirdly, evaluation can be performed by focusing on post-RFA contrast-enhancement, which might be misleading because of contrast-enhancement associated with post-RFA inflammation and contrast-enhancement due to residual tumour origin. Differentiation between these entities can be performed by their different morphological characteristics and contrast-enhancement patterns on multiphase-CT-scanning[[21, 22], but remains difficult.

A possible solution to detect residual tumour after RFA without being hindered by incomparable pre-RFA images and post-RFA images is to perform PET-CT. PET is reported to have a high diagnostic accuracy in detecting residual tumour after RFA compared to contrast-enhanced CT and even MRI, modalities which are more readily available[21, 23]. Until now, only few studies with small patient groups assessed the usefulness of PET-CT after RFA. Based on our study it might be that PET-CT is the preferred imaging modality to detect incomplete ablations in patients with discordant pre-RFA scans and post-RFA scans. A potential limitation might be a false-positive result because of glucose uptake associated with post-RFA inflammation in the early post-RFA period[24]. Another possibility to evaluate completeness of the ablation is to monitor ablation zone volume on consecutive CT-scans, since an ongoing decline in ablation zone volume on consecutive scans is highly predictive of complete ablation and because an increase in volume is associated with ASR[25]. However, this is only noticed later in the follow-up and not on the first post-procedural scan. Therefore, we recommend that in case of SiPS-id, patients undergo regular follow-up with multiphase CT-scanning every three months in the first two years after the RFA-procedure and biannually thereafter. Patients with SiPS-diff should be followed in similar fashion, but it might be advisablein these patients to perform additional PET-CT-scanning three to six months after the RFA-procedure, when the post-RFA inflammation has subsided and eventual glucose uptake can be attributed to residual tumour.

We report an ASR rate of 23% per patient and 21% on a tumour basis. Previously reported ASR rates vary widely between 1.8-55%[2, 3, 11, 26-28]. We found more ASR in tumours with SiPS-diff classified scans, CT-targeted RFA treated tumours and tumours with a diameter of >20 mm. Although some studies have shown a higher incidence of ASR with ablation margins <10mm [29-31], our findings are in line with that of others who have reported that ASR is not related to ablative margins[31, 32]. Unfortunately, authors often do not mention their evaluation methods, which may lead to contradictory reports because of the use of different techniques.

It has been reported that CT-targeted RFA is associated with a higher risk of ASR[2, 3, 12, 26, 28, 33-35], which is in accordance with our results. The most important explanation for the higher ASR rate in CT-targeted RFA is limited access to the tumour compared to open RFA, leading to inadequate ablation. Open RFA allows complete mobilization of the liver, better electrode accessibility, additional manoeuvres (Pringle) and tumour visibility (using intra-operative ultrasound). Especially relevant is our finding that despite the higher incidence of ASR in CT-targeted RFA, survival is not different from patients treated with open RFA. This can very

likely be explained by thorough post-procedural follow-up. By carefully monitoring patients, early detection of ASR offers the possibility of timely interventions such as repeat RFA or partial hepatectomy.

This study has certain limitations. Firstly, the newly introduced SiPS-classification system is used in a small patient population. We are planning to validate the SiPS-classification in a larger patient cohort. Secondly, we did not perform biopsies to confirm the diagnosis of ASR. However, the reason not to do so is well-founded - biopsies can be associated with tumour seeding. This risk outweighs the benefit of the procedure[36, 37]. Although SiPS was described in a relative small population encompassing 97 tumours, it is highly reproducible as reflected by the excellent intra-observer and substantial interobserver agreement.

In conclusion, lack of anatomical concordance between pre-RFA images and post-RFA images, CT-targeted RFA and index-tumour size >20 mm are independent risk factors associated with future ablation site recurrences. Anatomical concordance of pre-RFA images and post-RFA images, expressed in the Similarity of Positioning Score, is important in evaluating the RFA procedure. In discordant scans, no reliable judgement can be made about the completeness of ablation and thus whether an additional RFA is necessary. Therefore, it is associated with an indirect increased risk of future ablation site recurrences.

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# 3

# Increase in Volume of Ablation Zones during Follow-Up Is Highly Suggestive of Ablation Site Recurrence in Colorectal Liver Metastases Treated with Radiofrequency Ablation

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## Introduction

Radiofrequency ablation (RFA) is a well-established locally directed therapy which considerably improves survival of patients with unresectable liver metastases(1-6). Intensive post-procedural-follow-up is necessary since one of the yet unresolved limitations of RFA is the high incidence of ablation site recurrences (ASR). Reported rates of ASR vary widely between 2-60%(4,7). Early detection of ASR as well as other intrahepatic or extrahepatic recurrences is indicated to offer patients secondary interventions if possible. Although it is still unclear which imaging modality is preferable for post-RFA-follow-up, most institutions use multiphase contrast-enhanced computed-tomography (CT) or magnetic resonance imaging (MRI) with or without fluoride-radiolabeled-deoxy-glucose positron emission tomography (FDG-PET)(8,9). Successfully and insufficiently ablated metastases and intrahepatic recurrences have their own specific characteristics on post-RFA-CT-scans and MRI-scans. However, ASR of hypovascular metastases may have atypical appearances(10,11). In addition, detection of subtle changes in tumor-shape and tumor-size can be challenging with two-dimensional cross-sectional-imaging(12,13). Volumetric quantification of ablation zones (AZ) could theoretically lead to a more accurate assessment compared to two-dimensional measurements. Therefore, the aims of this study were (1) to test the hypothesis that volume-changes of AZs on successive post-RFA CT-scans of colorectal liver metastases are predictive of ASR and (2) to analyze the course of the AZ-volumes on successive CT-scans in the post-RFA-follow-up in relation to the development of ASR.

## Material and Methods

### Patients

The study was approved by our institutional review board. Consecutive patients who underwent open-RFA or percutaneous CT-targeted-RFA for colorectal liver metastases (CRLM) in the period from July 2000 until August 2009 were retrieved from a prospective database. One week after the RFA-procedure, a baseline control CT-scan was performed. Follow-up consisted of physical and biochemical examination (including carcino-embryonic antigen, CEA, as tumor marker) and CT-scanning every three months during the first two years and biannually thereafter. Patients were included when the pre-RFA-scan, post-RFA-scan and at least another scan afterwards was available. Patients were excluded if adequate scans for comparison were missing.

### RFA details

RFA was performed by using a RF 3000 TM Radio Frequency Ablation System (Boston Scientific, Boston, MA, USA). A LeVeen-electrode of 2, 3.5, 4 or 5 cm in diameter was used, depending on index-metastasis diameter. The RFA-electrode was positioned under ultrasonography in open RFA or under CT-targeting in CT-targeted procedures. RFA was applied according to the protocol of

the manufacturer. RFA was continued until the generation of radiofrequency waves was blocked by the rise in tissue impedance. Large metastases were treated by several overlapping positions of the deployed RFA-electrode. RFA was performed under general anaesthesia. Terminology used in this paper is in accordance to the guidelines by Goldberg et al(14).

**Standard of reference**

The standard of reference was defined by combining clinical, biochemical (CEA) and imaging data (CT and PET-CT) during follow-up. Biopsies for histological confirmation were not routinely performed. When RFA was performed concomitantly with resection, histology was obtained of the resected part of the liver. ASR was identified if the recurrence fulfilled the following criteria: (1) growth of a contrast-enhancing lesion within or in the border of the AZ and (2) the largest diameter of the lesion was in direct contact with the AZ. The latter prerequisite is to exclude outgrowth of pre-existing micrometastases in the close vicinity but not the border of the AZ. We prefer the term "ASR" over "local recurrence" or "local tumor progression" because the latter terms are confusing with respect to recurrence at the primary tumor-site, for instance pelvic recurrence in patients with rectal cancer.

**Imaging details**

Multiphase intravenous contrast enhanced CT scans were obtained using either a 16-slice or 64-slice multidetector-CT-scanner (Somatom Sensation 64, Siemens, Erlangen, Germany). One hundred and twenty cc of iodixanol 320mg I/ml (Visipaque 320, GE Healthcare, Chalfont St Giles, UK) was injected at a flow-rate of 4.0cc/sec. Images were obtained during arterial, portal-venous and delayed phases. FDG-PET-CT imaging was obtained when CT-imaging was inconclusive. All subjects were scanned in craniocaudal direction during inspiratory breath-hold. CT-images were acquired in a supine position using a 16x1.5 (16-slice) or 24x1.2 (64-slice) collimation, tube-potential 120kV, tube-current-time-product 130mAs, pitch 1, slice-thickness 2mm, reconstruction Kernel B30f and reconstruction-increment 1.5.

Imaging follow-up consisted of multiphase CT-imaging that was obtained one week after RFA (t1), then every 3 months during the first 2 years and every 6 months thereafter (t2-t5). Multiphase CT-imaging consisted of a series without contrast and series in the arterial, portal-venous and late phase. FDG-PET-CT was performed when CT-imaging was inconclusive.

**Volumetry**

One of the authors (2 years abdominal CT-experience) performed all measurements retrospectively and was unaware of the follow-up results. After transferring imaging-data, volumetry was performed on a Siemens Syngo workstation (version CT2007A) with the "Volume calculation" application. Volumes of interest (VOIs) were manually drawn around the contours of the metastasis and the AZ in the axial view in the portal-venous-phase on 2mm-thickness-slices. VOIs were drawn in

every other slice to minimize partial volume effects. The programme automatically interpolated between VOIs. Each interpolation was revised and corrected manually if necessary. Evaluation was started after finishing the definition of each individual VOI. Approximate volumes were calculated with automatic multiplication of the circumscribed areas by the CT-section-thickness. Volume-results were expressed in cubic centimetres ($cm^3$). Minimum and maximum density values, expressed in Hounsfield units (HU), were set between -50 to 200 for metastasis-volumes and -50 to 50 for AZ-volumes, according to the minimum and maximum HUs measured in each individual metastasis and AZ which were automatically calculated by the application. This was done for all measurements. Measurements were performed on the pre-RFA-scan, the first post-RFA-scan (t1) and available subsequent scans thereafter (t2-t5).

**Intraobserver-varibility and interobserver-variability**

To determine intra-observer and interobserver-variability, two radiologists performed all volumetry in triplo on a subset of 25 randomly selected pre-RFA and post-RFA scans from the patient database, with repetitive measurements at least two weeks apart.

**Calculations with volumes**

The ratio between the first post-RFA AZ-volume and metastasis-volume was defined as $V_{t1}/V_{metastasis}$-ratio, in which $V_{metastasis}$ is the index-metastasis-volume and $V_{t1}$ is the volume of the AZ at t1. Volumes of AZs at the subsequent time points t2-t5 were represented as percentages compared to $V_{t1}$, which was set at 100%. The proportional (volume) change of AZs between subsequent scans was the difference in volume-percentages between two successive time points. Negative values represented a decrease in volume, positive values represented an increase in volume.

**Statistical analysis**

Categorical variables were presented as numbers and percentages. Groups were compared using Chi-square-test or Fisher's exact test and included the following variables: growth (yes versus no); CT-scan-results (positive versus negative versus doubt) and PET-scan-results (positive versus negative). Continuous variables were expressed as median and interquartile range (IQR). Groups were compared by using Mann-Whitney-U-test. Receiver-operating-characteristic (ROC) curves and areas under the ROC-curve (AUC) were calculated to determine whether $V_{t1}/V_{metastasis}$-ratio and proportional change at t1-t2, t2-t3 and t3-t4 are predictive of ASR. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated for CT alone and CT-volumetry. Intraobserver-variability and interobserver-variability on volumetry were evaluated by using intraclass correlation coefficients (ICC). P-values $<0.05$ were considered statistically significant. Statistical analysis was performed by using the statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL).

## Results

### General characteristics and recurrence patterns

Table 1 summarizes general patient and metastasis characteristics. A total of 58 patients were included. There were 37 males and 21 females. Median age at the time of the RFA-procedure was 59 years (IQR 15.5). Fifty-eight patients with 117 metastases underwent 81 RFA-procedures. Twenty-four patients had more than one metastasis ablated during an RFA-procedure (range 2-7). Eighteen patients with 52 metastases had more than one RFA. Forty RFA-procedures were performed by laparotomy, 41 percutaneously under CT-targeting.

Recurrences, either intrahepatic, extrahepatic or both were seen in 50 patients (86%). ASR occurred in 17 patients (29%) and 27 AZs (23%). Intrahepatic and/or extrahepatic recurrence without ASR was seen in 35 patients (60%) with 78 AZs (67%). ASR without recurrence elsewhere was seen in 2 patients (4%) with 4 AZs (3%). Six patients (10%) with 12 AZs (10%) had neither recurrence nor ASR. Median time to ASR was 8 months (IQR 9). Median total follow-up time was 26 months (IQR 25). Median volumetric follow-up time was 9 months (IQR 8.5). The difference between total and volumetric follow-up is due to loss of AZs (see below in section "Follow-up of AZs).

### Metastases

Of the 117 metastases, 66 were treated by open-RFA (56%) and 51 by CT-targeted-RFA (44%). Median diameter of the index-metastases was 1.99 cm (IQR 1.62) and median index-metastasis-volume was 4.2cm$^3$ (IQR 12.5). Index-metastases of AZs with ASR were larger (2.45 cm) in diameter than those without ASR (1.85 cm, p=0.015). The volume of index-metastases of AZs with ASR was also larger (7.7cm$^3$) than the volume of those without ASR (3.3cm$^3$, p=0.014).

### Follow-up of AZs

Imaging was performed in all 117 AZs at t1 and t2. There were 95 AZs left at t3. Twenty-two AZs were lost between t2-t3 because of recurrent disease (n=16, these underwent either repeat-RFA, surgical resection or were referred for palliative chemotherapy), ASR (n=4, these were treated with either repeat-RFA, surgical resection or were referred for palliative chemotherapy in case of coexisting intrahepatic or extrahepatic recurrence) or other reasons (n=2). There remained 60 AZs at t4. Thirty-five AZs were lost between t3-t4 because of recurrent disease (n=19), ASR (n=11), other reasons (n=3) or because there were no subsequent scans (n=2). A total of 40 AZs remained at t5. Twenty AZs were lost between t4-t5 because of recurrent disease (n=8), ASR (n=6), other reasons (n=4) or because there were no subsequent scans (n=2). ASR occurred in 6 AZs at t5.

**Table 1.** Patient and metastasis characteristics.

| | Patients | Metastases |
|---|---|---|
| Number | 58 | 117 |
| Age, years: median (interquartile range) | 59 (15.5) | - |
| Male/female | 37/21 (64 %/ 36 %) | - |
| RFA-procedure* (81 procedures) | | |
| Open | 40 (49 %) | 66 (56 %) |
| CT-targeted | 41 (51 %) | 51 (44 %) |
| One RFA-procedure | 40 (69 %) | - |
| More than one RFA-procedure** | 18 (31 %) | - |
| Recurrence | 50 (86 %) | - |
| Intrahepatic recurrence | 14 (28 %) | - |
| Extrahepatic recurrence | 7 (14 %) | - |
| Intra- and extrahepatic recurrence | 29 (58 %) | - |
| Recurrence without ASR*** | 35 (60 %) | - |
| Intrahepatic recurrence | 8 (23 %) | - |
| Extrahepatic recurrence | 7 (20 %) | - |
| Intra- and extrahepatic recurrence | 20 (57 %) | - |
| ASR | 17 (29 %) | 27 (23 %) |
| ASR and site of additional recurrence | 15 (26 %) | 23 (20 %) |
| Intrahepatic recurrence | 6 (40 %) | 9 (39 %) |
| Extrahepatic recurrence | 0 (0 %) | 0 (0 %) |
| Both intra- and extrahepatic recurrence | 9 (60 %) | 14 (51 %) |
| ASR without recurrence elsewhere | 2 (3 %) | 4 (3 %) |

*RFA: radiofrequency ablation.
**More than one RFA was performed for the following reasons: ablation site recurrences (6 patients with 10 metastases) or intrahepatic recurrences other than ablation site recurrence (12 patients with 20 metastases).
***ASR: ablation site recurrence.

### Course of AZ-volumes

Figure 1 represents the volumes of AZs without ASR as percentage of the first post-RFA volume one week after RFA (t1), which was set at 100%. Figure 2 shows the same for AZs with ASR. An increase in volume was seen in 26 (96%) of the 27 AZs with ASR. However, one AZ (4%) with ASR at t2 showed no increase in volume, but ASR was "proven" by a hotspot on FDG-PET-imaging. Of note, none of the AZs without ASR increased in volume.

Median post-procedural volumes of AZs with and without ASR were 44.4cm$^3$ and 62.0cm$^3$, respectively. $V_{t1}/V_{metastasis}$-ratio was smaller in AZs with ASR (5.6) than in AZs without ASR (16.8, p=0.010) (Figure 3). The ROC-curve of $V_{t1}/V_{metastasis}$-ratio for occurrence of ASR showed an AUC of 0.66 (p=0.011, 95%-CI 0.54-0.79).

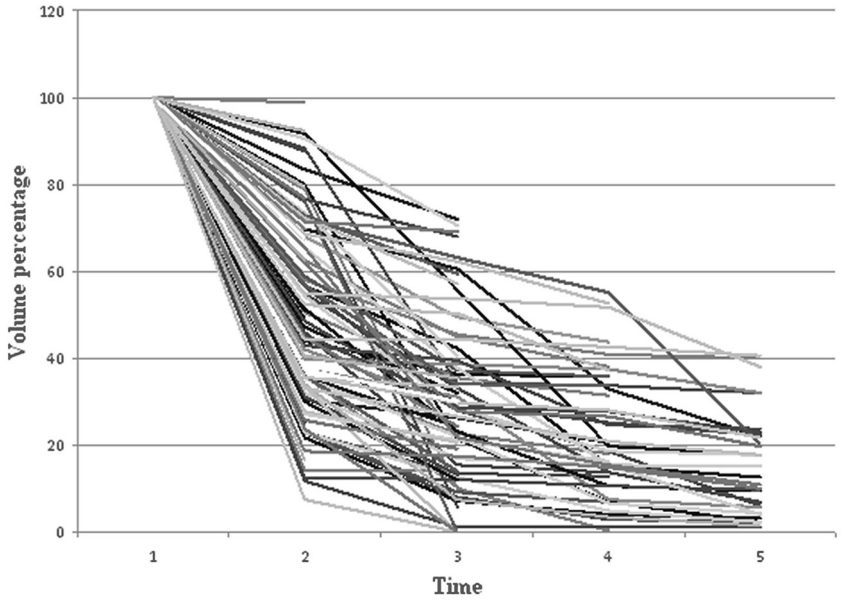


**Figure 1.Volume percentages in ablation zones (AZ) without ablation site recurrences (ASR).**
AZs without ablation site recurrences did not show growth. Some AZs became so small that volumetry was impossible to perform. The horizontal axis represents the different time points of measurement t1-t5, in which t1 is performed on the first post-RFA-scan (1 week post-RFA) and t2-t5 on the subsequent scans, performed at intervals of three months. The vertical axis represents the volume percentage in which the volume at t1 is set at 100%.

The proportional changes in AZs with ASR were all positive from t2-t3 and on and differed significantly from the proportional changes of AZs without ASR, which were all negative (all $p<0.001$) (Table 2). The ROC-curve of the proportional change at t1-t2 for occurrence of ASR showed an AUC of 0.54 ($p=0.583$, 95%-CI 0.42-0.66). For the proportional change at t2-t3 for the occurrence of ASR, the AUC was 0.85 ($p<0.001$, 95%-CI 0.74-0.97). The AUC for the proportional change at t3-t4 for the occurrence of ASR was 0.81 ($p=0.001$, 95%-CI 0.64-0.98).

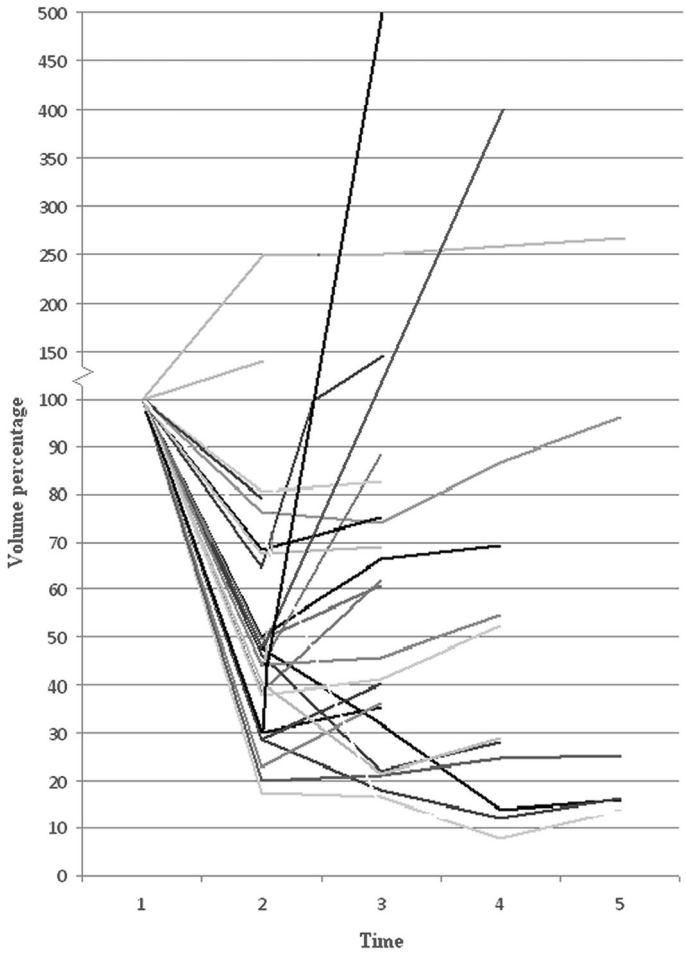


**Figure 2. Volume percentages in ablation zones (AZs) with ablation site recurrences (ASR).**
All AZs, except for one, showed an increase in volume percentage when there was an ablation site recurrence. An initial decrease was seen in AZs which developed ablation site recurrences later. The horizontal axis represents the different time points of measurement t1-t5, in which t1 is performed on the first post-RFA-scan (1 week post-RFA) and t2-t5 on the subsequent scans, performed at intervals of three months. The vertical axis represents the volume percentage in which the volume at t1 is set at 100%.

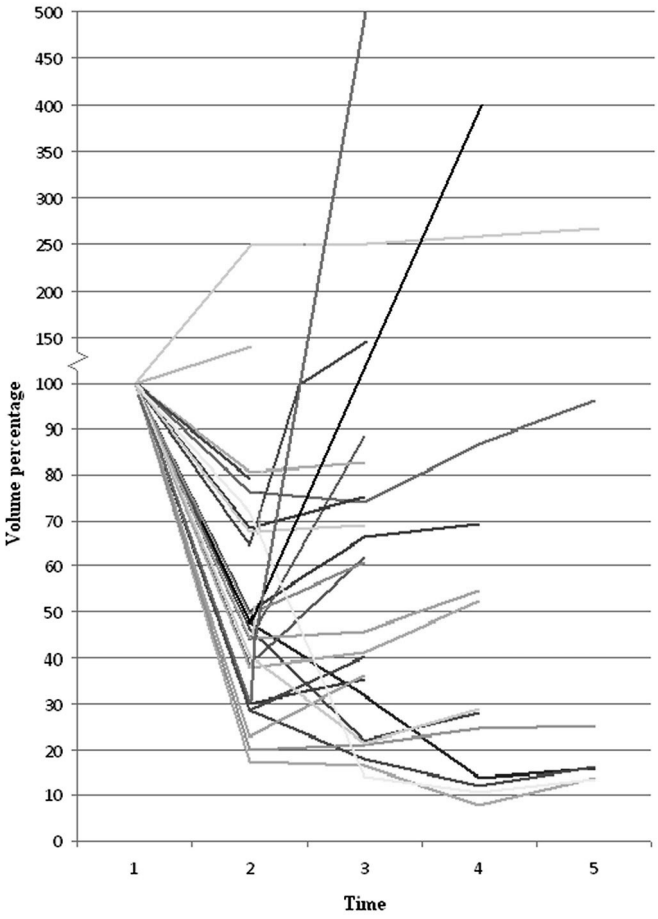


**Figure 3. Median $V_{t1}/V_{metastasis}$-ratio in AZs with and without ASR.**
The median $V_{t1}/V_{metastasis}$-ratio was smaller in AZs with ASR (p=0.010). Note: the scale on the y-axis is a logarithmic scale.





### Detection of ASR with conventional CT

Of the 27 AZs with ASR, 17 (63%) were identified correctly on follow-up-CT-scans, 7 (26%) were missed and 3 AZs (11%) were classified as doubtful. Additional PET-CT-scanning in these doubted AZs was positive, suggestive of an ASR. A total of 23 AZs with ASR (85%) underwent PET-CT-scanning, which was positive in all. PET-CT was not performed in 4 (15%) AZs with ASR. Table 3 shows a better diagnostic performance of CT-volumetry compared to CT alone (p=0.012).

**Table 2.** Absolute volumes and proportional volume changes in AZs with and without ASR.

| Volumes ($cm^3$) | ASR, median (IQR) | No ASR, median (IQR) | *p* |
|---|---|---|---|
| Metastasis volume | 7.7 (27.3) | 3.3 (7.0) | 0.014 |
| $V_{t1}/V_{metastasis}$ | 5.6 (20.1) | 16.8 (23.5) | 0.010 |
| Volume t1 | 44.4 (56.8) | 62.0 (82.5) | 0.928 |
| Volume t2 | 20.2 (31.2) | 22.4 (41.9) | 0.962 |
| Volume t3 | 28.6 (43.4) | 13.2 (22.9) | 0.006 |
| Volume t4 | 18.4 (42.1) | 12.4 (20.7) | 0.143 |
| Volume t5 | 14.2 (32.7) | 6.1 (18.5) | 0.277 |
| Volume change $\Delta_{\%}$* | ASR, median (IQR) | No ASR, median (IQR) | *p* |
| $\Delta_{\%}$ t1-t2 | -52.2 (41.5) | -51.1 (35.8) | 0.785 |
| $\Delta_{\%}$ t2-t3 | +2.5 (17.4) | -14.2 (16.4) | < 0.001 |
| $\Delta_{\%}$ t3-t4 | +5.8 (14.7) | -5.0 (8.4) | < 0.001 |
| $\Delta_{\%}$ t4-t5 | +4.4 (9.7) | -2.6 (5.2) | < 0.001 |

In this analysis, all AZs with ASR are included in the ASR-group, irrespective whether ASR is already present at the time point of measurement or will develop in the future (future-ASR).
* Proportional volume change ($\Delta_{\%}$).

### Intraobserver-variability and interobserver-variability

The ICC for intraobserver-variability for the first post-RFA-scan was 0.998 (95%-CI 0.996-0.999, p<0.001) and 0.987 for the subsequent scans (95%-CI 0.975-0.994, p<0.001). The ICC for interobserver-variability was 0.993 (95 %-CI 0.987-0.996, p<0.001).

**Table 3.** Diagnostic performance of conventional contrast-enhanced CT alone and CT-volumetry in the detection of ASR.

| | Sensitivity (95%-CI*) | Specificity (95%-CI) | PPV** (95%-CI) | NPV*** (95%-CI) | Accuracy |
|---|---|---|---|---|---|
| CT**** | 74 % (52-88) | 100 % (53-100) | 100 % (80-100) | 63 % (39-83) | 94 % |
| CT-volumetry | 96 % (79-100) | 100 % (70-100) | 100 % (84-100) | 92 % (62-100) | 99 % |

*95%-CI: 95%-confidence interval.
**PPV: positive predictive value.
***NPV: negative predictive value.
****CT: computer tomography.

## Discussion

Baseline control imaging and follow-up is mandatory to detect ASR in metastases treated with RFA. However, detection of ASR is not always straight-forward with CT and MRI. Three-dimensional assessment of AZs, for instance by volumetry, could help in detecting ASR. In the present study, we hypothesized that proportional (volume) changes of AZs within the first three months after RFA (t1-t2) are predictive of ASR. Proportional changes at t1-t2 were not predictive for the development of ASR. However, at t2-t3 and t3-t4, proportional changes were

indeed predictive of ASR. ASR is the result of new tumor growth in the border of an AZ or an insufficiently treated AZ in which viable metastasis is left. Theoretically, AZs with viable metastatic cells should have a smaller decrease in volume between two subsequent time points of measurement. Residual metastatic cells will continue to multiply and inhibit or slow down the volume-decrease of the AZ. An explanation that the proportional changes at t1-t2 were not predictive for ASR could be that the initial decrease in volume in this period, mainly due post-RFA effects, exceeds the volume-increase caused by growth of viable metastatic cells. After three months, AZs with ASR did increase in volume, probably because the volume-increase caused by the growth of ASR is larger than the volume decrease caused by the shrinkage of the AZ. Another explanation could be the relatively small number of ASR in this study.

It has been reported previously that AZs gradually become smaller because of shrinkage of the metastases and resolution of post-RFA-inflammation(10,11,15). In the present study, AZs without ASR invariably decreased in volume. Volumes of AZs with ASR increased in volume and the proportional changes from t2-t3 and on were predictive of ASR. One AZ with ASR did not increase in volume. This AZ was situated in segment 8 of the liver in a patient with previous right portal vein embolisation in who an extended right-sided hepatectomy was planned at a later moment. The coils from the embolisation-procedure produced scattering artefacts. This disturbed volumetry, since the contours of the AZ were badly visible and difficult to define. Currently, there are no coils available which do not produce artefacts on imaging. Volumetry should be used with caution in patients with possible artefact-causing procedures.

There are several strategies to detect ASR in the follow-up after RFA, but detection of ASR can be very difficult. Firstly, ASR can be detected by focusing on contrast-enhancement on imaging. Most institutions perform multiphase contrast-enhanced CT. MRI has also proved to be useful, especially when used with liver-specific contrast media, but is used less commonly(16,17). ASR may appear as a focal enhancing lesion within or around the AZ. However, contrast-enhancement may also be due to inflammation in the first months after RFA. Post-RFA inflammation presents as a hyperattenuating, smooth thin rim around the AZ. ASR appears as an irregular thick rim. Another distinguishing feature is the appearance of the rim on portal-venous images. Where ASR appears hypoattenuating, post-inflammational changes remain either hyperattenuating or iso-attenuating. Additionally, ASR can present as a nodular contrast-enhancing lesion at the periphery of the AZ or as an overall increase in size of the AZ, features which are not seen in post-RFA inflammation(11). Differentiation between these two entities is possible with these morphological characteristics on multiphase CT and MRI, but remains troublesome(11). Furthermore, detection of changes in shape and size of AZs requires a precise section-by-section comparison with previous scans, which can be difficult when organ positions and orientations differ between successive follow-up-scans, unfortunately a commonly encountered problem for which so far no solution has been found(16,18).

FDG-PET, especially in combination with CT, is regarded the best follow-up imaging modality in the early detection of ASR(8). Several studies demonstrated the superiority of FDG-PET to CT and MRI in post-RFA-follow-up(19,20). PET is able to detect ASR earlier than conventional imaging, because metabolic alterations are thought to occur before morphologic changes(21). However, PET has several limitations, namely its costs, unreliable results caused by the effects of chemotherapy(19) and the uselessness in PET-negative tumors(19). Additionally, PET has limitations in the early period after the RFA-procedure, because FDG-uptake may be due to inflammation and it has poor resolution and lack of anatomical references unless PET-CT is performed(19,20).

We found that the $V_{t1}/V_{metastasis}$-ratio was smaller in AZs with ASR. This has also been reported in a previous study in which this ratio was called necrosis-tumor-quotient and the conclusion was that the $V_{t1}/V_{metastasis}$-ratio could be used in the prediction of ASR(22). Although the AUC of the ROC-curve of the $V_{t1}/V_{metastasis}$-ratio for occurrence of ASR was not impressive in the present study, small $V_{t1}/V_{metastasis}$-ratios deserve attention, since they may be associated with ASR.

Volumetry has been used in several studies in the evaluation of AZs(8,12,13,23-25). Since manual volumetric quantification is time-consuming, most studies used semi-automated analyses. Nevertheless, volumetry takes relatively short time since AZs are small. In addition, semi-automated measurements are less reliable than hand-made measurements, as is the case with all automated evaluation methods in the field of radiology. However, the latter may be overcome in the future, since software is being continuously improved. Volumetry has important advantages over conventional post-RFA evaluation methods as provided by CT, MRI and PET. Firstly, where CT, MRI and PET are of limited value in the first months after RFA, volumetry could help to detect ASR, since ASR presented with an increase in volume of AZs even within this period. Secondly, volumetry provides three-dimensional information on AZs, whereas conventional modalities only reveal two-dimensional information. Thirdly, quantitative post-RFA evaluation methods are often hindered by differences in organ position between subsequent scans. This could result in unreliable measurements on AZs. Theoretically, volumetry is not influenced by organ position differences. The radiologist will be able to perform reliable volumetry on successive scans and interpret them correctly, even when the scans are incomparable. Fourthly, data derived from volumetry provide additional information, such as the $V_{t1}/V_{metastasis}$-ratio.

One of the limitations of this study is its retrospective design. Patients were retrieved from a prospective database, but volumetry of AZs were performed retrospectively. ASR was previously evaluated by measuring diameters of AZs and screening for contrast enhancement at the site of the ablated metastases on post-RFA CT-scans. As ASR was not detected with conventional evaluation methods of CT-scans as early as with volume volumetry, immediate treatment was not performed. Imaging was continued in these patients until ASR was detected with conventional CT-scans or FDG-PET/CT. Repeat-RFA or surgical resection was performed after confirmation of ASR by these imaging modalities if possible. Secondly, ASR was not proven by histology. However,

since it has been shown that the risk of tumor seeding outweighs the benefit of the procedure and diagnostic accuracy of radiological imaging is up to 90%, which is further increased by with serial CEA measurements(26,27), biopsies to confirm ASR are not performed routinely in our centre. Thirdly, very small volume-increases, for example less than 5%, could also be due to an individual measurement error. However, our finding that none of the AZs without ASR showed any increase in volume argues against this. Additionally, the high reproducibility of the measurements, as suggested by the intraclass correlation coefficients of the intra-observer and interobserver variability, shows that the margins of an individual measurement error are very narrow. A strong aspect of this study is the relative large number of AZ compared to previous studies concerning post-RFA-volumetry.

In conclusion, volumetry of AZs is useful because an increase in AZ volume during follow-up is highly suggestive of ASR. AZs without ASR decreased in volume without exception. AZs with ASR almost invariably increased in volume. A small $V_{t1}/V_{metastasis}$-ratio is seen more often in AZs with ASR. The proportional (volume) changes of AZs within the first three months after the RFA-procedure were not predictive of ASR, but thereafter there was a strong correlation between the proportional changes and ASR. Although manually performed volumetry is relatively time consuming, it should be performed in addition to conventional evaluation methods, since early detection of ASR offers patients the possibility of a secondary treatment.

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# 4

## Immediate post-procedural measurements on ablation zones after microwave ablation: the sooner is not always the better

Kele PG, De Jong KP, Van der Jagt EJ.

*Submitted*

## Abstract

**Aim**:
To determine whether immediate postprocedural contrast-enhanced CT-scanning is suitable in determining the effectiveness of microwave-ablation (MWA) in patients with liver tumours.

**Methods**:
Fifteen patients with 21 tumours underwent MWA. Assessment was performed on contrast-enhanced CT-scans immediately after the MWA-procedure and one week post-procedurally and consisted of measurements of volumes, diameters and Hounsfield-Unit-values of index-tumours and ablation-zones.

**Results**:
Median index-tumour-volume was 9.2cm3 (IQR 3.8-34.2), with a median diameter of 3cm (IQR 1.6-5.5) and mean density of 57HU (SD 17). Mean immediate post-procedural-volume was 89cm3 (SD 51) with a mean diameter of 6cm (SD 2.2) and mean density of 54HU (SD 11). Mean volume one week post-procedurally was 112cm3 (SD 64) with a mean diameter of 6.5cm (SD 2.5) and mean density of 43HU (SD 11).Volumes and diameters of ablation-zones one week post-procedurally were significantly larger than immediately post-MWA (both $p<0.001$). Densities of ablation-zones one week post-procedurally were significantly lower than immediate post-MWA densities ($p=0.027$). Eleven tumours appeared to be insufficiently ablated on immediate post-procedural qualitative assessment, confirmed in 9 on the scan performed one week post-MWA, of which eight underwent repeat-MWA.

**Conclusion**:
Immediate post-ablational assessment is unreliable, since dimensions of ablation-zones are changing significantly in the first week post-MWA.

## Introduction

Local ablative techniques are increasingly used in patients with unresectable liver tumours (1, 2). Radiofrequency ablation is a well established locally directed therapy in these patients. Microwave ablation (MWA) is a more recently introduced thermo-ablative technique. MWA is not yet widely used in Europe and the United States, but has been used for several years in Asian countries(3)(4). In contrast to surgical resection, no tissue is obtained after thermal ablation for histopathological examination to assess whether all tumour tissue has been destroyed. Therefore, post-procedural evaluation fully relies on imaging, usually performed with contrast-enhanced CT or MRI. Unfortunately, it is not known what the optimal time point is for the first post-procedural imaging. In the present author's center, a non-contrast enhanced CT-scan is performed routinely immediately after percutaneous ablation on which the ablative procedure is initially assessed. One week after thermal ablation, a protocol multiphase contrast-enhanced CT-scan is performed for assessment of the completeness of the ablative procedure. Immediate post-procedural contrast-enhanced imaging could have several advantages: (1) increased visibility of the ablation zones (AZs), which could lead to better evaluation of the ablative procedure, making the scan performed one week after the ablative procedure unnecessary, (2) higher detection of insufficiently ablated tumours, reducing the need for the patient for being rescheduled and undergoing general anesthesia again, since ablation can be continued in insufficiently treated tumours in the same session and (3) reduction of the radiation dose, as it is not necessary for the patient to undergo an additional multiphase CT-scan one week after the ablative procedure. In the present study, we report our initial experiences on the value of an immediate post-procedural contrast-enhanced CT-scan in the assessment of thermal ablative procedures.

## Material and Methods

### Patients

The study was approved by our institutional review board. Data of consecutive patients who underwent percutaneous CT-targeted MWA for benign and malignant liver tumours in the period from October 2010 until December 2011 were retrieved from a prospective database. Non-contrast enhanced CT-scanning was performed for intraprocedural monitoring of the MWA-procedure. Thereafter, a contrast-enhanced CT-scan (arterial or portal-venous phase, depending on the characteristics of the tumour) immediately at the end of the MWA-procedure was performed in all patients (t1). Multiphase CT-scanning – which consisted of a series without contrast and series in the arterial, portal-venous and late phase - was performed routinely one week after the MWA-procedure (t2). Follow-up of the patients consists of multiphase CT-scanning every three months during the first two years after the MWA-procedure and every six months thereafter, in addition to clinical and biochemical (carcino-embryonic antigen) examination in case of colorectal liver metastases.

**Microwave Ablation Details**

MWA was performed percutaneously under CT-targeting using the Acculis microwave tissue ablation (MTA) system and the Accu2i pMTA applicator (Microsulis Medical Ltd. Denmead, UK). Ablation was performed with the intention to create ablation zones which at least cover the tumour and a safety margin of 1 cm around the tumour in all planes. Terminology used in this paper is in accordance to the guidelines as proposed by Goldberg et al(5).

**Imaging details**

CT-scanning was performed on a 16-slice multidetector CT-scanner for the immediate post-MWA-scan. At the end of the MWA-procedure, a contrast-enhanced CT-scan was performed in the arterial phase in the case of hypervascular tumours and the portal venous phase in case of hypovascular tumours. A 64-slice multidetector-CT-scanner (Somatom Sensation 64, Siemens, Erlangen, Germany) was used for the protocol CT-scan one week after the MWA-procedure. Intravenous contrast was used, 120cc iodixanol 320mg I/ml (Visipaque 320, GE Healthcare, Chalfont St Giles, UK) with a flow-rate of 4.0cc/sec. All subjects were scanned in craniocaudal direction during inspiratory breath-hold. CT-images were acquired in a supine position using a 16x1.5 (16-slice) or 24x1.2 (64-slice) collimation, tube-potential 120kV, tube-current-time-product 130mAs, pitch 1, slice-thickness 2mm, reconstruction Kernel B30f and reconstruction-increment 1.5.

One of the authors (3 years liver CT-experience) performed all volume measurements manually on a Siemens Syngo workstation (version CT2007A) with the "Volume calculation" application. Volumes of interest (VOIs) were manually drawn around the contours of the index tumour and the AZs in the axial view on 2mm-thickness-slices. Volumetry on t2-scans was always performed in the portal-venous phase. Volume-results were expressed in cubic centimetres ($cm^3$). The Hounsfield Unit values (HU) of the ablation zone at t1 and t2 were measured on the non-contrast enhanced series.

**Statistical analysis**

Categorical variables were presented as numbers and percentages. Continuous variables were expressed as mean and standard deviation (SD) when they were normally distributed. In the case of non-normal distribution, continuous variables were presented as median and interquartile range (IQR). Groups were compared by using Wilcoxon signed rank test. *P*-values <0.05 were considered statistically significant. Statistical analysis was performed by using the statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL).

## Results

### Patient characteristics

Fifteen patients with 21 index tumours were included. Nine (60%) were male, 6 (40%) were female. Mean age at the time of MWA was 62 years (SD 13, range 37-82 years). A total of 10 patients (66 %) with 15 index tumours (72%) underwent MWA because of colorectal liver metastases. Three patients (20%) with 3 index tumours (14%) had MWA for hepatocellular carcinoma. MWA was performed for liver metastases of a neuroendocrine tumour of the pancreas in one patient (7%) with 2 index tumours (9%). One patient (7%) underwent MWA for a benign adenoma (5%) (Table 1). Median time of follow-up was 9 months (IQR 7-16).

### Volumetry

Figure 1 represents the course of the volumes of the index tumours and the AZs at t1 and t2, respectively. All AZs increased in volume between the two time points of measurement. Median index tumour volume was 9.2 cm3 (IQR 2.9-25.8), with a median diameter of 2.6 cm (IQR 1.5-5.5). Median density of the index tumours was 60 HU (IQR 46-73) (Table 1). Median volume of the AZs at t1 was 71 cm3 (47-152) with a median diameter of 5.8 cm (IQR 4.6-7.5). Median density of the AZs immediately after the MWA-procedure was 54 HU (IQR 48-62). Median volume at t2 was 98cm$^3$ (IQR 60-189) with a median diameter of 6.0 cm (IQR 4.8-8.4). Median density of the AZ one week after MWA was 46 HU (IQR 36-51). Median percentage of the volume of the AZ one week after the MWA compared to the immediate post-procedural volume was 124% (IQR 109-147). The volumes and diameters of the AZs at t2 were significantly larger than the t1-volumes and diameters ($p=0.026$ and $p<0.001$, respectively). Median densities (HU-values) of the AZs at t2 were significantly lower than the t1-densities ($p<0.001$) (Table 2).

**Table 1.** Patient and index tumour characteristics.

| | Patients (n=15) | Index tumours (n=21) |
|---|---|---|
| Male/female | 9 / 6 (60 % / 40 %) | - |
| Age in years (mean ± SD[a]) | 62 ± 13 | - |
| Indication for MWA[b] | | |
| - CRCLM[c] | 10 (66 %) | 15 (72 %) |
| - HCC[d] | 3 (20 %) | 3 (14 %) |
| - NET[e] | 1 (7 %) | 2 (9 %) |
| - Adenoma | 1 (7 %) | 1 (5 %) |
| Index tumour diameter in cm (median, IQR[f]) | - | 2.6 (1.5-5.5) |
| Index tumour volume in cm3 (median, IQR) | - | 9.2 (2.9-25.8) |
| HU-value (median, IQR) | - | 60 (46-73) |

Abbreviations used: [a]SD: standard deviation; [b]MWA: microwave ablation; [c]CRCLM: colorectal liver metastasis; [d]HCC: hepatocellular carcinoma; [e]NET: neuroendocrine tumour; [f]IQR: interquartile range.

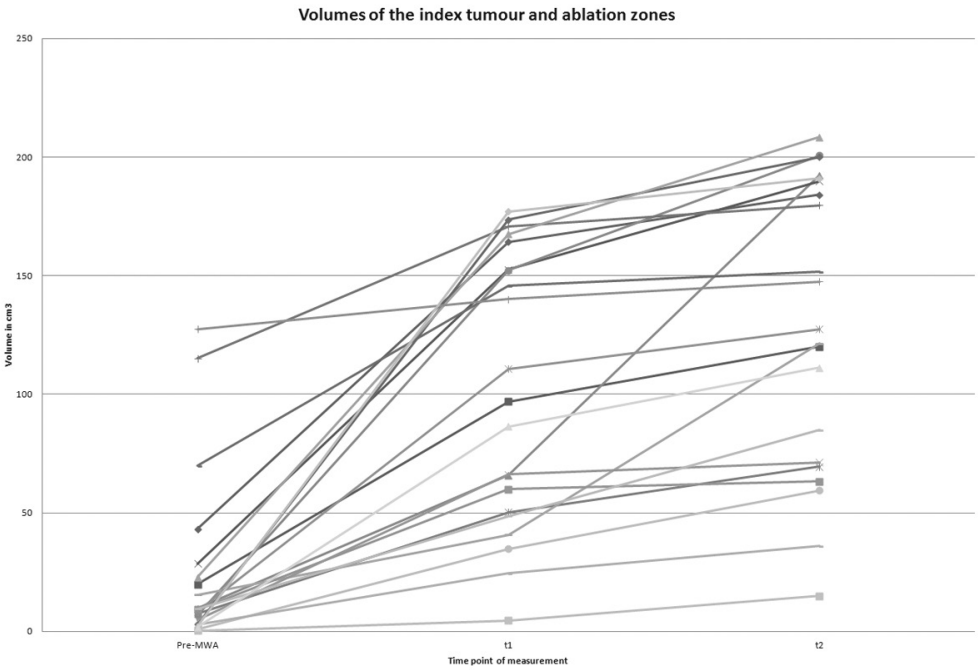


**Figure 1. Volumes of the ondividual index tumours and the ablation zones at t1 and t2.**
Volumes of the individual index tumours and the ablation zones at t1 and t2 in $cm^3$. The volumes of the ablation zones at t2 were significantly larger than the volumes at t1 ($p<0.001$).

**Table 2.** Volumetry.

| Ablation zone (n=21) | t1[a] | t2[b] | p-value |
|---|---|---|---|
| Diameter in cm (median, IQR[c]) | 5.8 (4.6-7.5) | 6.0 (4.8-8.4) | 0.026 |
| Volume in cm3 (median, IQR) | 71 (47-152) | 98 (60-189) | <0.001 |
| Percentage of index tumour volume (median, IQR) | 678 % (434-1791) | 1093 % (470-3745) | <0.001 |
| Percentage volume of t1 (median, IQR) | 100 % | 124 % (109-147) | - |
| Mean HU-value[d] (median, IQR) | 54 (48-62) | 46 (36-51) | <0.001 |

Abbreviations used: [a]t1: immediate post-procedural scan; [b]t2: scan one week after MWA; [c]IQR: interquartile range; [d]HU: Hounsfield Unit.





## Discussion

The present study reports on the usefulness of an immediate post-procedural contrast-enhanced CT-scan in the assessment of the success of thermal ablation compared to the protocol multiphase CT-scan as performed one week post-procedurally. Volume and diameters of the AZs on the t2-scans were larger than volumes and diameters on the t1-scans. Additionally, the densities of the AZs on the t2-scans were lower compared to the t1-scans. These differences between the t1-scan and t2-scans indicate that quantitative assessment of the AZ cannot be performed reliably immediately after MWA.

Imaging after thermal ablation is the cornerstone in the evaluation of the success of the procedure as well as in the follow-up. The recommended time point of the first post-ablation scan is not well determined and varies from immediately after ablation to one month post-procedurally(6). Unfortunately, there is no universally accepted optimal post-procedural imaging protocol. The first baseline control scan is important to assess whether there is a need of a re-intervention in the case of tumour residue. Ablation margins and volumes of the AZ are measured on the first post-procedural scan and compared to the dimensions or volumes of the index tumour. This is important to compare the size and/or volume of the AZ on subsequent scans. It has been shown that subtle changes - visually imperceptible but detectable with detailed measurements such as volumetry - could point toward ASR (7). Therefore, it is important to have quantitative information on AZs documented for comparing these values on subsequent scans. Based on the findings of this preliminary study, we recommend that measurements on AZs should only be obtained more delayed after ablation – for example one week after thermal ablation – because the size of AZs is changing after the first post-procedural week. The value of delayed post-procedural imaging has also been reported in the literature (8).

Tissue damage caused by thermal ablation involves a spectrum of processes. These include (1) dehydration of tissue; (2) enzyme deactivation; (3) rupture and aggregation of cell membranes and (4) vasoconstriction and intravascular coagulation. The response to thermal ablation varies per type of tissue, in which the heat effects on collagen and tissue architecture are determining factors (e.g. collapse around air filled spaces in the lung contrary to the solid tissue architecture of the liver)(9). Dehydration of tissue treated with thermal ablation is believed to cause tissue contraction. In the present study, AZs increased in volume on CT-scans performed one week post-procedurally compared to the volumes on the immediate post-procedural scans. In the literature, an increase in the size of the AZ on imaging performed a few days after thermal ablation has been observed(6, 9, 10). Therefore, immediate post-procedural imaging may underestimate the original volume and diameter of treated tissue and measurements should not be performed on these scans. The delayed increase in the size of the AZ may be contributed to rehydration of the AZ or due to edema caused by inflammatory reactions. Another explanation might be delayed cell destruction in the border of the AZ. Since less heat is delivered in the periphery of the AZ, tissue destruction in the border may not be immediately realized but occur later, as these cells were also exposed to damaging temperatures. Unfortunately none of these hypotheses are neither confirmed nor reprobated.

A decrease in the density or Hounsfield Unit (HU) value of the AZs after thermal ablation compared to the HU-value of the index tumour has been described previously(11) and indicates the presence of necrosis. In contrast, prefunded tissue – as viable tumour cells – will not show a large decrease in HU-value. To our knowledge, it is not knowm whether CT-scanning generates different HU-values of AZs immediately after the procedure versus those obtained one week after the procedure. The difference between the HU-values at t1 and t2 might be caused by

progression of the ablation-induced necrosis or the resorption of coagulated blood within the AZs, which has a higher HU-value than the necrotic tissue within the AZ.
In conclusion, quantitative assessment of AZs by measuring volumes and diameters should not be performed on immediate post-procedural images, since these parameters increase significantly in the first week post-procedurally. Therefore, quantitative assessment of thermal ablational techniques should be performed on imaging performed at least a few days to one week after the procedure.

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# PART 2

# 5

# Early hepatic regeneration index and completeness of regeneration at 6 months after partial hepatectomy

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## Abstract

**Background:**
The liver is known to regenerate following partial hepatectomy (PH), but little is known about the timing and completeness of regeneration relative to the resected volume. This study examined whether liver volume regeneration following PH and its completeness 6 months after surgery is related to the resected volume.

**Methods:**
A consecutive series of patients undergoing PH were included. All patients underwent preoperative computed tomography (CT) before and 7 days after surgery. Additional scans were performed 6 months after operation. Preoperative total liver volume (TLV), resected volume, future liver remnant (FLR) and liver remnant (LR) volumes were measured on CT images by freehand drawing of regions of interest in the portal venous phase on 2-mm thick slices. Regeneration indices were calculated at 7 days ($RI_{early}$) and 6 months ($RI_{total}$) using the formula 100 × (LR volume – FLR volume)/FLR volume. Patients were classified into five groups based on resected volume as a percentage of TLV: 0–19, 20–39, 40–59, 60–69 and at least 70 per cent in groups 1–5 respectively.

**Results:**
Ninety-one patients were enrolled. $RI_{early}$ varied from 11 to 63 per cent in groups 1–5 ($P$ < 0.001). $RI_{early}$ did not increase linearly with increasing resection volume and a plateau was seen from group 3 and above. In contrast, $RI_{total}$ was related linearly to resected volume; values ranged from 21 to 233 per cent in groups 1–5 ($P$ < 0.001). At 7 days, LR volume represented 97, 87, 70, 58 and 41 per cent of TLV in groups 1–5. At 6 months, respective values were 102, 99, 87, 82 and 91 per cent.

**Conclusion:**
Early postoperative liver volume regeneration was not related linearly to resected volume. At 6 months after surgery, RI was related linearly to resected volume, but LRs had not yet regenerated to preoperative TLV.

## Introduction

The liver is unique in its remarkable capacity to regenerate after surgery. At a molecular level, liver regeneration begins almost immediately after partial hepatectomy (PH). It involves an orchestrated interplay of signalling events, including growth factors, cytokines and transcription factors[1]. Microscopically, liver regeneration consists of a time-dependent replication of different types of cell[1] and ultimately results in macroscopic liver regeneration, with a volume increase after PH.

The volume increase can be assessed by imaging techniques such as computed tomography (CT) or magnetic resonance imaging (MRI)[2]. CT is the most commonly used method, with well proven accuracy in the evaluation of liver volumes[3]. Studies using CT-based volumetry have shown that the liver regenerates rapidly and that regeneration is most pronounced in the first 7–10 days after surgery, with high regeneration indices (RIs)[4,5]. It is presently unclear when regeneration is complete. Some authors have suggested that regeneration is complete after 3 months, but others have proposed that it continues up to 1 year after surgery[1,5–12]. Furthermore, it has been shown that complete regeneration, to 100 per cent of the initial liver volume, is often not reached. The liver regenerates to only 80–85 per cent of the initial volume after large resections, such as (extended) right-sided hemihepatectomies[5–12].

It is well known that liver regeneration depends on various factors, such as underlying parenchymal diseases, age and sex. Little is known about the process of liver regeneration after resection of different amounts of liver tissue. There is an obvious relationship between the resected liver volume and the amount of liver tissue that needs to regenerate, which implies that the resected volume may trigger the regeneration process after PH[9–11]. Studies evaluating liver regeneration have focused on right-sided hemihepatectomies or left lateral segmental resections. However, there is considerable interpatient variability in the size of the various liver segments. This means that the resected volume after a particular type of PH may differ substantially between patients[13]. The aim of this study was to evaluate whether the amount of liver regeneration, expressed as the RI at 1 week and 6 months after surgery, is proportional to the resected volume after PH. In addition, the completeness of liver regeneration at 6 months after PH was studied.

## Methods

Patients included in this study were all enrolled in a randomized controlled multicentre trial comparing the use of fibrin sealants *versus* no sealant on the liver resection surface after PH (registration number ISRCTN85205641; http://www.controlled-trials.com) between May 2006 and June 2010. All patients underwent CT 1 week after surgery to objectively analyse resection surface-related fluid collections, in accordance with the protocol. Patients eligible for the present study on liver regeneration were those included in the randomized study who underwent an

anatomical liver resection in the present authors' unit, had a preoperative CT scan available, and provided informed consent. Indications for liver resection were malignant or benign liver tumours or donor hemihepatectomies. Patients with pre-existing chronic liver disease and/or liver dysfunction were excluded. This study was conducted in compliance with national legislation as well as guidelines from the local medical ethics committee.

All patients underwent CT before and 1 week after surgery. Thereafter, CT was performed when indicated. Patients were classified into five groups according to the resected volume, calculated as a percentage of total non-tumorous liver volume: group 1, 0–19 per cent of total liver volume; group 2, 20–39 per cent; group 3, 40–59 per cent; group 4, 60–69 per cent, and group 5, at least 70 per cent.

**Imaging and volumetry**

Patients underwent multiphase CT before surgery (t0), 1 week after surgery (t1) and 6 months after operation (t2) when indicated. CT was performed using a multidetector scanner (Somatom Sensation 64; Siemens, Erlangen, Germany) with the use of intravenous contrast (120 ml iodixanol 320 mg I/ml; Visipaque™ 320, GE Healthcare, Chalfont St Giles, UK).

Volume measurements on CT images were performed by one investigator with 2 years' experience in abdominal CT-volumetry, supervised by two other investigators (a radiologist with 25 years' experience in abdominal radiology and an experienced hepatobiliary surgeon). After transferring imaging data, volumetry was performed on a workstation (Syngo version CT 2007A; Siemens). Regions of interest (ROIs) were drawn manually in the axial view on 2-mm thick slices in the portal venous phase. The volumes were calculated based on the surface of the ROIs multiplied by the slice thickness. The following contours were outlined: total liver, excluding vena cava and gallbladder; the part of the liver to be resected according to the Couinaud classification and on the basis of the hepatic (vascular) anatomy; tumour(s); and postoperative liver remnants on t1 and t2 scans. Approximate volumes were calculated by automatic multiplication of the circumscribed areas by the CT section thickness.

To determine intraobserver variability and interobserver variability, two investigators performed all volume measurements in triplicate on 25 presurgical and postsurgical scans, with repeat measurements at least 2 weeks apart. The weight of the resected part was measured at pathological anatomical examination and was considered the 'gold standard'. Results of the CT-volumetry were then correlated with specimen weight.



**Calculation of regeneration indices**

After volumetry, tumour volume was subtracted from total preoperative liver volume and resected volume. All calculations involving total preoperative liver volumes and resected volumes were performed after subtraction of tumour volumes. The following variables were calculated: resected liver volume as a percentage of total volume without tumour volume, calculated as $100 \times V_{resection}/$

$V_{total}$ in which $V_{resection}$ is the resected volume and $V_{total}$ is total preoperative liver volume; future liver remnant (FLR) volume ($V_{FLR}$), calculated as ($V_{total} - V_{resection}$); percentage FLR, calculated as $100 \times V_{FLR}/V_{total}$; RI between t0 and t1 ($RI_{early}$), calculated as $100 \times (V_{LRt1} - V_{FLR})/V_{FLR}$ in which $V_{LRt1}$ is the liver remnant volume at t1; RI between t0 and t2 ($RI_{total}$), calculated as $100 \times (V_{LRt2} - V_{FLR})/V_{FLR}$ in which $V_{LRt2}$ is the liver remnant volume at t2. Complete regeneration was defined as a $V_{LRt2}$ of at least 95 per cent of total preoperative liver volume, as there is a measuring error in CT-volumetry of 5–10 per cent.

### Statistical analysis

Continuous variables are presented as median (interquartile range), unless stated otherwise. Comparisons of continuous variables between groups were performed with Kruskal–Wallis test. $\chi^2$ test or Fisher's exact test, as appropriate, was used for analysis of categorical variables. Associations between resected volumes and RIs were determined using Spearman's rank correlation test. Intraobserver and interobserver variability were determined using the intraclass correlation coefficient. $P < 0.050$ was considered statistically significant. All statistical analyses were performed using SPSS® version 16.0 (SPSS, Chicago, Illinois, USA).

## Results

A total of 91 patients undergoing PH were included in the study. Demographic and surgical characteristics are presented in *Table 1*. There were 50 men and 41 women. Median age at the time of PH was 62 (52–68) years and 75 patients (82 per cent) were older than 50 years.

### Liver volume measurements and correlation with specimen weight

All patients had undergone CT at t1, a median of 7 (7–7) days after surgery. Sixty-four patients had a t2 scan, at a median of 6 (5–7) months after operation. Twenty-seven patients did not have a t2 scan for the following reasons: postoperative follow-up was performed with other imaging modalities (ultrasound or MRI, 18 patients), progressive metastatic disease (2), end of follow-up of benign tumours (2), living donor (3) and death (2). There was no significant difference in timing of t2 scans between the five patient groups ($P = 0.463$).

**Table 1.** Patient characteristics overall and for patients grouped according to resected liver volume

| | All patients ($n$ = 91) | Group 1 ($n$ = 21) | Group 2 ($n$ = 26) | Group 3 ($n$ = 19) | Group 4 ($n$ = 19) | Group 5 ($n$ = 6) |
|---|---|---|---|---|---|---|
| Age (years)* | 62 (52–68) | 62 (52–69) | 59 (49–69) | 62 (59–69) | 62 (55–67) | 56 (43–66) |
| Sex ratio (M : F) | 51 : 40 | 11 : 10 | 13 : 13 | 10 : 9 | 12 : 7 | 4 : 2 |
| Body mass index (kg/m$^2$)* | 25 (23–29) | 28 (23–31) | 26 (23–29) | 24 (22–29) | 26 (23–29) | 24 (23–25) |
| Body surface area (m$^2$)* † | 2.0 (1.8–2.1) | 2.1 (1.8–2.2) | 2.0 (1.8–2.2) | 1.8 (1.8–2.1) | 2.1 (1.9–2.2) | 2.0 (1.8–2.2) |
| Indication for surgery | | | | | | |
| Malignant tumours | 76 (84 ) | 15 (71 ) | 20 (77 ) | 17 (89 ) | 19 (100 ) | 5 (83 ) |
| Benign tumours | 12 (13 ) | 5 (24 ) | 4 (15 ) | 2 (11 ) | 0 (0) | 1 (17 ) |
| Living donor | 3 (3 ) | 1 (5 ) | 2 (8 ) | 0 (0) | 0 (0) | 0 (0) |
| Previous chemotherapy‡ | 7 (8 ) | 3 (14 ) | 0 (0) | 2 (11 ) | 1 (5 ) | 1 (17 ) |
| Co-morbidity | | | | | | |
| Cardiovascular | 46 (51 ) | 13 (62 ) | 12 (46 ) | 8 (42 ) | 11 (58 ) | 2 (33 ) |
| Diabetes | 11 (12 ) | 3 (14 ) | 2 (8 ) | 3 (16 ) | 3 (16 ) | 0 (0) |
| Pulmonary | 9 (10 ) | 3 (14 ) | 3 (12 ) | 2 (11 ) | 1 (5 ) | 0 (0) |
| Steatosis (%)§ | | | | | | |
| None | 38 (42) | 8 (38 ) | 8 (30 ) | 12 (63 ) | 8 (42 ) | 2 (33 ) |
| < 30 | 47 (52) | 12 (57 ) | 16 (62 ) | 5 (26 ) | 11 (58 ) | 3 (50 ) |
| > 30 | 6 (7) | 1 (5 ) | 2 (8 ) | 2 (11 ) | 0 (0) | 1 (17 ) |

Values in parentheses are percentages unless indicated otherwise; *values are median (interquartile range). Patients were grouped by resected liver volume, expressed as a percentage of preoperative total non-tumorous liver volume: group 1, 0–19 per cent; group 2, 20–39 per cent; group 3, 40–59 per cent; group 4, 60–69 per cent; and group 5, 70 per cent or more. †Calculated by means of the Mosteller formula as $\sqrt{}$ ((height [cm] × weight [kg])/3600). §Determined by histopathological examination of the resected liver. There were no significant differences between groups.

*Table 2* shows the resected liver volumes in relation to types of PH and volume measurements are summarized in *Table 3*. Overall median preoperative total liver volume was 1529 (1328–1724) $cm^3$, with no significant differences between groups ($P$ = 0.481). Median tumour volume was 43 (14–209) $cm^3$ and was not significantly different among groups ($P$ = 0.087). FLV differed according to the extent of resection ($P$ < 0.001).

The intraclass correlation coefficient for intraobserver variability was 0.998 (95 per cent confidence interval 0.996 to 0.999) for the first postoperative scan ($P$ < 0.001) and 0.987 (0.975 to 0.994) for the second scan ($P$ < 0.001). The intraclass correlation coefficient for interobserver variability was 0.875 (0.354 to 0.981) ($P$ = 0.005). There was a good correlation between measured volume and actual weight of the resected specimen (Spearman's correlation coefficient 0.975, $P$ < 0.001) (*Fig. S1*, supporting information).

**Table 2.** Types of partial hepatectomy in relation to resected liver volume

| | No. of patients | Group 1 ($n$ = 21) | Group 2 ($n$ = 26) | Group 3 ($n$ = 19) | Group 4 ($n$ = 19) | Group 5 ($n$ = 6) |
|---|---|---|---|---|---|---|
| Right-sided | 36 | 0 (0) | 4 (11 ) | 12 (33 ) | 16 (44 ) | 4 (11 ) |
| Extended right-sided | 11 | 0 (0) | 2 (18 ) | 4 (36 ) | 3 (27 ) | 2 (18 ) |
| Left-sided | 12 | 4 (33 ) | 7 (59 ) | 1 ( 8 ) | 0 (0) | 0 (0) |
| Extended left-sided | 1 | 0 (0) | 0 (0) | 1 (100 ) | 0 (0) | 0 (0) |
| Bisegmentectomy | 31 | 17 (55 ) | 13(42 ) | 1 (3 ) | 0 (0) | 0 (0) |

Values in parentheses are percentages. Patients were grouped by resected liver volume, expressed as a percentage of preoperative total non-tumorous liver volume: group 1, 0–19 per cent; group 2, 20–39 per cent; group 3, 40–59 per cent; group 4, 60–69 per cent; and group 5, 70 per cent or more.

### Regeneration indices and completeness of regeneration

*Fig. 1* summarizes the RIs for each patient group. Median $RI_{early}$ for all groups combined was 36 (12–56) per cent. $RI_{early}$ increased significantly as resections became larger (Spearman correlation coefficient 0.702, $P$ < 0.001). A plateau was observed for group 3 and higher (more than 40 per cent of total liver volume resected), indicating that $RI_{early}$ did not correlate linearly with the amount of liver tissue resected. Median $RI_{total}$ was 52 (24–107) per cent overall, and was significantly higher for the groups with larger resections ($P$ < 0.001). In contrast to $RI_{early}$, $RI_{total}$ correlated linearly with the amount of liver tissue resected (Spearman correlation coefficient 0.705, $P$ < 0.001).

For all groups combined, the median FLR was 61 (39–80) per cent of the total preoperative liver volume. The median liver remnant volume was 81 (61–93) per cent of total preoperative liver volume at t1 and 92 (82–102) per cent at t2. Thirty patients (47 per cent) reached their original preoperative liver volume by t2. The percentage of patients whose livers reached complete regeneration in the five groups was 85, 58, 29, 15 and 0 per cent respectively. *Fig. 2* shows the median liver volumes as a percentage of preoperative total liver volume at t1 and t2 for each group.

**Table 3.** Liver volumes and regeneration indices

| | Group 1 ($n$ = 21) | Group 2 ($n$ = 26) | Group 3 ($n$ = 19) | Group 4 ($n$ = 19) | Group 5 ($n$ = 6) | $P$* |
|---|---|---|---|---|---|---|
| Tumour volume($cm^3$) | 20 (9–208) | 65 (16–353) | 132 (32–442) | 43 (24–96) | 30 (8–40) | 0.087 |
| Total liver volume ($cm^3$) | 1524 (1285–1813) | 1591 (1364–1737) | 1474 (1293–1637) | 1641 (1395–1815) | 1624 (1490–1749) | 0.481 |
| Resected non-tumour volume ($cm^3$) | 246 (183–273) | 410 (330–537) | 722 (660–860) | 1103 (933–1155) | 1228 (1095–1363) | <0.001 |
| Future liver remnant volume, t0 ($cm^3$) | 1278 (1072–1493) | 1087 (885–1266) | 709 (593–817) | 557 (480–687) | 377 (326–435) | < 0.001 |
| Liver remnant volume, t1 ($cm^3$) | 1475 (1280–1683) | 1278 (1121–1577) | 918 (858–1 254) | 925 (856–1035) | 649 (531–872) | < 0.001 |
| Liver remnant volume, t2 ($cm^3$) | 1678 (1307–1934) | 1433 (1205–1631) | 1295 (1147–1626) | 1218 (1031–13) | 1336 (1043–1630) | 0.035 |
| $RI_{early}$ | 11 (6–22) | 18 (7–26) | 49 (37–60) | 66 (40–88) | 63 (38–145) | < 0.001 |
| $RI_{total}$ | 21 (13–25) | 35 (13–45) | 91 (67–114) | 113 (96–155) | 233 (233–241) | < 0.001 |

Values are median (interquartile range). Patients were grouped by resected liver volume, expressed as a percentage of preoperative total non-tumorous liver volume: group 1, 0–19 per cent; group 2, 20–39 per cent; group 3, 40–59 per cent; group 4, 60–69 per cent; and group 5, 70 per cent or more. t0, before surgery; t1, 1 week after surgery; t2, 6 months after surgery; $RI_{early}$, early regeneration index 1 week after surgery; $RI_{total}$, total regeneration index 6 months after surgery. *Kruskal–Wallis test.

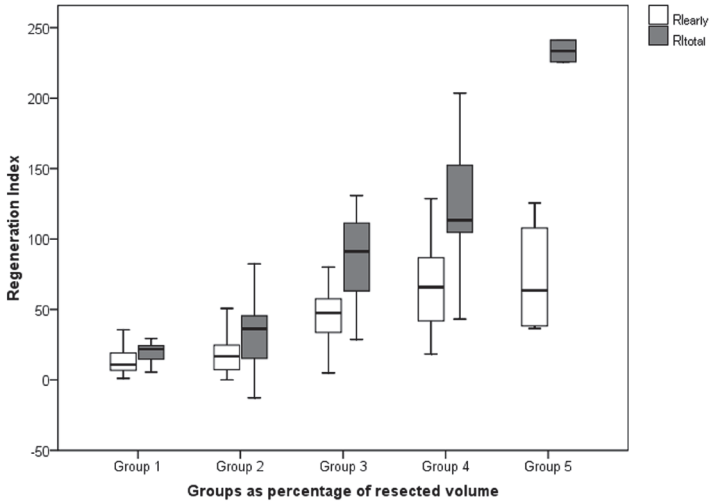


**Figure 1.** Box-and-whisker plots showing regeneration indices ($RI_{early}$, regeneration in first week, between t0 and t1; $RI_{total}$, regeneration in first 6 months, between t0 and t2) in relation to resected liver volume, expressed as a percentage of preoperative liver volume: group 1, 0–19 per cent; group 2, 20–39 per cent; group 3, 40–59 per cent; group 4, 60–69 per cent; and group 5, 70 per cent or more. Median values (line within box), interquartile range (box) and range (error bars) are shown. $P < 0.001$ (Kruskal–Wallis test).

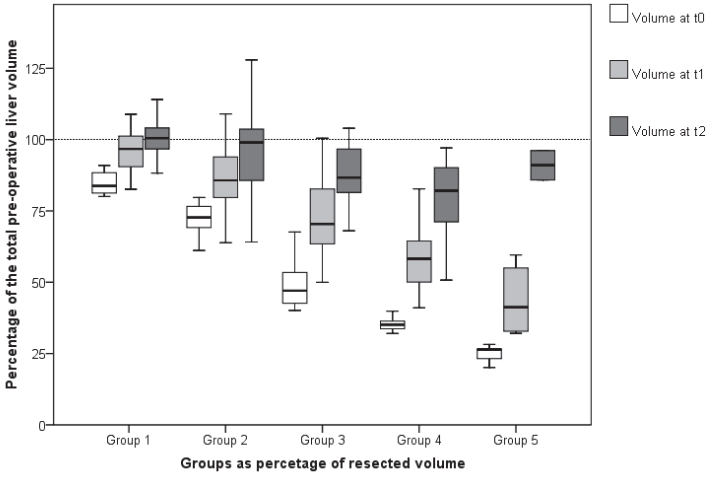


**Figure 2.** Box-and-whisker plots showing future liver remnant volumes at t0, and liver remnant volumes at t1 (1 week after surgery) and t2 (6 months after surgery) as a percentage of total preoperative liver volume. Patients were grouped by resected liver volume, expressed as a percentage of preoperative total non-tumorous liver volume: group 1, 0–19 per cent; group 2, 20–39 per cent; group 3, 40–59 per cent; group 4, 60–69 per cent; and group 5, 70 per cent or more. Median values (line within box), interquartile range (box) and range (error bars) are shown. The dotted line represents total preoperative liver percentile volume, set at 100 per cent.

## Discussion

In the present study, the RIs for liver volume regeneration were assessed at 1 week ($RI_{early}$) and 6 months ($RI_{total}$) after PH in relation to the amount of resected liver tissue, and the completeness of liver regeneration was studied at 6 months. The two main findings were that $RI_{early}$ was relatively low in patients who underwent a large liver resection (and thus had a relatively small liver remnant), and that liver regeneration was not (yet) complete by 6 months after surgery in patients who underwent a large liver resection, in contrast to patients who underwent a relatively small liver resection.

Previous studies have shown that liver regeneration occurs rapidly after PH, whether performed in living donors or for the resection of malignant liver tumours[4,5,8–10,12,14]. Many of these studies evaluated liver regeneration at a later time in the postoperative course, 1–6 months after PH[5–12]. Data on early liver regeneration (1 week after resection) are scarce. Reported early RIs range from 28 to 64 per cent[4,5,11]. RIs in the later postoperative course are considerably lower, suggesting that the first week after operation is quantitatively important in the process of liver regeneration[5,9,10,12]. The discordance between reported RIs could be explained by different types of hepatectomy performed as well as by differences in amount of liver parenchyma resected. The pattern of liver regeneration observed after left-sided hepatectomy may not reflect what occurs after right-sided hepatectomy. Besides anatomical differences in resections, the resected volume also varies according to type of PH. Equally, it has been shown that there is significant interpatient variation in liver volumes[13]. This implies that resected volumes in individual patients undergoing right-sided hepatectomy may vary considerably. For example, a right-sided hepatectomy does not necessarily always involve 60 per cent of total preoperative liver volume, but may vary between 20 per cent to more than 70 per cent. Thus, RIs may not be comparable purely according to type of PH, especially when tumour size is taken into account. The present study also showed considerable interpatient variability in resected volumes among those undergoing the same type of PH. This was why liver regeneration was studied based on resected volume rather than on type of PH.

In the present study, $RI_{early}$ was 11 per cent in the smallest resection group and 63–66 per cent in the groups with the largest resections, which were mainly right-sided hepatectomies. The latter is in line with findings from previous studies in which liver regeneration was studied after right-sided hepatectomy[4,5,12]. Although the $RI_{early}$ increased as resections became larger, a plateau was seen for patients who underwent resection of 40 per cent or more of total preoperative liver volume. An explanation for this could be that the regeneration process is of secondary importance to the metabolic demands of the patient. It is conceivable that a sufficient amount of liver parenchyma needs to remain active to preserve appropriate liver function. When the FLR is small, relatively more remnant liver cells are needed to support the metabolic needs. Cells that are undergoing mitosis do not have an active function in hepatic metabolism and detoxification[15,16]. Conversely,

cells that are fulfilling metabolic and detoxifying functions cannot enter the mitotic phase. If metabolic and detoxifying functions of the liver prevail over the process of liver regeneration – at least until there are enough hepatocytes to carry out both functions – regeneration will be relatively delayed after larger resections. The present data suggest that the early liver regeneration reaches a plateau phase when 40 per cent or more of the total liver volume is resected. In other words, early liver regeneration is less efficient when larger volumes are resected. Later on, however, there seemed to be a catch-up growth after larger resections.

Complete regeneration was found in only 47 per cent of the patients at 6 months after surgery. Complete liver regeneration at this time point occurred mainly in patients who had smaller resections. No patient who underwent resection of 70 per cent or more of total preoperative liver volume reached complete regeneration by 6 months after operation. It has been shown that the FLR regenerates up to 80–90 per cent of total preoperative liver volume for right-sided hepatectomies after 6 months and 92–97 per cent for left lateral segmental resections[5,6,8–10]. It is possible that not all patients reached complete regeneration by 6 months after surgery because complete regeneration take longer than this. Additionally, volume increase could continue beyond the time point chosen in the present study; this seems a topic worthy of future research.

The present study used CT for the evaluation of liver volumes. CT is a well established non-invasive method for evaluating liver (vascular) anatomy and estimating segmental volumes[2,3]. Several types of error could affect liver volume measurements. However, the excellent correlation between estimated volume of resected liver and its actual weight in the present study suggests that the preoperative volumes were estimated accurately. The intraobserver and interobserver variability showed that volume measurements by CT are reliable and reproducible.

This study has certain limitations. First, several reported factors that could influence liver regeneration, such as steatosis, preservation of the middle hepatic vein, portal venous flow and spleen size, were not taken into account specifically because there was no significant difference in age, sex, body mass index, body surface area, preoperative chemotherapy and presence of steatosis between the five patient groups. Second, the increase in liver volume does not necessarily reflect liver regeneration. Parenchymal oedema, vascular engorgement and eventual inflammation, which are seen in the first phase of the regeneration process, could have influenced the measured liver volume. Last but not least, volume regeneration does not necessarily reflect functional recovery of the liver.





**Disclosure**

The authors declare no conflict of interest.

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# 6

# The Impact of Hepatic Steatosis on Liver Regeneration After Partial Hepatectomy

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*Accepted in Liver International*

## Abstract

**Background & Aim**:
Experimental studies in animals have suggested that liver regeneration is impaired in steatotic livers. However, few studies have focused on the impact of steatosis in patients undergoing partial hepatectomy (PH). This study aims to determine the role of steatosis on liver regeneration in humans following PH.

**Methods**:
Eighty-eight patients undergoing PH were included in the study. All patients underwent CT-scanning of the liver preoperatively and 7 days after surgery. Additional CT-scans were performed 6 months postoperatively. Pre-operative and postoperative volumes of the total liver (TLV), future liver remnant (FLR) and liver remnant (LR) were measured on CT-scans. Regeneration-indices (RI) were calculated at 7 days and 6 months using the formula: (Volume LR- Volume FLR) / Volume FLR*100%. Based on histological examination of the resected part of the liver, patients were classified into 3 groups: (1) no steatosis, (2) mild steatosis (1-29%) and (3) moderate-to-severe steatosis (≥30%).

**Results**:
The early RI (at day 7) was 40%, 24%, and 20% for patients in group 1, 2 and 3, respectively. Late RI (at 6 months) was 81% for group 1, 44% for group 2, and 22% for group 3 (p=0.019). At 7 days, the LR represented 79%, 80% and 79% of the TLV for groups 1-3. At 6 months, this was 93%, 92% and 79%, respectively.

**Conclusion**:
Although early RI after PH did not differ in patients with or without steatosis, the late RI in patients with moderate-to-severe-steatosis was lower, suggesting that late liver regeneration is impaired in these patients.

## Introduction

Hepatic steatosis is characterized by lipid accumulation within hepatocytes. It is a common finding in human biopsy specimens and is estimated to affect up to 30% of the Western population(1,2). It is reasonable to assume that its prevalence will further increase due to the current obesity epidemic. Hepatic steatosis can lead to non-alcoholic steatohepatitis, cirrhosis and development of hepatocellular carcinoma. In addition, it is reported to be a risk factor for postoperative complications, although reports are contradicting(3,4). The regenerative capacity of steatotic livers is less well established. Studies on liver regeneration and steatosis which have been performed in animal models have suggested that steatosis impairs liver regeneration(5)-(6). In humans, these studies are scarce. Most of the few existing reports have focused on regeneration in living donors for liver transplantation, but again, the results are contradictory(3,7-11). Although most of these studies have suggested that regeneration is not impaired by steatosis, it should be noted that moderate-to-severe steatosis is generally a contraindication for living donation in relative contrast to partial hepatectomy for liver tumors. Therefore, living donors are not an ideal population to study the impact of steatosis on liver regeneration(3,12). To the best knowledge, there is no study which assesses the impact of steatosis on liver regeneration in patients undergoing elective partial hepatectomy for liver tumors. Therefore, the aim of this study was to determinate whether liver regeneration in patients after partial hepatectomy for liver tumors is associated with the degree of hepatic steatosis.

## Material and Methods

### Patients

All patients included in this study were identified retrospectively from the prospective database of the FRESCO-trial (Efficacy of fibrin sealant in reducing resection surface related complications after partial liver resections, registration number ISRCTN85205641). The FRESCO-trial was a multicentre randomized controlled trial on the efficacy of fibrin sealant in reducing resection surface related complications after partial liver resections(13). All patients underwent protocolized CT-scan one week after surgery in order to objectively analyze resection surface related fluid collections. Thereafter, CT-imaging was performed when clinically indicated. Patients included in the present study on liver regeneration met the following criteria: (1) an anatomical liver resection was performed in our center, (2) at least two CT-scans were available (preoperatively and at 7 days after surgery). Indications for liver resection were malignant or benign liver tumors. Liver function tests were assessed before surgery and daily thereafter until discharge. Parameters for liver injury used for the analyses in this study were alanine aminotransferase (ALT, normal range <45 U/L) and aspartate aminotransferase (AST, normal range <40 U/L). Liver function parameters used for the analyses in this study were total bilirubin (TB, normal range ≤17

μmol/L) and prothrombin time (PT, normal range 9-12 seconds). Patients were classified in three groups based on the degree of steatosis assessed by histological examination of the resected liver specimen. Group 1 consisted of patients without steatosis, in group 2 were patients with mild steatosis (1-29%), and group 3 consisted of patients with moderate-to-severe steatosis (≥30%). All histological examination were performed by one experienced hepatopathologist (ASHG), who was unaware of the regeneration data. The FRESCO study protocol was approved by the medical ethical committee of our hospital and all patients gave their written informed consent. The study was conducted in compliance with national legislation as well as guidelines from our medical ethical committee.

**Imaging Details and Volumetry.**

Patients underwent multiphase CT-scanning before surgery, one week and 6 months after surgery. CT-scanning was performed on a multidetector CT-scanner (Somatom Sensation 64, Siemens, Erlangen, Germany) with the use of intravenous contrast (120ml iodixanol 320mg I/ml, Visipaque 320, GE Healthcare, Chalfont St Giles, UK).

Volumetry on CT-images was performed by one investigator (PK) with 2 years experience in abdominal CT-imaging, supervised by two other investigators (EJJ, a radiologist with 25 years experience in abdominal radiology and MTB, an experienced hepatobiliary surgeon). After transferring imaging data, measurements were performed on a workstation (Siemens Syngo version CT 2007A). Volumes of interest were manually drawn in the axial view on 2-mm-thickness slices in the portal-venous phase around the following contours: (1) the total liver with exclusion of the vena cava and the gallbladder, (2) the intended part of the liver to be resected according to the Couinaud-classification and on the basis of the hepatic (vascular) anatomy, (3) the tumor(s) and (4) the postoperative liver remnants at one week and 6 months after surgery. Approximate volumes were calculated with automatic multiplication of the circumscribed areas by the CT-section-thickness. Results were presented in cubic centimetres ($cm^3$) in a table. To determine intra- and interobserver-variability, two investigators performed all volume measurements in triplicate on 25 pre-surgical and post-surgical scans, with repetitive measurements at least 2 weeks apart.

**Calculations of Regeneration Indices.**

After volumetry, the tumor volume was subtracted from the total pre-operative liver volume and the resected volume. All calculations with total pre-operative liver volumes and resected volumes were performed without tumor volumes. The following variables were calculated: (1) the percentage of the resected liver volume without tumor volume, calculated as $V_{resection}$/ TLV*100% in which $V_{resection}$ is the volume of the resected part and TLV is the total pre-operative liver volume; (2) the volume of the future liver remnant ($V_{FLR}$), calculated as (TLV – $V_{resection}$); (3) the percentage of the FLR, calculated as $V_{FLR}$ / TLV*100%; (4) the regeneration index between the pre-operative CT-scan and the CT-scan at 7 days after surgery (early RI), calculated as ($V_{LRt1}$–

$V_{FLR}$)/$V_{FLR}$*100% in which $V_{LRt1}$ is the volume of the liver remnant at one week postoperatively and $V_{FLR}$ is the volume of the future liver remnant; (5) the regeneration index between the pre-operative CT-scan and the CT-scan at six months after surgery (late RI), calculated as ($V_{LRt2}$ – $V_{FLR}$)/$V_{FLR}$*100% in which $V_{LRt2}$ is the volume of the liver remnant at six months postoperatively; (6) the early regeneration, which is the volume of the liver remnant at one week postoperatively as percentage of the total pre-operative liver volume, calculated as $V_{LRt1}/V_{total}$*100% and (7) the late regeneration, which is the volume of the liver remnant at six months postoperatively as percentage of the total pre-operative liver volume, calculated as $V_{LRt2}/V_{total}$*100%.

**Histopathological assessment of hepatic steatosis.**
All resected specimens were subjected to pathological-anatomical examination. Speciments were weighted. The degree of steatosis was quantified as the percentage of hepatocytes containing fat droplets on conventional haematoxylin and eosin-stained slides and defined as none (0%), mild (1-29%%) and moderate-to-severe (≥30%) by a pathologist specialized in liver pathology. This was performed in sections of the liver distant from tumors.

**Statistical analysis.**
Categorical variables were presented as numbers and percentages, continuous variables were presented as median and interquartile range. Continuous variables were analyzed with Kruskal-Wallis test. Categorical variables were compared by using Chi-square test or Fisher's exact test, as appropriate. Intra- and interobserver-variability on volume-measurements were evaluated by using intraclass-correlation-coefficients (ICC). A *p*-value less than 0.05 was considered statistically significant. All analyses were performed with the statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL).

## Results

**General Patient Characteristics.**
A total of 88 patients who underwent partial hepatectomy were included in the study. There were 49 males (56%) and 29 females (44%). Demographic and surgical characteristics of the patients are presented in Table 1. Median age at the time of surgery was 62 years (IQR 53-68 years). There were 13 patients (15%) younger than 50 years, 75 patients (85%) were older. According to the histological assessment of hepatic steatosis, 35 patients (40%) were classified in group 1, 47 (53%) were assigned to group 2 and 6 patients (7%) were classified in group 3.

**Table 1.** General patient characteristics.

| | Group 1<br>No steatosis<br>(n=35) | Group 2<br>Mild steatosis<br>(n=47) | Group 3<br>Moderate-to-severe steatosis<br>(n=6) | *p*-value |
|---|---|---|---|---|
| Age (median, IQR[a], years) | 62 (56-69) | 63 (55-68) | 51 (32-61) | 0.061 |
| Sex, male / female | 18/17 (51% / 49%) | 27/20 (57% / 43%) | 4/2 (67% / 33%) | 0.747 |
| Body Mass Index (median, IQR, kg/m$^2$) | 23.4 (22.4-26.0) | 26.4 (24.4-29.6) | 28.1 (27.6-39.2) | 0.001 |
| Indication for surgery | | | | 0.270 |
| - Malignant tumors | 30 (86 %) | 42 (89 %) | 4 (67 %) | |
| - Benign tumors | 5 (14 %) | 5 (11 %) | 2 (33 %) | |
| Prior chemotherapy | | | | 0.816 |
| - Yes | 2 (5 %) | 5 (11 %) | - | |
| - No | 33 (95 %) | 42 (89 %) | 6 (100 %) | |
| Comorbidity | | | | |
| Pulmonary | 3 (9 %) | 5 (11 %) | 1 (17 %) | 0.736 |
| Cardiovascular | 22 (62 %) | 23 (49 %) | 1 (17 %) | 0.094 |
| Diabetes | 3 (9 %) | 7 (15 %) | 1 (17 %) | 0.512 |
| Type of hepatectomy | | | | 0.077 |
| - Right-sided | 23 (66 %) | 22 (47 %) | 2 (33 %) | |
| - Left-sided | 6 (17 %) | 7 (15 %) | - | |
| - Bisegmentectomy | 6 (17 %) | 18 (38 %) | 4 (66 %) | |

[a]IQR: interquartile range.

**Table 2.** Liver volumes

| Median (IQR[a]) | Group 1<br>No steatosis<br>(n=35) | Group 2<br>Mild steatosis<br>(n=47) | Group 3<br>Moderate-to-severe steatosis<br>(n=6) | *p*-value |
|---|---|---|---|---|
| TLV[b] | 1474 (1231-1724) | 1550 (1367-1698) | 2093 (1942-2593) | 0.002 |
| Resected volume | 690 (336-901) | 556 (271-933) | 794 (507-1126) | 0.333 |
| % Resected volume of TLV | 48 % (25-63) | 32 % (20-62) | 30 % (23-61) | 0.613 |
| $V_{FLR}$[c] | 783 (601-1000) | 995 (593-1265) | 1539 (678-1903) | 0.064 |
| $V_{LRt1}$[d] | 1099 (897-1280) | 1155 (938-1476) | 1764 (1076-2193) | 0.073 |
| $V_{LRt2}$[d] | 1361 (1117-1573) | 1433 (1177-1655) | 1653* (1410-1986) | 0.425 |
| $\%V_{LRt1}$ of TLV[e] | 79% (61-91) | 80 % (61-94) | 79 % (54-86) | 0.858 |
| $\%V_{LRt2}$ of TLV[e] | 93 % (86-102) | 92 % (78-101) | 79 % (68-91) | 0.168 |

Abbreviations used: [a]IQR: interquartile range; [b]TLV: Total pre-operative liver volume; [c]$V_{FLR}$: Volume of the future liver remnant; [d]$V_{LRt1}$ and $V_{LRt2}$ are the volumes of the liver remnants measured at one week and six months after surgery; [e]$\%V_{LRt1}$ and $\%V_{LRt2}$ are the volumes of the liver remnants measured at one week and six months after surgery as percentages of the pre-operative total liver volume, representing the completeness of liver regeneration at the measured time points.

* One patient had a decrease in liver volume on the second postoperative scan. Postoperative resorption and normalization of the vascular engorgement and tissue edema could explain this finding.

**Intra-observer and Interobserver Variability.**

The intraclass-correlation-coefficient for intraobserver-variability was 0.998 for the first postoperative scan (95%-CI 0.996-0.999, $p<0.001$) and 0.987 for the second scan (95%-CI 0.975-0.994, $p<0.001$). The intraclass-correlation-coefficient for interobserver-variability was 0.875 (95%-CI 0.354-0.981, $p=0.005$).

**Regeneration Indices and Degree of Hepatic Steatosis.**

All patients underwent protocol CT-scanning after a median of 7 days after surgery (IQR 7-7 days). Sixty-four patients (70%) had a scan six months postoperatively, performed after a median of 6 months after surgery (IQR 5-7 months). Twenty-four patients did not have a scan at 6 months for the following reasons: post-operative follow-up was performed with other imaging modalities than CT-scanning (i.e. ultrasound or magnetic resonance imaging, n=18); progressive metastatic disease (n=2); end of follow-up in the case of a benign tumor (n=2) and patient death (n=2). There was no significant difference in timing of the CT-scan at 6 months between the three patient groups. Liver volume measurements are presented in Table 2. Before surgery, the total liver volume was significantly different between the groups ($p<0.001$). There were no significant differences between the volumes of the resected part, FLR and liver remnants at one week and 6 months postoperatively (Table 2).

Early RI at one week was 40%, 24% and 20% for groups 1-3, respectively and was not significantly different. Late RI at 6 months was 81%, 44% and 22% for groups 1-3, respectively and did differ significantly (p=0.021) (Figure 1).

At one week after surgery, the liver remnant represented 79%, 80% and 79% of the preoperative total liver volume for groups 1-3, respectively without significant difference (early regeneration). At 6 months after surgery, liver volumes were 93%, 92% and 79% of the preoperative volume, respectively and did not differ significantly (late regeneration) (Figure 2).

**Biochemical Profiles**

Perioperative changes in serum total ALT, AST, bilirubin and prothrombin time in the three groups are depicted in Figure 3. There were no significant differences in these parameters of liver injury and liver function among the three groups.

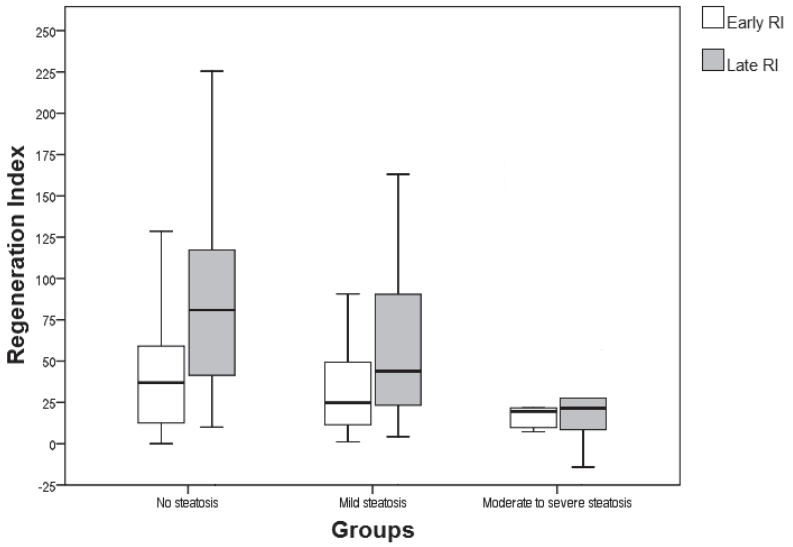


**Figure 1.**
Box-and-whisker plots representing the early (one week) and late (6 months) regeneration indices for the patient groups based on the degree of hepatic steatosis. The lower quartile (25$^{th}$ percentile), median and the upper quartile (75$^{th}$ percentile) are represented by the bottom, the line and the top of the box. The smallest and largest observations are represented by the ends of the whiskers (5$^{th}$ percentile and 95$^{th}$ percentile, respectively). Regeneration indices are declining as the degree of steatosis increases. The early RI did not differ significantly ($p$=0.292). The late RI was significantly different between the groups ($p$=0.021).

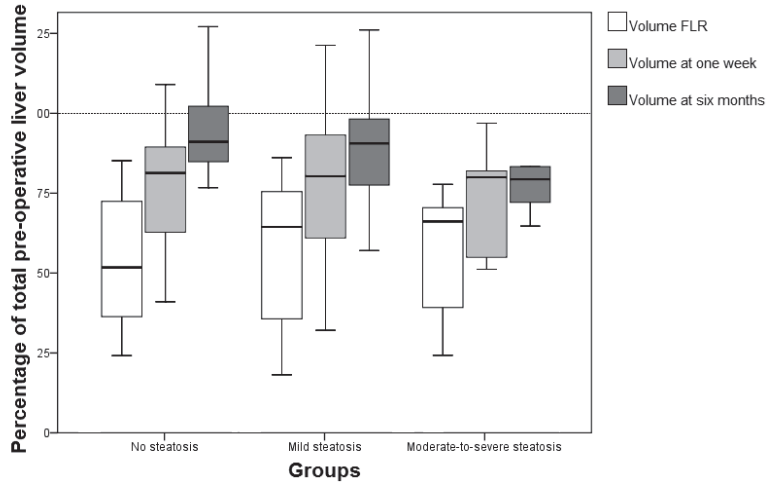


**Figure 2.**
Box-and-whisker plots of the volumes of the future liver remnant (FLR) and liver remnants one week and six months postoperatively as percentage of the total pre-operative liver volume, representing the completeness of liver regeneration at the different time points. The lower quartile (25$^{th}$ percentile), median and the upper quartile (75$^{th}$ percentile) are represented by the bottom, the line and the top of the box. The smallest and largest observations are represented by the ends of the whiskers (5$^{th}$ percentile and 95$^{th}$ percentile, respectively).The total pre-operative liver volume was set at 100 %. There were no significant differences between the groups.

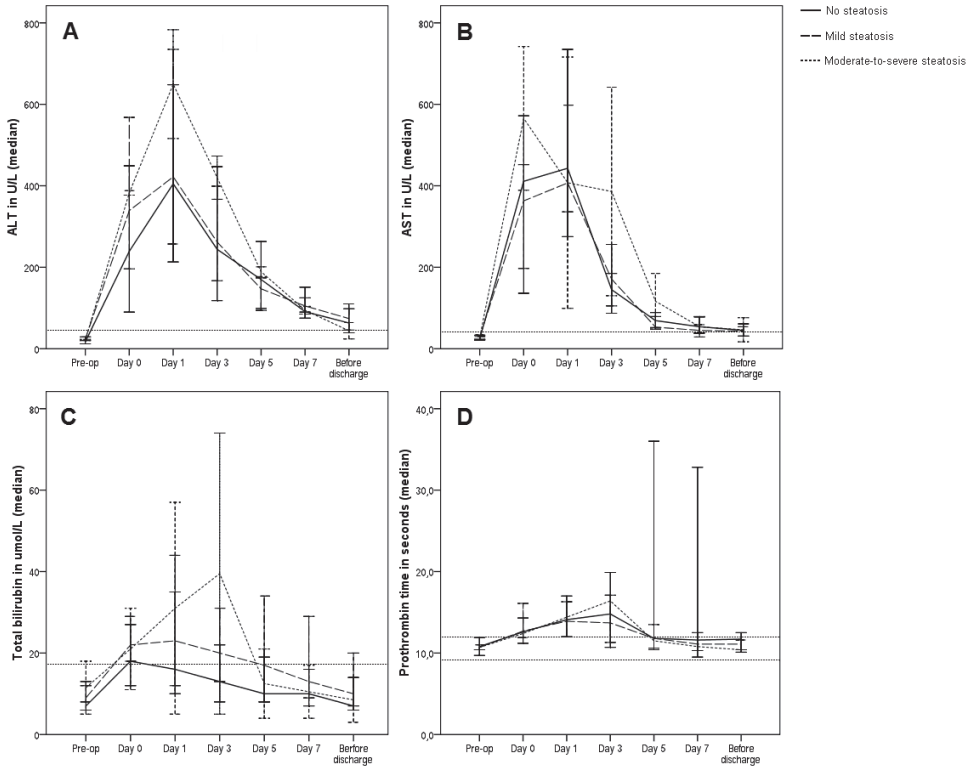


**Figure 3. Serum levels of alanine aminotransferase, aspartate aminotransferase, total bilirubin and prothrombin time.**

Median peri-operative changes in levels of alanine aminotransferase (ALT, panel A) in U/L, aspirate aminotransferase (ALT, panel B) in U/L, total bilirubin levels in µmol/L (panel C) and prothrombin time in seconds (panel D). The smallest and largest observations are represented by the ends of the error bars ($5^{th}$ percentile and $95^{th}$ percentile, respectively). Levels at day 0 were assessed immediately after surgery. The horizontal dotted lines represent the normal values for each parameter. There was no significant difference in pre-operative and postoperative serum levels of ALT, AST, bilirubin and prothrombin times in between the groups.

## Discussion

The present study assessed liver regeneration in patients with and without steatosis who underwent partial hepatectomy. We found no significant differences in the early regeneration index (early RI, one week postoperatively), although the early RI tended to be lower with increasing degrees of steatosis. However, the late regeneration index (late RI, 6 months postoperatively) did differ significantly between the three patient groups with lower late RIs in patients with moderate-to-severe steatosis. This finding suggests that liver regeneration is impaired by the presence of steatosis > 30%. Liver injury markers and liver function parameters did not differ significantly between the patient groups, as reflected by the serum levels of AST, ALT, total bilirubin and

prothrombin time, but patients with moderate-to-severe steatosis seemed to have worse postoperative biochemical profiles. Our results suggest that large resections should be performed cautiously in these patients, because their regeneration response is impaired by the presence of steatosis.

Hepatic steatosis is an increasingly common encountered condition. Experimental models which have assessed the impact of steatosis on liver regeneration have shown that the regenerative capacity of the liver is affected by the presence of steatosis(5,6). Severe steatosis is associated with an impaired regeneration response, mainly due to an excessive pro-inflammatory cytokine response and insufficient antioxidant response which predisposes the hepatocytes to extensive necrosis. This results in increased hepatocellular damage, which affects the regenerative capacity of the steatotic liver after hepatectomy(6). However, results from human studies, mostly performed in the setting of living donor liver transplantation, are contradicting(3,7-11). Reported RIs tended to be lower in mildly steatotic livers, especially in the first three months after surgery. However, most studies failed to reach statistical significance, probably because of the small sample size of the steatotic patient groups, as steatosis is generally an exclusion criterion for donor candidates. Additionally, the degree of steatosis in these studies did not exceed 30%. This could have influenced the results, since impairments in liver regeneration are expected to become more pronounced when there is clinically significant steatosis (generally regarded as ≥30%). Furthermore, differences in general health and age between living donors and patients with liver tumors makes it difficult to compare liver regeneration in these patients. Lastly, disparities in the definition of steatosis and different histologic techniques used in the assessment of steatosis can also contribute to these discrepancies. It is difficult to extrapolate regeneration data of healthy living donors to patients undergoing elective partial hepatectomy. Therefore, it remains unknown whether clinically significant steatosis has a negative influence on the regenerative capacity of the liver in the latter population. The present study, performed in patients who underwent elective hepatectomy, showed a tendency towards a lower early RI in patients with moderate-to-severe steatosis, but this was not significant. The late RI was significantly different between the patient groups with lower late RIs in patients with moderate-to-severe steatosis. We hypothesize that hypertrophy of cells together with the presence of postoperative vascular engorgement and tissue edema could have resulted in falsely larger volumes of the liver remnants one week after partial hepatectomy, leading to less pronounced differences in the early RI between the patient groups. This resolves within a few weeks postoperatively, thus the late RI is not affected by this phenomenon and is in fact more strictly related to true cellular multiplication-related volume growth. Our results suggest that large resections should be performed cautiously in these patients, because their regeneration response is impaired by the presence of steatosis.

An increasing number of oncological patients undergo chemotherapy prior to resection in order to downgrade the stage of their (metastatic) disease. Certain types of agents used for chemotherapy – for example such irinotecan, oxaliplatin and 5-fluorouracil - are well-known risk-

factors for chemotherapy-induced hepatic steatosis(14-16). The impact of chemotherapy on liver regeneration is still unclear, but it is assumable that any type of injury induced by pre-operative chemotherapy negatively affects postoperative liver regeneration. Seven patients received pre-operative chemotherapy in our study and none of them had moderate-to-severe steatosis. Thus, the presence of steatosis and the resulting impaired regeneration response in patients with moderate-to-severe steatosis cannot be explained by pre-operatively administered chemotherapy.
It has been described previously that patients with steatosis have worse postoperative biochemical markers for liver injury and liver function(4,12,17-20). In the present study, parameters of liver injury and liver function did not differ significantly between the patient groups. Although not statistically significant, there were higher peaks in serum ALT, AST, total bilirubin and prothrombin time in patients with moderate-to-severe steatosis. This suggests that the presence of steatosis has a negative influence on postoperative hepatic biochemistry. One explanation that the present study did not reach statistical significance on biochemical changes could be that the number of patients with moderate-to-severe steatosis was too low. Another explanation could be the wide distribution of the biochemical parameters at the time points of measurement (Figure 3).
The present study has certain limitations. Firstly, specific factors which could influence liver regeneration such as preservation of the middle hepatic vein, the portal venous flow and spleen size were not taken specifically into account. Secondly, the increase in liver volume does not necessarily reflect liver regeneration. Parenchymal edema, vascular engorgement and eventual inflammation which are seen in the first phase of the regeneration process could have influenced the measured liver volume. Lastly, volume regeneration does not necessarily reflect functional recovery of the liver.
In conclusion, hepatic steatosis impairs liver regeneration, as is reflected by the declining regeneration indices in patients with an increasing degree of steatosis. The clinical data of this study are in line with previous experimental animal data that hepatic steatosis is an important risk factor for impaired liver regeneration after partial hepatectomy.

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# 7

# Postoperative Decrease in Platelet Count Is Related to Liver Regeneration After Partial Hepatectomy

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*Submitted*

## Abstract

**Aim**:
To study whether changes in postoperative platelet count is associated with liver regeneration in patients after partial hepatectomy (PH).

**Methods**:
Ninety-one patients undergoing PH were included in the study. All patients underwent pre-operative CT-scanning and protocol CT-scanning 7 days postoperatively. Liver regeneration was assessed by volumetry of the pre-operative total liver volume (TLV) and postoperative liver remnants on CT-scans. After assessing the regeneration rate with respect to the amount of the resected liver volume, patients were classified in three groups according to their regeneration-rate: (1) poor regeneration (regeneration rate≤25$^{th}$ percentile); (2) intermediate regeneration (regeneration rate between 25$^{th}$-75$^{th}$ percentile) and (3) high regeneration (regeneration rate≥75$^{th}$ percentile). Platelet count was assessed pre-operatively and daily thereafter until discharge. The change in post-operative platelet count was defined as the difference between pre-operative and post-operative platelet counts. Platelet counts were presented as percentage of the pre-operative value.

**Results**:
Pre-operative platelet counts did not differ between the patient groups. Postoperative platelet counts decreased in all patients with a nadir at day 3 postoperatively and increased thereafter. At day 3 postoperatively, changes in platelet-count differed significantly between patient groups ($p=0.049$). Changes in platelet-count were significantly different at day 3 postoperatively in group 3 compared to group 1 ($p=0.032$) and group 2 ($p=0.028$).

**Conclusion**:
Platelet counts decrease in the first three days after PH and restore thereafter. Patients with high regeneration rates have stronger decreases in platelet counts compared to patients with poor regeneration, suggesting that more platelets are consumed in patients with high regeneration.

## Introduction

Among all organs, the liver is unique due its remarkable capacity of regeneration after injury or partial resection. The liver can tolerate resections of as much as 75-80% of its volume with the prerequisite that there is no underlying parenchymal disease[1, 2]. Liver regeneration occurs rapidly after partial hepatectomy (PH) and is related to the amount of liver volume resected[3]. Hepatic regeneration is an orchestrated interplay of signaling events, consisting of growth factors, cytokines and transcription factors. Platelets, which contain multiple growth factors, are thought to be directly involved in liver regeneration. Several experimental studies in rodents have demonstrated a major role for platelets in liver regeneration[4-6]. It has been shown that platelets are recruited at the site of injury in direct contact to the hepatocytes to exert their positive effects in liver regeneration [7, 8]. Experimentally induced thrombocytopenia results in a markedly reduced proliferative activity in the liver. On the other hand, trombocytosis is associated with accelerated liver regeneration. It has been shown that platelets enter liver tissue quickly after a liver resection in mice. These platelets have been shown to enter the space of Disse which enables direct contact with the hepatocytes. This mechanism is likely required to stimulate liver regeneration by a mechanism involving release of various growth factors that are stored within platelet granules In humans, it has been shown that a low postoperative platelet count is associated with delayed functional recovery of the liver. Trombocytopenic patients have increased markers of liver injury, a higher degree of liver dysfunction and higher mortality[9]. It has been reported that platelet count decreases after PH and that this decrease is related to the extent of resection[10, 11]. A decrease in platelet count has also been described after liver transplantation[12]. Since both liver regeneration and the postoperative platelet count depend on the amount of the liver volume resected, we hypothesized that the decrease in postoperative platelet count is associated with the degree of liver regeneration. Specifically, we hypothesized that those patients with the largest drop in platelet count would have the highest regenerative response, since the drop in platelet count, at least partly, would reflect platelet accumulation and release of platelet-derived growth factors within the liver remnant. A direct relationship between postoperative platelet count and liver regeneration has never been studied or proven in humans. Therefore, the aim of this study was to evaluate whether the decrease in postoperative platelet count is related to liver regeneration in patients after PH.





## Material and methods

**Patients.**

Patients included in this study were all enrolled in a randomized controlled multicenter trial comparing the use of fibrin sealants versus no sealants on the liver resection surface after PH (Controlled trial number ISRCTN85205641[13]) between May 2006 and June 2010. All

patients underwent per protocol CT-scanning one week postoperatively in order to objectively analyze resection surface related fluid collections. Patients eligible for the present study on liver regeneration participated in the randomized study and (1) underwent an anatomical liver resection in the present authors' unit, (2) had an available pre-operative CT-scan and (3) provided informed consent. Indications for liver resection were malignant or benign liver tumours or donor hemihepatectomies. Patients with pre-existing liver cirrhosis were excluded. All patients underwent pre-operative and per protocol CT-imaging one week after surgery. Thereafter, CT-imaging was performed when indicated. Platelet counts (normal range 150-350x$10^9$g/L) were assessed before surgery, immediately after surgery upon arrival at the intensive care unit (day 0), then at subsequent days until discharge. To evaluate the change in platelet count, pre-operative platelet count was set at 100% and postoperative platelet counts were calculated as percentage of the pre-operative platelet count. Hemoglobin (normal range 8.7-10.6 mmol/ L for men and 7.5-9.9 mmol/L for women) and hematocrit (0.420-0.520 volume percentage for men and 0.370-0.470 volume percentage for women) were assessed pre-operatively and then daily until discharge. Liver function tests were assessed before surgery and daily thereafter until discharge. Liver function parameters used for the analyses in this study were total bilirubin (TB, normal range ≤17 μmol/L) and prothrombin time (PT, normal range 9-12 seconds). Parameters for liver injury used for the analyses in this study were aspartate aminotransferase (AST, normal range <40 U/L) and alanine aminotransferase (ALT, normal range <45 U/L). This study was conducted in compliance with national legislation as well as guidelines from our medical ethical committee.

**Imaging Details and Volumetry.**

Patients underwent multiphase CT-scanning before surgery and, one week postoperatively and thereafter when indicated. The second CT-scan used in this study was the CT-scan performed 6 months postoperatively. CT was performed on a multidetector CT-scanner (Somatom Sensation 64, Siemens, Erlangen, Germany) with the use of intravenous contrast (120ml iodixanol 320mg I/ml, Visipaque 320, GE Healthcare, Chalfont St Giles, UK).

Volumetry on CT-images were performed by one investigator (PK) with 2 years experience in abdominal CT-imaging, supervised by two other investigators (EJJ, a radiologist with 25 years experience in abdominal radiology and MTB, an experienced hepatobiliary surgeon). After transferring imaging data, measurements were performed on a workstation (Siemens Syngo version CT 2007A). Volumes of interest were manually drawn in the axial view on 2-mm-thickness slices in the portal-venous phase around the following contours: (1) the total liver with exclusion of the vena cava and the gallbladder, (2) the intended part of the liver to be resected according to the Couinaud-classification and on the basis of the hepatic (vascular) anatomy, (3) the tumor(s) and (4) the postoperative liver remnants. Approximate volumes were calculated with automatic multiplication of the circumscribed areas by the CT-section-thickness.

**Calculations of the Regeneration Index.**

Pre-operative liver volumes and resected volumes were adjusted by subtracting tumour volumes. The following variables were calculated: (1) percentage of resected liver volume, calculated as $100*V_{resection} / V_{total}$ in which $V_{resection}$ is the resected volume and $V_{total}$ is total pre-operative liver volume; (2) volume of future liver remnant ($V_{FLR}$), calculated as ($V_{total} – V_{resection}$); (3) percentage of the future liver remnant, calculated as $100*V_{FLR} / V_{total}$; and (4) regeneration index, calculated as $100*(V_{LR}–V_{FLR})/V_{FLR}$ in which $V_{LR}$ is the liver remnant volume one week postoperatively and $V_{FLR}$ is the future liver remnant volume.

Since liver regeneration is dependent on the amount of resected liver tissue, regeneration indices need to be evaluated based on the volume of the resected part of the liver. A patient with a large resection will have a larger regeneration index than a patient with a small resection, which does not mean that the latter has poor regeneration. Therefore, patients were classified in 5 groups, according to the amount of resected liver tissue. Group 1 consisted of patients who underwent a resection of <20% of their total preoperative liver volume, group 2 were patients with a resection of 20-39%, group 3 consisted of patients with a resection of 40-59%, group 4 were patients with a resection of 60-69% and group 5 consisted of patients with a resection of ≥70% of their total pre-operative liver volume. Median regeneration indices were calculated for each patient group separately with interquartile ranges. Within each of these 5 groups, patients were categorized as having poor regeneration (regeneration index≤25$^{th}$ percentile), intermediate regeneration (regeneration index between the 25$^{th}$ and 75$^{th}$ percentile) and high regeneration (regeneration index≥75$^{th}$ percentile). Thereafter, all patients with poor, intermediate and high regeneration from the subgroups were rejoined in three large patient groups. Thus, there are three patient groups in total. Group 1 consisted of patients with poor regeneration (regeneration index≤25$^{th}$ percentile), in group 2 were patients with intermediate regeneration (regeneration index between the 25$^{th}$ and 75$^{th}$ percentile) and group 3 consisted of patients with high regeneration (regeneration index≥75$^{th}$ percentile). Changes in platelet count were analyzed in these three patient groups.

**Statistical analysis.**

Categorical variables were presented as numbers and percentages, continuous variables were presented as mean ± standard deviation (SD) or median and interquartile range (IQR). Continuous variables were analyzed with Student's t-test (intergroup) or ANOVA (between-group) with Bonferroni post-hoc test in case of normal distribution. Mann-Whitney-U-test (intergroup) or Kruskal-Wallis test (between-group) were used for non-parametrically distributed variables. Categorical variables were compared by using Chi-square test or Fisher's exact test, as appropriate. *P*-values <0.05 were considered statistically significant. All analyses were performed with the statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL).

## Results

**General Patient Characteristics.**

Ninety-one patients were included in this study. Demographic and surgical characteristics of these patients are presented in Table 1 and Table 2, respectively. Median intra-operative blood loss was 750 ml in the patient group with poor regeneration (IQR 375-1150), 600 ml in the patient group with intermediate regeneration (IQR 300-1050) and 400 ml in the patient group with high regeneration (IQR 250-825) ($p$=0.396). Intra-operative red blood cell transfusion was used in a total of 11 patients (12 %), of which 3 patients (15 %) with poor regeneration, 7 patients (15 %) with intermediate regeneration and 1 patient (5 %) with high regeneration ($p$=0.684). There were no patients with intra-operative platelet transfusion. Postoperative red blood cell transfusion was used in a total of 2 patients, of which 1 patient with intermediate regeneration and 1 patient with high regeneration ($p$=0.713). A total of 44 patients (48 %) suffered from postoperative complications. Complications were seen in 11 patients (52 %) with poor regeneration, 24 patients (49 %) with intermediate regeneration and 9 patients (43 %) with high regeneration ($p$=0.886). Infectious complications occurred in 6 patients (29 %) with poor regeneration, 17 patients (35 %) with intermediate regeneration and 7 patients (33 %) with high regeneration ($p$=0.955). Bile leakage was seen in 3 patients (14 %) with poor regeneration, 8 patients (16 %) with intermediate regeneration and 2 patients (10 %) with high regeneration ($p$=0.921). Liver failure occurred in 1 patient (5 %) with poor regeneration, 6 patients (12 %) with intermediate regeneration and no patient with high regeneration ($p$=0.258). Cardiovascular complications occurred in 1 patient (5 %) with poor regeneration and 1 patient (2 %) with intermediate regeneration. No patient with high regeneration suffered from cardiovascular complications (p=0.713). Multiple complications occurred in one patients with intermediate regeneration.

**Table 1.** Demographic characteristics.

| | All patients | Poor regeneration Group 1 (n=21) | Intermediate regeneration Group 2 (n=49) | High regeneration Group 3 (n=21) | *p*-value |
|---|---|---|---|---|---|
| Sex | | | | | 0.568 |
| • Male | 51 (56 %) | 12 (57 %) | 27 (55 %) | 11 (52 %) | |
| • Female | 40 (44 %) | 9 (43 %) | 22 (45 %) | 10 (46 %) | |
| Age* | 62 (52-68) | 61 (49-68) | 61 (52-67) | 65 (52-69) | 0.414 |
| Body Mass Index* | 25.2 (23.0-29.0) | 26.4 (23.3-29.3) | 26.9 (23.5-28.4) | 23.5 (22.3-29.2) | 0.355 |
| Prior chemotherapy | 7 (8 %) | - | 7 (11 %) | - | 0.041 |
| Indication for surgery | | | | | 0.218 |
| • Malignant | 76 (84 %) | 20 (95 %) | 40 (82 %) | 16 (76 %) | |
| • Benign | 12 (13 %) | 1 (%) | 8 (%) | 3 (%) | |
| • Living donor | 3 (3 %) | - | 1 (%) | 2 (%) | |
| Comorbidity | | | | | |
| • Cardiovascular | 46 (51 %) | 11 (52 %) | 22 (45 %) | 13 (62 %) | 0.466 |
| • Diabetes | 11 (12 %) | 5 (24 %) | 5 (10 %) | 1 (5 %) | 0.194 |
| • Pulmonary | 9 (10 %) | 3 (14 %) | 4 (8 %) | 2 (10 %) | 0.735 |

Catergorical variables are expressed as number and percentage. Continuous variables are expressed as median (interquartile range)* or mean ± standard deviation**.

**Table 2.** Surgical characteristics.

| | All patients | Poor regeneration Group 1 (n=21) | Intermediate regeneration Group 2 (n=49) | High regeneration Group 3 (n=21) | *p*-value |
|---|---|---|---|---|---|
| Steatosis | | | | | |
| • No steatosis | 38 (42 %) | 8 (38 %) | 19 (39 %) | 11 (52 %) | 0.662 |
| • < 30% steatosis | 47 (52 %) | 11 (52 %) | 26 (53 %) | 10 (48%) | |
| • > 30% steatosis | 6 (6 %) | 2 (10 %) | 4 (8 %) | 0 | |
| Type of partial hepatectomy | | | | | 0.749 |
| • (Extended) right-sided hepatectomy | 47 (52 %) | 11 (52 %) | 26 (53 %) | 10 (48 %) | |
| • (Extended) left-sided hepatectomy | 13 (14 %) | 2 (10 %) | 6 (12 %) | 5 (24 %) | |
| • Bisegmentectomy | 31 (34 %) | 8 (38 %) | 17 (35 %) | 6 (28 %) | |
| Resection group | | | | | 1.000 |
| • 0-19 % | 21 (22 %) | 5 (24 %) | 11 (23 %) | 5 (24 %) | |
| • 20-39 % | 26 (29 %) | 6 (28 %) | 14 (29 %) | 6 (28 %) | |
| • 40-59 % | 19 (21 %) | 5 (24 %) | 10 (20 %) | 4 (19 %) | |
| • 60-69 % | 19 (21 %) | 4 (19 %) | 10 (20 %) | 5 (24 %) | |
| • ≥ 70 % | 6 (7 %) | 1 (5 %) | 4 (8 %) | 1 (5 %) | |
| Total pre-operative liver volume* | 1554 (1328-1737) | 1737 (1528-2035) | 1550 (1340-1720) | 1401 (1189-1639) | <0.001 |
| Resected volume* | 639 (322-901) | 636 (334-1067) | 650 (280-896) | 639 (276-861) | 0.564 |
| Future liver remnant** | 947 ± 423 | 1061± 404 | 959 ± 467 | 803 ± 287 | 0.136 |
| Pre-operative platelet count** | 259 ± 75 | 243 ± 70 | 263 ± 81 | 267 ± 62 | 0.504 |
| Intra-operative blood loss* | 600 (300-1050) | 800 (450-1275) | 600 (300-1000) | 425 (213-888) | 0.396 |
| No. of patients requiring RBC-transfusion | | | | | |
| • Intraoperative | 11 (12 %) | 3 (14 %) | 7 (11 %) | 1 (5 %) | 0.684 |
| • Postoperative < 24 hr | 2 (2 %) | 0 (0 %) | 1 (2 %) | 1 (5 %) | 0.713 |
| All complications | 44 (48 %) | 11 (52 %) | 24 (49 %) | 9 (43 %) | 0.886 |
| • Infectious | 30 (33 %) | 6 (29 %) | 17 (35 %) | 7 (33 %) | 0.955 |
| • Bile leak | 13 (14 %) | 3 (14 %) | 8 (16 %) | 2 (2 %) | 0.921 |
| • Liver failure | 7 (8 %) | 1 (5 %) | 6 (12 %) | 0 (0 %) | 0.258 |
| • Cardiovascular | 2 (2 %) | 1 (5 %) | 1 (2 %) | 0 (0 %) | 0.713 |
| Platelet count after 6 months** | 211 ±77 | 241 ±92 | 186 ±66 | 232 ±77 | 0.147 |

Catergorical variables are expressed as number and percentage. Continuous variables are expressed as median (interquartile range)* or mean ± standard deviation**.

Total serum bilirubin, prothrombin time, AST, ALT, haemoglobin and hematocrit not differ significantly between the groups before and after surgery (all $p>0.05$) (Figure 1).
The volume of the part to be resected, the future liver remnant and the liver volumes at one week and 6 months postoperatively did not differ significantly between the groups. Median regeneration index in the patient group with poor regeneration was 6.1 % (IQR 3.4-27), in the patient group with intermediate regeneration this was 25 % (IQR 12-51) and in the patient group with high regeneration, the median regeneration index was 57 % (IQR 35-90) ($p<0.001$).

**Course of the postoperative platelet count.**

Pre-operatively, there were no significant differences in platelet count among the regeneration index-based patient groups ($p=0.504$) (Table 1). Figure 2 shows the postoperative course of the platelet count as percentage of the pre-operative value. Platelet counts decreased postoperatively until day three postoperatively. Thereafter, platelet counts increased to above the pre-operative value and normalized at six months postoperatively. Platelet counts differed significantly between the groups at postoperative day 3 ($p=0.049$). On intergroup analysis, group 3 had significantly lower platelet counts at postoperative day 3 compared to group 1 ($p=0.032$) and group 2 ($p=0.028$). Immediately after surgery and on postoperative day 1 and 5, platelet counts were lower in group 3 than in group 1, but these differences did not reach statistical significance ($p=0.072$, $p=0.078$ and $p=0.066$, respectively). Figure 3 emphasizes the nadir of the platelet count at postoperative day 3 in patients with poor, intermediate and high regeneration.

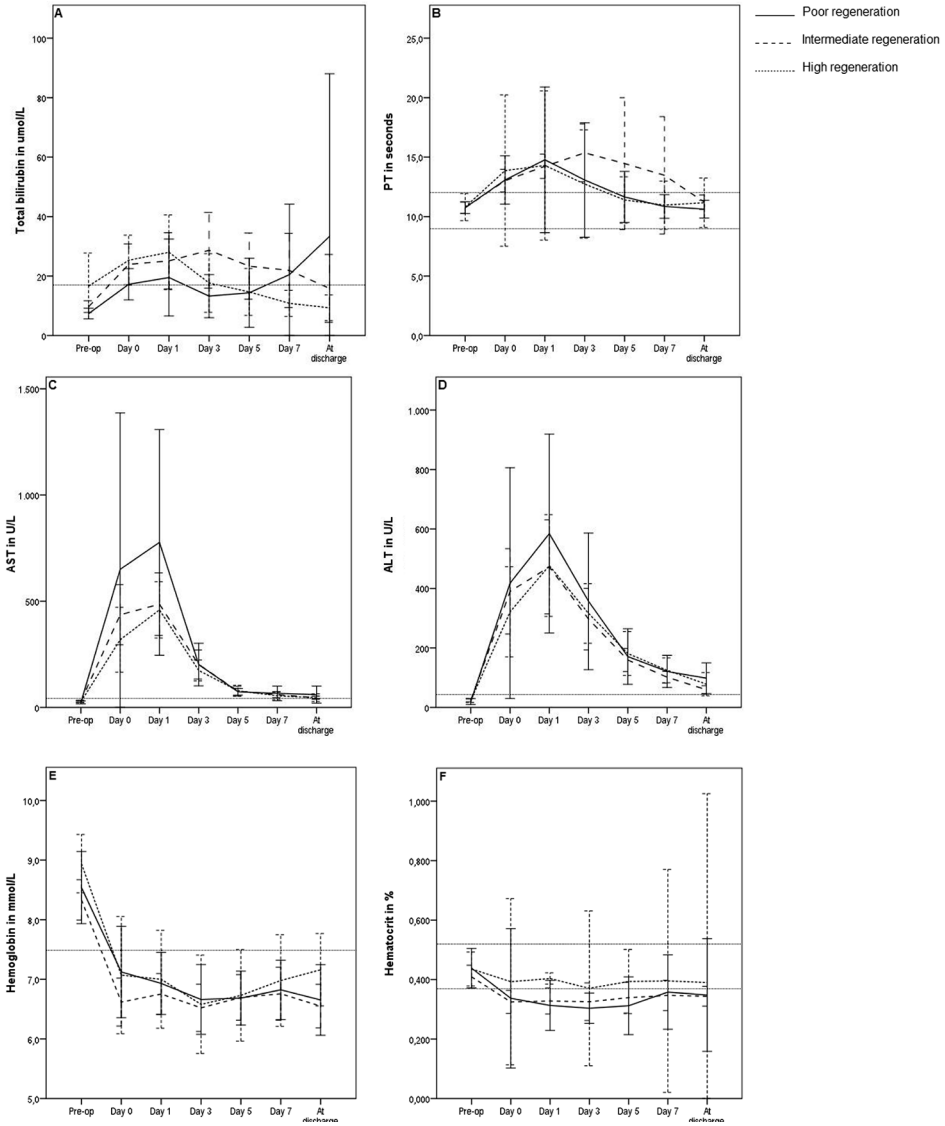






**Figure 1.**

Median peri-operative changes in levels of total bilirubin levels in µmol/L (panel A), prothrombin time in seconds (panel B), aspirate aminotransferase (AST, panel C) in U/L, alanine aminotransferase (ALT, panel D) in U/L, hemoglobin in mmol/L (panel E) and hematocrit in volume percentage (panel F). Patients were classified according to the regeneration index (group 1: poor regeneration index, continuous line, group 2: intermediate regeneration index, coarsely dotted line and group 3: high regeneration, finely dotted line). Pre-op: pre-operative platelet count, 0-7 immediate post-operatively-day 7 after surgery and at discharge is the platelet count at discharge. The smallest and largest observations are represented by the ends of the error bars (5$^{th}$ percentile and 95$^{th}$ percentile, respectively). Levels at day 0 were assessed immediately after surgery. The horizontal dotted lines represent the normal values for each parameter. There were no significant differences in pre-operative and postoperative serum levels of total bilirubin, prothrombin time, AST, ALT, haemoglobin and hematocrit between the groups.

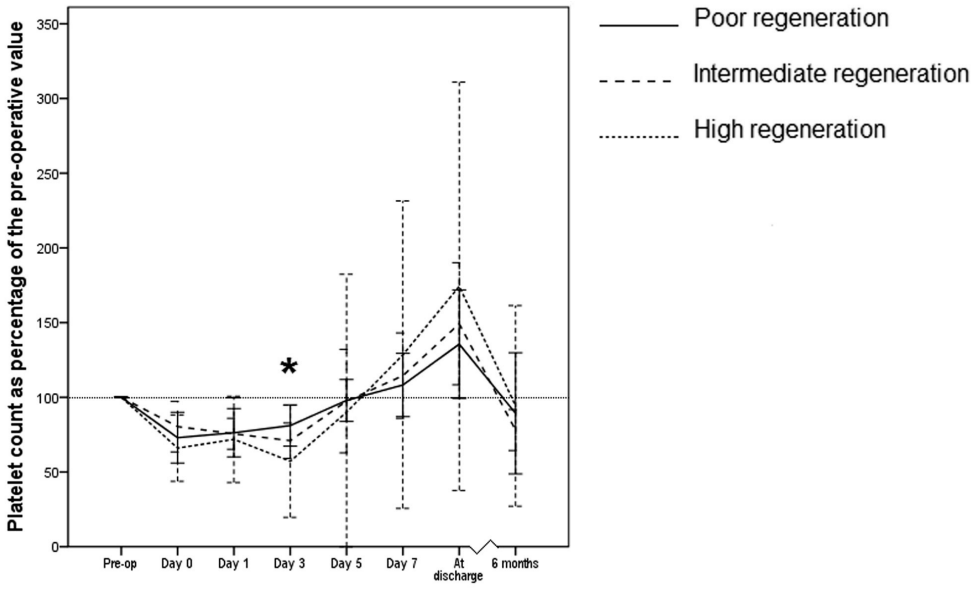


**Figure 2.**
Postoperative course of the mean platelet count expressed as percentage of the pre-operative value. The smallest and largest observations are represented by the ends of the error bars (5$^{th}$ percentile and 95$^{th}$ percentile, respectively). Patients were classified according to the regeneration index (group 1: poor regeneration index, continuous line, group 2: intermediate regeneration index, coarsely dotted line and group 3: high regeneration, finely dotted line). Pre-op: pre-operative platelet count, 0-7 immediate post-operatively-day 7 after surgery, at discharge is the platelet count at discharge and 6 months is 6 months postoperatively. A decrease was seen in all groups, but was most pronounced in the patient group with high regeneration. The decrease in platelet count was significant at postoperative day 3 ($p$=0.049) in all groups (marked with an asterisk). On intergroup analysis, group 3 had a greater decrease compared to group 1 and 2 ($p$-values were 0.032 and 0.028, respectively).

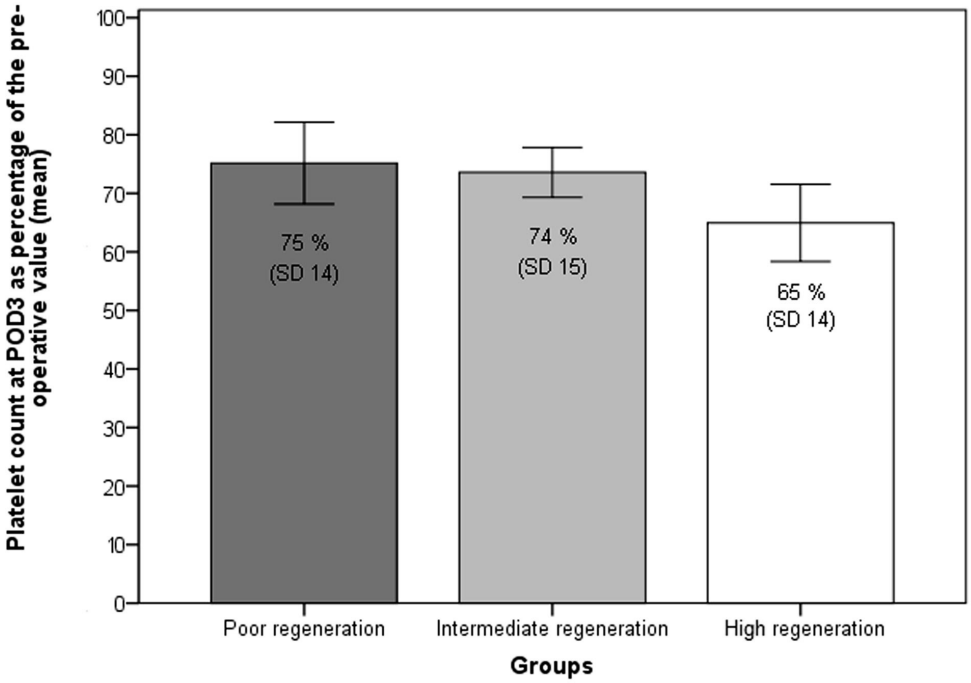


**Figure 3.**
Histogram of the mean platelet count (expressed as percentage of the pre-operative values) at postoperative day 3 in the three patient groups with poor, immediate and high regeneration. The smallest and largest observations are represented by the ends of the error bars ($5^{th}$ percentile and $95^{th}$ percentile, respectively).

## Discussion

The present study evaluated whether there is a relationship between the change in postoperative platelet count and liver regeneration in patients after partial hepatectomy (PH). We found an immediate decrease in platelet count after resection which continued until postoperative day 3. From then on, platelet counts increased to above the pre-operative value and normalized six months postoperatively. Patients with high regeneration had a significantly larger decrease in their platelet count at postoperative day 3 compared to patients with poor and intermediate regeneration. This suggests that a larger decrease in postoperative platelet count is associated with better regeneration.

The liver responds to injury - for example PH - by regeneration of the lost volume. Liver regeneration is a complex process, which involves an orchestrated interplay of cytokines, growth factors and transcription factors. Liver regeneration starts within one day after resection in response to the loss of tissue[2]. Firstly, hepatocytes begin with replication. Thereafter other cell types follow, such as endothelial cells and Kuppfer cells. Liver regeneration continues until one

year after surgery when an appropriate amount of liver tissue is restored, approximating a normal liver/body mass ratio to enable proper organ function. There is a positive correlation between the amount of resected liver tissue and liver regeneration[4, 14, 15] Therefore, the magnitude of liver regeneration needs to be adjusted to the amount of resected liver[3].

Platelets have hemostatic, thrombotic, inflammatory and secretory functions, but their role in hepatic pathophysiology only gained interest in the past decade. Platelets contain numerous growth factors, such as serotonin, hepatocyte growth factor, platelet derived growth factor, vascular endothelial growth factor, epidermal growth factor, tissue growth factor and insulin-like growth factor, which have been implicated in liver regeneration [5, 7, 8]. It has been shown in experimental models in rodents that platelets actively translocate into Disse's spaces after partial hepatectomy. Platelets accumulate in the liver by migrating from sinusoidal spaces into Disse's spaces through the fenestration of the sinusoidal cells[7]. Normally, flattened sinusoidal endothelial cells are fenestrated and the diameter of these pores is smaller than that of the platelets. The fenestrae become larger immediately after partial hepatectomy, allowing the active translocation of platelets into Disse's spaces trough the fenestrae and thus direct contact between platelets and hepatocytes[7, 8]. In vitro, it has been demonstrated that this contact between hepatocytes and platelets is necessary for the release of the growth factors and cytokines from the platelets and their direct delivery to the hepatocytes to promote liver regeneration[7, 8, 16]. Thrombocytopenic rodents show an impaired regeneration response in experimental studies[17]. In contrast, thrombocytotic rodents show accelerated liver regeneration[18]. When platelets are massively recruited in the remnant liver, one expects a decrease in postoperative platelet count. Although never related to liver regeneration, a postoperative decrease in platelet count of 30-55% in human has been reported in the literature[6, 10, 11, 16]. The present study is the first in which the decrease in postoperative platelet count was shown to be related to the degree of liver regeneration. The results showed that patients with high regeneration had the largest decrease in postoperative platelet count. Therefore, these findings are in line with previous studies in experimental animal models which have shown that platelets have a pivotal role in liver regeneration by migrating into the remnant liver to exert their positive effects. Based on the literature discussed above, we thus hypothesize that the stimulating role of platelets in liver regeneration depends on intraparenchymal delivery of growth factors stored within platelet granules, although this mechanism has not yet been formally proven in vivo. Based on the findings described in the present study, we furthermore speculate that a greater reduction of platelet count is associated with an increased platelet influx into the remnant liver, with a consequently higher platelet-dependent proliferative response, but also this mechanism requires experimental verification.

Platelet-derived serotonin is thought to be one of the candidate substances which are important in liver regeneration, since serotonin, or 5-hydroxytryptamine (5HT), is a potent mitogen which is involved in remodeling of tissue[4, 5, 19]. Platelets take up serotonin from the circulation by

serotonin transporters for internal storage[20]. About 95% of all serotonin found in blood is stored in platelets. In vivo, platelet-derived serotonin is responsible for postoperative liver regeneration by stimulating mitosis of hepatocytes via the 5-HT2A and 2B serotonin receptor subtypes which are present in hepatocytes [17]. Therefore, a close proximity between hepatocytes and platelets is likely needed[5]. Serotonin-antagonists or depletion both inhibit liver regeneration, suggesting that serotonin acts directly on the liver. However, more recently, the role of platelet-derived serotonin in liver regeneration has been challenged. Rodents deficient in the serotonin transporter SERT which have substantially decreased levels of serotonin in their platelets showed no difference in liver regeneration compared with controls, suggesting that serotonin is not relevant for liver regeneration [20]. Unfortunately, studies on the role of serotonin in liver regeneration are scarce and contradicting. Therefore, the precise mechanisms involved in platelet-mediated liver regeneration remain unknown.

It has been reported previously that a low post-operative platelet count measured just after the procedure was associated with delayed liver function recovery and with poor outcome after a liver resection[9]. These results are not necessarily in contrast with the results of the present study. The previous study suggested that a minimal platelet count is required for efficient platelet-mediated liver regeneration. In other words, in those patients with severe postoperative thrombocytopenia, there may be an insufficient translocation of platelets and platelet-associated growth factors into the liver remnant. In the present study, instead of examining the absolute platelet count, we have expressed the platelet count as percentage of preoperative platelet count, and found that a larger decrease in platelet count at day 3 was associated with a better outcome, which may be explained by a larger platelet influx with consequent release of platelet-derived growth factors in the liver remnant. This mechanism may be defective in those patients with a low platelet count immediately after surgery, but in the present study, none of the patients had an immediate post-operative platelet count of <100.000/µl.

In conclusion, this study shows a decrease in platelet count during the first three days after partial hepatectomy, which resolves within one week after surgery. The decrease in platelet count is stronger in patients with a high regeneration index compared to patients with poor regeneration, suggesting that more platelets are consumed when there is a high regeneration index during the process of liver regeneration.



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# 8

# Influence of preoperative chemotherapy on CT volumetric liver regeneration following right hemihepatectomy

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*Submitted*

## Abstract

**Background:**
An increasing number of patients undergo major liver resection following preoperative chemotherapy. Liver regeneration may be impaired in these patients, predisposing them to postoperative liver dysfunction. The aim of the present study was to evaluate the effects of preoperative chemotherapy on liver regeneration after partial liver resection.

**Methods:**
Patients planned for right hepatectomy either with (group B) or without (group A) prior chemotherapy were identified retrospectively from a prospective multi-institutional database created in the conduct of a national RCT investigating resection surface related complications. Prior chemotherapy was not an inclusion or exclusion criterion of the trial. Future remnant liver volume (FRLV) was calculated by measuring total functional liver volume (total liver volume – tumour volume) and resection specimen on preoperative CT-scans. Remnant liver volume after 7 days ($V_{RLV7days}$) was measured on scheduled postoperative CT scans. The early regeneration index 7 days after surgery ($RI_{early}$) was calculated as [($V_{RLV7days}$ - FRLV) / FRLV]* 100%. Data are expressed as median [interquartile range].

**Results:**
A total of 72 patients undergoing right hemihepatectomy were enrolled, 45 in group A and 27 in group B. In the whole group the liver remnant showed a 58% [4-202] increase in volume of the FRLV at day 7 [2-13 days]. The $RI_{early}$ was not significantly different between group A and B, 60% [5-202%] and 50% [4-126%], respectively ($p$=0.47). However, patients who had received more than 6 cycles of chemotherapy had significantly less early liver regeneration than patients treated with 6 or less cycles of chemotherapy.

**Conclusion:**
Preoperative chemotherapy does not seem to have a negative impact on early liver regeneration after partial liver resection, provided less than 6 cycles of chemotherapy are given.

## Introduction

Liver resection is the only potentially curative treatment for patients with colorectal rectal liver metastases (CRLM). Unfortunately, due to local irresectability, extra-hepatic disease and/or co-morbidity only 15-20% of the patients with CRM are eligible for liver resection. In an attempt to increase resectability rates, a number of combined surgical and chemotherapy strategies are increasingly applied for patients with CRLM [1-3]. Neo-adjuvant chemotherapy can change the surgical options and downstage patients who initially present with non-resectable disease [4]. In patients with initially resectable disease secondary tumours can be downsized in order to achieve a radical margin with a smaller resection [5]. The most commonly applied chemotherapy is oxaliplatin combined with capecitabine or folinic acid (leucovorin)/fluorouracil (5-FU) [6, 7]. In some patients, targeted molecular therapy (bevacizumab or cetuximab) is added to this regimen to achieve higher clinical response rates [8, 9].

Unfortunately, there is a clinical paradox, since preoperative chemotherapy may improve surgical options, but chemotherapy also has considerable hepatotoxic effects [10, 11]. Oxaliplatin can cause vascular changes in the liver such as sinusoidal obstruction syndrome, which increases morbidity after liver resection [11, 12]. 5-FU (insert ref Peppercorn Br J Cancer 1998;77:2008-11) and irinotecan (insert ref Parikh HH J Gastrointest Surg 2003; 7:1082-88) regimens can lead to steatosis of the liver and consequently increase the risk of impaired liver regeneration and postoperative liver failure. However, the exact effect of preoperative chemotherapy on liver regeneration after major liver resection in humans is unknown. The vast majority of liver regeneration occurs within the first week after major liver resections [13]. The aim of the present study was to investigate whether preoperative chemotherapy impairs liver regeneration in patients undergoing major liver resection. To that purpose CT- based liver volumetry was used before and 7 days after straight forward anatomical right hemihepatectomy.

## Method

### Patients

All patients undergoing anatomical right hemihepatectomy were identified retrospectively from the prospective database of the FRESCO-trial (title: "Efficacy of fibrin sealant in reducing resection surface related complications after partial liver resections", registration number: ISRCTN85205641). The FRESCO-trial was a multi center randomized controlled trial on the efficacy of fibrin sealant in reducing resection surface-related complications after partial liver resections [14]. In this trial, patients were intraoperatively randomized between application of fibrin sealant or no application of fibrin sealant. Primary endpoint of the study was the incidence of resection surface-related complications after partial liver resection. Part of the study was a CT scan at day 7 after liver resection to evaluate resection surface-related complications. Patients

were eligible to participate in this trial irrespective of whether they had had preoperative chemotherapy, and this factor did not play a role in the randomization procedure.
To investigate the effect of preoperative chemotherapy on liver regeneration in the present study, all patients who had undergone a right hepatectomy only, and had had pre- and postoperative scans as per protocol in the FRESCO-trial were divided into two groups: patients who were not treated with preoperative chemotherapy before undergoing liver resection (group A, no chemotherapy group) and patients who were treated with chemotherapy, either in an adjuvant or neoadjuvant setting, before undergoing liver resection (group B, chemotherapy group). The following information was recorded: type of chemotherapy regime, number of cycles and the date of last chemotherapy administration. The influence of chemotherapy-surgery interval and the number of chemotherapy cycles were analyzed in different subanalysis.

**Operative procedure.**
Liver resection was performed as detailed elsewhere [15]. In short, laparotomy was performed by bilateral subcostal incision, followed by intraoperative ultrasonographic assessment of the liver. Once resectability had been confirmed, mobilization of the liver was performed to prepare for hepatic parenchymal transection. In all these right hepatectomies, transection followed Cantlie's line from the top of the gallbladder, paralleling the middle hepatic vein straight to the suprahepatic inferior caval vein. In all patients the middle hepatic vein remained in situ with the liver remnant. There were subtle variations (e.g. type of incision) in the surgical procedures because these patients were operated by different surgeons in different centres, which is inevitable in a multicentre randomized controlled trial.

**CT imaging details.**
As part of the FRESCO-trial protocol, all patients had a contrast-enhanced triphasic CT-scan in their routine preoperative assessment and also a scheduled CT-scan 7 days after liver resection. All subjects were scanned in craniocaudal direction during inspiratory breath-hold. CT-images were acquired in a supine position. The CT-scanning protocol was predefined and agreed between centres in the context of the FRESCO-trial.



**Volume measurements.**
Two investigators (S.D.; P.K.) with considerable experience in abdominal CT-imaging volumetry performed all measurements. This was supervised by a specialist consultant abdominal radiologist (E.J.v.d.J.) and a hepatobiliary surgeon (M.d.B.). After transferring imaging data, volume measurements were performed on a Siemens Syngo workstation (version CT 2007A) with the "Volume calculation" application. Scans were retrieved from the individual patients in various centres, transferred and then all measured in the University Medical Centre of Groningen.
Regions of interest (ROIs) were manually drawn in the axial view on 2-mm slices in the portal

venous phase. ROIs were drawn in every other slice to minimize partial volume effects. The volume programme automatically interpolated between two pending ROIs. Each interpolation was revised and corrected manually if necessary. After finishing the definition of each individual ROI, the evaluation procedure was started. Approximate volumes were calculated with automatic multiplication of the circumscribed areas by the CT slice thickness. The vena cava and the gallbladder were excluded from the ROIs. For definition of the anatomical segments Couinaud's classification was used.

On the preoperative scan, total liver volume (TLV), the volume of the resection specimen ($V_{resection}$) and tumour volume ($V_{tumour}$) were measured. $V_{resection}$ was outlined according to Couinaud's classification and on the basis of hepatic (vascular) anatomy. Remnant liver volume after 7 days ($V_{RLV7days}$) was measured on the postoperative scan (figure 1).

**Calculations of volumes.**

Functional total liver volume ($TLV_{func}$) was calculated as: TLV - $V_{tumour}$. Future remnant liver volume (FRLV) was calculated as: TLV - $V_{resection.}$ The early regeneration index 7 days after surgery ($RI_{early}$) was calculated as: [($V_{RLV7days}$ - FRLV) / FRLV]* 100%. The functional resection percentage ($Funct_{Resection}$%) was calculated as: [($V_{resection}$ - $V_{tumour}$)/ $TLV_{func}$]*100%.

**Volumetric analysis Syngo®.**

For assessment of the accuracy of volumetric measurements of the liver with Syngo®, volumes of resection specimens were compared with actual weights of the resection specimens if these were available. The resection weights were measured in only two of the seven participating medical centres as this was not part of the FRESCO-trial protocol. This was done immediately after liver resection: weights of resection specimens were recorded in the operating theatre or at arrival on the pathology department. The actual weights of the resection specimens remained blinded to the investigators conducting CT-volumetry.

**Postoperative complications.**

Postoperative complications were registered according to the Clavien-Dindo score in the prospective database [16].

**Liver function and liver cell damage markers.**

Patients were admitted to the hospital one day preoperatively and routine blood tests were performed by the clinical chemistry department in the individual hospital. Routine blood tests for liver function and liver cell damage were performed on preoperative day 1, on postoperative day 0, 1, 3, 5, 7 and on the day of discharge. The levels of alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), gamma-glutamyl transpeptidase (γGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin total and prothrombin time (PT) were assessed.

**Ethics.**

The study was approved by the medical ethical committee of the University Medical Center of Groningen and conducted according to the Declaration of Helsinki. All patients gave written informed consent.

**Statistics.**

All data are expressed as median (interquartile range). To compare different subgroups the nonparametric Mann-Whitney *U*-test was applied. Dichotomous data were compared using Fisher's exact test. Multiple group comparisons for continuous data were done by Kruskal-Wallis test, with Dunn's post hoc test. A p value <0.05 was considered to indicate statistical significance. Statistical analysis was performed using Prism 4.0 for Windows (Graphpad software, Inc, San Diego, CA).

## Results

**Patients.**

Three hundred and ten patients were included in the FRESCO-trial of which 102 underwent a right hepatectomy. In 30 patients it was not possible to perform a complete CT-volumetric analysis (i.e. no adequate preoperative or postoperative CT-scan). This left 72 patients (35 male; 37 female) undergoing right hepatectomy for benign (n=7) or malignant liver tumours (n=65) for the final analysis. Twenty seven out of 72 patients (37.5%) had been treated preoperatively with chemotherapy and 45 out of 72 patients (62.5%) did not receive preoperative chemotherapy (group A) (figure 1).

There were no significant differences between groups in baseline characteristics (table 1). The vast majority of patients in group B (59.3%) received preoperative chemotherapy consisting of oxaliplatin, capecitabine and bevacizumab. The other patients in group B (40.7%) received a variety of other chemotherapy regimens (table 2). 18.5% of the patients in group B had adjuvant chemotherapy as part of treatment of the primary colorectal tumour and 81.5% of the patients in group B received neoadjuvant chemotherapy to treat the liver metastases. There was no significant difference in intraoperative blood loss between group A 750 mL [200-3,780 mL] and group B 800 mL [250-5,000 mL] ($p$=0.57). There was also no significant difference in operation time between group A and B: 310 min [150-675 min] and 320 min [150-600 min], respectively ($p$=0.49).

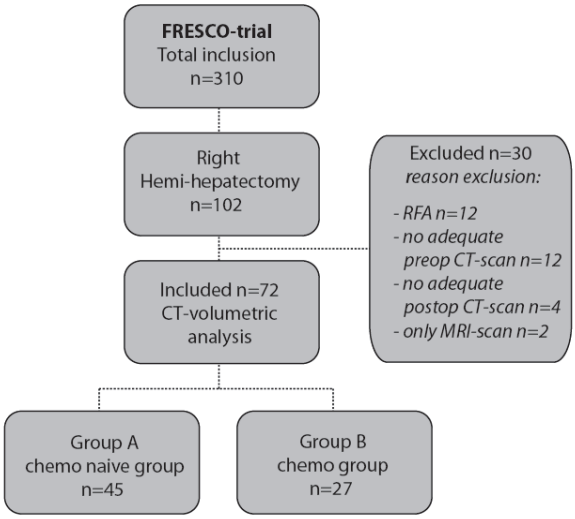


**Figure 1.** Flowchart of the study.

**Table 1.** Preoperative patient characteristics*

| | **Group A** No preoperative chemo (n = 45) | **Group B** Preoperative chemo (n= 27) | *p-value* |
|---|---|---|---|
| Age (years) | 61 (33-78) | 63 (40-79) | 0.70 |
| Sex | 19 M, 26 F | 16 M, 11 F | 0.22 |
| AST (IU/L) | 28 (7-58) | 26 (21-49) | 0.60 |
| ALT (IU/L) | 26 (7-69) | 28(13-71) | 0.35 |
| LDH (IU/L) | 206 (142-519) | 226 (154-663) | 0.37 |
| γGT (IU/L) | 48 (10-431) | 46 (17-204) | 0.66 |
| ALP (IU/L) | 93 (42-323) | 89 (57-365) | 0.86 |
| Bilirubin Total (μM) | 9 (4-43) | 12 (5-24) | 0.22 |
| C-Reactive Protein (mg/l) | 4.4 (1.0-88.0) | 4.0 (1.0-48.0) | 0.57 |
| Leucocytes (10Eg/L) | 6.8 (4.0-11.0) | 6.7 (3.0-10.0) | 0.13 |
| Albumin (g/L) | 45 (16-97) | 44 (38-46) | 0.30 |
| INR | 1.0 (0.9-1.0) | 1.0 (0.9-1.0) | 0.80 |
| PT (seconds) | 10.6 (10.0-14.0) | 10.7 (10.0 -13.0) | 0.51 |

*) Data are presented as median (interquartile range)
Abbreviations used: INR= International Normalized Ratio; PT=prothrombin time

**Table 2.** Specification of the chemotherapeutic strategies

| Group B, n=27 | Median (range) |
|---|---|
| Number of cycles | 6 (2-12) |
| Duration of chemotherapy (weeks) | 15 (5-31) |
| Time between chemotherapy and surgery (months) | 3 (1-24) |
| Type of chemotherapy: | Number (percentage of total) |
| Oxaliplatin, Capecitabine, Bevacizumab | 16 (59.3%) |
| Capecitabine | 3 (11.1%) |
| Capecitabine, Bevacizumab | 2 (7.4%) |
| 5-FU, Leucovorin, Oxaliplatin, Bevacizumab | 2 (7.4%) |
| 5-FU, Leucovorin, Oxaliplatin | 2 (7.4%) |
| 5-FU, Leucovorin | 1 (3.7%) |

**CT-measured liver volumes.**

The weight of the resection specimen as measured in 47 patients was 882 g [533-1760 g]. A strong significant correlation was found between the resection weight and resection volume measured with Syngo® ($p<0.0001$; $r=0.92$) (figure 2). In the total series (group A+B) the preoperatively calculated TLV, $V_{resection}$ and $V_{tumour}$ were 1591 mL [958-3002 mL]; 995 mL [541-1890 mL] and 34 mL [1-1468 mL], respectively. The $TLV_{func}$ and FRLV were 1527 mL [906-2378 mL] and 594 mL [321-1470 mL],respectively. $V_{tumour}$ was significantly larger in group A compared to group B: 38 mL [1-1467 mL] *vs.* 19 mL [1-93 mL] ($p<0.05$) (table 3).

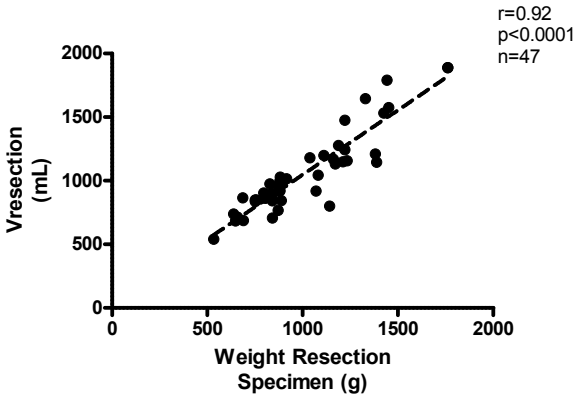


**Figure 2.** Correlation between volume measured with Syngo® and resection weight measured in the operating theatre.

**Table 3.** CT-volumetry of livers and tumours*

| N= 72 | Group A<br>No preoperative chemo<br>(n=45) | Group B<br>Preoperative chemo<br>(n=27) | *p-value* |
|---|---|---|---|
| TLV (mL) | 1595 (1145-3002) | 1550 (958-2266) | 0.36 |
| $TLV_{Func}$ | 1513 (968-2378) | 1570 (906-2177) | 0.57 |
| $V_{tumour}$ (mL) | 38 (1-1467) | 19 (1-93) | 0.04 |
| $V_{resection}$ (mL) | 1000 (682-1890) | 967 (541-1473) | 0.36 |
| $Funct_{Resection}$% | 59 (23-80) | 63 (42-77) | 0.10 |
| FRLV (mL) | 613 (378-1470) | 565 (321-975) | 0.25 |
| $V_{RLV7days}$ (mL) | 951 (574-1721) | 895 (460-1281) | 0.15 |
| $RI_{early}$ (%) | 60 (5-202) | 50 (4-126) | 0.47 |

Data are presented as median (interquartile range)

***Early liver regeneration.***

The $RI_{early}$ at day 7 in the whole group of 72 patients was 58% [4-202%]. The $RI_{early}$ was not significantly different between group A and B, (60 [5-202%] and 50 [4-126%] respectively; $p$=0.47) (table 3, figure 4A). In group B the time interval between chemotherapy and the liver resection was 3 months [1-24 months] (table 2). There was no significant difference in liver regeneration between patients who underwent liver resection within 3 months after chemotherapy (n=11) and patients who underwent liver resection after 3 or more months (n=16), 61 [4-84%] and 49 [10-126%] respectively; $p$=0.57). There was also no significant difference in liver regeneration between patients who underwent liver resection within 3 months after chemotherapy (n=11) and patients who did not receive chemotherapy (n=45), 61 [4-84%] and 60 [5-202%] respectively; $p$=0.x).

It can be argued that if the time interval between the last cycle of preoperative chemotherapy and liver surgery is more than 6 months the chance is small that chemotherapy has still an effect on liver regeneration. To analyse this, a subanalysis was performed in which patients with a chemotherpay-surgery interval of more than six months (n=5) were included in the group of patients who did not receive chemotherapy (n=45) and this group was then compared to the group of patients who received surgery within 6 months after chemotherapy (n=22). Again there was no significant difference in liver regeneration between these two groups: 51 [4-126%] and 59 [4-201%] respectively; $p$=0.71).

The $RI_{early}$ was significantly lower in patients who had had more than 6 cycles of chemotherapy (n=5) compared to patients who were treated with 6 or less cycles of chemotherapy (n=22) (37 [10-40%] and 62 [4-126%] respectively; $p$<0.05) (figure 4B). The time interval between chemotherapy and liver resection was not significantly different between patients who had received more than 6 cycles of chemotherapy compared to patients who were treated with 6 or less cycles of chemotherapy, (5 months [2-24 months] and 3 months [1-6 months] respectively; $p$=0.13).

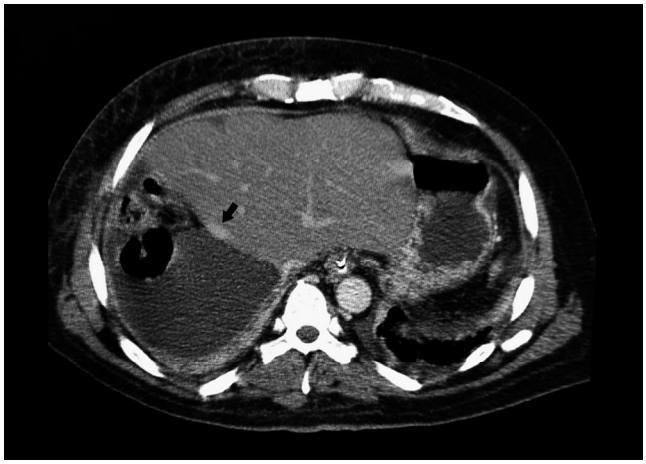

**Figure 3.** An outline of the remnant liver volume after 7 days ($V_{RLV7days}$) measured on the postoperative scan. Arrow shows the middle hepatic vein.

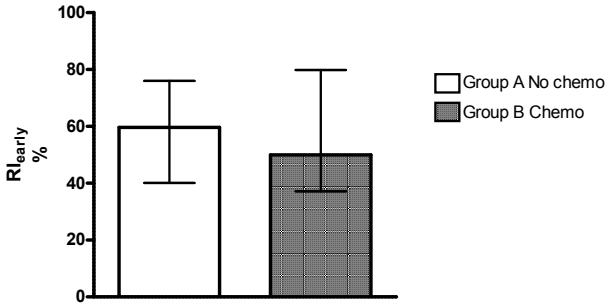


**Figure 4. A** $RI_{early}$, as a marker of early regeneration in patients without preoperative chemotherapy (group A) and in patients treated with preoperative chemotherapy (group B). Data are medians and interquartile ranges.





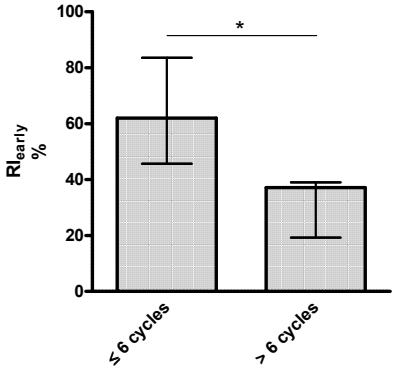


**Figure 4. B** $RI_{early}$, as a marker of early regeneration in patients who were treated with more than 6 cycles of chemotherapy versus those who were treated with 6 or less cycles of chemotherapy (*$p$<0.05).

Some patients did not have a postoperative CT-scan at day 7, but at an earlier or later time point. This deviation from the original protocol was usually related to early functional recovery of the patient and early discharge from the hospital. There was no significant difference between groups in the number of days between the operation and the first postoperative CT-scan (group A: 7 [2-13 days] vs. group B: 7 [3-13 days], $p$=0.24). However, in order to exclude a potential difference in liver regeneration due to difference in time (surgery/CT-scan interval), the $RI_{early}$ was also calculated and compared in patients who had a CT-scan exactly on postoperative day 7. In group A 26 patients and in group B 15 patients had a CT-scan exactly on postoperative day 7. In this sub-analysis the $RI_{early}$ was also not significantly different between group A and B, (58 [4-202%] and 73 [22-126%] respectively; p=0.84).

As mentioned $RI_{early}$ in patients who had more than 6 cycles of chemotherapy was significantly lower compared with patients receiving 6 or less cycles and this remained different when only patients who had a CT-scan strictly on postoperative day 7 were analyzed, (37 [28-38%] and 78 [22-126%] respectively; $p$<0.05).

In order to investigate the potential negative impact of bevacizumab on liver regeneration, the $RI_{early}$ in patients treated with preoperative bevacizumab was compared with patients who had not received bevacizumab. The $RI_{early}$ was not significantly different between patients (n=20) that had received bevacizumab and patients (n=7) who had not (56 [4-126%] and 38 [9-85%] respectively; $p$=0.18).

***Postoperative complications.***

There was no significant difference between groups in postoperative complications, length of hospital stay or 30-day mortality (table 4).

***Liver function and liver cell damage markers.*** In both groups ALT, AST, LDH, bilirubine and PT levels were postoperatively significantly increased compared to baseline ($p$<0.05). At the day of discharge ALT, AST, LDH, bilirubin and PT levels were normalized and comparable to baseline levels in both groups. Levels of γGT were significantly increased at postoperative day 7 and on the day of discharge compared to baseline. There was also a significant increase of ALP on the day of discharge compared to baseline in both groups (figure 5 A-G).

There were no significant differences in liver function and liver cell damage markers on the day before surgery between groups. Postoperatively, there were also no significant differences in liver function and liver cell damage markers between groups (table 1, figure 5 A-G).

**Table 4:** Postoperative complications

| | Group A N=45 No preoperative chemo | Group B N=27 Preoperative chemo | *p* |
|---|---|---|---|
| **Minor Complications**[#] | | | |
| Total | 17 | 9 | 0.80 |
| Grade I | 7 | 1 | 0.24 |
| Grade II | 10 | 8 | 0.58 |
| **Major Complications**[#] | | | |
| Total | 10 | 7 | 0.78 |
| Grade III a-b | 7 | 3 | 0.73 |
| Grade IV a-b | 2 | 1 | 1.0 |
| Grade V | 1 | 3 | 0.15 |
| Total | 27 | 16 | 1.0 |
| 30-day mortality | 1 (2.2%) | 3 (6.7%) | 0.11 |
| Length of hospital stay | 11 (4-83) | 10 (5-55) | 0.33 |
| Length of intensive care stay | 1 (0-51) | 1 (0-21) | 0.07 |

[#] graded according to the Clavien–Dindo classification [16]

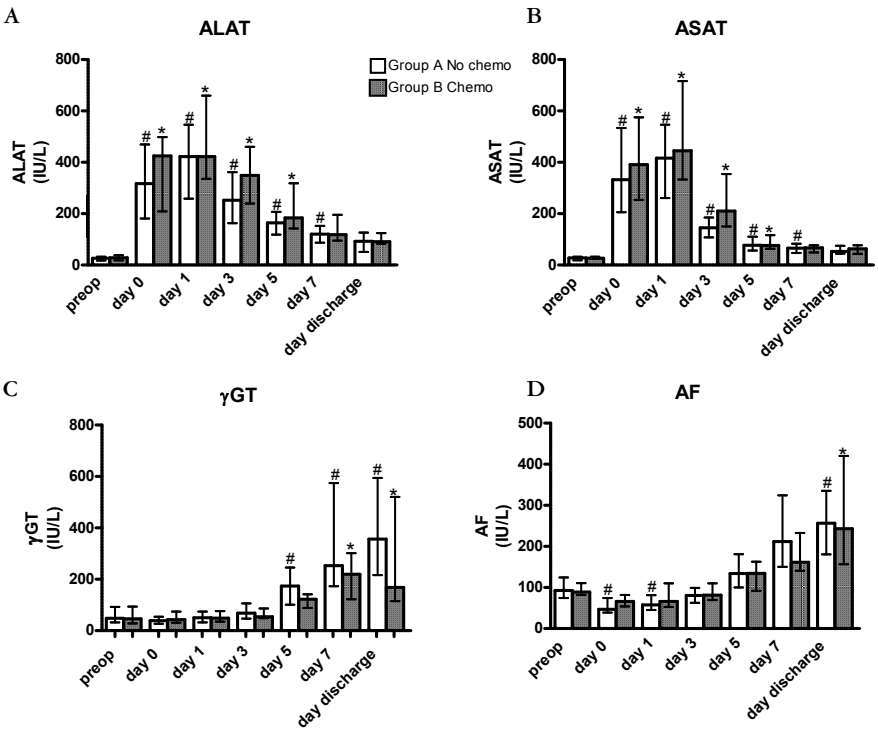


**Figure 5.A-D** Postoperative plasma levels of ALAT, ASAT, γGT and ALP as markers of liver cell damage. Group A [#]$p<0.05$ compared to preoperative baseline level; group B *$p<0.05$ compared to preoperative baseline level. No significant differences between groups. Data are medians and interquartile ranges.

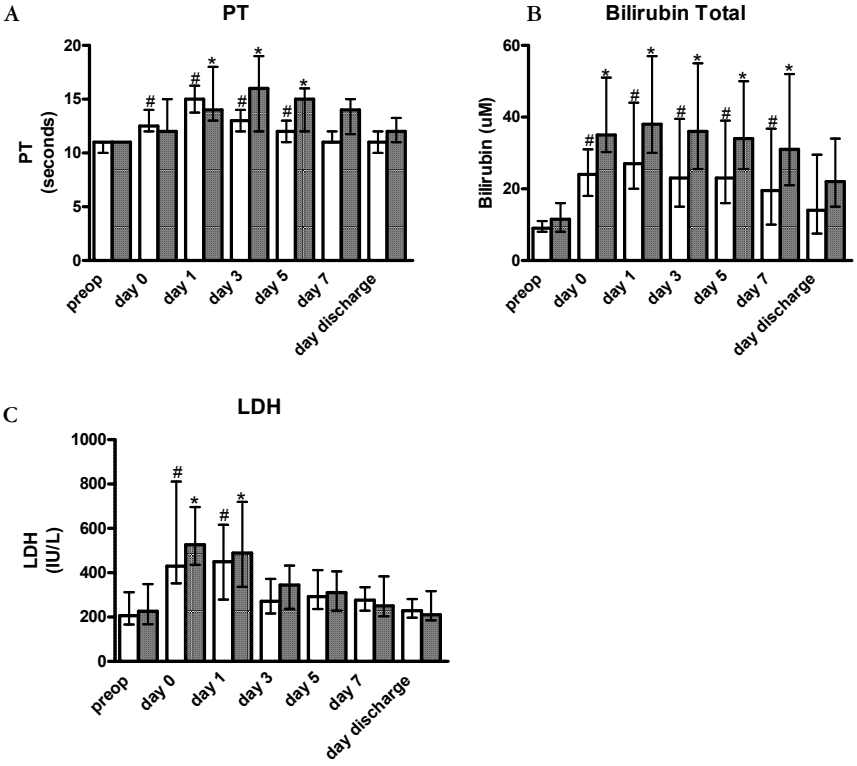


**Figure 6.A-C** Postoperative plasma levels of PT and bilirubin, and LDH as markers of liver function and liver cell damage, respectively. Group A # $p<0.05$ compared to preoperative baseline level; group B *$p<0.05$ compared to preoperative baseline level. No significant differences between groups. Data are medians and interquartile ranges.

## Discussion

This study aimed to investigate whether preoperative chemotherapy impairs liver regeneration in patients undergoing major liver resection. To that purpose CT-based liver volumetry was used before, and 7 days after right anatomical hepatectomy. This study indicates that chemotherapy prior to major liver resection has no negative impact on early liver regeneration. However, patients who had received more than 6 cycles of chemotherapy had significantly less early liver regeneration than patients treated with 6 or less cycles of chemotherapy.

There are only few studies that have focused on early liver regeneration. Most of these studies measure the early liver regeneration index in living donor for liver transplantation and the results in these studies vary, presenting different regeneration indices after one week [13, 17, 18]. The data of the present study showed that approximately 58% of liver regeneration takes place in the first week after resection of the right lobe. These results are in accordance with results from Zappa *et al.*[17] who reported a regeneration index of 56% in the first week in patients undergoing

right hepatectomy. Although several studies warn for the hepatotoxic effects of preoperative chemotherapy on liver regeneration in liver resection patients [10-12, 19], data on the exact effect of preoperative chemotherapy on liver regeneration in humans are scarce. To our knowledge the present study is the first study that analyses the effect of preoperative chemotherapy on liver regeneration in a large group of patients after major liver resection. All patients underwent an anatomical right hemihepatectomy and there were no major differences in mobilization and transection of the liver. Therefore these patients were comparable and the protocol of this randomized controlled trial provided the opportunity to investigate early liver regeneration in an adequate and valid human study model. Since there is no reliable marker to assess liver regeneration in humans, CT- volumetry is an attractive approach. The CT-volumetric method with Syngo®, a professional radiological software program used in the present study, was shown to be valid and reliable as evidenced by the excellent correlation between resected weight and volume. This method was comparable with other recently validated CT-volumetry methods [20, 21].
The type of chemotherapy and also the number of cycles of chemotherapy that are given to patients often varies. One would expect that patients have worse liver regeneration with an increasing number of cycles of chemotherapy because their liver is longer exposed to hepatotoxic compounds. This hypothesis was confirmed in the present study as a smaller $RI_{early}$ was measured in patients who were treated with more than 6 cycles of chemotherapy compared with those who were treated with 6 or less cycles. Impaired early liver regeneration in these patients is probably also associated with an increased risk of complications after liver resection. In line with this Karoui *et al.* found that preoperative chemotherapy was an independent predictive factor of postoperative morbidity after liver surgery especially when an increasing number of cycles was given to patients [22]. In order to reduce the risk of impaired liver regeneration and postoperative morbidity in patients who need preoperative chemotherapy, it would be better to minimize the number of cycles of chemotherapy if possible instead of completing the chemotherapeutic regimen. These data also provide evidence that treating patients with initially resectable disease with more than 6 cycles of chemotherapy is not advisable due to the increased risk of impaired early liver regeneration.
Contradictive results have been reported by several researchers regarding the potentially negative impact of bevacizumab on liver regeneration. Aussilhou *et al.* [23] have shown that bevacizumab impairs liver regeneration after preoperative portal vein embolization. On the other hand Gruenberger *et al.* [24] showed that neoadjuvant bevacizumab does not affect liver regeneration 3 months after resection. The same group of authors also showed in another study that when bevacizumab is combined with oxaliplatin, bevacizumab protects the liver against sinusoidal obstruction syndrome [25]. Data of the present study showed that the preoperative administration of bevacizumab did not impair early liver regeneration after major liver resection.
The present study has some limitations. It was assumed that percentage early liver volume increase assessed with CT-volumetry reflects liver regeneration and also liver function, however

part of the liver volume increase may be caused by edema or hepatic swelling due to high portal pressure induced by the concentrated flow of the entire stream of portal blood toward the small remnant liver [26]. There were no significant differences between group A and B in traditional liver function and liver cell damage markers preoperatively and postoperatively in the first week. Yet, it has to be taken into account that these are relatively crude markers [27]. In this context, it is worthwhile mentioning some recent reports using a combination of CT volumetric analysis and the LIMAx-function test [28, 29]. Investigating recovery of liver function with LIMAx-test in the first week after liver resection in patients with or without preoperative chemotherapy in future studies may be an interesting future topic.

In conclusion, this study shows that preoperative chemotherapy does not seem to have a negative impact on early liver regeneration after partial liver resection. However there was a significantly lower regeneration capacity in patients who were treated with more than 6 cycles of chemotherapy. This study shows that strategies combining preoperative chemotherapy with liver surgery are safe, based on liver regeneration in the first week, provided less than 6 cycles of chemotherapy are given.

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# General discussion and conclusions

## Part one: Thermal ablation.

Although liver surgery is considered the gold standard in the treatment of malignant liver tumors, minimally invasive therapies have gained interest in the past decades. Local thermal ablative therapies offer an attractive treatment in patients who are not suitable for surgical resection. The main goal of these locally directed therapies is to achieve a survival rate similar to that of partial hepatectomy[1-4]. However, this is an issue which is still debated. Additionally, locally directed therapies are accompanied with less post-treatment morbidity. Thus, these offer great advantages over surgical resection.

The main problem with thermal ablation is the unacceptably high incidence of ablation site recurrence, which is the result of incomplete ablation. In the literature, ablation site recurrence rates vary widely, depending on the patient population and the definition of ablation site recurrences[2, 3, 5-8]. Chapter 2 and 3 describe a series of patients who underwent radiofrequency ablation for colorectal liver metastases. The ablation site recurrence rates in both series approximate 20 % and are at the lower limit of what is reported in the literature. In line with previous studies, we found that the approach of the procedure – percutaneous versus open - and tumor size – tumors larger than 3 cm in diameter - are main risk factors associated with a higher ablation site recurrence rate. Interestingly, the ablation margin was not a risk factor for ablation site recurrence. However, it should be noted that ablation site recurrences did not occur with the ablation margins greater than 1 cm. This could point towards the importance of the size of the ablation margins.

In **Chapter 2**, the concept of a "Similarity Positioning Score" is introduced. This two-point scale, obtained by visual assessment, describes the comparability of the pre-operative scan and the first post-operative scan, which is the baseline control scan used in the further follow-up. Well-comparable scans were assigned the score "SiPS-identical", whereas incomparable scan were classified as "SiPS-different". When measuring ablation margins by comparing pre-treatment and post-treatment scans, it can be noticed sometimes that the liver (and other abdominal organs) has totally different positions on both scans. These are the SiPS-different scans. The dimensions of the tumor and the ablation zone may differ, so that measurements on such incomparable scans become highly unreliable. The distance in a certain direction from the border of the ablation zone to the border of the liver may be larger than the distance from the border of the tumor to the border of the liver. These "negative" margins are often encountered on SiPS-different scans, thereby hampering the radiologist to give a well-founded judgment on the success of the ablative procedure. The results in chapter two show that SiPS is an important risk factor for ablation site recurrences, not because incomparable scans are directly related to ablation site recurrences, but because of the lack of a reliable statement on the success of the ablative procedure. Unfortunately, it is impossible to obtain two exactly similar images even within one multiphase scan series, let alone after manipulation in the abdomen, as is the case after thermal ablation. Additionally, the normal respiratory related movements of the liver due to variations in diaphragm position may

contribute to these position differences. Motion tracking systems and mathematical models which correct for organ position differences may reduce the incomparability of two scans. However, they are not widely used in general radiology and until then, these patients should be followed up thoroughly.

Follow-up after thermal ablation is performed with clinical, biochemical and imaging procedures at regular intervals. Imaging consists of multiphase CT-scanning in order to detect ablation site recurrences as soon as possible. The interval at which imaging is performed, varies between centers. In the University Medical Center Groningen (UMCG), patients undergo CT-scanning every three months in the first two years after the procedure and thereafter biannually. Unfortunately, it may be very difficult to detect ablation site recurrences, especially in early phases when the ablation site recurrence is very small and eventual post-procedural inflammatory changes are present, which may be confused with ablation site recurrences[9]. Additionally, when subsequent scans are incomparable – SiPS may also apply to subsequent follow-up scans – it might be extremely difficult to compare these scans section-by-section. PET-CT is regarded the best follow-up modality in the detection of ablation site recurrences, but is not used routinely because of its drawbacks – mainly its costs[1011, 12]. A more simple method to detect early changes in the size of an ablation zone is by measuring its volumes on successive scans and compare the results of these measurements. The use of volumetry in the follow-up after radiofrequency ablation is studied in **Chapter 3**. Volumetry showed to be a valuable tool in the follow-up of after radiofrequency ablation with a detection rate of ablation site recurrences of 96 %. For comparison, conventional two-dimensional evaluation with CT showed a detection rate of 63 % and ablation site recurrence was doubted in 11 %. Thus, the detection rate of ablation site recurrences with conventional CT is at best 74 %. Volumetry provides highly detailed three-dimensional information on ablation zones, whereas all other imaging modalities, including PET-CT, only reveal two dimensional information. Additionally, it is not hindered by organ position differences. However, volumetry has two main drawbacks. Firstly, volumetry can be disturbed by artifacts, for example produced by the presence of coils after portal vein embolisation. This was the case in the only one ablation zone with ablation site recurrence which was not detected with volumetry in chapter 3. Therefore, volumetry should be used with caution in patients who underwent possible artifact-causing procedures. Secondly, it is relatively time-consuming to perform. However, since ablation zones are relatively small, it is recommended to perform volume measurements in addition to conventional evaluation methods, since the earlier ablation site recurrences are detected, the sooner patients can be offered a secondary treatment.

As shown in Chapter 2 and 3, imaging is the cornerstone in the follow-up after thermal ablation. After thermal ablation, imaging is performed at regular intervals. A well-documented post-procedural baseline scan is the starting point in the follow-up of patients who underwent thermal ablative procedures. This means that quantitative and qualitative assessment should be performed

vigorously on this scan. It is not well known what the ideal time point is to perform the baseline scan. Centers have their own protocol on when to perform the first scan after ablation[13]. The timing of this baseline scan varies from immediately at the end of the procedure to up to a month post-procedurally[13]. In the UMCG, patients are scheduled for the baseline scan one week after thermal ablation. When thermal ablation is performed with laparotomy, ultrasound is performed after the procedure to assess the completeness of the ablational procedure. In case of percutaneous CT-guided ablation, a non-contrast enhanced CT-scan is performed immediately after the thermal ablative procedure for a rough first evaluation of the ablation. Extensive evaluation is only performed on the protocol scan one week post-procedurally. In our own experience, the success of radiofrequency ablation can be reasonably estimated on immediate post-procedural non-contrast enhanced scans. With the introduction of microwave ablation, the same assumption was made: immediate post-procedural non-contrast enhanced scans should also be suitable for gross evaluation of the completeness of the microwave ablative procedure. Unfortunately, it became clear that ablation zones created by MWA were more difficult to visualize than ablation zones created by RFA on immediate post-procedural non-contrast enhanced scans. This resulted in a higher incidence of incomplete ablations than we were used to with RFA. These incomplete ablations became only visible on the baseline scan performed one week post-procedurally. Therefore, the question rose whether an immediate post-procedural contrast enhanced scan is of additional value in the initial evaluation of the success of a microwave ablation procedure. The results are discussed in **Chapter** 4. Ablation zones were evaluated quantitatively. Quantitative assessment included measurements of ablation margins, volumes and Hounsfield units on the immediate post-procedural scan. The same was performed on the protocol scan one week post-procedurally. Quantitative assessments of both scans were then compared to see whether significant changes occur in the first post-procedural week. The protocol CT-scan performed one week post-procedurally was used as gold standard. Thus, the evaluation could only be performed retrospectively, since the protocol CT-scan one week post-procedurally had to become available. The results in Chapter 4 show that immediate postprocedurally performed quantitative measurements were highly unreliable because ablation zones increased in volume in the first week after the MWA-procedure. Ablation margins, volumes and diameters of the ablation zone changed significantly within this period. Therefore, quantitative assessment should not be performed immediately after thermal ablation. Thus, the baseline scan performed one week post-procedurally is indispensable for the quantitative baseline control values, which should be used in the further follow-up. We recommend this time point of measurement, since the volumes of ablation zones are not stationary in time[14]. Ablation zones show an increase in volumes in the first post-procedural week to a maximum size and after three months, a decrease in volume is seen. Although the results in Chapter 4 are observations from microwave ablation procedures, there are no reasons to assume that there are differences for radiofrequency ablation, since tissue damage is afflicted according to the same principles.

In conclusion, imaging after thermal ablation is highly important for the post-procedural follow-up. Incomparability between the pre-operative and baseline control scan hinders evaluation of the success of thermal ablative procedures, since ablation margins cannot be reliably assessed. Volume measurements should be performed at any time point in the follow-up, since subtle changes can be detected with volumetry which otherwise remain undetectable with the naked eye. After thermal ablation, quantitative assessment should only be done on scans performed one week post-procedurally, since shape and size of ablation zones change significantly within this period.

## Part two: Liver regeneration.

Partial hepatectomy is increasingly performed in patients with liver tumors. With the availability of improved imaging techniques, it is now possible to carefully select patients with tumors restricted to the liver or with limited extrahepatic disease. Pre-operative chemotherapy may lead to downstaging of the tumor load in patients who would otherwise be unsuitable candidates for surgical resection, but is associated with hepatotoxicity[15, 16]. Improved intra-operative and postoperative management has improved the safety of hepatic surgery. These factors together have lead to expansion of the indications of partial hepatectomy. The main limitation of partial hepatectomy is that there needs to remain a sufficient amount of functional liver, since extensive resection may lead to liver failure and death within a few days after surgery. Several strategies which may increase liver volume and function of the future liver remnant before resection are developed with the intention to make hepatic surgery safer. The most commonly used technique is portal vein embolisation. There are several experimental pharmacologic approaches, of which the effectiveness has to be established yet[15].

After partial hepatectomy, the liver regenerates in response to lost tissue. There are several studies on liver regeneration, but most have been conducted in living donors[17, 18, 19, 20, 21, 22, 23]. Patients were selected based on the type of partial hepatectomy, instead of the amount of resected volume. Since there is a considerable interpatient variability in the size of the various segments, the amount of resected volume may differ between patients with the same type of partial hepatectomy[24]. Additionally, the regenerated volume is thought to be related to the amount of resected volume[20, 21, 25]. Therefore, liver regeneration should be measured against the amount of liver tissue resected. This is studied in **Chapter 5**. The patients enrolled in the study on liver regeneration were patients included in the FRESCO-trial, a study on the benefits of fibrin sealant on the resection surface. A pre-operative CT-scan was available and all patients all underwent a protocol CT-scan exactly 7 days after partial hepatectomy. The pre-operative total liver volume, the volume of the part to be resected and eventual tumor volumes were measured on the pre-operative CT-scan. With these data, the volume of the future liver remnant could be estimated. Tumor volumes were subtracted from the total pre-operative liver volume and the volume of the part to be resected, since the tumor does not contribute to the effective liver volume. With these data, patients were classified

in 5 groups, based on the extent of the resection. Volumes of the liver remnant were measured on two time points: (1) exactly one week after partial hepatectomy and (2) 6 months after partial hepatectomy if there was a CT-scan available at this time point. Liver regeneration was expressed in two "regeneration indices", namely an early regeneration index (one week postoperatively) and a total regeneration index (6 months postoperatively). These regeneration indices were calculated as the increase in liver volume, with respect to the volume of the future liver remnant as measured on the pre-operative scan. The results in Chapter 5 show that regeneration indices become higher as resections become larger. Interestingly, the early regeneration index shows a plateau in patients with a resection of 40 % or more of their total pre-operative liver volume. An explanation for this finding may be that the process of liver regeneration is of secondary importance to the metabolic demands of the patient. Patients with a resection of 40 % or more have a relatively small liver remnant. There remain less hepatocytes to preserve appropriate liver function. Cells which are actively replicating – undergoing mitosis – do not take part in hepatic metabolism. Vice versa, cells fulfilling metabolic and detoxifying functions cannot enter the mitotic phase. Thus, metabolic functions prevail over the process of liver regeneration until there are enough hepatocytes to carry out both functions properly and regeneration is delayed after larger resections. This hypothesis is supported by the total regeneration index. The finding that the total regeneration index increases steadily with larger resections points towards a catch-up growth in patients with major partial hepatectomies. Although liver regeneration seems to be highly efficient, the majority of patients did not reach their total pre-operative liver volume. This was especially the case in patients with large resections. It might be that the time point of six months post-surgically was chosen too soon and that volume increase continues beyond this time point. Since it is not known when liver regeneration is finished, it seems a topic worthy of future investigation to assess whether liver volume increases beyond the time point of six months after partial hepatectomy.

It is well known that liver regeneration depends on various factors. Age, sex and the presence of underlying parenchymal disease affect the process of liver regeneration. Cirrhosis is a well-known parenchymal disease which is a risk factor for impaired regeneration. A more common parenchymal condition is steatosis. The incidence of hepatic steatosis is thought to be increasing due to the current obesity epidemic. Hepatic steatosis is not as innocent as was previously assumed. It can lead to non-alcoholic steatohepatitis, cirrhosis and the development of hepatocellular carcinoma. Additionally, some studies have found increased post-operative complications in patients with steatosis[2627-29]. Most studies on liver regeneration are performed in animal models[30, 31]. Studies on the impact of steatosis on liver regeneration in humans are scarce and most studies concerned on living donors[19, 20, 28, 32, 33]. Most studies have suggested that regeneration is not impaired by the presence of steatosis. The main problem with these studies is that they were conducted in living donors. Clinically significant steatosis is generally a contraindication for living donation, in contrast to partial hepatectomy for liver tumors. Therefore, we aimed to determinate whether liver regeneration is impaired by the presence of steatosis in patients undergoing elective partial

hepatectomy. The results are discussed in **Chapter 6**. The same patient data were used as in chapter 5, but now with the exclusion of the living donors. Patients were classified in 3 groups according to the degree of steatosis and regeneration indices were compared between the groups. There were no differences in the amount of resected volume and other factors that might have influence on liver regeneration between the patient groups. Of note, no patient with clinically significant steatosis had received chemotherapy prior to partial hepatectomy. This is an important issue, since chemotherapy is a well known risk factor for induction of hepatic steatosis[34]. The only significant difference was the BMI, which is known to be related to hepatic steatosis. The results in Chapter six show that although the early regeneration index is not significantly affected by hepatic steatosis, the total regeneration index was significantly lower in the presence of steatosis. This was most pronounced in patients with clinically significant steatosis. Animal models show that steatosis is associated with an excessive pro-inflammatory cytokine response and insufficient antioxidant response, which predispose hepatocytes to extensive necrosis. This leads to increased hepatocellular damage, which affects the regenerative capacity of steatotic livers after partial hepatectomy[30, 31]. It is assumable that this also applies to human livers. The study in Chapter 6 is the first which reports on liver regeneration and hepatic steatosis in patients with elective partial hepatectomy. Since steatosis was associated with a lower regeneration response, extensive liver resections should be performed with caution in patients with hepatic steatosis.

Following partial hepatectomy, liver regeneration is initiated by a complex series of growth factors and cytokines. Platelets have gained interest in their role in liver regeneration because they contain multiple growth factors[35-37]. Experimental models have shown that thrombocytopenia result in decreased proliferative activity in the liver[35, 38, 39]. Thrombocytosis is associated with accelerated liver regeneration. In humans, it has been showed that a low post-operative platelet count was an independent predictor for delayed recovery of liver function[40]. In **Chapter 7**, the decrease in post-operative platelet counts was studied in association with the regeneration response. The same patient data was used as in Chapter 5. Firstly, patients were classified in five groups, according to the extent of the resection (see Chapter 5). Within each patient group, patients were assigned as having a poor, intermediate or high regeneration response. Thereafter, all patients were joined into three patient groups, according to their regeneration response: poor, intermediate or high. Platelet counts were assessed pre-operatively, upon arrival at the intensive care unit and then daily until discharge. The decrease in postoperative platelet count, expressed as percentage of the pre-operative platelet count, was compared in these three groups. In all groups, platelet count decreased in the first three postoperative days, but the decrease was most pronounced in the patient group with high regeneration. The question rises whether this finding means that platelets have a role in liver regeneration. It has been reported that there is a decrease in platelet count after partial hepatectomy[39, 41-43]. However, this has never been linked to liver regeneration. Postoperatively, there is entrapment of platelets at the site of injury. It may be that platelets could

exert their regeneration-potentiating effect directly at the site they are needed. Large resection surfaces could theoretically lead to entrapment of more platelets than small resection surfaces. However, the results in Chapter 7 are corrected for this bias, because patients were classified in having poor, intermediate or high regeneration responses according to the amount of resected volume. A larger decrease in postoperative platelet counts in patients with a high regeneration response suggests a link between platelets and liver regeneration. The mechanism beyond this finding has to be clarified in future research.

Partial hepatectomy is nowadays mostly performed in patients with primary and secondary malignant liver tumours. Although only a minority of patients are candidates for surgery, criteria for respectability are changing, partly due to innovations of cytotoxic agents and improvements of chemotherapeutic regimens. A major side effect of chemotherapy is hepatotoxicity, resulting in increased postoperative morbidity and mortality[15, 16]. Injuries to the liver parenchyma induced by pre-operatively administered chemotherapy could theoretically have a negative effect on postoperative liver regeneration. However, the effect of pre-operative chemotherapy on liver regeneration has not been studied well in human. **Chapter 8** studies the impact of pre-operative chemotherapy on early liver regeneration. Patients enrolled in the FRESCO-trial who underwent right hemihepatectomy were included. Volumetry and calculation of the early regeneration index were performed as described in Chapter 5. There was no difference between the regeneration index in patients with or without pre-operative chemotherapy. However, patients who received more than 6 cycles of chemotherapy had significantly smaller early regeneration indices than patients who received less than 6 cycles of chemotherapy. It has been reported that an increasing number of pre-operative chemotherapy increases the risk of postoperative morbidity and mortality[44]. Together with the results of Chapter 8, it is advisable to minimize the number of chemotherapeutic cycles prior to surgery to lower the risk of increased postoperative morbidity, mortality and impaired liver regeneration. This especially applies to patients undergoing large resections.

In conclusion, liver regeneration highly depends on the amount of resected liver volume. The early regeneration index increases as resections become larger. The early regeneration index – one week after partial hepatectomy – plateaus in patients with a resection of 40 % or more of their pre-operative total liver volume. However, the total liver regeneration index – 6 months after partial hepatectomy – shows a steady increase in regeneration indices with larger resections. This indicates that patients with larger resections show a catch-up growth after an initial delay in liver regeneration. Liver regeneration is affected by hepatic steatosis and large resections should be performed with caution in patients with this condition. The role of platelets in liver regeneration in humans has to be elucidated, but patients with a high regeneration response show a larger decrease in postoperative platelet counts compared to patients with intermediate and poor regeneration.

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# Summary

The aim of this thesis is twofold. Part one concerns on imaging after thermal ablation. Part two discusses liver regeneration after liver surgery.

## General introduction.

Among all organs, the liver is a unique because of its ability to regenerate after injury, for example partial hepatectomy. This regenerative capacity was already recognized in ancient history and has been mentioned in Greek mythology. Liver surgery has traditionally been performed in battle field injuries. Because the liver is a highly perfunded organ, massive hemorrhage is the major risk in liver surgery. Therefore, patients often did not survive liver surgery. The first well-documented partial hepatectomy dates from 1887. Thereafter, larger studies followed soon. However, liver surgery remained risky with a high mortality. This changed with improvement of surgical techniques and postoperative care. A major breakthrough was the anatomical segmentation of the liver by the French surgeon Couinaud in 1957. He devided the liver into eight segments, each with a seperate blood and biliary supply. When these anatomical distributions are taken into account during partial hepatectomy, blood loss and bile leakage could be reduced significantly in liver surgery. Liver surgery is nowadays mostly performed in patients with malignant liver tumors and is regarded as the therapy of first choice. Benign liver tumors are less often treated surgically. Most malignant liver tumors are metastases from a primary tumor elsewhere in the body. Colorectal cancer is one of the most common types of cancer and metastasizes often to the liver. Unfortunately, the majority of patients (80-90 %) with colorectal liver metastases are no candidates for surgical treatment because of the extent of the disease. For these patients, other treatment options need to be sought. The aim of these secondary treatments is to destroy the tumors, but to spare liver tissue. Thermal ablation is one of the most widely used forms of this type of secondary therapies. A needle - the electrode – is inserted in the tumor under image guidance. Subsequently, the electrode is heated, for example by radiofrequency waves as in radiofrequency ablation or electromagnetic waves as in microwave ablation. Tissue in the vicinity of the electrode is destroyed by this heating. Radiofrequency ablation and more recently microwave ablation are the most common forms of thermal ablation. Thermal ablation can be performed by opening the abdomen (laparotomy), by keyhole surgery (laparoscopy) or through the skin (percutaneous). In thermal ablation by laparoscopy and laparotomy, localization of the tumor and placement of the electrodes is performed by using ultrasound. For percutaneous thermal ablation, ultrasound can be used, but the needles are usually placed under computed tomography guidance (CT). Imaging is also essential to monitor the progress of treatment. Additionally, imaging is used at the end of treatment to check whether the ablation zone overlaps the tumor. The major advantage of thermal ablation is that it is associated with less postoperative morbidity than liver surgery. The major drawback of thermal ablation is that the cure rate is significantly lower than after liver surgery, especially because the tumor often seems to recur in or around the ablation zone. These

are the feared ablation site recurrences. The incidence of ablation site recurrences varies widely in the literature from 2 % to 55 %. It is important that ablation site recurrences are detected at an early stage, because the sooner they are discovered, the better the chances are for successful additional treatment. After thermal ablation, patients will undergo intensive follow-up. This is performed by regular physical, biochemical and imaging studies. Imaging is mostly performed with multi-phase CT examination. This means that firstly a scan without contrast is obtained: the non-contrast scan. Thereafter, contrast is administered intravenously to the patient and scanning is performed on three time points after contrast administration, namely the arterial phase, the portal venous phase and the late phase. In some centers, magnetic resonance imaging (MRI) or positron emission tomography (PET), with or without with CT is performed.

## Part 1: Imaging after thermal ablation.

In contrast to liver surgery, after thermal ablation no tissue is obtained for pathological-anatomical examination. After partial hepatectomy, the pathologist investigates whether the resection margins are tumor-free. This is not possible after thermal ablation, since no tissue is removed. Destroyed tissue remains in situ. Postprocedural imaging is the only way to assess the completeness of thermal ablation. During percutaneous thermal ablation, multiple non-contrast CT scans are performed to monitor the progress of the procedure. If the clinician is convinced that the procedure is completed, a last non-contrast CT scan is performed to see if the tumor overlaps the ablation zone completely. When this is the case, the ablation is finished. If it appears that the ablation zone does not fully enclose the tumor, the procedure is continued until the ablation zone is large enough. This last non-contrast scan is a rough first check. Thereafter, patients will be followed at regular intervals with imaging studies. Follow-up imaging is usually performed with multiphase CT scans, although in some centers MRI or PET are used instead. The first multiphase CT-scan is performed one week after thermal ablation. This baseline control scan is used to determine definitively whether the treatment has been successful or not. The radiologist looks for contrast enhancement which may indicate the presence of residual tumor in or around the ablation zone. In addition, the diameter of the ablation zone and ablation margins are measured, analogous to the size of the tumor-free resection margin after liver surgery. These dimensions are used as comparative material for subsequent scans which will be performed in the follow-up. To obtain these measurements, the preprocedural scan is compared with the baseline postprocedural control scan. The main issue is that a very precise, section-by-section comparison is required for the measurements. The liver must be situated in (nearly) the same position on both scans. Unfortunately, it appears quite often that this is not the case. Measurements of the ablation zone on such incomparable scans are unreliable, when the liver has different positions on both scans. These differences in position of the liver may be explained by the fact that the liver is situated just below the diaphragm. Respiratory movements result in rotational position

differences of the liver. Although an abdominal CT scan is always performed in full inspiration, the depth of inspiration often differs between two scans. As a result, the liver may be positioned differently on both scans. **Chapter two** discusses that these positional differences have significant influence on the reliability of measurements and that they are a major risk factor for ablation site recurrences. In this chapter, a scoring system is introduced – the Similarity Postitioning Score (SIPS). This score classifies the preprocedural and postprocedural CT-scans as well comparable or badly comparable (SiPS-identical and SiPS-different). Measurements performed on SiPS-different scans will give unreliable results. SiPS is also a risk factor for ablation site recurrences. Of course, SIPS does not cause directly ablation site recurrences. If the preprocedural and postprocedural images are not well comparable, no reliable judgment can be given on the success of the ablation. Thus, only time will learn whether the procedure was actually successful: whether ablation site recurrences will appear in the months after the ablation or not. Unfortunately, this problem has to be overcome yet. A possible solution would be the use of specialized software that mathematically corrects for differences in the position of the liver on the preprocedural and postprocedural images. Such images are "converted" by recalculation in order to project the liver in an equal position on both scans. Another possible solution is the so-called "respiratory motion tracking system" where the depth of inspiration is monitored by sensors and an image is created at the appropriate depth. These methods are used mainly in the radiotherapy, but not for this purpose in radiology. Perhaps this is worthy for future investigation.

After this first postprocedural scan, imaging is performed regularly in the follow-up. Patient undergo multiphase CT-scanning every three months during the first two years after treatment and thereafter biannually. The radiologist evaluates the ablation zone and compares the images with previous scans. Normally, ablation zones become smaller with time. One of the signs of an ablation site recurrence is that the ablation zone increases in size. Ablation site recurrences will usually result in slow growth of the ablation zone, especially in its early stages. This growth is barely visible to the naked eye. Other features of ablation site recurrences are specific contrast-enhancement patters, but these can be easily missed when the ablation site recurrence is small. Early detection of ablation site recurrences is extremely important. **Chapter three** describes whether volume measurements of ablation zones can help in the early detection of ablation site recurrences. Volumetry provides three-dimensional information: length, width and height. Measurements of the diameter of the ablation zone provides only two-dimensional information: length and width. The results show that ablation site recurrences are better detected with volume measurements than with two-dimensional measurements on ablation zones or by focusing on contrast-enhancement patterns. All but one ablation zones with ablation site recurrences showed growth. With two-dimensional measurements, this growth was detected at a later stage or not at all. Ablation zones without ablation site recurrences became invariably smaller. Addtionally, volume measurements are not affected by position differences between two scans. It is therefore recommendable to perform volumetry in addition to the conventional

measurements on diameters of ablation zones in each patient who underwent thermal ablation. Recently, the principle of microwave ablation has been introduced. The procedure is performed percutaneously under CT-guidance. Postprocedurally, patients undergo the same follow-up as after radiofrequency ablation. Just like after percutaneous radiofrequency ablation, the protocol in the UMCG was to perform non-contrast CT scans during and at the end of the procedure. Unfortunately, it appeared that the ablation zone was very badly visible on the non-contrast scans with microwave ablation, in contrast to the ablation zones created with radiofrequency ablation. This is especially a main problem for the last scan performed immediately after the procedure, because it could hardly be determined whether the ablation zone enclosed the tumor or not. This resulted in more incomplete ablations than the specialists were used to. **Chapter four** investigates whether a contrast-enhanced CT-scan results in improved assessment of the ablation zones at the end of the microwave ablation procedure. The assessment of the immediate post-procedural contrast-enhanced scan was performed quantitatively by measuring diameters and volumes of ablation zones. These assessments were also done on the standard baseline multi-phase CT scan performed one week after the thermal ablation. The results of both scans were compared with each other. Quantitative assessment – measurements – should not be performed on the immediate contrast-enhances postprocedural images. Since the size of the ablation zone changes significantly within the first week after treatment, results of measurements on the immediate postprocedural scans are unreliable. The first measurements are very important for the further follow-up of the patient because they are compared with subsequent studies. For quantitative assessment, the scan one week after the procedure remains important. Based on the results of chapter four it is therefore recommended that quantitative assessment should not be performed on the immediate postprocedural scan. Instead, measurements should be performed on the scan which is made one week after the ablation, because the dimensions of the ablation zone are changing substantially during this period.

## Part two: Liver regeneration after partial hepatectomy.





The liver is an unique organ due to its remarkable capacity to regenerate after partial hepatectomy. The process of liver regeneration has been studied extensively in experimental models. There are relatively few studies on liver regeneration in humans. Existing studies are performed in living donors, which are the ideal population to study liver regeneration on, since these individuals are young and in good health. After all, major comorbidities are contra-indications to participate as living donor. The majority of patients undergoing elective partial hepatectomy have liver tumors and many of them are oncological patients. These patients are older and the incidence of major comorbidities is higher in this patient group. Therefore, it is important to have knowledge on the process of liver regeneration in these patients, since they are the largest patient group undergoing partial hepatectomy. Additionally, previous studies did not take the size of the resected part in

account. Liver regeneration was studied according to the type of partial hepatectomy, with the assumption that with each type of partial hepatectomy, approximately the same volume of tissue is removed. Since there is considerable interindividual variability in the size of the liver segments, volumes of the resected part of the liver may differ substantially between patients with the same type of partial hepatectomy.

**Chapter five** discusses liver regeneration in patients after partial hepatectomy. The amount of regenerated volume is expressed in an early and a late regeneration index, for which the measurements are obtained on CT-scans one week and six months after surgery. The results show that the amount of regenerated liver tissue is higher after larger resections. Although the early regeneration index is increasing with larger resections, a plateau phase is observed in patients with a resection of 40 % or more of their total pre-operative liver volume. Liver regeneration is not efficient after major resections in the early post-operative phase. However, the late regeneration index increases steadily with larger resections, which indicates that patients with large resections show a catch-up growth in the late post-operative phase. The liver will not regenerate to its original pre-operative total liver volume, especially not after major resections.

Liver regeneration is well known to depend on different factors, such as sex, age and the presence of major co-morbidities, especially diabetes, cardiovascular and pulmonary diseases. The liver can only regenerate sufficiently when the liver parenchyma is healthy. Liver cirrhosis is a well-known condition of the liver parenchyma which negatively affects liver regeneration after partial hepatectomy. Another far more common parenchymal disease of the liver is the fatty liver – hepatic steatosis. Steatosis was previously regarded as relatively harmless. However, it has been recognized that hepatic steatosis may lead to non-alcoholic steatohepatitis, cirrhosis and even hepatocellular carcinoma. Steatosis is found to affect up to 30 % of the patients who underwent a liver biopsy for any reason. Since obesity is a major risk factor for steatosis, it is assumable that the incidence of steatosis will increase with the obesity epidemic. Steatosis can be classified roughly in three degrees: no steatosis (0 %), mild steatosis (1-29 %) and moderate-to-sever steatosis (30 % or more). The percentages represent the percentage of hepatic cells with fat droplets. Clinically relevant steatosis is regarded as steatosis of 30 % or more. It has been reported from experimental models that steatosis has a negative influence on liver regeneration after partial hepatectomy, but the results are contradicting. Human studies on the influence of steatosis on liver regeneration do not show any negative influence of steatosis on liver regeneration. It should be noticed that these studies have been performed in living donors. Since steaosis is a relative contra-indication for being a living liver donor, individuals with clinically significant steatosis are generally excluded from living donation to warrant proper function of the graft in the recipient as much as possible. Therefore, the results from these studies may not be extrapolated gratuitously to the patient population with elective partial hepatectomy, since steatosis is not a general contra-indication for surgery in these patients. The influence of hepatic steatosis on liver regeneration is studied in **chapter six**. Regeneration indices, as calculated in chapter five, are compared in patients without

steatosis, mild steatosis and moderate-to-severe steatosis. Factors which could have influenced liver regeneration, especially the size of the resected part, did not differ between the patient groups. Interestingly, no patient with moderate-to-severe steatosis had received chemotherapy pre-operatively. This is important, because certain chemotherapy regimens are notorious as they can induce steatosis. The early regeneration index did not differ significantly between the groups, although it tended to be lower in patients with mild and moderate-to-severe steatosis. The total regeneration index was significantly lower in patients with any degree of steatosis. Thus, it can be concluded from these results that the presence of hepatic steatosis has a negative impact on liver regeneration after partial hepatectomy. Therefore, large resections should be performed with caution in these patients, since the regenerative capacity of the liver is impaired.

The process of liver regeneration is an orchestrated interplay of signaling events, cytokines and growth factors. Platelets are thought to play an important role in liver regeneration, since they contain multiple growth factors. Experimental studies showed that thrombocytopenia is associated with poor regeneration. On the other hand, thrombocytosis leads to a high regeneration response. In human studies, a low postoperative platelet count was associated with a delayed recovery of liver *function* and a higher postoperative mortality. The relationship between platelets and liver *regeneration* has not been studied in humans. **Chapter seven** discusses relationship between the course of the postoperative platelet count and liver regeneration. Patients were classified in three groups: (1) poor regeneration response, (2) intermediate regeneration response and (3) high regeneration response. The amount of resected volume is taken into account with this classification, as described in chapter 5. The results show a general decrease in postoperative platelet count with a nadir at day 3 postoperatively. However, patients with a high regeneration response have a stronger decrease in postoperative platelet count and show a significantly lower platelet count at day three postoperatively than patients with a poor or intermediate regeneration response. The question rises whether this finding shows a causal relationship between liver regeneration and platelets, which has been found in experimental studies. This is a topic worthy of future research.

Liver resection is mostly performed in oncological patients. The majority of the patients with liver tumours are no suitable candidates for surgery, mainly because irresectable disease. Advances in chemotherapeutic agents and regimens have changed the resectability criteria by downstaging the extent of the disease. Patients who were initially classified as non-resectable can undergo surgery as a result of a proper response to chemotherapy by shrinkage of the tumours. A major side effect of chemotherapy is its hepatotoxicity. Pre-operative chemotherapy has been associated with higher postoperative morbidity and mortality. Theoretically, chemotherapy could have influence on liver regeneration. The effect of chemotherapy on liver regeneration is studied in **Chapter eight**. The early regeneration index, as described in chapter 5, was calculated for patients who underwent a right hemihepatectomy and compared between patients who received pre-operative

chemotherapy and patients who did not. There was no difference between the early regeneration indices between these two patient groups. However, within the patient group with pre-operative chemotherapy, patients who received more than 6 cycles of chemotherapy had a smaller early regeneration index than patients who received less than 6 cycles of chemotherapy. It is advisable to minimize the number of chemotherapeutic cycles prior to surgery, since more cycles will lead to an impaired regeneration response.

# Nederlandse samenvatting

Het doel van het dit proefschrift is tweeledig. In het eerste deel wordt beeldvorming na thermale ablatie van levertumoren besproken. In het tweede deel wordt ingegaan op het proces van leverregeneratie na leverchirurgie.

## Algemene introductie.

De lever is een uniek orgaan vanwege zijn vermogen tot regeneratie na kwetsuren, bijvoorbeeld na leverchirurgie. Dit regenererende vermogen werd al in de oudheid onderkend en zelfs in de Griekse mythologie genoemd. Operaties aan de lever zijn al vroeg verricht, waarbij het meestal ging om verwondingen bij soldaten. Omdat de lever een zeer goed doorbloed orgaan is, is verbloeding het grote gevaar bij chirurgische ingrepen aan de lever. Om deze reden overleefde de patiënt de ingreep meestal niet. De eerste goed gedocumenteerde operatie aan de lever stamt uit 1887. Enkele jaren later volgden de eerste studies met grotere aantallen patiënten. Leverchirurgie bleef echter een riskante onderneming met een hoge mortaliteit. Dit veranderde toen de operatieve technieken en postoperatieve zorg verbeterden. Een grote doorbraak was de anatomische indeling van de lever door Franse chirurg Couinaud in de jaren vijftig van de twintigste eeuw. Hij deelde de lever op in acht segmenten met elk zijn eigen bloed- en galvoorziening. Door bij leveroperaties rekening te houden met deze verdelingen, kon het bloedverlies en gallekkage flink teruggebracht worden. Leverchirurgie wordt tegenwoordig vooral uitgevoerd bij patiënten met maligne levertumoren. Benigne levertumoren worden veel minder vaak chirurgisch behandeld. De meeste maligne levertumoren zijn metastasen van een primaire tumor elders in het lichaam. Met name het colorectaal carcinoom, één van de meest frequent voorkomende soorten kanker, metastaseert naar de lever. Helaas is er bij het grootste deel van de patiënten met colorectale levermetastasen geen chirurgische behandeling mogelijk vanwege de uitgebreidheid van de metastasering. Hierbij moet gedacht worden aan 80-90 % van de patiënten die niet meer met een operatie geholpen kunnen worden. Om deze patiënten toch te kunnen helpen, is er naar andere behandelmogelijkheden gezocht. Hierbij is het doel de tumoren te vernietigen, maar zoveel mogelijk leverweefsel te sparen. Thermale ablatie is één van de meest toegepaste vormen van dit soort alternatieve therapieën. Hierbij worden de tumoren met een naald – de elektrode - aangeprikt. Vervolgens wordt de elektrode verhit. Weefsel in de buurt van de elektrode wordt door deze verhitting gedood. Radiofrequente ablatie en meer recent microwave ablatie zijn de meest toegepaste vormen van thermale ablatie. Bij thermale ablatie kunnen de elektroden middels door het openmaken van de buik (laparotomie), door een kijkoperatie (laparoscopie) of door de huid (percutaan) worden geplaatst. Bij thermale ablatie via laparotomie en een laparoscopie wordt de tumor met behulp van echografie gelokaliseerd en aangeprikt. Bij percutane thermale ablatie kan de tumor ook met echo worden gevonden en aangeprikt, maar meestal wordt hiervoor computer tomografie (CT) gebruikt. Beeldvorming dient er ook voor om de voortgang van de behandeling te kunnen monitoren en om aan het einde van de behandeling te controleren of de ablatiezone de

tumor overlapt. Het grote voordeel van thermale ablatie is dat er minder complicaties optreden dan na leverchirurgie. Het grote nadeel van thermale ablatie is dat de genezingskans een stuk lager is dan na leverchirurgie, vooral omdat de tumor maar al te vaak terug blijkt te komen in of om de ablatiezone. Dit zijn de beruchte lokaalrecidieven. Getallen over hoe vaak lokaal recidieven voorkomen, wisselen erg in de literatuur, namelijk van 2 % tot 55 %. Belangrijk is om deze lokaal recidieven in een vroeg stadium op te sporen, want hoe eerder ze ontdekt worden, des te groter de kans dat er aanvullende behandeling mogelijk is. Om dit te kunnen waarborgen, ondergaan patiënten na thermale ablatie intensieve follow-up. Dit gebeurt door regelmatige poliklinische bezoeken, waarbij lichamelijk, laboratorium- en beeldvormend onderzoek verricht worden. Beeldvorming vindt meestal plaats door middel van multifase CT-onderzoek. Dit houdt in dat er eerst een scan zonder contrast wordt gemaakt: de blanco scan. Daarna wordt er contrast toegediend aan de patiënt en op drie vaste tijdstippen na toediening gescand, namelijk de arteriële fase, de portaal veneuze fase en de late fase. in sommige gevallen magnetic resonance imaging (MRI) of positron emissie tomografie (PET), al dan niet gecombineerd met CT.

## Deel 1: Beeldvorming na thermale ablatie.

Anders dan na leverchirurgie wordt bij thermale ablatie geen weefsel verkregen voor pathologisch-anatomisch onderzoek. Na een leverresectie controleert de patholoog of de snijvlakken tumorvrij zijn. Bij thermale ablatie is dat niet mogelijk; er wordt immers geen weefsel weggehaald, maar weggebrand wat vervolgens in het lichaam van de patiënt achterblijft. Beeldvorming na thermale ablatie is dus het enige middel om te beoordelen of de behandeling succesvol is of niet. Bij percutane thermale ablatie wordt er gedurende de procedure regelmatig een blanco CT-scan gemaakt om de voortgang van de procedure te monitoren. Als de behandelaar meent dat de procedure klaar is, wordt er nog een laatste CT-scan gemaakt om te zien of de ablatiezone de tumor volledig overlapt. Is dit het geval, kan de ablatie gestaakt worden. Indien blijkt dat de ablatiezone de tumor niet volledig omvat, wordt de ablatie voortgezet net zolang tot de ablatiezone groot genoeg is. Deze laatste scan zonder contrast is voor een grove eerste controle. Hierna volgt de follow-up. Patiënten ondergaan na thermoablatie op regelmatige tijdstippen beeldvormend onderzoek. Beeldvorming wordt meestal verricht middels multifase CT-scans met contrast. In sommige centra wordt ook wel MRI verricht. De eerste CT-scan na de thermale ablatie wordt één week na de procedure verricht en is belangrijk om te bepalen of de behandeling succesvol is geweest of niet. Op de eerste postprocedurele scan wordt gekeken naar de aanwezigheid van contrastaankleuring wat kan wijzen op een tumorrest in de ablatiezone. Daarnaast worden de afmetingen van de ablatiezone en ablatiemarges gemeten, analoog aan de tumorvrije afstand tot het resectievlak bij leverchirurgie. Deze afmetingen worden gebruikt als vergelijkingsmateriaal met afmetingen van de ablatiezone op latere scans. Voor deze metingen wordt de preprocedurele scan vergeleken met deze eerste postprocedurele scan. Het grote probleem is dat er een zeer

precieze vergelijking nodig is van beide CT-scans. De lever moet in dezelfde positie liggen op beide scans. Helaas blijkt dat maar al te vaak niet het geval te zijn waardoor de metingen aan de ablatiezone niet betrouwbaar zijn. Hierdoor kan niet goed worden beoordeeld of de ablatiezone de tumor volledig overlapt of niet. Deze verschillen in positie van de lever zijn vaak toe te dichten aan het feit dat de lever zich vlak onder het diafragma bevindt. Ademhalingsbewegingen en de diepte van de ademhalingsbewegingen zorgen ervoor dat de lever meebeweegt, wat voornamelijk rotationele positieverschillen geeft. Hoewel een abdominale CT-scan altijd wordt gemaakt in inspiratiestand, kan niet altijd dezelfde diepte van inademing gegarandeerd worden. Hierdoor is het mogelijk dat lever op de ene scan net iets anders gedraaid ligt dan op de andere scan. Dat deze kleine verschillen in positie de metingen sterk beïnvloeden en een risicofactor vormen voor het optreden van lokaal recidieven, wordt in **hoofdstuk 2** besproken. In dit hoofdstuk is een scoresysteem geïntroduceerd - de Similarity Positioning Score (SiPS) - dat de beelden van voor en na de ablatie indeelt in goed of slecht vergelijkbaar (SiPS-identical en SiPS-different). Het blijkt dat metingen bij patiënten met slecht vergelijkbare beelden onbetrouwbare resultaten opleveren. Daarnaast treedt er vaker een lokaal recidief op. Natuurlijk heeft de SiPS-score geen directe relatie met het al dan niet optreden van een lokaal recidief. Echter, als de beelden niet goed vergelijkbaar zijn, kan er geen betrouwbaar oordeel gegeven worden over het succes van de ablatie. Dan is het afwachten of de procedure daadwerkelijk geslaagd is of niet: of er wel of geen lokaal recidief optreedt in de maanden na de ablatie. Helaas kan er weinig gedaan worden aan dit probleem. Een mogelijke oplossing zou het gebruik van gespecialiseerde software kunnen zijn, dat corrigeert voor de positieverschillen van de lever: beelden worden zodanig "omgerekend" dat ze op gelijke positie geprojecteerd worden op het scherm van de radioloog. Een andere mogelijke oplossing is het zogenaamde "respiratory motion tracking systeem" waarbij de diepte van de ademhaling wordt gevolgd door sensoren en op het juiste moment een afbeelding wordt gemaakt. Deze methoden vinden hun toepassing voornamelijk in de radiotherapie, maar nog niet voor dit doeleinde in de radiologie. Wellicht is dit iets voor de toekomst.

Na deze "éénweeksscan" wordt er in de follow-up regelmatig beeldvorming verricht. De patiënt ondergaat elke drie maanden gedurende de eerste twee jaar na de behandeling een multifase CT-scan. Daarna wordt er daarna halfjaarlijks een multifase CT-scan verricht. De radioloog beoordeelt de ablatiezone en vergelijkt de beelden met voorgaande scans. Normaal gesproken worden ablatiezones met de tijd kleiner. Één van de tekenen van een lokaal recidief is dat de ablatiezone in omvang toeneemt. Meestal groeit een ablatiezone ten gevolge van een lokaal recidief zeer langzaam, met name in het beginstadium. Deze groei is voor het blote oog niet tot nauwelijks waarneembaar. Andere kenmerken van lokaal recidieven zoals contrastaankleuring, kunnen in dit vroege stadium ook gemakkelijk gemist worden. Het is juist erg belangrijk dat een lokaal recidief vroeg herkend wordt, want hoe eerder het ontdekt wordt, des te groter is de kans op behandelmogelijkheden. In **hoofdstuk drie** wordt beschreven of volumemetingen van ablatiezones kunnen bijdragen in de vroege detectie van lokaal recidieven. Immers, volumes

geven driedimensionale informatie: lengte, breedte en diepte. Tweedimensionale beelden geven alleen informatie over lengte en breedte. De resultaten tonen dat met het meten van volumes van de ablatiezones lokaal recidieven beter ontdekt worden dan bij tweedimensionale metingen aan ablatiezones of door op contrastaankleuring te letten. Vrijwel alle alblatiezones met een lokaal recidief vertoonden groei. Deze groei werd niet ontdekt met tweedimensionale metingen of pas in een later stadium. Ablatiezones zonder lokaal recidief werden zonder uitzondering kleiner. Daarnaast hebben positieverschillen van de lever tussen twee verschillende scans geen invloed op volumemetingen. Het is dan ook aan te raden om bij elke patiënt na thermale ablatie niet alleen tweedimensionaal, maar ook driedimensionaal – dus volumes - te meten.
Recent is het principe van microwave ablatie geïntroduceerd. De behandeling wordt percutaan, CT-geleid verricht en patiënten ondergaan na afloop dezelfde follow-up als na radiofrequente ablatie. Net als na percutane radiofrequente ablatie werd er in het UMCG in eerste instantie na de behandeling nog een blanco CT-scan gemaakt om te controleren of de ablatiezone de tumor overlapt. Helaas is gebleken dat op deze blanco scan de ablatiezone na microwave ablatie vaak heel slecht zichtbaar is, in tegenstelling tot de ablatiezones gecreëerd na radiofrequente ablatie. Dit vormde een groot probleem, omdat niet goed beoordeeld kon worden of de ablatie wel volledig is geweest en resulteerde in veel incomplete ablaties. In **hoofdstuk vier** wordt onderzocht of de ablatiezones beter te beoordelen zijn als er aan het einde van de microwave ablatieprocedure een scan *met* contrast wordt gemaakt. De beoordeling van deze direct postprocedurele scan met contrast geschiedde kwantitatief, dus door metingen van de diameters en volumes van de ablatiezones. Deze beoordelingen werden ook gedaan op de standaard multifase CT-scan één week na de thermale ablatie. Beide scans werden met elkaar vergeleken. Kwantitatieve beoordeling moet echter niet op de direct postprocedurele beelden verricht worden. De afmeting van de ablatiezone verandert dusdanig in de eerste week na de behandeling, dat metingen geen betrouwbare resultaten geven. Ablatiezones namen namelijk significant toe in grootte in de eerste week na de ablatie. De eerste metingen zijn juist van belang voor de verdere follow-up van de patiënt; de afmetingen van de ablatiezone moeten immers worden vergeleken met voorgaande onderzoeken. Hiervoor is de scan gemaakt één week na de procedure onmisbaar. Op basis van de resultaten van hoofdstuk vier wordt dan ook aangeraden om metingen van diameters en volumes niet op de direct postprocedurele scan te verrichten. Daarvoor moet men de scan gebruiken die één week na de ablatie is gemaakt, omdat de afmetingen van de ablatiezone sterk veranderen in deze periode.



## Deel 2: Leverregeneratie na partiële hepatectomie.

De lever is een uniek orgaan vanwege zijn vermogen tot regeneratie na chirurgie. Het proces van leverregeneratie is uitgebreid bestudeerd in proefdieren. In de mens zijn er relatief weinig studies verricht naar leverregeneratie. Beschikbare humane studies zijn verricht in levende donoren. Op

zich is dit een ideale populatie om leverregeneratie te bestuderen, omdat het gezonde, jonge mensen zijn. Immers, relevante comorbiditeiten vormen een contra-indicatie om als levende donor te kunnen fungeren. Electieve partiële hepatectomieën worden voornamelijk uitgevoerd bij mensen met levertumoren, waarvan oncologische patiënten de grootste groep vormen. Deze populatie is meestal een stuk ouder en relevante comorbiditeiten komen bij hen vaker voor. Kennis omtrent het proces van leverregeneratie bij deze mensen is dus van belang, omdat zij de grootste groep patiënten vormen die partiële hepatectomie ondergaan. Daarnaast is er in de tot nu toe gepubliceerde studies geen rekening gehouden met de grootte van het gereseceerde stuk lever. Leverregeneratie is bestudeerd naar soort partiële hepatectomie, waarbij er vanuit gegaan werd dat bij een bepaalde resectie steeds dezelfde hoeveelheid weefsel wordt weggehaald. Dit blijkt niet zo te zijn, daar er aanzienlijke interindividuele variatie bestaat in de grootte van de verschillende leversegmenten. Het volume van het gereseceerde stuk lever na een rechtszijdige partiële hepatectomie kan dus tussen patiënten onderling verschillen.

In **hoofdstuk vijf** is uiteengezet hoeveel de lever regenereert na partiële hepatectomie. Deze hoeveelheid is uitgedrukt in een vroege en een late regeneratie index, respectievelijk één week en zes maanden postoperatief. De resultaten tonen dat er meer weefsel regenereert naarmate er een groter volume wordt gereseceerd. Echter, de vroege regeneratie index toont een plateaufase bij resecties van 40 % of meer van het totale pre-operatieve levervolume. Regeneratie in de eerste week na de operatie stagneert dus bij grote leverresecties. De totale regeneratie index vertoont wel een stijgende lijn bij toenemende grootte van de partiële hepatectomie. Daaruit kan men concluderen dat patiënten met een grote leverresectie een inhaalgroei vertonen in de latere postoperatieve fase. De lever groeit in de meeste gevallen niet meer terug naar het originele pre-operatieve totale levervolume, vooral niet na grote resecties.

Leverregeneratie hangt af van verschillende factoren, zoals geslacht, leeftijd en comorbiditeiten, met name diabetes, cardiovasculaire aandoeningen en pulmonale problemen. Een lever kan alleen goed regenereren als het leverweefsel gezond is. Levercirrose is een bekende en beruchte aandoening van het leverparenchym dat leidt tot verminderde regeneratie na partiële hepatectomie. Leververvetting of steatose is een andere, veel vaker voorkomende aandoening van het leverparenchym. Steatose werd voorheen gezien als een relatief onschuldige conditie van het leverweefsel, maar het blijkt dat een vette lever kan leiden tot niet-alcoholische steatohepatitis, cirrose en zelfs hepatocellulair carcinoom. Steatose wordt bij ongeveer 30 % van de patiënten gevonden waarbij om welke reden dan ook een leverbiopt wordt verricht. Aangezien obesitas een belangrijke risicofactor voor steatose vormt en het een toenemend maatschappelijk probleem is, is het aannemelijk dat de incidentie van steatose ook zal stijgen. De mate van steatose kan grofweg worden ingedeeld in drie groepen: (1) geen steatose (0%), milde steatose (1-29 %) en matig tot ernstige steatose (30 % of meer). De percentages slaan op het percentage cellen in een biopt of preparaat met vetdruppels. Klinisch relevante steatose wordt gezien als steatose van 30 % of meer. Uit dierexperimenteel onderzoek is gebleken dat steatose leidt tot verminderde regeneratie

na partiële hepatectomie, maar de resultaten van de verschillende studies zijn tegenstrijdig. Studies bij de mens naar de invloed van steatose op leverregeneratie tonen geen negatieve invloed van steatose op de leverregeneratie. Hierbij dient wel aangetekend te worden dat deze studies bij levende donoren zijn verricht. Omdat alleen mensen met gezond leverweefsel als donor mogen fungeren, worden levers van kandidaat-donoren die te steatosisch blijken te zijn, uitgesloten om een goede functie van het transplantaat in de ontvanger te waarborgen. Hierbij is teveel vet in de lever al snel teveel, ook als het klinisch voor de donor niet van belang is. Steatose dat echt van klinisch belang is komt dus in deze groep niet voor. De resultaten uit deze studies zijn niet te door te voeren op de algemene patiëntengroep die een electieve partiële hepatectomie ondergaat, omdat bij deze patiënten steatose geen contraindicatie is voor partiële hepatectomie.
In **hoofdstuk zes** wordt de invloed van steatose op leverregeneratie besproken. Patiënten zijn ingedeeld in drie groepen, namelijk geen steatose, milde steatose en matig tot ernstige steatose. De regeneratie indices, zoals gebruikt in hoofdstuk 5, zijn vergeleken tussen de drie groepen. Er was geen verschil in grootte van de resectie en andere factoren die van invloed zouden kunnen zijn op de leverregeneratie binnen de drie groepen. Interessant is hierbij te vermelden dat geen van de patiënten met matig tot ernstige steatose pre-operatief chemotherapie heeft ontvangen, daar chemotherapie bekend om staat dat het steatose kan induceren. De vroege regeneratie index was niet significant verschillend tussen de groepen, hoewel er een neiging was naar een lagere regeneratie index bij patiënten met milde en matig tot ernstige steatose. De totale regeneratie index was echter wel significant lager bij patiënten met milde en matig tot ernstige steatose. Er kan dus worden geconcludeerd dat de aanwezigheid van steatose wel degelijk een negatief effect heeft op de leverregeneratie. Grote resecties moeten met voorzichtigheid worden uitgevoerd bij deze patiënten omdat de lever minder goed in staat is om te regenereren.
Het proces van leverregeneratie is een goed georganiseerd samenspel van verscheidene signalerende factoren, cytokines en groeifactoren. Binnen de leverregeneratie wordt een belangrijke rol toegedicht aan bloedplaatjes, omdat zij vele groeifactoren bevatten. Uit dierexperimenteel onderzoek is gebleken dat trombocytopenie tot een verminderde regeneratierespons leidt. Trombocytose zorgt juist voor een goede regeneratierespons. Uit humane studies is gebleken dat een laag bloedplaatjesaantal een vertraagd herstel van de lever*functie* geeft na de operatie en leidt tot hogere mortaliteit. De relatie tussen bloedplaatjes en lever*regeneratie* is tot op heden nog niet in de mens bestudeerd. In **hoofdstuk zeven** wordt uiteengezet hoe het postoperatieve verloop van het aantal bloedplaatjes is in relatie tot de leverregeneratie. Patiënten werden in drie groepen ingedeeld: (1) een slechte regeneratierespons, (2) een intermediaire regeneratierespons en (3) een goede regeneratierespons. Bij deze indeling is rekening gehouden met de grootte van de resectie, zoals beschreven in hoofdstuk 5. De resultaten tonen dat het plaatjesaantal in alle patiënten postoperatief daalt met een dieptepunt op de derde dag na de operatie. Daarna neemt het aantal bloedplaatjes weer toe. Echter, patiënten met een goede regeneratierespons vertonen een sterkere daling van het bloedplaatjesaantal en dit is significant op dag 3 na de operatie. De

grote vraag rijst nu natuurlijk of dit gegeven een causaal verband aantoont tussen leverregeneratie en bloedplaatjes, wat ook gevonden is in dierexperimenteel onderzoek, of dat dit op toeval berust. Dit is een interessant onderwerp voor toekomstig onderzoek.

Leverresectie wordt voornamelijk verricht bij oncologische patiënten. Slechts een klein deel van patiënten met levertumoren komt in aanmerking voor chirurgische behandeling, voornamelijk vanwege de uitgebreidheid van de tumoren. Ontwikkelingen op het gebied van chemotherapie hebben ervoor gezorgd dat het toedienden van chemotherapie het aantal tumoren kan reduceren, zodat patiënten die eerst als niet-resectabel werden beoordeeld, alsnog chirurgische behandeling kunnen ondergaan. Één van de bijwerkingen van chemotherapie is hepatotoxiciteit door schade van het cytostatisch middel aan de levercellen. Pre-operatieve chemotherapie is geassocieerd met hogere postoperatieve morbiditeit en mortaliteit. Theoretisch zou chemotherapie ook het proces van leverregeneratie kunnen beïnvloeden. Dit wordt onderzocht in **hoofdstuk acht**. De vroege regeneratie index, zoals beschreven in hoofdstuk 5, werd berekend bij patiënten met een rechtszijdige hemihepatectomie. Deze werd vergeleken tussen patiënten die pre-operatief wel of geen chemotherapie hebben ondergaan. Er was geen verschil in de vroege regeneratie indices bij deze twee groepen patiënten. Echter, binnen de groep patiënten die chemotherapie hebben ondergaan, hadden patiënten die meer dan 6 cycli chemotherapie hadden ontvangen een lagere regeneratie index dan patiënten die minder dan 6 cycli chemotherapie kregen. Het is dan ook aan te raden dat het aantal cycli pre-operatieve chemotherapie tot een minimum beperkt wordt bij patiënten die leverresectie ondergaan, omdat meer chemotherapie leidt tot een verminderde regeneratierespons.

# Dankwoord

Hoewel promoveren soms een eenzame aangelegenheid kan zijn, komt een proefschrift niet tot stand zonder de hulp van anderen. Een aantal personen wil ik graag in het bijzonder bedanken.

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# List of Publications

**Lack of anatomical concordance between pre-ablation and post-ablation CT-images: a risk factor related to ablation site recurrence.**
*Accepted in International Journal of Hepatology.*
Oral presentation at the European Congress of Radiology 2010, Vienna.
Oral presentation at the 9th Annual Meeting of the International Cancer Imaging Society, Salzburg, Austria (2009).

**Increase in Volume of Ablation Zones during Follow-Up Is Highly Suggestive of Ablation Site Recurrence in Colorectal Liver Metastases Treated with Radiofrequency Ablation.**
*JVIR 2012 Apr;23(4):537-44.*
Oral presentation at the 10th Annual Meeting of the International Cancer Imaging Society, Edinburgh, United Kingdom (2010).

**Immediate post-procedural measurements on ablation zones after microwave ablation: the sooner is not always the better.**
*Submitted.*
Oral presentation at the 12th Annual Meeting of the International Cancer Imaging Society, Oxford, United Kingdom (2012).

**Early Hepatic Regeneration Index and Completeness of Regeneration at Six Months After Partial Hepatectomy.**
*British Journal of Surgery 2012 Aug;99(8):1113-9.*
Oral presentation at the European Congress of Radiology 2012, Vienna, Austria.

**The Impact of Hepatic Steatosis on Liver Regeneration After Partial Hepatectomy.**
*Accepted in Liver International.*

**Postoperative Decrease in Platelet Count Is Related to Liver Regeneration After Partial Hepatectomy.**
*Submitted.*

**Influence of preoperative chemotherapy on CT volumetric liver regeneration following right hemihepatectomy.**
Second author.
*Submitted.*

**Diffusion Weighted Imaging in The Liver.**
*World J Gastroenterol 2010 April;16(13):1567-1576.*

# Curriculum Vitae

Petra Serbanescu-Kele was born on February 26th 1982 in Tîrgu Secuiesc, Romania. She attended high school at the Christelijke Scholengemeenschap Hondsrug College in Emmen and graduated in 2000.

In 2000, she attended the faculty of Medical Biology at the University of Amsterdam for one year with completing her propaedeutics. In 2001, she started her study in Medicine at the Academical Medical Center at the University of Amsterdam. She graduated as a Medical Doctor in November 2007.

From July 2008, she started working as research-physician at the department of Radiology at the University Medical Center of Groningen, University of Groningen and enrolled a PhD program. This resulted in the thesis you are currently reading, in which imaging after thermoablation and liver regeneration after partial hepatectomy are discussed. She attended several congresses for oral presentations of her scientific work, among which two European Congresses of Radiology and three annual meetings of the International Cancer Imaging Society.
In October 2013, she will start her residency in Psychiatry. Although she will not continue her medical career in Radiology, she will always be kindly disposed to this field of medicine.