M.G. van Vledder

Liver Surgery

Imaging and Image Guided Therapies

Liver Surgery: Imaging and Image Guided Therapies.

Mark G van Vledder

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Introduction

1.1

Imaging and Liver Surgery

Imaging and Liver Surgery

The liver is the most common site of malignant tumors in patients worldwide, including both primary (hepatocellular carcinoma) and metastatic liver cancer (eg colorectal liver metastases). Complete resection or thermal ablation of all tumor deposits currently offers the only potentially curative treatment for patients with a malignancy of the liver.

Historically, the liver was considered an organ too fragile and prone to bleeding for elective surgery. Increasing insight in the hepatic vascular and biliary anatomy and the introduction of antisepsis and anesthesia allowed pioneers like Langenbruch and Keene to start performing partial liver resections in the late nineteenth century[1,2]. Ongoing insight in the hepatic anatomy -like the division of the liver in eight distinct anatomic segments by Couinaud- further encouraged surgeons to perform liver surgery for hepatic neoplasms[3]. However, until late in the twentieth century, the absence of any form of non-invasive diagnostic imaging severely hampered the application of liver resection at a large scale.

Since the introduction of transcutaneous B-mode ultrasonography (US) in the late sixties [4], physicians have been able to visualize anatomical and pathological structures in the liver in a non-invasive way[5]. This allowed for a more detailed knowledge of the hepatic anatomy and diagnosis of a liver tumor in an earlier stage, thus enabling better pre-operative staging, safer procedures and better post-operative outcomes. The development and introduction of contrast enhanced CT and multiple detector row scanning (MDCT) [6] and contrast enhanced magnetic resonance imaging (MRI) [7] have further contributed to the way in which patients with a liver tumor are being diagnosed and treated nowadays. In addition, the use of ultrasonography during surgery –intraoperative ultrasound (IOUS)- has allowed surgeons to detect additional tumor deposits and visualize the hepatic anatomy in great detail and more important, at the time of surgery[8]. Currently, the diagnosis, treatment and follow-up of patients with a liver tumor is unthinkable without modern medical imaging. Ongoing technological developments, the introduction of new and more effective chemotherapeutic agents and the increasing use of minimally invasive treatment modalities such as radiofrequency ablation (RFA) are continuously altering the utility of these imaging modalities and the way we use them. This thesis explores and investigates the role of modern imaging for the liver surgeon in terms of lesion detection, treatment monitoring and guidance and patient selection.

Intraoperative Ultrasound in Liver Surgery

The first part of this thesis will investigate the value of IOUS in the surgical treatment of colorectal liver metastases in the modern era. In the early eighties, intraoperative ultrasound was introduced by Maakuchi et al. as a superior method to detect liver tumors when compared to pre-operative imaging such as transcutaneous ultrasound and hepatic artery arteriography [8]. Dedicated intraoperative probes were developed allowing for a full examination of the liver and many surgeons reported on the additional value of intraoperative ultrasound with regard to real time assessment of the hepatic anatomy and lesion detection[9,10]. When used in the right way, IOUS was described to change the surgical procedure in up to two thirds of all patients based on new findings[11]. More recently, it was speculated that the ongoing development of multidetector row computed tomography (MDCT), contrast enhanced magnetic resonance imaging (MRI) and 5-flourodeoxyglucose positron emission tomography (5FDG-PET) has lessened the addional value of IOUS, identifying additional metastatic deposits in less than 3% of all patients [12,13]. While this might be true to some extend, ultrasound likely remains an important tool for the liver surgeon and its additional value probably depends on several patient and tumor related factors[14]. For instance, the increasing use of more aggressive pre-operative chemotherapeutic regimens in patients with liver metastases[15] and the rising incidence of hepatic steatosis (or, non-alcoholic fatty liver disease, NAFLD)[16] have been shown to significantly impact on the diagnostic value of pre-operative imaging [17-19] and might re-enforce the use of intra-operative ultrasound.

Local Ablative Therapies and Imaging in Liver Surgery

The second part of this thesis focuses on the application of radiofrequency ablation (RFA) for the treatment of colorectal liver metastases and other tumors. Radiofrequency ablation is a technique that uses conductive heat to induce tissue necrosis. During radiofrequency ablation of a liver tumor, a probe is inserted in

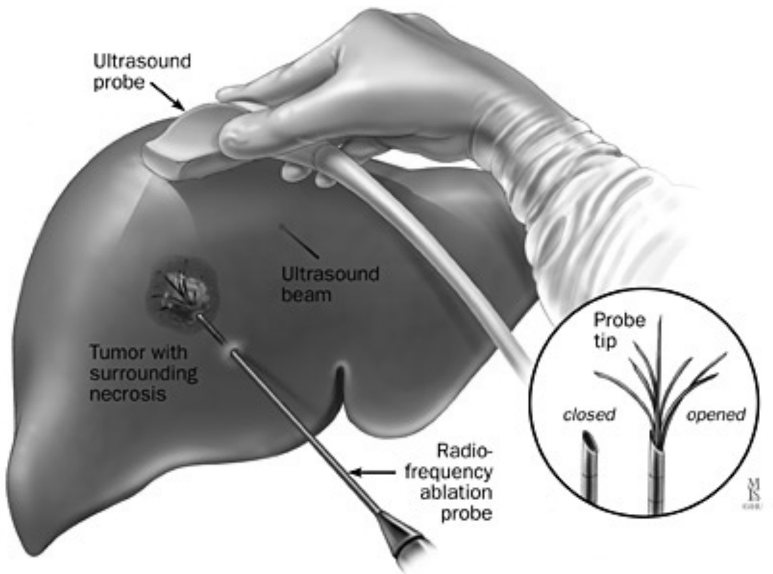

Figure 1.1: Schematic drawing of an intra-operative ultrasound probe on the liver during radiofrequency ablation treatment of a liver tumor

the tumor and a quickly alternating current is send through the tissue (figure 1.1). This causes a rise in temperature up to 100 degrees Celsius around the probe, leading to dehydration and ultimately coagulative necrosis in the targeted tissue. This can be performed either with an opened abdomen, using laparoscopy or via a percutaneous route. RFA was introduced by the end of the twentieth century as an alternative for surgical resection of liver tumors. Since then, authors have reported on the application of this technique for the treatment of a variety of liver tumors, including hepatocellular carcinoma[7], colorectal liver metastases[6], liver metastases from other primary tumors (eg neurendocrine tumors, breast cancer)[1,20] and benign liver tumors (eg hepatic adenoma)[21]. While initially reserved for patients with extensive disease not amenable for resection and patients with co-existing liver disease or co-morbidities precluding major liver resection, some authors have made a plea for the use of RFA in patients with resectable HCC and CRLM based on series with favourable outcomes after RFA[5,22,23], although other studies conflict with these results stating that RFA is associated with higher rates of recurrence and death and should thus be reserved for those patients unable to undergo liver resection. Regardless, little discussion exists regarding the increased risk of local recurrence after RFA when compared to surgical resection of a liver tumor, requiring repeat treatment in many cases and potentially resulting in decreased recurrence-free and overall survival.

In a meta-analysis from 2005, local recurrence rates after RFA of CRLM were described to be around 15%. In this analysis by Mulier et al, the reported rates of local recurrence ranged from 4% to 60% [21,24,25]. Some of these differences might be explained by patient selection and surgeon experience, but there are several other reasons for ablation to fail (table 1.1). The most important reason is probably the inability to precisely monitor and document complete margin-negative tumor destruction using current technology and guide treatment accordingly (eg perform a re-ablation of unablated tissue). While ultrasound is now the most commonly used imaging modality used for intra-operative guidance and monitoring of local ablative therapies, the value of conventional B-mode ultrasonographic assessment of the success of ablation has been criticized and questioned [26,27]. In part two of this thesis, outcomes after resection combined with RFA and local recurrence rates after RFA of CRLM will be investigated. Also, the

TABLE 1.1: Reasons for ablation to fail

1	Effectiveness of tumor destruction
2	Unpredictability of ablation size and shape
3	Difficulty with probe guidance to target location
4	Imprecise monitoring of ablation zone during therapy
5	Difficulty documenting adequate margin

value of intra-operative ultrasound and ablation parameters to monitor treatment and determine the ablative margin after RFA will be focused on. In addition, the development, experimental evaluation and implementation of a novel ultrasound based technique (ultrasound elasticity imaging) will be described. At last, the ability of RFA to treat hepatocellular adenomas will be investigated.

Pre-operative Imaging, Body Composition and Outcome in Liver Surgery

The third part of this thesis will focus on a novel application of pre-operative cross sectional imaging in cancer patients. To identify patients with a high risk of peri-operative morbidity and death after hepatic surgery, the surgeon nowadays has to rely on clinical judgement of a patient's functional reserve and clinical measures such as BMI and weight. These have been judged to be subjective and imprecise for the prediction of adverse events after surgery. In addition, current clinical patient and tumor related factors such as tumor number, size and growth tell us little about the biology of a liver tumor, its response to treatment and the prognosis of the patient. Recent evidence shows that cancer cachexia or muscle wasting (sarcopenia) might be a strong prognostic factor in patients with several types of cancer[28]. Unfortunately, cachexia can be hard to measure since BMI or obesity is usually maintained even when extensive muscle wasting is present. Recently, several reports have been published describing the utility of CT-based measurements of body composition and its correlation with outcome in cancer patients (Figure 1.2). Prado et al showed that a decrease in skeletal muscle mass (sarcopenia) as measured on a single CT slide at the third lumbar

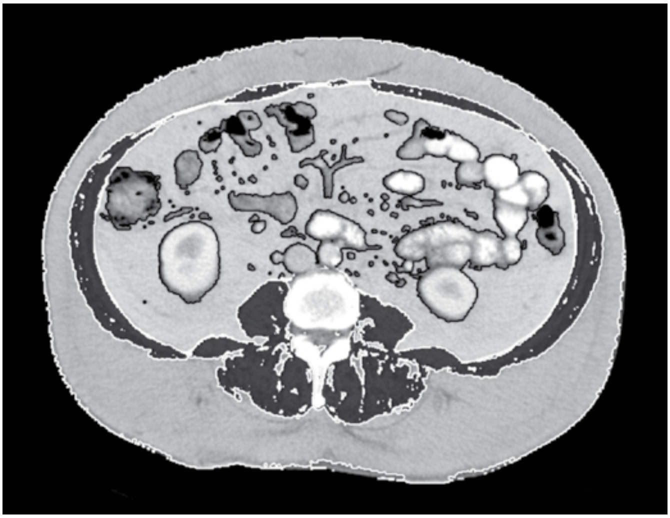

Figure 1.2: Segmented single slice CT-image showing different compartiments of subcutaneous visceral adipose tissue, visceral adipose tissue and skelettal muscle mass

vertebra significantly worsens survival in patients with malignant tumors of the gastrointestinal and respiratory tract undergoing chemotherapy[29]. The same has been shown for patients undergoing liver transplantation and patients with pancreatic cancer[30,31]. The important implication of these data is obvious: If a measurable depletion of muscle mass, either as a result of cancer cachexia or pre-existent, truly has a significant impact on survival in patients undergoing surgery for various malignancies, optimalisation of these patients by improving appetite and intake and converting their catabolic state into an anabolic state prior to surgery might lead to improved outcomes. Experimental studies have already confirmed this hypothesis[32]. In this thesis, it was investigated whether pre-operative radiological studies can aid the clinician to diagnose sarcopenia in patients undergoing liver resection for colorectal liver metastases.

Outline of the thesis

In **chapter 2.1** the additional value of IOUS in terms of the detection of additional colorectal liver metastases not seen pre-operatively is investigated. It is hypothesized that even with modern pre-operative imaging, IOUS detects metastases that were not visualized during pre-operative staging and significantly contributes to the subsequent treatment of patients with CRLM. Moreover, patient and tumor related factors that might influence the additional value of IOUS are investigated.

In **chapter 2.2** we investigate the impact of various histopathological liver and tumor characteristics on the echogenicity of CRLM. Most importantly, the impact of hepatic steatosis on the echogenicity of CRLM is investigated. While steatosis is generally considered to impair the quality of ultrasonography, its presence might also alter the relative echogenicity of liver metastases, making them more visible and thus increasing the utility of IOUS when compared to pre-operative imaging in patients with steatosis.

Pre-operative chemotherapy alters the sensitivity of pre-operative imaging through the development of steatosis and also causes CRLM to shrink to a size that they disappear on pre-operative imaging studies, making it hard to detect these tumors during surgery. In **chapter 2.3** the incidence of disappearing metastases after pre-operative chemotherapy, the intraoperative detection rate and the impact on recurrence-free and overall survival when left untreated is investigated.

In **chapter 3.1** the incremental value of RFA in patients with unresectable CRLM is investigated in a large multi-institutional cohort. More specifically, the rates of recurrence and survival in patients undergoing liver resection combined with radiofrequency ablation is investigated. Also, it is described how the addition of RFA to surgical resection can expand the eligibility criteria for surgical treatment in patients with CRLM.

Chapter 3.2 focuses on a per-lesion analysis with an attempt to identify parameters that may identify those patients who might derive the greatest benefit from ablative therapy and be offered a curative-intent approach potentially comparable to extirpative therapy. In addition, through a prospective observational trial which assesses intraoperative parameters of ablation, it is attempted to define a level of predictable efficacy during a procedure, analogous to operative histologic analysis of pathologic margin status for liver resection.

Further expansion of the application of RFA is investigated in **chapter 3.3**, where the use of RFA for the treatment of hepatocellular adenoma's in described. While a benign tumor in itself, its capacity for growth and concomitant rupture and potentially lethal bleeding often requires preventive treatment. With liver resection often being a highly invasive treatment for patients with these type of benign tumors, RFA could offer a much less invasive way of treating these patients.

Large animal models offer a controlled environment to investigate novel interventional imaging modalities in vivo. However, no useful tumor bearing model is currently available to test and investigate novel imaging methods for ablation guidance and monitoring in the liver. Therefore, **chapter 3.4** describes the development of a hepatic pseudotumor for image guided surgical and interventional research.

Chapter 3.5 describes the use of ultrasound elasticity imaging to document the ablative margin after intra-operative RFA of a liver tumor more accurately. Ultrasound elasticity imaging uses the elastic properties of tissue by documenting and substracting mechanically compressed and uncompressed ultrasound images. Tissue elasticity is then calculated and visualized by calculating relative pixel movement between both images. Since normal liver tissue, tumorous tissue and ablated tissue have a different elasticity, it is hypothesized that ablation margins can be visualized using this new method. This will be demonstrated in various clinical and animal experiments.

In **chapter 4.1 and chapter 4.2** the influence of a depletion of skeletal muscle mass (sarcopenia) as well as the presence of visceral obesity as measured by preoperative CT imaging on the short term and long term outcomes of patients undergoing liver resection for CRLM are investigated.

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2

Intraoperative Ultrasound

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Adapted From: *Factors Determining the Sensitivity of Intraoperative Ultrasonography in Detecting Colorectal Liver Metastases in the Modern Era*

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2.1

Intraoperative Ultrasound And Liver Surgery

Abstract

Background: With improved preoperative cross-sectional imaging, the added clinical value of intraoperative ultrasonography (IOUS) in the detection of colorectal liver metastases (CRLM) is unclear. Specifically, the ability of IOUS to detect additional liver metastases during surgery and its relationship between clinical and lesion specific ultrasonographic characteristics remains ill-defined. The purpose of the current study was to determine the association of clinical parameters and tumor echogenicity with the ability of IOUS to detect occult CRLM.

Methods: 213 patients undergoing surgical exploration and IOUS for CRLM between 1998-2009 were included in the study. All patients underwent preoperative multi-detector CT imaging and lesion detection was compared to those identified by IOUS. In addition, early (< 6 months) intrahepatic recurrence was used as a surrogate for residual disease (e.g. metastases that were undetected on initial IOUS). The influence of various characteristics on the rate in which additional metastases were detected and the rate of early intrahepatic recurrence was examined.

Results: Overall, IOUS detected additional liver metastases in 10% of patients (n=22). Detection of additional metastases was significantly higher in patients with multiple (≥4) tumors ($p<0.001$) and hypoechoic tumors ($p=0.007$). Of 153 patients undergoing resection only, 17 (11%) had an early intrahepatic recurrence. This was more common in patients with isoechoic metastases during IOUS (p=0.03).

Conclusions: Even with the use of modern cross-sectional preoperative imaging, IOUS detects additional liver metastases in 10% of patients. In addition, the sensitivity of IOUS for detecting occult CRLM is highly dependent on the number and echogenicity of detected tumors.

Introduction

Approximately half of the 150,000 patients diagnosed yearly with colorectal cancer in the United States develop metastases to the liver[1]. Roughly 20% to 30% of these patients are candidates for liver resection or ablation with curative intent. With modern surgical techniques and advanced chemotherapeutic regimens, 5-year survival is now reported to exceed 50% [2-4]. Complete resection or ablation of all liver lesions is mandatory in order to obtain long term disease-free and overall survival, and can often only be achieved when all disease is visualized intraoperatively.

Intraoperative ultrasound (IOUS) has long been known to be of benefit for the detection of colorectal liver metastases (CRLM) in patients undergoing liver surgery. In particular, IOUS allows the surgeon to detect additional small CRLM not seen on preoperative cross-sectional imaging. Detection of additional disease may change the surgical approach to allow complete extirpation of all disease and may contribute to improved outcomes.. Historic reports identify additional detection rate to be as high as 10-20% [5-7]. More recent reports, however, suggest that with improvements preoperative imaging, including multi-detector helical CT scan and MRI, the added benefit of IOUS at metastasThis study examines the sensitivity of IOUS during hepatic surgery for colorectal metastases. Specifically, we aim to evaluate the added ability of IOUS to detect additional lesions in patients undergoing modern preoperative imaging, including multi-detector contrast-enhanced CT and MRI imaging. In addition, we examined clinicopathologic and ultrasonographic features which may predict differences in IOUS sensitivity.

Patients and Methods

Between January 1998 and January 2009, 325 patients underwent surgical exploration with IOUS for planned resection and/or tumor ablation for CRLM with curative intent at Johns Hopkins University School of Medicine, Baltimore, Maryland. Of these, 213 patients fulfilled the following inclusion criteria and were included in the study: (1) preoperative contrast-enhanced CT or MRI; (2) complete IOUS images digitally captured, recorded, and available for review (3) Follow-up > 6 months. Clinicopathologic data were collected for all patients, including gender, age, tumor number, size of the largest tumor, preoperative chemotherapy use, and details about surgical treatment. Analysis of early intra-hepatic recurrence was performed on a subgroup of 153 resected patients, after excluding patients that underwent resection with ablation, ablation only or a non-therapeutic laparotomy (n=60). All data were collected prospectively in a database and the study was approved by the Institutional Review Boards at the Johns Hopkins Hospital.

Preoperative Imaging

All patients underwent one or more preoperative contrast-enhanced multi-detector CT imaging within 60 days prior to surgery. Those with outside studies were reviewed for quality by a Johns Hopkins radiologist and repeated at our institution if considered not optimal. Some patients (n=97) also underwent preoperative contrast-enhanced MRI imaging at the discretion of the treating clinicians and 105 patients underwent preoperative PET or PET/CT imaging. In patients receiving preoperative chemotherapy, cross-sectional imaging both prior to and after therapy was assessed and findings included in the analysis.

Intraoperative Assessment and Ultrasonography

IOUS was performed by a single hepatobiliary surgeon using a 4.0-8.0 mHz curvilinear transducer (Phillips ATL HDI 5000) using an standard protocol as previously described by our group [10]. Following mobilization of the liver, all known metastatic sites were visualized by IOUS and measured and captured in both transverse and sagittal planes (index lesions). The liver was then systematically assessed for additional metastases, both by inspection and palpation, as well as by IOUS. Additional lesions detected only by IOUS were defined as those not detected on preoperative imaging or by operative inspection/palpation. Satellite lesions, defined as small lesions adjacent to or near a dominant metastasis, were not counted as additional lesions. Capsular dimpling or focal fibrosis, with or without concomitant ultrasonographic findings was scored as non-IOUS detection. All detected metastases were confirmed histologically by resection or biopsy. Benign lesions (cysts or hemangiomas) were confirmed by IOUS or biopsy and excluded. When additional lesions were detected intraoperatively, preoperative CT and MRI scans were retrospectively reviewed to confirm their absence in these studies. Lesions identified retrospectively on cross-sectional imaging were scored as detected preoperatively.

Ultrasonographic characteristics, including relative tumor echogenicity, were determined retrospectively using prospectively collected digital ultrasound images on a per lesion basis. Images were randomly coded and scored for echogenicity relative to the surrounding liver parenchyma (0 hypoechoic; 1 isoechoic; 2; hyperechoic) by two reviewers blinded to the clinical information. For patients with more than one lesion, the echogenicity was based on the median echogenicity score for that patient.

Follow-up for Early Intrahepatic Recurrence

In order to establish a surrogate for lesions potentially missed on IOUS, *early intrahepatic recurrence* (EHR) was used. Postoperatively, all patients underwent serial contrast-enhanced CT imaging every 3-6 months. EHR was defined as evidence of a new lesion identified within the remnant liver on cross-sectional imaging within 6 months of liver operation. True local recurrences (adjacent to resection margin) and patients undergoing tumor ablation were excluded from this analysis.

Statistical Analysis

All statistics were computed using Stata 10.1 (College station, TX) and SPSS 17 (Chicago, IL). Analysis of factors associated with the detection of additional lesions and early intra-hepatic recurrence was performed using Chi-squared and Fishers exact test for categorical variables and ANOVA or Kruskall Wallis tests for continuous variables. Analysis of overall recurrence was done using the log-rank test. Odds ratios and their 95% confidence intervals were calculated using logistic regression analysis. A *p*-value of <0.05 was considered to be statistically significant.

Results

Patients

Two-hundred and thirteen eligible patients were included in the analysis (table 2.1.1). The median age was 60 years (range 23-90) and 120 patients (56%) were male. The median number of preoperatively detected tumors was 2 (range 2-15), while the median size of the largest preoperatively detected tumor was 3

TABLE 2.1.1: Clinical and morphological characteristics of 213 patients

Variable	
Median Age (range)	60 (23-90)
Sex	
Male	120 (56)
Female	93 (44)
Tumor number	
1	105 (49)
2-3	62 (29)
≥ 4	46 (22)
Median Size largest tumor in cm (range)	3 (1-16)
Node status primary tumor	
Positive	143 (67)
Negative	70 (33)
Missing	10
Disease-free interval	
< 1 year	145 (69)
≥ 1 year	68 (31)
Pre operative CEA	
< 100 ng/ml	189 (88)
≥ 100 ng/ml	24 (12)
Pre operative chemotherapy	
None	115 (54)
5-FU	22 (10)
Irinotecan	38 (18)
Oxaliplatin	38 (18)
Surgical Strategy	
Liver resection only	153 (72)
Liver resection and ablation	43 (20)
Ablation Only	10 (5)
Non Therapeutic Laparotomy	7 (3)
Echogenicity of index lesion	
Hypoechoic	90 (42)
Isoechoic	91 (43)
Hyperechoic	31 (15)

CEA: Carcinoembryonic Antigen

cm (range 1-16 cm). 98 patients (46%) underwent preoperative chemotherapy for their metastatic disease. Resection alone was performed in 153 patients (72%), whereas 43 patients (20%) underwent resection combined with ablation and 10 patients underwent operative ablation alone (5%). Seven patients (3%) had an aborted procedure and non-therapeutic laparotomy due to previously unrecognized more advanced disease.

Tumor echogenicity varied among different patients but were generally highly consistent within the same patient. Hypoechoic tumors were found in 90 patients (42%) whereas hyperechoic or brighter tumors were found in 32 patients (15%). Metastases scored as isoechoic relative to the surrounding liver were found in 91 patients (43%) (figure 2.1.1).

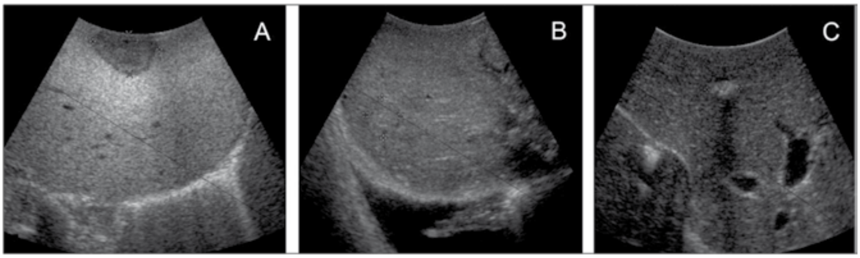

FIGURE 2.1.1: Examples of differences in tumor echogenicity by IOUS. Figure A: hypoechoic lesion; figure B: isoechoic lesion; and figure C: hyperechoic lesion.

Intraoperative Detection of Additional Liver Metastases

Additional metastases were detected during surgery in 35 patients (16%), leading to a change in surgical strategy in 19 patients (9%). In 22 patients (10%), these metastases were only detected by IOUS (table 2.1.2). Among those patients with new lesions found by IOUS, the average number of additional metastases found was 2 (range 1-6). All additional lesions detected by IOUS were 1.5 cm or less in diameter, the smallest of which measured 4 mm. The only clinical factor associated with detection of additional metastases with IOUS alone was the number of preoperatively detected lesions. Specifically, in patients with 4 or more preoperatively detected metastases, there was a 26% chance of finding additional lesions on IOUS compared to 11% in those with 2-3 known lesions, and only 3% in those with solitary tumors ($p<0.001$). Viewed differently, the probability

TABLE 2.1.2: Factors associated with the intraoperative detection of additional hepatic metastases with IOUS.

Variable	Additional lesions detected by IOUS (n=22; 10%)	No additional lesions detected (n=191; 90%)	*p*-value
Echogenicity			
Isoechoic	3 (3)	88 (97)	-
Hypoechoic	16 (18)	74(82)	0.004
Hyperechoic	3 (9)	29 (91)	0.19
Size Largest Tumor (mean, cm)			
< 5cm	16 (11)	130 (89)	0.66
> 5 cm	6 (9)	61 (91)	
Preoperative Tumor Number			
1	3 (3)	102 (97)	
2-3	7 (11)	55 (89)	0.04
≥4	12 (26)	34 (74)	<0.001
Disease-free Interval			
< 1 year	17 (12)	129 (88)	0.36
≥ year	5 (7)	62 (93)	
Preoperative Chemotherapy			
Yes	12 (12)	89 (88)	0.48
No	10 (9)	102 (91)	
Response to Preoperative Chemotherapy			
CR / PR	6 (14)	51 (86)	0.33
SD / PD	6 (11)	28 (89)	
No available data		10	
Pre-operative Imaging			
MRI	11 (11)	86 (89)	0.66
No MRI	11 (9)	105 (91)	
FDG-PET	12 (11)	93 (89)	0.60
No FDG-PET	10 (9)	98 (91)	
Interval Between Last Scan and Surgery (mean, days)			
≤ 30 days	15 (12)	114 (88)	0.44
> 30 days	7 (8)	77 (93)	
Surgical Time Period			
1998-2003	16 (11)	128 (89)	0.59
2004-2009	6 (9)	63 (91)	

CR: complete response. PR: partial response. SD: stable disease. PD: progression of disease of finding additional lesions was more than 5 times higher in patients with ≥4 preoperatively detected metastases when compared to patients with <4 known metastases (OR 5.54 95%CI 2.21 – 13.9; $p<0.001$).

Detection of additional metastases by IOUS was significantly impacted by the echogenicity of the known index lesions. In particular, patients in whom the known metastases were found to be isoechoic, the probability of finding additional lesions was significantly lower than when known disease was scored as either hypo- or hyperechoic (OR 0.18 95% CI 0.05-0.63; p=0.007). In patients with known disease that was hypoechoic, additional metastases were found in 16/90 (18%) patients, whereas additional lesions were found in only 3/91 (3%) among those with index lesions that were isoechoic (OR 0.16 95%CI 0.04-0.56; $p=0.004$) (figure 2.1.2). Additional lesions were found in 3/32 (9%) patients with hyperechoic lesions, a difference that did not reach statistical significance (p=0.19).

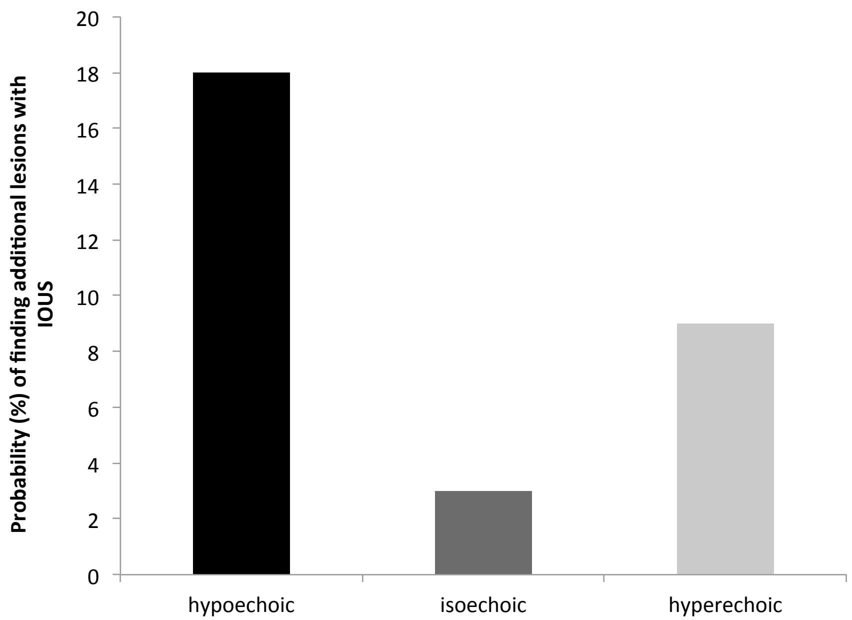

Figure 2.1.2: The probability of detecting additional liver metastases using IOUS by the echogenicity of the index or known lesions.

TABLE 2.1.3: Factors associated with early (<6 month) intrahepatic recurrence

Variable	Early intrahepatic recurrence n=17 (11%)	No early intrahepatic recurrence n=136 (89%)	p-value
Echogenicity			
Isoechoic	12 (18)	55(82)	-
Hypoechoic	3 (5)	57 (95)	0.034
Hyperechoic	2 (8)	24 (92)	0.23
Size Largest Tumor (mean, cm)			
< 5cm	11 (11)	92 (89)	0.80
> 5 cm	6 (12)	44 (88)	
Preoperative Tumor Number			
1	10 (10)	85 (90)	
2-3	4 (10)	37 (90)	0.89
≥4	3 (18)	14 (82)	0.40
Disease-Free Interval			
< 1 year	14 (14)	49 (86)	0.14
≥ 1 year	3 (6)	87 (94)	
Preoperative Chemotherapy			
Yes	11 (17)	55 (83)	0.06
No	6 (7)	81 (93)	
Response to Preoperative Chemotherapy			
CR + PR	2 (9)	20 (81)	0.35
SD + PD	7 (18)	32 (82)	
No available data	2	3	
Pre-operative Imaging			
MRI	12 (13)	78 (87)	0.29
No MRI	5 (8)	58 (93)	
FDG-PET	8 (9)	77 (91)	0.46
No FDG-PET	9 (13)	59 (87)	
Interval Between Last Scan and Surgery (mean, days)			
> 30 days	7 (11)	54 (89)	0.91
≤ 30 days	10 (11)	82 (89)	
Postoperative Adjuvant Chemotherapy			
Yes	6 (9)	63 (87)	0.39
No	11 (13)	73 (91)	

CR: complete response. PR: partial response. SD: stable disease. PD: progression of disease

No difference in the IOUS detection rate was observed based on age, gender, disease-free interval, primary tumor stage, or size of the largest lesion. Similarly, no difference was seen in detection rate in those who did or did not receive preoperative chemotherapy, or when comparing chemotherapy responders vs. non-responders. Neither the interval between the last CT scan and surgery (< 30 days vs. > 30 days) nor the additional use of preoperative MRI or PET had an impact on the probability of detecting additional lesions. Also, the chance of finding additional metastases with IOUS did not statistically significantly differ among patients undergoing surgery before or after 2004. Patients in whom additional lesions were detected were significantly more prone to either undergo resection combined with ablation or non-therapeutic laparotomy compared to those without new identified lesions ($p<0.001$).

Early Intrahepatic Recurrence

Seventeen patients (11%) developed an EHR within 6 months of surgery as detected on follow-up imaging (table 2.1.3). Interestingly, the probability of developing such a recurrence was significantly impacted by the tumor echogenicity seen on IOUS. Overall, patients with isoechoic disease had a significantly higher probability of developing an EHR compared to those with non-isoechoic disease (OR 3.24 95% CI 1.14-9.02; $p=0.03$). More specifically, patients with isoechoic metastases had an 18% probability of developing an EHR compared to those with hypoechoic tumors, in whom early hepatic recurrences occurred in only 5% of patients (OR 0.24 95% CI 0.06-0.90; p=0.034) and hyperechoic tumors, in which EHR occurred in 8% (OR 0.38 95% CI 0.79-1.84; p=0.23).

In contrast, patients with isoechoic tumors did not have a statistically significantly increased rate of late (> 6months) intrahepatic recurrence (p=0.108) or overall recurrence-free survival (p=0.63) when compared to patients with hypo- or hyperechoic tumors (figure 2.1.3). Other clinicopathologic features were not found to predict EHR, including tumor size, tumor number, primary stage, the use of or response to preoperative chemotherapy, the additional use of preoperative MRI or PET, or the time period in which the patient underwent surgery. Similarly, we did not find differences in early recurrence between those who did or did not have additional lesions found during IOUS.

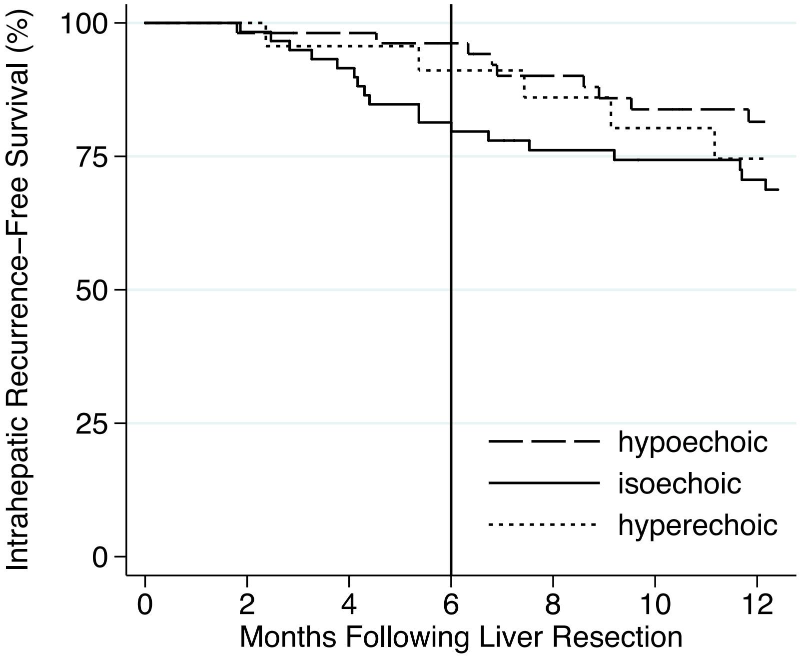

Figure 2.1.3: Kaplan Meier Curve demonstrating intrahepatic recurrence-free survival in the first year following resection of hepatic colorectal metastases, based by echogenicity (hypo-, iso-, and hyper-). Early intrahepatic recurrence is defined as within 6 months (line).

Discussion

Intraoperative ultrasonography remains an important component of hepatic surgery. In addition to providing detailed visualization of intrahepatic anatomy and guidance for needle biopsy or ablation, IOUS has long been considered important for improved intrahepatic staging and lesion detection in patients undergoing liver surgery for colorectal metastases [5-7]. However, recent literature has challenged the sensitivity of IOUS for detecting otherwise occult disease in our modern era of high quality preoperative cross-sectional and functional imaging [7-9]. Moreover, the reasons for variability in IOUS sensitivity have not been well studied [11,12]. The present study was designed to specifically address these concerns by examining the sensitivity of IOUS in a contemporary population treated in a single high-volume academic medical center.

In this study, we found that IOUS detects additional liver metastases in 10% of patients undergoing surgery for colorectal liver metastases, even with the use of state-of-the-art preoperative imaging. We found that the additional use of preoperative MRI, PET, or PET/CT did not impact on the detection rate of IOUS. Previous reports suggest the value of preoperative PET in these patients resides in the ability to detect extrahepatic sites of disease and therefore would not be expected to significantly affect the IOUS detection rate[13] . Based on these findings, we feel the probability is sufficiently high to conclude a clinically significant benefit for improved staging, even when modern preoperative imaging is utilized.

Some have suggested a diminishing role of IOUS with the use of modern preoperative imaging. In one study, the investigators found IOUS influenced the surgical strategy in only 2% of all patients after preoperative PET imaging [8]. In another study, additional colorectal liver metastases were found in only 2.6% of patients after undergoing adequate preoperative staging with CT and MRI [9]. Other studies support the continued value of IOUS in detection of colorectal liver metastases. Conlon et al. reported in patients undergoing preoperative MRI that IOUS changed surgical management in 18% of cases and detected additional lesions on 7 of 80 patients [14]. Other studies have similarly reported superiority of IOUS when benchmarked to preoperative high-resolution CT, MR, and PET/CT [12,14-16]. Such discrepancies may be in part explained by patient selection and extent to which surgical therapy is altered based on IOUS findings. In addition, the quality and sensitivity of IOUS is likely dependent on various factors, including the ultrasound equipment used, surgeon experience at ultrasonography, and rigorousness of the IOUS protocoWe found the probability of finding additional metastases varied considerably based on specific clinical and ultrasonographic features. Patients with multiple known tumors were found to have a considerably higher chance of identifying additional lesions; 26% in those with four or more known metastases. While this finding is predictable, the clinical implications are important. In particular, as increasingly aggressive surgical approaches are being offered to patients with more extensive liver metastases, the utility of IOUS may play an increasingly important role in order to identify and treat all macroscopic disease.

Echogenicity of the index or known metastases was found to significantly impact on the ability to detect additional lesions by IOUS. Hypoechoic index lesions were associated with an added detection rate of 18%. In contrast, when the known disease is isoechoic in character, the expectation of identifying additional lesions is extremely low (3%). These findings are not unexpected as it likely directly relates to the conspicuity of these small otherwise occult lesions. We have previously reported the high consistency in echogenicity between metastases within the same patient [10]. Therefore, one might anticipate an improved detection rate of conspicuous (e.g. hypoechoic or hyperechoic) small lesions in patients with conspicuous known disease.

In order to determine factors associated with failure to identify otherwise occult liver metastases, it is useful to identify lesions potentially missed by IOUS. While this true denominator is impossible to know precisely, we attempt here to examine this question using the development of an early radiologically detectable recurrence as a surrogate for a lesion that may have been small but macroscopically present during surgery and missed on IOUS. Indeed, we found that such recurrences more closely correlate with IOUS sensitivity (i.e. echogenicity) than other more established clinicopathologic prognostic factors. This finding further validates the association between echogenicity and lesion conspicuity and IOUS sensitivity.

An increasing number of patients undergoing surgical therapy of colorectal liver metastases receive preoperative chemotherapy. High response rates offer potential conversion from unresectable to resectable status [18-22]. Even in initially resectable patients, use of neoadjuvant systemic chemotherapy is being used with increasing frequency to identify responders and potentially improve outcomes.[23]. Radiologic complete responses, however, may be associated with increased difficulty in finding all original sites of disease during surgery. Recent studies have found that residual viable disease is present in many of these sites and identifying and resecting all original sites of disease is important [24,25]. Importantly, identification of the "disappearing lesion" is often not possible during surgery, potentially confounding the surgical approach [26]. In this study, we did not find a significant correlation between the use of preoperative chemotherapy and IOUS sensitivity. Specifically, we did not find what might be expected: a potentially diminished detection rate in chemotherapy responders due to reduction in tumor size. Moreover, chemotherapy-associated steatosis often reduces image quality of IOUS theoretically further reducing detection rate[27-29]. However, it is possible that preoperative chemotherapy may increase detection rate in some cases. Responding tumors can be associated with capsular retraction or tumor calcification, potentially making them more evident. In addition, the presence steatosis paradoxically increases the probability for lesions to be relatively hypoechoic and therefore more conspicuous[30]. Therefore, the impact of preoperative chemotherapy on IOUS sensitivity can be variable and therefore difficult to define in this study.

Practically, this study may provide some guidance for the hepatic surgeon. In patients undergoing planned resection in which index lesions are conspicuous, particularly when hypoechoic, conducting IOUS carefully and completely will often yield detection of additional metastases and likely improved outcome. In patients with known disease that is found to be isoechoic in appearance, particularly when few in number, the utility of aggressively seeking out additional disease is limited.

In summary, our findings demonstrate that IOUS detects additional metastases in up to 10% of patients undergoing surgery for CRLM, despite high-quality preoperative imaging. IOUS should remain an important part of the standard of care for patients undergoing surgery for colorectal liver metastases. IOUS sensitivity was found to be strongly associated with tumor echogenicity as well as known tumor number. These findings have both technical and prognostic implications regarding the role of IOUS in the surgical management of hepatic colorectal metastases.

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Adapted From: *The Effect of Steatosis on Echogenicity of Colorectal Liver Metastases on Intraoperative Ultrasonography*

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2.2

Intraoperative Ultrasound And Hepatic Steatosis

Abstract

Background: Steatosis is observed with increasing frequency in patients undergoing hepatic surgery for colorectal liver metastases (CRLM), in part due to the increased use of preoperative chemotherapy. The impact of steatosis on the ability to detect CRLM using intraoperative ultrasound is ill-defined. Therefore, the association of relative tumor echogenicity and hepatic steatosis in patients undergoing resection of CRLM was investigated.

Methods: Prospective data, including IOUS images and clinicopathological information, were collected in 191 lesions from 126 patients undergoing liver surgery for CRLM between 1998-2008. IOUS images were blindly reviewed and scored for echogenicity (hypo-, iso-, or hyperechoic). Additionally, a histopathologic review of the non-tumorous liver was performed and the extent of steatosis was scored and correlated to tumor echogenicity.

Results: 49 patients (39%) of patients undergoing surgery were found to have mild to severe steatosis. Of the 191 total CRLM visualized by IOUS, 91 (48%) were found to be hypoechoic, 65 (34%) isoechoic, and 35 (18%) hyperechoic. In patients with steatosis, lesions were significantly more likely to be hypoechoic when compared to patients without steatosis (OR 4.17 95% CI 1.81-10.0, p=0.001). Echogenicity was independent of the etiology of steatosis or of response to chemotherapy.

Conclusions: The echogenicity of CRLM was significantly impacted by the presence of liver steatosis, with decreased echogenicity and increased conspicuity of lesions in spite of overall poorer image quality. These findings might reinforce the utility of IOUS in identifying additional CRLM in patients undergoing surgical therapy, even in patients with fatty livers.

Introduction

Hepatic steatosis is the most common liver disease in the United States and perhaps worldwide, affecting 17-33% of individuals in the general population[1,2]. Such fatty liver disease is commonly associated with obesity, type II diabetes mellitus, and other conditions promoting insulin resistance and free fatty acid accumulation in the liver. Importantly, the use of fluoropyrimidine-based chemotherapy for colorectal cancer is associated with pathologic changes to the liver parenchyma, including hepatic steatosis[2-4].

Steatosis is associated with changes in the hepatic parenchymal appearance on ultrasonography[5,6]. This feature can even be used as a non-invasive tool for diagnosing the condition using transcutaneous ultrasonography[7,8] However, the ultrasound appearance of a fatty liver is manifested as increased echogenicity and decreased image quality due to signal attenuation, highlighting a potential concern for diminished ability of ultrasound to detect metastases within a fatty liver. In particular, intraoperative ultrasonography (IOUS), an essential tool used for the detection of liver tumors, may be affected by steatosis. This point is particularly relevant in patients undergoing hepatic surgery for colorectal liver metastases, where steatosis is more common and lesion detection is paramount for successful surgical therapy[3,9]. Therefore, the objective of the current study was to determine the impact of hepatic steatosis on the ultrasound appearance of hepatic colorectal metastases. More specifically, we sought to evaluate the effect of the fatty liver on lesion echogenicity.

Patients and Methods

Data on the IOUS appearance of colorectal liver metastases were collected prospectively on 196 patients who underwent resection at Johns Hopkins Hospital between 1998 and 2008 by a single surgeon (MAC) with extensive experience in hepatic ultrasonography. Of these, 126 patients had complete histopathological slides available and were thus included. The study was approved by the Johns Hopkins Institutional Review Board.

Prospectively collected data, including IOUS images and standard demographic and clinicopathologic data were analyzed retrospectively, including patient demographics; type, duration and response of preoperative chemotherapy according to the response evaluation criteria in solid tumors (RECIST)[10]; tumor size, number and location; operative information; body mass index (BMI); and tumor echogenicity. IOUS of the liver was performed in each case using a 4.0 to 8.0-MHz curvilinear transducer probe (Phillips ATL HDI-5000). Operative imaging was performed according to an established protocol as previously described by our group[11,12]. Metastases were imaged in both the longitudinal and transverse planes with standard preset ultrasound settings, including: the depth of field, focal zone, gain setting, scan orientation, and image size. In each case, all evaluable IOUS images were digitally recorded and coded randomly. Analysis of images was conducted by two blinded reviewers and graded for echogenic appearance in relation to the surrounding liver: hypoechoic, isoechoic, or hyperechoic.

For this study, archival histopathology slides were retrieved and histologic evaluation was performed by an experienced hepatopathologist (M.T.) who was unaware of the clinical data. Specimens were examined by routine microscopy with hematoxylin/eosin and Masson trichrome stained slides. In all instances, the pathologic findings of the non-tumorous hepatic parenchyma remote from the cauterized margin was examined and scored. Steatosis was determined as minimal (0 to 5%), mild (5-30%), moderate (30-60%) or severe (>60%). Tumors were scored for the presence of extracellular mucin, intratumoral microcalcifications and pathologic response to chemotherapy. More specifically, the percentage of (residual) viable tumor cells and tumor necrosis within each lesion was estimated and scored as those with major pathologic response (<20% viable tumor cells) and minor or no response (> 20% viable tumor cells).

All statistics were computed using Stata 10.1 (College station, TX) and SPSS 16 (Chicago, IL). Summary statistics were obtained using Chi-squared and Fishers exact test for categorical variables and ANOVA for continuous variables. Correlation between steatosis and echogenicity was investigated per lesion using univariate and multivariate multinomial regression. The data was clustered per patient to correct for variance in the number of lesions per patient. Relative risk ratios (RRR) and their 95% confidence intervals were calculated with $p<0.05$ considered statistically significant.

Results

Patient Characteristics and Tumor Echogenicity

Two-hundred and seventy-nine lesions were resected in 126 patients. Of these, 191 lesions had both complete IOUS images for review and adequate linked pathologic data available, and were included in the final analysis. Table 2.2.1 shows the clinicopathologic features of the patient cohort. There were 65 (51.5%) men with a median age of 60 years (range 22 -90). Diabetes was present in 15.1% of patients and 30 patients (25%) had a BMI >30. The median number of hepatic metastases per patient was two (range 1- 14), with 47% solitary, 31% with 2-3 metastases, and 22% with ≥4 lesions resected. The median size of the largest tumor was 4.0 cm (range 1-16 cm). Fifty-nine patients (46.8%) were treated with chemotherapy for

TABLE 2.2.1: Clinical and morphologic features of the 126 patients

Median age (range)	60 (22-90)
Gender	
Female	61 (48.5)
Male	65 (51.5)
No. of tumors	
1	59 (46.7)
2-3	39 (31.0)
> 3	28 (22.2)
Median size largest tumor in cm (range)	4.0 (1-16)
Preoperative chemotherapy	
None	67 (53.2)
Fluoropyrimidine alone	15 (11.9)
Irinotecan-based	20 (15.9)
Oxaliplatin-based	24 (19.0)
BMI kg/m2	
< 30	90 (71.4)
≥ 30	30 (23.8)
Missing values	6 (4.8)
Diabetes	
Yes	19 (15.1)
No	107 (84.9)
Echogenicity	
Hypoechoic	55 (43.7)
Isoechoic	50 (39.7)
Hyperechoic	21 (16.6)

Data are presented as number (percentage) of patients unless otherwise indicated.

TABLE 2.2.2: Distribution of clinical and morphologic features among groups by echogenicity per patient

Variable	Hypoechoic N=55	Isoechoic N=50	Hyperechoic N=21	P-value
BMI (n=126)				
< 30 cm/m2 (n=90)	40 (72.7)	37(70.0)	15 (71.4)	0.89
≥ 30 cm/m2 (n=30)	12 (21.8)	13 (26.0)	5 (23.8)	
Missing values (n=6)	3 (5.4)	2 (4.0)	1 (4.8)	
History of diabetes mellitus (n=126)				
Yes (n=19)	9 (16.1)	8 (15.7)	2 (10.6)	0.43
No (n=107)	46 (83.9)	42 (84.3)	19 (89.4)	
No. of Tumors (n=126)				
1 (n=59)	24 (43.6)	22 (44.0)	13 (61.9)	0.17
2-3 (n=39)	14 (25.5)	19 (38.0)	6 (28.4)	
□ 4 (n=28)	17 (30.9)	9 (18.0)	2 (9.5)	
Pre-operative chemotherapy (n=126)				
None (n=67)	29 (52.7)	28 (56.0)	10 (47.6)	
Fluoropyrimidine alone (n=15)	6 (10.9)	6 (12.0)	3 (14.3)	0.98
Irinotecan-based (n=20)	10 (18.2)	6 (12.0)	4 (19.0)	
Oxaliplatin based (n=24)	10 (18.2)	10 (20)	4 (19.0)	
Radiologic Response (n=59)				
Complete or Partial response (n=31)	14 (53.8)	12 (54.5)	5 (45.5)	0.94
No response or Progressive disease (n=22)	9 (34.6)	8 (36.4)	5 (45.5)	
Missing values (n=6)	3 (11.5)	2 (9.1)	1 (9%)	

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared)

their metastatic disease prior to surgery. In these patients, a partial or complete radiological response was observed in 31 (53%) patients. 13 (22%) patients had a complete radiological response in at least one lesion, of which nine metastases were available for analys

The distribution of clinical features among echogenicity groups is summarized in Table 2.2.2. Overall, fifty-five patients (44%) were found to have hypoechoic metastases, 50 patients (40%) had isoechoic lesions and 21 patients (16%) had hyperechoic lesions. No difference in echogenicity was observed in patients with increased number or size of largest metastases. Tumor echogenicity was not significantly different between those with or without diabetes, nor was a difference seen in those with obesity. Tumor echogenicity was not significantly different among those receiving preoperative chemotherapy, nor did it differ by chemotherapy type or radiologic response.

Histologic Features

Microscopic assessment of the non-tumorous liver revealed the presence of steatosis (>5%) in 49 patients (39%), including mild steatosis in 33 patients (26%) and moderate or severe steatosis in 16 patients (13%) (Table 2.2.3). Only two patients were found to have steatohepatitis (1.6%). Thirty-six patients (29%) had evidence of sinusoidal dilatation, with moderate to severe sinusoidal dilatation seen only in five patients (4%). Of the 191 individual lesions evaluated, 45 tumors (22%) had evidence of microcalcifications on histology. This finding was more common in patients who received preoperative chemotherapy when compared to those receiving no chemotherapy (9% vs. 37%, $p<0.001$).

Tumor Echogenicity and Steatosis

The influence of histologic abnormalities of the surrounding liver on tumor echogenicity is summarized in Table 2.2.4. Specifically, patients with steatosis were more frequently found to have hypoechoic lesions (67%) compared with isoechoic (22%) or hyperechoic (11%) lesions. In contrast, patients without steatosis were found to have hypoechoic metastases in only 33% of cases, with 43% appearing as isoechoic and 24% as hyperechoic. ($p<0.001$). These hypoechoic tumors were frequently more conspicuous, in spite of the diminished image quality and definition seen in steatotic livers (figure 2.2.1). Evidence of sinusoidal dilatation was not associated with differences in tumor echogenicity. The extent of steatosis significantly impacted the relative echogenicity of CRLM (figure 2.2.2). The probability of finding hypoechoic tumors was 60% in those with mild steatosis (RRR 2.91;,95% CI 1.33-6.37 $p<0.007$, vs. no steatosis) and increased to over 90% in those with moderate-to-severe steatosis (RRR 21.6, 95% CI 4.74-98.0 $p<0.001$, vs. no steatosis).

TABLE 2.2.3: Distribution of pathologic characteristics per patient and per lesion

Variable	Patients, No. (%) N=126	Lesions, No. (%) N=191
Steatosis		
No steatosis (<5%)	77 (61.1)	110 (57.6)
Mild steatosis (5-30%)	33 (26.2)	58 (30.4)
Moderate to severe steatosis (>30%)	16 (12.7)	23 (12.0)
Sinusoidal dilatation		
Yes	36 (28.6)	47 (24.6)
No	90 (71.4)	144 (75.4)
Microcalcifications		
Yes	27 (21.4)	45 (23.6)
No	96 (76.2)	141 (73.8)
Missing values	3 (2.4)	5 (2.6)
Mucin (>10%)		
Yes	27 (21.4)	30 (15.7)
No	99 (78.6)	161 (84.3)
Pathologic response to chemotherapy		
(no chemotherapy)	67 (53.2)	94 (49.2)
Complete response	3 (2.4)	5 (2.6)
Major response	24 (19.0)	36 (18.8)
Moderate or no response	32 (25.4)	56 (29.3)

TABLE 2.2.4: Influence of the surrounding liver histology on relative echogenicity of individual lesions.

	Hypoechoic (n=91)	Isoechoic (n=65)	Hyperechoic (n=35)
Any steatosis (≥5%)			
Yes (n=81)	55(67.9)	18(22.2)*	8(9.9)**
No (n=110)	36 (32.7)	47(42.7)	27(24.6)
Sinusoidal Dilatation			
Yes (n=47)	19 (40.4)	18(38.3)	10(21.3)
No (n=144)	72(50.0)	47(32.6)	25(17.4)

* Hypoechoic vs isoechoic: RRR 3.98, 95% CI 1.87-8. 47, p<0.001

** Hypoechoic vs hyperechoic: RRR 5.18 95% CI 1.68-15.8, p=0.004

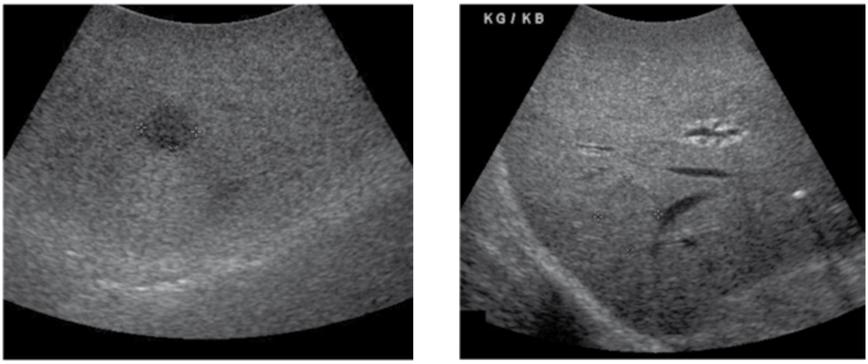

Figure 2.2.1: Intraoperative ultrasound images of hepatic colorectal metastases. A small hypoechoic lesion imaged in a surrounding liver with mild steatosis (left); an isoechoic lesion identified in a liver without histologic evidence of steatosis (right).

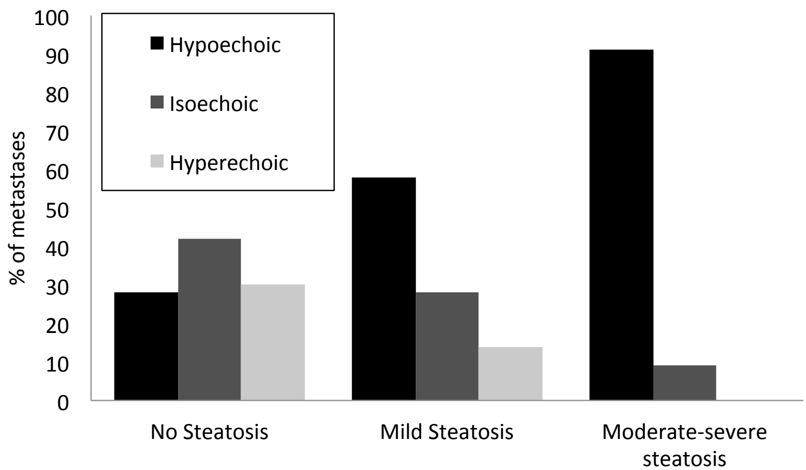

Figure 2.2.2: Correlation between tumor echogenicity the extent of hepatic steatosis. Histologic extent of steatosis: none (<5%), mild (5-30%), and moderate-to-severe (>30%). The probability of finding hypoechoic tumors was increased in those with mild steatosis (RRR 2.91 95% CI 1.33-6.37, p=0.007) and moderate-to-severe steatosis (RRR 21.6 95% CI 4.74-98.0, p<0.001) vs. no steatosis.

The effect of various histopathologic characteristics, including steatosis and microcalifications, on tumor echogenicity was evaluated using both univariate and multivariate clustered multinominal regression models. On univariate analysis, the presence of steatosis was the only parameter found to be independently associated with finding hypoechoic vs. isoechoic tumors (RRR 3.98, p=0.001) (Table 2.2.5). No other features, including microcalcifications, mucin, tumor size or location, preoperative chemotherapy use, or chemotherapy response were associated with a hypoechoic tumor appearance. In contrast, the finding of hyperechoic lesions compared to isoechoic ones was only associated with the presence of tumor microcalcifications (RRR 4.76, p=0.003). Tumor size, number, location, use of or response to preoperative chemotherapy, mucin, sinusoidal dilatation, or steatosis was not associated with hyperechogenicity. When adjusting for all other factors, steatosis (RRR 4.24; *p*=0.001) and calcifications (RRR 4.33;*p*=0.003) remained the only independent factors associated with echogenicity in the multivariate analysis.

We also sought to determine if the association between steatosis and tumor echogenicity was due to effect of preoperative chemotherapy on the tumor itself rather than only presence of steatosis. Specifically, when the percentage

Table 2.2.5: Univariate analysis of histological factors influencing the echogenicity of colorectal liver metastases on IOUS

	Hypoechoic vs. Isoechoic		Hyperechoic vs. Isoechoic	
	Relative Risk Ratio (95% CI)	*p*-value	Relative Risk Ratio (95% CI)	*p*-value
Steatosis > 5%	3.98 (1.87-8.47)	0.001†	0.77 (0.27-2.22)	0.63
Microcalcifications (yes)	0.52 (0.22-1.25)	0.14	4.76 (1.89-12.5)	0.001††
Extracellular Mucin (yes)	0.46 (0.19-1.08)	0.07	2.33 (0.57-9.50)	0.24
Sinusoidal dilatation (yes)	0.68 (0.28-1.69)	0.42	0.96 (0.34-2.67)	0.93
Preoperative chemo (yes)	1.09 (0.53-2.27)	0.81	2.22 (0.89-5.56)	0.09
Tumor size (cm)	0.47 (0.15-1.46)	0.19	0.87 (0.21 -3.54)	0.85
Pathological Tumor response (Major)	1.06 (0.36-3.09)	0.91	1.37 (0.35-5.37)	0.64

† RRR 4.24 (95%CI 1.82-9.90) *p*=0.001 after adjusting for all other variables
†† RRR 4.33 (95% CI 1.62-11.6) *p*=0.003 after adjusting for all other variables
All others: Not significant in multivariate analysis (p>0.05)

of hypoechoic lesions was compared between steatotic patients who received preoperative chemotherapy to those who did not, no difference was seen (66% vs. 76%, p=0.16). Similarly, tumor echogenicity was comparable, regardless of the attributed etiology of the steatosis.

Finally, we investigated the influence of pre-operative chemotherapy on the echogenicity of CRLM and the ability of IOUS to detect colorectal liver metastases. A total of nine liver metastases showed a complete radiological response on pre-operative imaging studies and were only detected intra-operatively. These were hypoechoic in almost all instances (n=8; 89%) with only one lesion being isoechoic. Moreover, six of the nine metastases with a complete radiological response on pre-operative imaging were detected in a liver with steatosis (>5%), of which three metastases were found in a liver with moderate (30-60%, n=2) or severe steatosis (>60%, n=1).

Discussion

IOUS is well known as an important tool for accurately staging liver tumors and it frequently impacts intraoperative decision making[13-20]. However, tumor echogenicity and image quality can have a significant bearing on the sensitivity and usefulness of IOUS for the operative management of hepatic colorectal metastases [15,21]. Steatosis, in particular, is associated with increased signal attenuation and decreased overall detection sensitivity of liver tumors [22] . The liver parenchyma appears brighter in the presence of steatosis and intrahepatic vascular structures appear less defined (figure 2.2.1). One might hypothesize, then, that the conspicuity of hepatic colorectal metastases may be diminished in the presence of fatty liver disease. Yet, little data exist regarding the IOUS image characteristics in such patients. Such data has important implications regarding the ability to visualize and detect metastases during planned liver resection.

In our study, we found a strong correlation between hepatic steatosis and the relative tumor echogenicity of CRLM. Specifically, isoechoic tumors (which are often difficult to distinguish from the surrounding liver) were found in only 22% of patients with steatosis, half the percent of those without steatosis. These findings are likely explained by the ultrasonographic characteristics of a fatty liver. When hepatic fat content increases, the parenchyma becomes brighter (hyperechoic) due to an increased sonographic attenuation. Because one might expect the ultrasound characteristics of the tumors themselves (i.e. absolute echogenicity) not to change with hepatic steatosis, metastases then become relatively more hypoechoic when compared to the surrounding liver tissue in the presence of steatosis.

The presence of non-alcoholic fatty liver disease (NAFLD) was common in our study population of patients undergoing liver resection of colorectal metastases (39%). These demographics reflect the general population within the U.S., with a prevalence of steatosis in western countries ranging from 3-23%. Moreover, rising rates of obesity and diabetes will likely increase the rates of NAFLD in surgical patients in the future[23]. Numerous authors have also reported on the correlation between preoperative chemotherapy and the development of steatosis in various degrees of severity [24-26]. In one study, 18.3% of patients undergoing liver surgery for colorectal cancer and treated with preoperative chemotherapy had moderate to severe steatosis, compared to only 3.4% of patients that received no chemotherapy. In another report, steatosis was observed in 24% of patients treated with preoperative irinotecan and oxaliplatin [26].

Our results are important for several reasons. First, when a grossly steatotic appearing liver is found during surgery, one should anticipate hypoechoic lesions on IOUS when screening for additional tumors. Previously, we have shown that echogenicity is consistent among tumors within the same patient [11]. This finding is supported by the correlation found here between echogenicity and steatosis, affecting all tumors within the same patient similarly. Furthermore, we have shown that the fraction of tumors with decreased echogenicity diminishes with the extent of steatosis, likely reflecting increased ultrasound signal attenuation with increasing fatty change.

The association between tumor appearance and steatosis was found to occur regardless of the etiology of the liver injury. Patients with chemotherapy-induced steatosis were found to have comparable proportion of hypoechoic tumors when compared to those with steatosis resulting from other causes. While echogenicity was not found to be influenced overall by preoperative chemotherapy use of tumor response to chemotherapy, it is likely the presence of the steatosis which influences the tumor appearance. These findings have important implications regarding the sensitivity of IOUS in these patients. While the use of preoperative chemotherapy has significant potential advantages in patients undergoing liver resection, some have raised concern that the associated steatosis may negatively impact on the ability to visualize metastases on pre-operative CT-imaging and intra-operative ultrasound[27,28]. This study suggests that steatosis results in fewer isoechoic lesions and likely increased tumor conspicuity. Smaller otherwise occult lesions may paradoxically become more visible and easier to detect in a fatty liver. This is supported by the fact that of the nine metastases that were no longer visible pre-operative imaging after responding to pre-operative chemotherapy, eight metastases were hypoechoic and a majority of these metastases was found in livers with some degree of steatosis. While we did not further address the influence of echogenicity on the sensitivity of IOUS for the detection of (occult) CRLM in this study, this could be an important subject for future investigation.

In summary, we found that the echogenicity of CRLM was significantly impacted by the presence of liver steatosis, resulting in a decreased echogenicity and increased conspicuity of lesions in spite of overall poorer image quality. These findings reinforce the utility of IOUS in visualizing hepatic metastases in patients undergoing surgical therapy for advanced colorectal cancer, even in those with fatty livers.

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2.3

Peri-operative Imaging and Chemotherapy

Abstract

Background: With increasing efficacy of preoperative chemotherapy for colorectal cancer, more patients will present with one or more disappearing liver metastases (DLM) on preoperative cross-sectional imaging.

Patients and Methods: A retrospective review was conducted evaluating the radiological response to preoperative chemotherapy for 168 patients undergoing surgical therapy for colorectal liver metastases at Johns Hopkins Hospital between 2000 and 2008.

Results: 40 patients (23.8%) had one or more DLM, accounting for a total of 127 lesions. In 22 patients (55%), all DLM sites were treated during surgery. Of the 17 patients with unidentified, untreated DLM, ten patients (59%) developed a local recurrence at the initial site, half of which also developed recurrences in other sites. While the intrahepatic recurrence rate was higher for patients with DLM left in situ (p=0.04), the 1-, 3-, and 5-year overall survival rate was not significantly different for patients with DLM left in situ (92.3%, 63.5% and 63.5% respectively) when compared to patients with a radiological chemotherapy response in whom all original disease sites were surgically treated (92.3%, 70.8% and 46.2% respectively) (p=0.66).

Conclusions: DLM were frequently observed in patients undergoing preoperative chemotherapy for liver metastases. Survival was comparable in patients with untreated DLM, in spite of high intrahepatic recurrence rates seen in these patients. Therefore, aggressive surgical therapy should be considered in patients with marked response to chemotherapy, even when all DLM sites cannot be identified.

Introduction

Approximately half of all patients diagnosed with colorectal cancer will develop liver metastases during the course of their disease. For these patients, hepatic resection offers the best chance for long-term survival, with reported 5-year survival rates over 50%[1-5]. An increasing number of patients are treated with systemic chemotherapy prior to liver resection, either administered as neoadjuvant treatment for initially resectable disease or in attempt to convert patients with unresectable disease into surgical candidates[6,7]. With the introduction of new chemotherapeutic regimens and targeted therapies, the radiologic response rates are high with an increasing number of patients showing a disappearance of one or more liver metastases on preoperative cross-sectional imaging [8,9]. Previous reports have shown variable results with regard to the proportion of these disappearing liver metastases (DLM) that represents a true complete remission (e.g. a complete pathological response or a durable remission on repeat imaging), ranging from 17-69%[8-11]. The current paradigm is therefore to aim for complete resection or ablation of all areas in the liver where disease was observed prior to chemotherapy, perhaps including resection of regions in which disease originally occurred but cannot be found intraoperatively. However, limitations in the ability to visualize or locate these DCM intraoperatively may result in leaving these metastases untreated [9].

Current studies which have reported on the fate of missing metastases have primarily focused on per-lesion analyses, whereas information regarding patient-specific findings, management, and outcomes in those with DLM is limited. Therefore, in this study, we aimed to determine the proportion of patients developing DLM following preoperative chemotherapy, characterize the fate of these DLM during surgery, and to investigate the impact of undetected, untreated DLM on recurrence-free and overall survival.

Methods

Patients

A retrospective analysis was conducted of 366 patients undergoing curative intent surgery for colorectal liver metastases at Johns Hopkins Hospital between

January 1, 2000 and December 31, 2008 in order to identify those who developed DLM following chemotherapy. 189 (51.6%) received preoperative chemotherapy. Of these, 21 were excluded (non-therapeutic laparotomy or incomplete staged procedure, n=10; incomplete imaging data available, n=9; non-surgical locoregional treatment prior to liver surgery, n=2), leaving 168 patients included in the study. The protocol was approved by the Johns Hopkins Hospital Institutional Review Board.

Preoperative Chemotherapy

Various preoperative chemotherapy regimens were utilized in these patients for their metastatic disease. 149 patients (88.7%) underwent only a single regimen of chemotherapy prior to surgery, and 21 patients (11.3%) received two or more lines of preoperative chemotherapy, excluding prior use for adjuvant therapy of primary disease. These included oxaliplatin-based, irinotecan-based, or fluoropyrimide monotherapy (5-fluorouracil or capecitabine) regimens. Targeted biologic therapies (bevacizumab and/or cetuximab) were incorporated preoperatively in 69 patients. The average number of chemotherapy cycles administered before surgery was 6.0 (SD 3.68). In 54 patients (32.1%), chemotherapy was initiated for initially unresectable disease (conversion-intent). Reasons for initially unresectable disease included distribution of bilateral metastases in 39 patients, size or location in 9 patients or suspected unresectable extrahepatic disease in 6 patients. In the other 114 patients (67.9%), resectable disease was observed prior to chemotherapy and chemotherapy was administered as a neoadjuvant strategy. When multiple regimens were used, the active preoperative regimen was defined as the last regimen to which the patient responded or that which was administered immediately prior to liver surgery. Determination of resectability and timing to proceed to surgery was left to the discretion of the treating physicians as part of a multidisciplinary management team. In general, resectability was defined as the ability to completely resect all metastatic sites while leaving sufficient volume of the hepatic remnant (>20-30%) and adequate remnant vascular/biliary inflow and vascular outflow.

Imaging

All patients were initially staged prior to chemotherapy using contrast-enhanced multi-detector computerized tomography (CT). PET or PET/CT was utilized selectively at the discretion of the treating physicians. Imaging following chemotherapy was performed using CT in the majority of patients, with contrast-enhanced MRI only obtained in 22 (13%) patients. The majority of imaging studies were performed at Johns Hopkins Hospital. All imaging studies were reviewed by experienced radiologists and hepatic surgeons, and repeated when considered inadequate. Post-chemotherapy imaging was all conducted within 60 days of surgery. A disappearing liver metastasis (DLM) was defined as that in which no radiologically visible lesion or abnormality was seen at a site initially identified as a liver metastasis. Postoperative surveillance for recurrence was determined using CT, PET or MRI every 3-6 months, at the discretion of the treating physician. If a DLM was identified and left surgically untreated, follow-up imaging studies were examined specifically for in-situ recurrence as determined by comparison to the initial CT.

Hepatic Surgery

All patients underwent open surgical exploration with curative intent. Intraoperative assessment included examination for extrahepatic metastatic disease as well as careful visualization and palpation of the mobilized liver. Intraoperative ultrasound (IOUS) was performed by the hepatobiliary surgeon using a 4.0-8.0 MHz curvilinear transducer (Phillips ATL HDI 5000) based on a standardized protocol [12]. All known metastatic sites were known to the surgeon, including information regarding location and number of original and persistent lesions, as well as DLM. Findings and IOUS imaging of regions of interest were documented. The goal of surgery when possible was to completely resect or ablate all sites of disease found during surgery as well as originally detected sites prior to chemotherapy. In five patients, preoperative right portal vein embolization or ligation was applied to allow for an adequate remnant liver volume after resection. Resection was combined with radiofrequency ablation (RITA-XL or XLie, Angiodynamics, Queensbury, NY) in 53 patients and microwave ablation (Microsulis Inc.) in one patient.

Histopathologic Examination

Resected specimens were serially sectioned in 0.5 cm slices and examined for metastatic deposits. Regions within the resected liver in which intraoperative lesions were identified or where prior metastases were felt to be present were pointed out to the pathologist for identification. Samples embedded and fixed in paraffin, sliced, stained with hematoxylin and eosin were examined microscopically for the presence of metastatic colorectal cancer. A complete pathological response was defined as the absence of any viable tumor cells at the sites of macroscopically visible tumors or if no evidence of any tumor was found at the site of previously identified DLM.

Statistics

Statistical analysis was performed using Stata 10.0 (Collegetown, TX). Summary statistics were obtained with established methods using χ^2 squared test and Fishers exact test for categorical data and student's *t*-test for continuous data. Factors predictive of the development of one or more DLM were investigated using univariate and multivariate logistic regression analysis. Differences in recurrence-free and overall survival were calculated with the log rank test and Kaplan Meier curves. A *p*-value of < 0.05 was considered statistically significant.

Results

Patient and Tumor Characteristics

Clinicopathological and morphologic characteristics of the 168 patients are summarized in table 2.3.1. The majority of patients included were male (n=94; 55.9%) with a median age of 57 years (range 23-84 years). At the time of resection of the primary tumor, 114 patients (67.9%) were found to have nodal metastases. Diagnosis of metastatic disease was synchronous with the primary tumor in 128 patients (76.2%). Eighty-seven patients (51.6%) had bilateral disease at the time of presentation with a median number of metastases of 2.0 (range 1-24). Sixty-one patients (36.3%) had a solitary metastasis before chemotherapy, 54 patients (32.1%) had 2 or 3 metastases, and 53 patients (31.6%) had 4 or more metastases prior to chemotherapy.

TABLE 2.3.1: Clinicopathologic and morphologic characteristics of 168 patients treated with chemotherapy prior to surgery

Variable	N=168
Median age in years (range)	57 (23-84)
Gender	
Male	94 (55.9)
Female	74 (44.1)
Diagnosis of liver metastases	
Synchronous	128 (76.2)
Metachronous	40 (23.8)
Node status primary	
Positive	114 (67.9)
Negative	47 (27.9)
Missing values	7 (4.2)
Median tumor number pre-chemotherapy (range)	2 (1-24)
Median size largest tumor in cm pre-chemotherapy (range)	3 (1-17)
Disease spread	
Bilateral	87 (51.6)
Unilateral	81 (48.2)
Indication for Chemotherapy	
Neoadjuvant	114 (67.9)
Conversion	54 (32.1)
Preoperative chemotherapy regimen	
Fluoropyrimidine monotherapy	15 (8.9)
Irinotecan-based	55 (32.7)
Oxaliplatin-based	96 (57.2)
FOLFOXIRI	2 (1.2)
Bevacizumab or Cetuximab	
Yes	69 (41.1)
No	99 (58.9)
Radiological Response (RECIST)	
Complete	11 (6.5)
Partial	88 (52.4)
Stable disease / Progressive disease	68 (40.5)
Missing data	1 (0.6)

Incidence and Predictors of DLM

Forty (23.8%) patients were observed to have a total of 127 DLM at the time of surgery. (Table 2.3.2) When compared to patients in whom all original disease sites were still visible, patients with one or more DLM presented more often with synchronous disease (OR 8.02; p=0.006) and initially unresectable disease (OR 4.90; p<0.001). Moreover, DLM were more common in patients with 4 or more metastases (25/53; 47.2%) when compared to patients with 3 or less metastases (15/115; 13.0%) detected prior to chemotherapy (OR 5.95; p<0.001). While no correlation between the last regimen of preoperative chemotherapy and the probability of developing DLM was seen, patients with a complete radiological response in one or more metastases received more cycles of preoperative chemotherapy (7.7±5.1 courses) than their counterparts without DLM (5.5±3.1 courses; OR 1.14; p=0.01). On multivariate analysis of factors predictive for the development of a complete radiologic response in one or more metastases, only tumor number > 3 (OR 13.1; p<0.001) and the number of courses of preoperative chemotherapy (OR 1.18; p=0.03) had an independent association with the development of one or more DLM. In addition, the median size of metastases prior to chemotherapy was significantly smaller in metastases that disappeared (median 1.0 cm; range 0.3 – 3.5 cm) when compared to the size of metastases that

TABLE 2.3.2: Univariate and multivariate logistic regression analysis of factors associated with the development of one or more DLM

Variable	OR	95% CI	p-value	OR	95% CI	p-value
Gender (male)	0.95	0.46-1.94	0.89	-		
Age	0.99	0.97-1.03	0.73	-		
Synchronous presentation	8.02	1.84-34.9	0.006	3.90	0.77-21.6	0.13
Positive node status	0.91	0.41-1.98	0.80			
Tumor number > 3	5.95	2.77-12.8	<0.001	13.1	3.50-49.3	<0.001
Pre-operative chemotherapy						
5-Fluoropyrimidine only	-	-	-			
Irinotecan-based regimen	1.12	0.27-4.60	0.88	-		
Oxaliplatin-based regimen	1.33	0.34-5.12	0.67	-		
FOLFOXIRI	4.00	0.19-84.2	0.37	-		
Biological added	1.84	0.89-3.77	0.09	2.25	0.75-6.73	0.15
Total number of cycles	1.14	1.03-1.28	0.013	1.18	1.02-1.37	0.03
Initially unresectable disease	4.90	2.31-10.4	<0.001	1.76	0.52-6.01	0.36

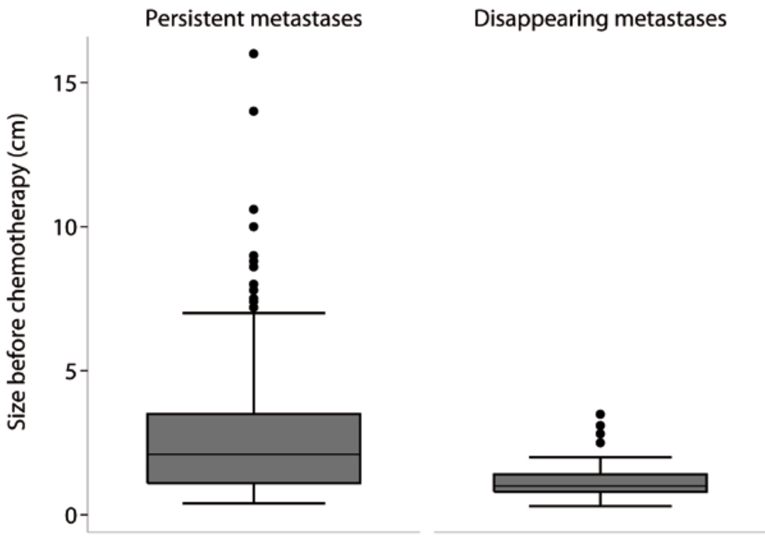


Figure 2.3.1: Box plot comparing the size of metastases prior to chemotherapy among those that radiologically disappeared versus those that remained visible following chemotherapy. Median diameter (range): Persistent = 2.1 cm (0.4–16 cm) vs. 1.0 cm (0.3–3.5 cm) ($p<0.001$).

did not disappear during chemotherapy (median 2.1 cm; range 0.4-16) ($p<0.001$) (figure 2.3.1).

Intraoperative Detection and Management of DLM

In 18 of the 40 patients (45.0%) with one or more DLM, all sites of metastatic disease identified prior to chemotherapy were detected during surgery, and in all cases, all sites were resected or ablated (Figure 2.3.2). In 22 patients with DLM (55.0%), detection of all DLM was not achieved during surgery. Of these, five patients underwent resection of regions in which the original tumors existed, all of which were achieved by incorporating these sites in a hemihepatectomy. In no cases was a separate resection performed of an undetected DLM.

Seventeen patients (42.5%) had DLM that were not detected and remained untreated during surgery. The median number of untreated metastases in these patients was 2 [range 1-11]. Specifically, in eight patients, one DLM was left untreated, in 4 patients 2 DLM were left untreated and in 3 patients 3 DLM were

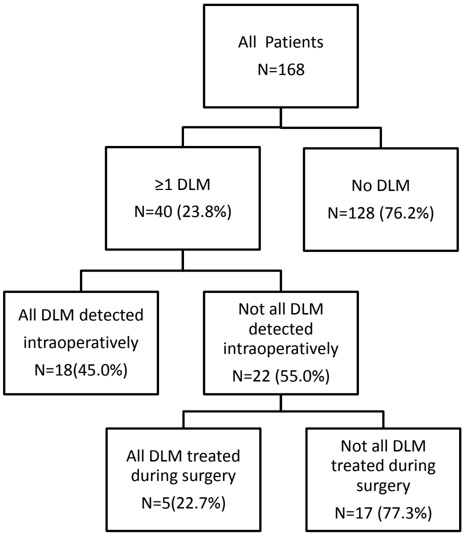


Figure 2.3.2: Flowchart depicting the findings and management of patients with one or more disappearing liver metastases (DLM).

left untreated. In two patients, ten and eleven metastases were left untreated respectively. The characteristics of these patients are outlined in table 2.3.3. Patients in which DLM were left untreated were more likely to have unfavorable prognostic factors when compared to patients in which all original disease sites were treated. More specifically, all patients with untreated DLM presented with synchronous disease and eleven of these patients (64.7%) had more than 4 metastases diagnosed prior to chemotherapy. Also, the majority of these patients (n=13; 76.5%) were initially considered unresectable, and only became surgical candidates after a significant response to chemotherapy (all $p<0.05$).

Recurrence -Free and Overall Survival

Thirteen (76.5%) of the 17 patients with DLM that were left untreated developed an intrahepatic recurrence. Moreover, in 10 of these 13 patients (76.9%), this intrahepatic recurrence was observed at the site of an untreated DLM. While five of these 10 patients (50.0%) developed concomitant intra- or extrahepatic recurrences (intrahepatic n= 1, extrahepatic n=2, both intrahepatic and extrahepatic n=2), in five patients recurrence was truly local and limited to the site of an untreated DLM. All of these patients successfully underwent repeat

TABLE 2.3.3: Characteristics of patients with residual untreated DLM versus all original sites treated after surgery.

Variable	Residual untreated DLM n=17 (10.0%)	All original sites treated n=151 (90.0%)	*p*-value
Gender			
Male	11 (64.7)	83 (54.9)	0.44
Female	6 (35.3)	68 (45.1)	
Diagnosis of liver metastases			
Synchronous	17 (100)	111 (73.5)	0.015
Metachronous	-	40 (26.5)	
Primary nodal status			
Positive	8 (47.1)	106 (73.6)	0.023
Negative	9 (52.9)	40 (26.4)	
Unknown (n=7)	0	7	
Tumor number pre-chemotherapy			
1	0	61 (40.4)	0.001
2-3	6 (35.3)	48 (31.8)	
≥ 4	11 (64.7)	42 (27.8)	
Indication for Chemotherapy			
Neoadjuvant	4 (23.5)	110 (72.9)	<0.001
Conversion	13 (76.5)	41 (27.2)	
Complete Pathological Response in any CLRM			
Yes	12 (70.6)	129 (85.4)	0.131
No	4 (23.5)	17 (11.3)	
RFA only	1 (5.9)	5 (3.3)	
Resection Margin			
R0	15 (88.2)	134 (88.8)	0.78
R1	1 (5.9)	12 (7.9)	
RFA only	1 (5.9)	5 (3.3)	
Postoperative Adjuvant Chemotherapy			
None	8 (47.1)	70 (46.4)	0.185
Systemic	6 (35.3)	72 (51.4)	
Intra-arterial	3 (17.7)	9 (6.4)	

surgical treatment for this recurrent disease. Of the five patients in whom three or more DLM were left behind, all recurred in within the liver with a median time to recurrence of 7 months [range 4-14]. In two of these patients, recurrence was limited to the site of a DLM and repeat surgery was performed.

When compared to patients in whom all original disease sites were surgically treated, patients with untreated DLM had a significantly higher rate of intrahepatic recurrence. More specifically, 1- and 3-year intrahepatic recurrence-free survival rates were 40.2% and 16.1% for patients with untreated DLM (median; 11 months) compared to 69.4% and 35.3% for those patients in which all original disease sites were treated (median; 20 months) (p=0.04) (figure 2.3.3a). In addition, 1- and 3-year any site recurrence-free survival were 33.1% and 13.2% in patients with untreated DLM (median=10 months) and 60.6% and 23.1% in patients in which all original disease sites were treated (median=15 months) (p=0.06) (figure 2.3.3b).

We then investigated the impact of untreated DLM on overall survival. The median overall survival was 45 months for the entire cohort, corresponding to a 93.2%, 59.0% and 40.0% 1-, 3- and 5-year overall survival. For patients in which DLM sites were left untreated, 1-, 3-, and 5-year survival rates were 93.8%, 63.5% and 63.5% respectively with a median survival of 65 months. For those in which all original disease sites were treated, 1-, 3- and 5-year survival rates were 93.1%, 58.5% and 37.5% respectively with a median survival of 45 months. When comparing those groups, no statistically significant difference in overall survival was observed (logrank; p=0.31) (figure 2.3.4a).

To adjust for the potential prognostic influence of a radiological response to chemotherapy on overall survival, a stratified survival analysis was performed in a subgroup of patients with a complete or partial radiological response to chemotherapy (n=99). When comparing overall survival for patients with untreated DLM (median; 65 months) and patients in which all disease sites diagnosed prior to chemotherapy were treated (median; 54 months), no statistically significant difference was found with corresponding 1-, 3- and 5-year survival rates for patients without untreated DLM of 92.3%, 70.8% and 46.2% respectively and 93.8%, 63.5% and 63.5% respectively for those with untreated DLM (p=0.66) (figure 2.3.4b).

Analysis of True Complete Response

The true complete response rate of DLM lesions was examined by determining both the complete pathological response in resected lesions as well as the durable remission in those lesions left in situ. Of the 126 DLM observed, 69 (54.7%) were

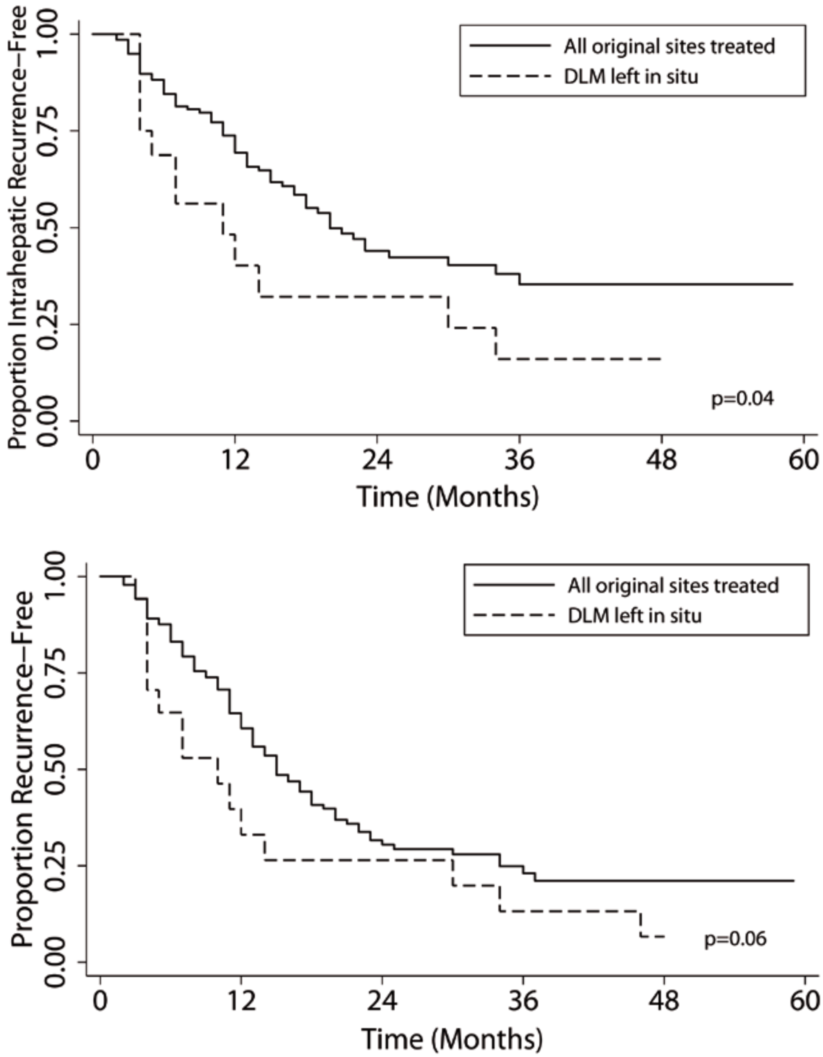
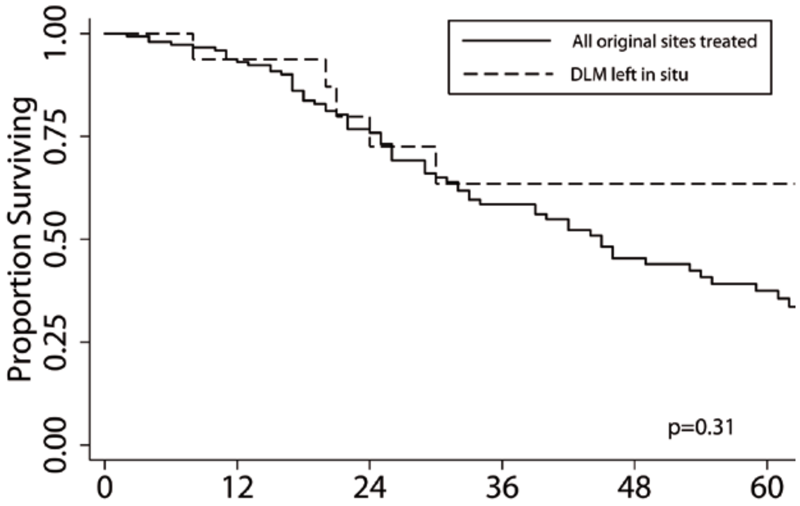


Figure 2.3.3: (A) Kaplan Meier curve of intrahepatic recurrence-free survival in patients with untreated DLM when compared to patients in whom all original disease sites were resected. (B) Kaplan Meier curve of any site recurrence-free survival in patients with untreated DLM when compared to patients in whom all original disease sites were resected.



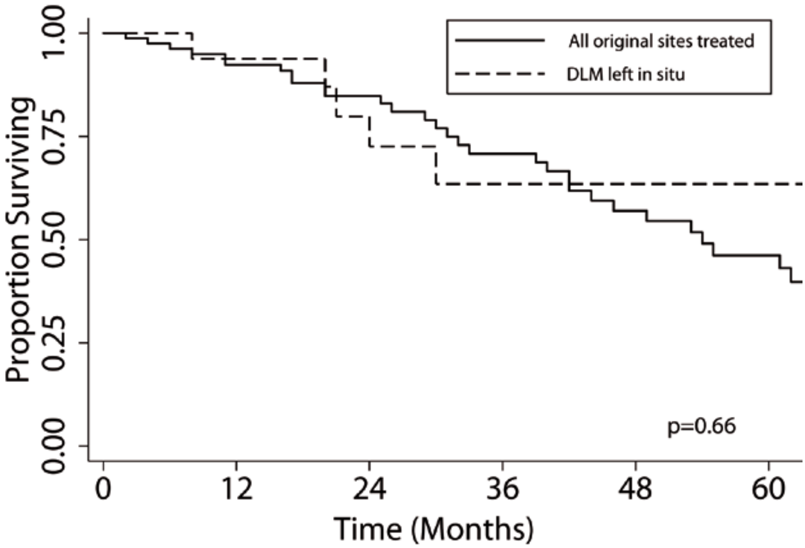


Figure 2.3.4: (A) Kaplan Meier curve of overall survival in patients with untreated DLM when compared to patients in whom all original disease sites were resected. (B): Kaplan Meier curve showing overall survival in 99 patients with a complete or partial radiological response (RECIST) to preoperative chemotherapy stratified by the presence of untreated DLM.

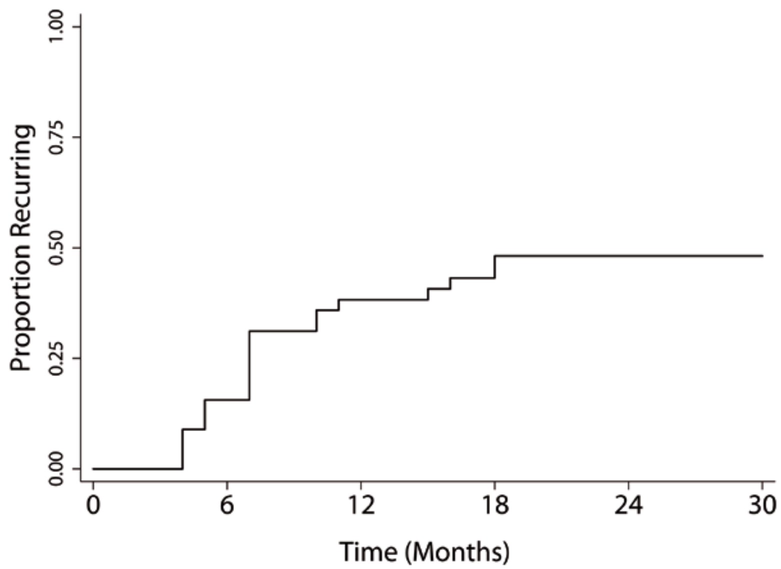


FIGURE 2.3.5: Kaplan–Meier plot illustrating the proportion of undetected DLM recurring locally when left untreated

detected during surgery and concomitantly treated (resection n=55; ablation n=14). Of the 55 DLM that were detected and resected (excluding ablated lesions), 19 metastases (34.5%) showed a complete pathological response. In contrast, complete pathological response was observed in 7 of the 12 DLM (58.3%) that were not detected during surgery but were incorporated in the resection of one hemiliver. Of the 45 DLM that were left untreated, 24 (53.3%) did not recur during a median follow-up of 20 months (range 7-88) (figure 2.3.5). Therefore, a true complete response was observed in 50 of the 112 DLM available for analysis (44.6%).

Discussion

In this study, one or more disappearing liver metastases were found to occur in 23.8% of patients receiving preoperative chemotherapy. We found that only approximately half of these could be indentified during surgery and if so, all of these sites were able to be resected or ablated. In 42.5% of these patients, one or more DLM remained untreated at the time of surgery, resulting in potential increased risk of intrahepatic recurrence. However, overall survival did not seem to be significantly impacted in these highly selected patients and was comparable when compared to patients in whom all original disease sites were detected.

The number of patients developing DLM in our study was higher than that reported in other series [9]. This likely reflects an aggressive policy towards our patients with initially unresectable disease and tendency towards surgical therapy if a radiological response is observed [13]. Indeed, many patients that developed DLM in this study were considered initially unresectable (61%), with utilization of longer duration and more aggressive chemotherapeutic regimens. In addition, the majority of the patients had multiple metastases, increasing the probability of developing DLM.

In this study, we found that patients with multiple tumors and those undergoing longer duration of chemotherapy were associated with a higher risk of developing DLM. More than 60% of patients with at least one DLM had four or more metastases prior to chemotherapy. In addition, small metastases (median size 1-cm) were more likely to disappear. These findings are not surprising but may be useful when planning use of chemotherapy prior to plan surgical therapy in order to avoid a complete radiologic response when possible. When a patient is initially resectable and the intent of chemotherapy is as a neoadjuvant approach, limiting the duration may be prudent. Small tumors in sites which may prove to be problematic if not operatively detectable following a response may be considered for initial surgical intervention. In initially unresectable patients in whom preoperative chemotherapy is being employed to convert to a resectable status, careful serial imaging is important, proceeding to surgical therapy as soon as resectability is achieved rather than waiting for maximum response. In addition, marking a small tumor which is in a potentially difficult location with a radiologically placed fiducial can be considered, either prior to chemotherapy [14] or using post-response marking based on the initial imaging studies.

We found that upon surgical exploration, including IOUS, an identifiable lesion was found in 55% of the metastases that had disappeared on cross sectional-imaging. The rate of intraoperative DLM detection rate found in our study was higher than that in most other reported series. Benoist et al. reported only 20 of

66 lesions (31%) with complete radiologic response could be found operatively [9]. Tanaka et al reported a 36% operative detection rate of DLM. [11] Reasons for these differences are likely multifactorial, in part related to the choice of imaging technique and perhaps time lapse between chemotherapy and surgery. In addition, lesions located near the surface or which become more conspicuous on IOUS may be detected more easily. Several studies have established the importance of detecting and identifying all macroscopic disease when possible in order to offer improved outcome for patients undergoing surgical therapy of colorectal metastases[8,9,11]. Implementation of preoperative chemotherapy may have a variable effect on the ability to detect and therefore treat all sites of disease. While in some cases reduction in tumor size may limit detection, changes in lesion echogenicity may paradoxically improve detection rate in other situations[15-17].

A true complete response, either no viable disease on pathologic assessment or a durable local remission of an unresected site, was observed in 43% of DLM in our study. Some studies have reported true complete responses in excess of 50% of cases, but these have included those receiving regional intrahepatic chemotherapy [8,10,11].[16] Benoist et al reported complete durable responses to be found in only 17% of lesions with radiologic complete response. Likely, observed differences may reflect various confounding factors, including chemotherapy duration and choice of agents, as well as differences in the waiting period between the development of DLM and surgical intervention. Yet, with the higher observed rate of true complete responses seen in this and other studies using aggressive chemotherapeutic regimens, the dogma that viable disease exists in most DLM might be reconsidered.

An important question arises regarding the optimal management of patients in which DLM occur. Given the relatively low rate of true complete pathological responses in these DLM and the high rate of intrahepatic recurrences observed in patients with untreated DLM, we still recommend that complete surgical treatment of all original sites should be done when possible, even if undetected intraoperatively. When a lesion cannot be identified, incorporation of the original sites into the hepatectomy should be done when possible. Such “blind” resections may include a major hepatectomy, for example, when lesions were originally contained within one hemiliver, even if persistent sites can be treated with limited resection or ablation. However, this may not be safe or possible in all cases. We found that in such patients, leaving undetected lesions untreated can still be associated with reasonable long-term outcome when repeat resection or ablation of an isolated local recurrence is possible[18].

The retrospective design of the current study presents some limitations to the analysis which necessitate some tempering of definitive conclusions based on these findings. Imaging methodology had evolved over the study period. In addition, while management decisions were based on radiologic assessment at the time, this study did not incorporate a systematic re-review of the cross-sectional studies.

In summary, disappearing metastases were commonly observed in patients receiving preoperative chemotherapy. With increasingly aggressive multimodality strategies being offered to patients with advanced colorectal cancer, including liver resection following chemotherapy, this is likely to become an increasingly common problem facing the hepatic surgeon. Anticipating the occurrence of DLM in patients with small, multiple metastases may alter management strategies regarding choice and duration of chemotherapy before surgery. When DLM develop, one can anticipate finding and treating these lesions in many cases with careful intraoperative assessment. In those circumstances in which all sites cannot be identified and when incorporation of undetected original sites in a resection is not safe or possible, leaving them behind can be considered in selected cases. However, these untreated sites have a high risk of in situ recurrence and therefore we advise that one must only consider surgical therapy for those in whom all original sites can be treated, either at the time of initial surgery or when a recurrence occurs after initial liver surgery.

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3

Local Ablative Therapies and Imaging

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Adapted From: *Therapeutic Efficacy of Combined Intraoperative Ablation and Resection for Colorectal Liver Metastases.*

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3.1

Resection and RFA for Colorectal Liver Metastases

Abstract

Background: Only 10-25% of patients presenting with colorectal liver metastases (CRLM) are amenable to hepatic resection. By combining resection and ablation, the number of patients eligible for surgery can be expanded. We sought to determine the efficacy of combined resection and ablation for CRLM.

Methods: Between 1984-2009, 1425 patients who underwent surgery for CRLM were queried from an international multi-institutional database. Of these, 125 patients underwent resection combined with ablation as the primary mode of treatment.

Results: Patients presented with a median of 6 lesions. The median number of lesions resected was 4; the median number of lesions ablated was 1. At last follow-up, 84 patients (67%) recurred with a median disease-free-interval of 15 months. While total number of lesions treated (HR: 1.47; p=0.23) and number of lesions resected (HR: 1.18; p=0.43) did not impact risk of intrahepatic recurrence, the number of lesions ablated did (HR: 1.36; p=0.05). Overall 5-year-survival was 30%. Survival was not influenced by the number of lesions resected or ablated (both p>0.05).

Conclusions: Combined resection and ablation is associated with long-term-survival in a subset of patients, however recurrence is common. The number of lesions ablated increases risk of intrahepatic recurrence but does not impact overall survival.

Introduction

Colorectal cancer is the third most common type of cancer worldwide and the second most common cause of cancer-related death in North America and Western Europe.[1,2] Up to one-fourth of patients with colorectal carcinoma have synchronous hepatic metastasis at the time of presentation, while another one in five patients develops metachronous metastasis to the liver during the course of their disease.[3,4] The median survival of patients with unresectable colorectal liver metastases (CRLM) is 21 to 24 months.[5,6] When feasible, surgical resection is the gold standard in the treatment of CRLM. Following resection of CRLM, 5-year survival rates of up to 58% have been reported.[7-12]

Unfortunately, only 10-25% of patients with CRLM are amenable to hepatic resection. Many patients are not candidates for resection due to the number and distribution of the hepatic lesions. Due to recent advances in both surgical and more importantly, medical oncology, the criteria for resectability of CRLM have expanded.[13] Certain patients can be treated with pre-operative chemotherapy in order to decrease the tumor burden in the liver.[14,15] Other patients who may have an anticipated small future liver remnant may be candidates for portal vein embolization.[16,17] Still other patients who have multiple bilateral lesions may be candidates for a two-stage approach.[18] Another therapeutic option for patients with multiple intrahepatic CRLM can involve the combination of hepatic resection with ablation.

By utilizing combined modality approaches such as interstitial ablative techniques simultaneous with hepatic resection, the number of patients eligible for curative intent surgery may be expanded.[13] Most published data on CRLM and ablation have focused on outcomes comparing patients who exclusively underwent either resection versus ablation only.[19-21] The data on combining resection with ablation for the primary treatment of advanced CRLM have been more limited. Specifically, most previous data on combined resection and ablation for primary hepatic treatment of CRLM have come from single-institution series and were limited by small sample sizes (n <75 patients).[22-29] In addition, previous studies failed to examine pattern of disease recurrence relative to the number of lesions resected versus ablated.[30] As such, the purpose of the current study was to determine the therapeutic efficacy of combined resection and ablation for CRLM as well as determine factors predictive of survival in a large multicenter cohort of patients. In addition, we sought to identify those factors predictive of recurrence, with a particular emphasis on how the number of lesions ablated impacted the risk of recurrence.

Methods

Data on 1425 patients who underwent liver directed therapy for CRLM from 1984 to 2009 were indentified from an international, multi-institutional hepatobiliary database (Johns Hopkins Hospital, Baltimore, USA, Maastricht University Medical Centre, Maastricht, the Netherlands, Ospedale Mauriziano Umberto I, Turin, Italy and Cliniques Universitaires Saint-Luc, Brussels, Belgium). The study was approved by the Institutional Review Boards of the respective institutions. Patients who were operated on with a palliative intent, who had less than 6 months of follow-up, or who were lost to follow-up were excluded. Only patients who underwent initial hepatic resection combined with simultaneous intraoperative ablation as the primary mode of treatment for CRLM were included; patients who underwent percutaneous ablation were excluded. Only patients who were operated on with curative intent and who had planned complete extirpation / destruction of all known hepatic disease were included. A total of 125 (8.6%) patients were identified and were the subject of the current study.

At the time of surgery, all patients were treated with both resection and ablation during the same operation. At the time of laparotomy, following mobilization of the liver, intraoperative ultrasound was performed to identify and characterize the nature and location of the CRLM. In general, the surgical approach involved resection of the larger / dominant lesions combined with ablation of the lesser disease. Lesions were considered resectable if at least two adjacent hepatic segments could be spared, vascular inflow and outflow could be maintained and adequate biliary drainage could be preserved while maintaining an adequate liver remnant while achieving an anticipated R0 resection.[13,31] Resection was classified as a minor resection (<3 liver segments) or a major hepatic resection (≥3 liver segments).[32] For those CRLM that were considered unresectable, due to location, inadequate future liver remnant or proximity to vascular structures, ablation was utilized. In general, radiofrequency ablation (RFA) (n=101) was performed using a radiofrequency generator (RITA Model 1500X Rita Medical Systems, Inc, Fremont, California or RF 2000 with LeVeen; Radio Therapeutics, Mountain View, Corporation, California) and when applicable a saline-enhanced device (Starburst XL or XLi, Rita Medical Systems, Inc). A small minority of patients underwent cryoablation (Cryotech LC52000; Candela Laser, Wayland, Massachusetts) (n=21) or microwave ablation (Microsulis Tissue Ablation; Microsulis Medical Limited, Denmead, Hampshire, United Kingdom) (n=3). Ablation of CRLM was performed at the time of laparotomy according to a previously described standardized treatment approach.[33,34] In short, intraoperative ultrasonography was used to place the needle into the lesions to be treated. Ablation was only considered to be curative in intent when the probe could be optimally positioned under intraoperative ultrasound guidance, to achieve complete destruction of the tumor and at least a 1 cm zone of normal liver parenchyma. In most cases, postoperative cross-sectional imaging was obtained prior to discharge to ensure adequate ablation and moreover to establish a new baseline image for future follow-up.

All patients were followed regularly based on established algorithms at each respective institution. In general, follow-up in general consisted of outpatient evaluation along with appropriate cross-sectional (i.e. abdominal and thoracic computed tomography scan (CT-scan)) imaging as well as the serum tumor marker carcinoembryonic antigen (CEA) every 3 to 4 months following surgery up to 2 years and then every 6 months thereafter. Recurrence was defined as a lesion that was biopsy proven recurrent adenocarcinoma or a lesion that was deemed suspicious on cross-sectional imaging in the setting of an elevated CEA level.

Data collection

In addition to standard demographic data, the following data were collected for each patient: primary tumor characteristics (TNM-stage and location of primary tumor), CRLM details (details on hepatic metastases location, number and size), tumor marker CEA, treatment related variables and presence of extrahepatic metastases. Data on vital status and recurrence (including locations of recurrence) were noted. Recurrence was defined as intra- or extrahepatic.

Statistical Analyses

Summary statistics were obtained using established methods and presented as percentages or median values. Time to recurrence and survival were estimated using the nonparametric product limit method. Differences in survival were examined using the log-rank test. Factors associated with survival were examined using univariate and multivariate Cox regression analyses. The hazard ratio (HR) and the 95% confidence intervals (CI) were estimated and a *p*-value of less than 0.05 was considered significant. All statistical analyses were performed using SPSS version 17.0 (Chicago, Illinois).

Results

Patient and tumor characteristics

The patient and tumor characteristics of the 125 patients who underwent simultaneous resection plus ablation are detailed in Table 3.1.1. Over time, the combination of resection plus ablation was utilized in an increasing number of patients after 2000 ($p<0.001$). Among patients with metachronous disease, the median time from the primary tumor diagnosis to the development of liver metastasis was 13.9 months.

With regard to the extent of CRLM, about one-half of patients had bilateral disease (n=59; 47.2%). The median size of the largest lesion was 3.0 cm. The median number of treated CRLMs was 6 lesions. A small subset of patients (n=12; 9.6%) had extrahepatic metastatic disease at the time of liver directed surgery. The site of extrahepatic metastasis was pulmonary in most patients (n=5; 41.7%).

Overall, 109 individual patients (87.2%) received chemotherapy. Pre-operative chemotherapy was administered to 85 (68.0%) patients, whereas 74 (59.2%) patients received adjuvant chemotherapy. Among those subjects for which the exact pre-operative chemotherapy-regimen was known, 19 (22.4%) patients received monotherapy with 5-fluoruracil, while 26 (30.6%) patients received an oxaliplatin-based regimen and another 26 (30.6%) patients received an irinotecan-based regimen. Regarding adjuvant chemotherapy, among those cases for which the exact chemotherapy-regimen was known, 16 (21.6%) patients

TABLE 3.1.1. Patients and tumor characteristics

Variable	No. of patients (%), n=125
Patient characteristics	
Median age [range], y	59 [29-83]
Sex (male)	80 (64.0)
Primary Tumor Site	
Location of primary tumor, colon	89 (71.2)
AJCC T-stage, T3/T4	99 (79.2)
Lymph node disease	85 (72.6)
Hepatic Metastasis	
Presentation, synchronous	74 (59.2)
Size of largest metastasis (median [range]), cm	3.0 [0.4-9.2]
No. of metastasis (median [range])	6 [2-19]
Location (unilobular)	66 (52.8)
Details of surgical procedure	
Total number of resected tumors (median [range])	4 [1-16]
Total number of ablated tumors (median [range])	1 [1-8]
Number of ablated tumors per patient	
1	65 (52.0)
2	22 (17.6)
3	16 (12.8)
≥4	22 (17.6)
Extent of hepatic resection	
Minor hepatectomy	89 (71.2)
Major hepatectomy	36 (28.8)

received monotherapy with 5-fluoruracil, while 20 (27.0%) and 15 (20.3%) received oxaliplatin- or irinotecan-based regimens, respectively.

At the time of surgery, the majority of patients (n=89; 71.2%) underwent a minor hepatic resection (<3 segments) (Table 3.1.1). The majority of these patients underwent multiple minor resections. Overall, the median number of lesions resected was 4 [range, 1-6], whereas the median number of lesions ablated was 1 [range, 1-8]. The exact combinations of resection and ablation are summarized in Table 3.1.2. Patients who had ablation performed of only 1 lesion (n=65; 52.0%) were more likely to have undergone a major hepatic resection (n=26; 40.0%) compared with patients who had more than 1 lesion ablated (n=10; 16.7%) (p=0.004).

TABLE 3.1.2. Summary of the combinations of extent of hepatic resections and number of lesions ablated.

	No. of patients (%), n=125			
Extent of hepatic resection	*Number of lesions ablated per patient*			
	1 (*n*=65)	2 (*n*=22)	3 (*n*=16)	≥4 (*n*=22)
Minor hepatectomy (*n*=89)	39 (60.0)	16 (72.7)	13 (81.2)	21 (95.5)
Major hepatectomy (*n*=36)	26 (40.0)	6 (27.3)	3 (18.8)	1 (4.5)

Post-operative death within 90 days of treatment occurred in 2 (1.6%) patients, both of whom underwent a major hepatic resection combined with ablation. One patient developed portal vein thrombosis, liver failure, multi-system organ failure, and died on post-operative day 10. The second patient developed fulminant liver failure with associated intractable metabolic acidosis and died on post-operative day 14.

Recurrence and Overall Survival

Following liver directed surgery, 84 (67.2%) patients recurred after a median disease-free interval of 14.7 months. Overall, 3- and 5-year disease-free survival was 24.2% and 14.7%, respectively. At the time of last follow-up, the pattern of recurrence was intrahepatic only in 43 (34.4%) patients, extrahepatic only in 22 (17.6%) patients, and intra- and extrahepatic in 18 (14.4%) patients. Compared with patients who underwent resection only (n=1292) or ablation only (n=35), patients who underwent concomitant resection plus ablation had a worse disease-free survival (Figure 3.1.1). Patients who underwent resection plus ablation, however, also had an increased likelihood of other adverse clinopathologic factors. Specifically, patients who underwent combined resection and ablation more often presented with synchronous disease, a greater number of hepatic metastases, and bilateral hepatic involvement (all $p<0.05$). On univariate analysis, patients who had more than 1 lesion ablated had a higher risk of any-site recurrence compared with patients who had only 1 lesion ablated (HR=1.14; $p=0.04$). On multivariate analysis, after controlling for competing risk factors, the number of lesions ablated was no longer associated with any-site recurrence-free survival ($p=0.12$) (Table 3.1.3). Moreover, when these analyses were performed excluding the 12 patients who presented with concomitant extrahepatic disease, the same results were observed.

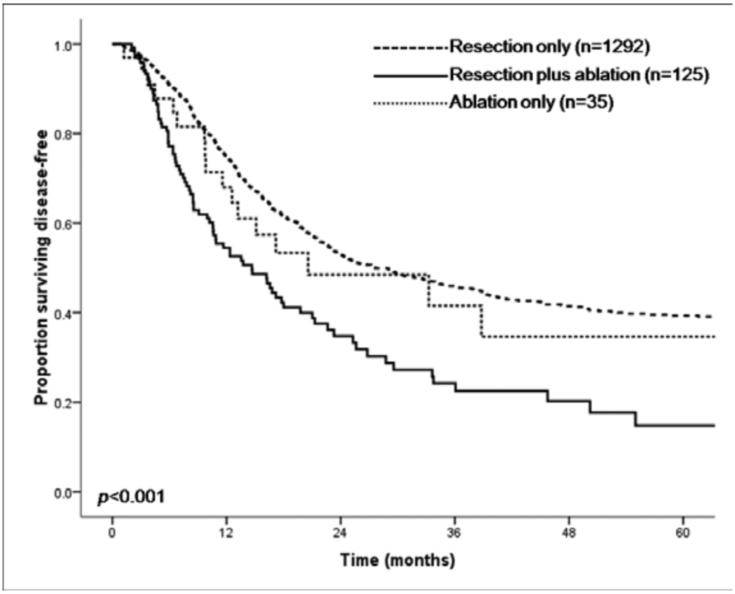

Figure 3.1.1. Kaplan Meier curves showing disease-free survival stratified by mode of curative intent surgery: combined resection and ablation versus resection only versus ablation only.

TABLE 3.1.3. Univariate and multivariate analyses of factors proposed to be associated with recurrence free survival

	Univariate analysis			*Multivariate analysis*		
Prognostic factor	**Hazard Ratio**	**95% CI**	***p*-value**	**Hazard Ratio**	**95% CI**	***p*-value**
Gender (male)	1.12	0.70-1.78	0.65	--	--	--
Location of primary tumor (rectum)	1.12	0.70-1.78	0.65	--	--	--
AJCC T-stage (T3 or T4)	1.08	0.55-1.10	0.83	--	--	--
Node positive primary tumor	1.73	1.03-2.91	0.040	1.86	1.07-3.24	0.029
Serum CEA >200 ng/mL	2.00	0.86-4.68	0.11	1.97	0.82-4.74	0.13
Synchronous presentation	1.23	0.79-1.91	0.37	--	--	--
Bilobar disease	1.02	0.66-1.58	0.91	--	--	--
Size of largest lesion	1.00	0.88-1.12	0.96	--	--	--
Number of CRLM resected	1.05	0.98-1.13	0.20	--	--	--
Number of CRLM ablated	1.14	1.00-1.29	0.043	1.11	0.97-1.27	0.12
Total number of CRLM treated*	1.05	0.99-1.11	0.072	--	--	--
Extent of hepatic resection	1.49	0.90-2.43	0.12	1.33	0.76-2.30	0.32
Presence of concomitant extrahepatic disease	1.83	0.87-3.84	0.11	1.59	0.73-3.47	0.24

* Not included in multivariate analysis due to collinearity

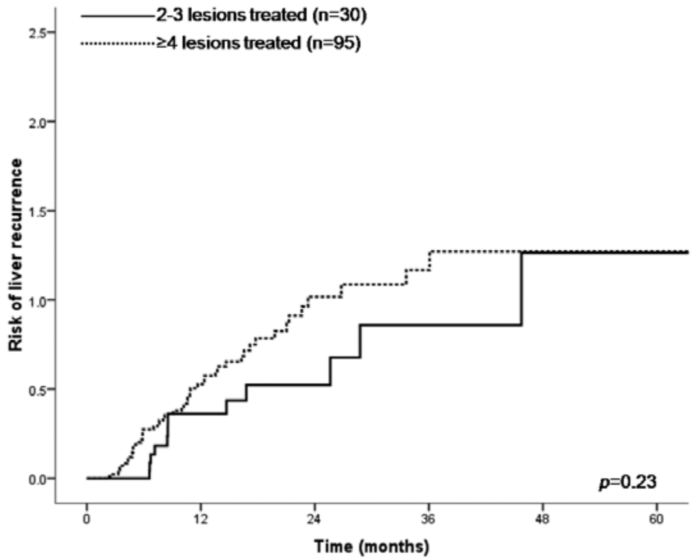

Figure 3.1.2. Hazard curves showing the risk of developing an intrahepatic recurrence, stratified by total number of lesions treated.

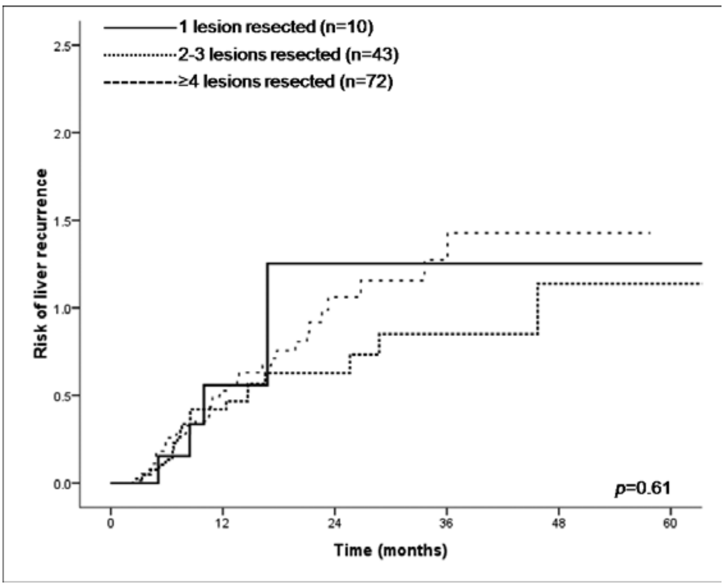

Figure 3.1.3. Hazard curves showing the risk of developing an intrahepatic recurrence, stratified by total number of lesions resected.

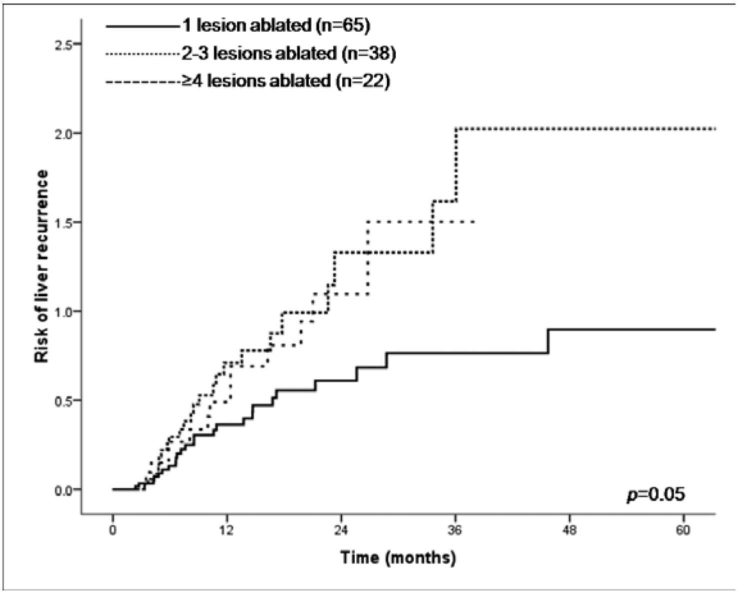

Figure 3.1.4. Hazard curves showing the risk of developing an intrahepatic recurrence, stratified by total number of lesions ablated.

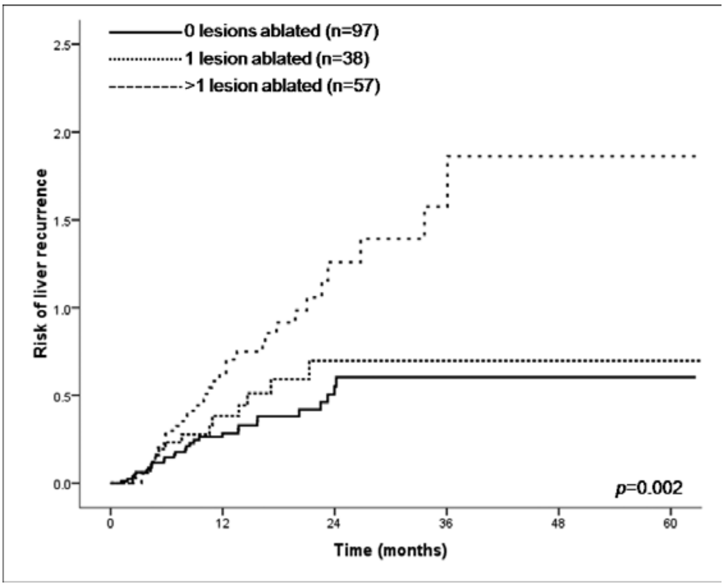

Figure 3.1.5. Hazard curve showing the risk of intrahepatic recurrence among patients with 4 or more lesions treated stratified by number of lesions ablated.

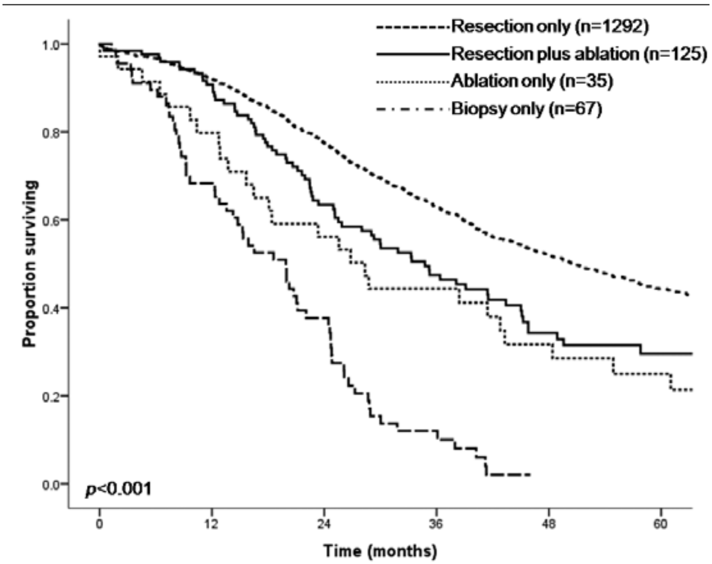

Figure 3.1.6. Kaplan Meier curves showing overall survival stratified by mode of curative intent surgery: combined resection and ablation versus resection only versus ablation only.

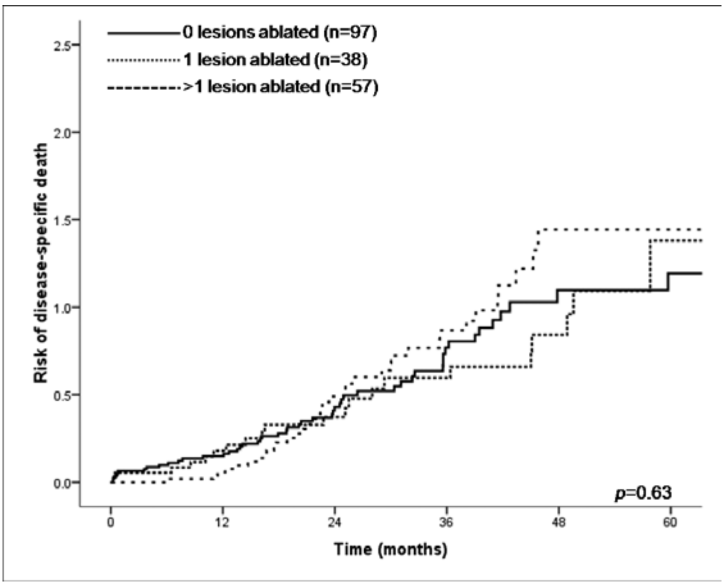

Figure 3.1.7. Hazard curve showing the risk of disease specific death among patients with 4 or more lesions treated stratified by number of lesions ablated.

Among all patients who underwent resection plus ablation, the total number of lesions treated (p=0.23) and the number of lesions resected (p=0.43) were not associated with risk of intrahepatic recurrence (Figure 3.1.2 and 3.1.3). In contrast, there was an associated trend with the number of lesions ablated and the risk of intrahepatic recurrence (HR: 1.36; p=0.05) (Figure 3.1.4). In examining the entire cohort of patients who had 4 or more lesions (n=192), the subgroup of patients who had 4 or more lesions treated with resection plus ablation (n=95; 77.6%) had a higher risk of intrahepatic recurrence than patients who had 4 or more lesions treated by resection only (n=97) (HR: 1.87; p=0.002)(Figure 3.1.5). However, among patients who had 4 or more lesions treated with resection plus ablation, the risk of intrahepatic recurrence was higher among patients who had more than 1 lesion ablated versus patients who had only 1 lesion ablated (HR: 1.93; p=0.04). In fact, the risk of intrahepatic recurrence was similar among patients who underwent resection alone versus resection plus a single ablation.

The overall median survival following liver directed surgery for patients treated with resection plus ablation was 34.8 months, with a 3- and 5-year overall survival of 47.3% and 29.5%, respectively. While overall survival was better among patients treated with resection only (median 50.5 months), patients who did not have

TABLE 3.1.4. Univariate analyses of factors proposed to be associated with overall survival

	Univariate analysis		
Prognostic factor	**Hazard Ratio**	**95% CI**	***p*-value**
Gender (male)	0.84	0.53-1.34	0.47
Location of primary tumor (rectum)	1.22	0.74-2.01	0.50
AJCC T-stage (T3 or T4)	1.07	0.51-2.23	0.87
Node positive primary tumor	1.71	0.98-3.00	0.060
Serum CEA >200 ng/mL	0.48	0.12-1.93	0.30
Synchronous presentation	0.83	0.52-1.31	0.43
Bilobar disease	1.12	0.70-1.79	0.42
Size of largest lesion	1.04	0.92-1.19	0.64
Number of CRLM resected	1.01	0.93-1.09	0.52
Number of CRLM ablated	1.12	0.99-1.27	0.080
Total number of CRLM treated	1.03	0.97-1.08	0.38
Extent of hepatic resection	1.54	0.90-2.63	0.11
Presence of concomitant extrahepatic disease	1.48	0.68-3.25	0.32

extirpation of their disease (i.e. biopsy only) had a worse survival compared with patients treated with resection plus ablation (19.9 months) (*p*=0.001) (Figure 3.1.6). Among those patients treated with resection plus ablation, the total number of lesions treated, the number of lesions resected and the number of lesions ablated were not associated with overall survival (all *p*>0.05) (Table 3.1.4). Excluding the 12 patients who had extrahepatic disease at time of presentation had no difference on these results.

To control for tumor number, a stratified analysis was then performed examining survival of patients who had 4 or more lesions. Among all patients with 4 or more lesions (n=192), patients treated with resection only (n=97) were noted to have a similar overall median survival 5-year survival, compared with patients who had resection plus ablation (35.6 months (30.3%) versus 31.9 months (25.0%), respectively) (both *p*>0.05). Patients who had 4 or more lesions treated with resection plus ablation had no difference in 5-year survival whether 1 lesion was ablated (25.1%) versus more than one lesion ablated (23.7%) (*p*=0.63)(figure 3.1.7).

Discussion

Combining resection with ablation has been proposed as a means to increase the number of patients with CRLM eligible for liver-directed therapy as it may allow the surgeon to remove the bulk of disease while ablating any smaller residual disease. While data on the combination of resection and ablation is important, very limited data exist in the literature. Most studies are single institution series comprising a small numbers of patients.[22-29] Our data show that combined resection plus ablation is a relatively infrequent therapeutic approach to patients with CRLM (8.6%). The reason for this is probably multi-factorial but undoubtedly reflects, in part, the authors' collective inclination to resect CRLM when possible. The current study is to our knowledge, one of the largest series of patients treated with combined resection and ablation specifically for CRLM. Perhaps more importantly, the current study not only examined recurrence and overall survival, but also investigated the impact of ablation number on outcome.[30] In treating patients with combined resection and ablation, the relative impact of an increasing number of ablations has not been well-defined. Our data suggest that an increasing number of ablations increased the risk of intrahepatic recurrence, but did not impact overall survival. De Haas et al. had previously reported that R1-margin status following surgical resection of CRLM was associated with risk of intrahepatic recurrence, but not disease-specific death.[35] The current data are important because, for the first time, we report that similarly for ablation of CRLM, the number of ablations is associated with an increased risk of intrahapetic recurrence, but not a worse survival.

There has been concern that surgery for extensive hepatic disease may be associated with increased peri-operative mortality. Previous publications, however, have shown that ablation combined with hepatic resection does not necessarily increase peri-operative mortality.[33,36] Resection plus ablation is generally well tolerated and comparable with the mortality associated with resection alone.[29,33] Our data would corroborate the low mortality associated with resection plus ablation as our reported post-operative mortality was only 1.6%. However, it is important to note that two patients did die following resection plus ablation. In both cases, ablation was combined with a major hepatic resection (i.e. right hepatectomy) and the patients succumbed to liver failure. Other investigators have similarly reported death secondary to liver failure in patients undergoing resection combined with ablation.[28,33,37] Similar to considering a major extended hepatic resection, when combining resection of the right liver with ablation of the left liver, the anticipated size of the *viable* remnant liver must be considered in light of the risk of possible liver insufficiency.
In the past, 4 or more colorectal liver metastasis were considered a relative contra-indication to surgery.[38,39] Now few surgeons would consider tumor number alone to be a contra-indication to surgery.[40] Managing patients with multiple hepatic metastases can be challenging and it is this subset of patients where combined therapy with resection plus ablation is most applicable. Although patients treated with combined resection plus ablation have a worse long-term survival compared with patients treated with resection alone, these patients also had a greater number of hepatic metastases, as well as an increased risk of other adverse clinicopathologic factors. As such, comparison of these groups may be inappropriate and lead to unreliable causal inferences, as the two groups are inherently not comparable.[41] Therefore, to help control for tumor number, a stratified analysis was performed to examine survival. Among all patients with 4

or more lesions, patients treated with resection only had a similar 5-year survival compared with patients who were treated with resection plus ablation. Perhaps more interestingly, we found that among patients who had 4 or more lesions treated with resection plus ablation there was no difference in survival whether 1 lesion was ablated versus more than one lesion ablated. As such, when planning the surgical approach for patients with multiple lesions, the relative number of lesions to be resected versus ablated does not appear to impact overall survival. Rather, in patients with multiple tumors and advanced CRLM, the inherent tumor biology of the underlying disease is more likely to be the important factor dictating long-term outcome.

Recurrence among patients who underwent resection combined with ablation was common (actuarial 5-year disease-free survival: 14.7%). While on multivariate analysis the number of lesions treated or ablated was not associated with any-site recurrence, the number of lesions ablated did impact the risk of intrahepatic recurrence. Interestingly, among patients who had 4 or more lesions treated with resection plus ablation, patients who had more than 1 lesion ablated had nearly a two-fold increased risk of intrahepatic recurrence compared with patients who had only 1 lesion ablated. Other investigators have suggested that the use of ablation relative to resection may increase the risk of intrahepatic recurrence while not impacting overall survival.[10] The current study provides data that specifically defines the relation of multiple ablations with the increased risk of intrahepatic recurrence.

The current study did not specifically examine the rate of "true" local intrahepatic failure at the site of ablation. Rather, we only reported the incidence of "any-site" intrahepatic recurrence. The local recurrence rate of ablation has previously been well documented.[42-45] Given the multi-center, international nature of the current study, re-review of cross-sectional imaging to document "true" local recurrence was not feasible. In addition, the goal of the current study was not to determine rates of local recurrence following ablation. As with virtually all retrospective analyses, selection criteria and surgical technique could not be standardized. However, the international, multi-instutional nature of our study instead provides a comprehensive "real-world" generalizeable experience of how resection combined with ablation is being used at major hepatobiliary centers worldwide.

In conclusion, although resection remains the gold standard treatment in most patients, a subset of patients with CRLM may benefit from hepatic resection and simultaneous ablation. While patients may derive a long-term survival benefit, recurrence is common. The number of ablations performed did not appear to impact long-term survival, but an increasing number of ablations was associated with an increased risk of intrahepatic recurrence.

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Under review

3.2

Operative Radiofrequency Ablation And Intraoperative Ultrasound

Abstract

Objective: To prospectively investigate the value of intraoperative ablation parameters to predict local recurrence after radiofrequency ablation (RFA) of colorectal liver metastasis (CRLM).

Background: While tumor ablative therapies such as RFA can offer potential benefit for patients with CRLM, the ability to determine efficacy of therapy intraoperatively and predict local failure remain poor.

Methods: 135 patients undergoing surgical RFA for CRLM from 2000 to 2009 were evaluated and data with regard to tumor size and location were collected. Among these, 54 patients were included in a prospective observational trial and data on intraoperative ablation parameters were collected. An intraoperative ablation score (A-status) was developed to predict long-term local control.

Results: 110 metastases were included in the prospective study. Tumor size >3cm (HR 5.78; $p<0.001$) and proximity to vasculature (HR 3.08; $p<0.001$) were associated with an increased risk of LR. Operative assessment scored ablations as A0, A1, and A2 in 76%, 18%, and 5%, respectively. Among these, the 1-year LR rates were 11.4 %, 41.3% and 66.6% respectively ($p<0.001$). When applied to tumors ≤3 cm and not in immediate proximity to vascular structures, an A0 ablation was associated with a 1- and 2-year local recurrence rate of 8.9% compared to a 14.3% and 46.4% 1-year and 2-year LR rate among A1/A2 lesions ($p=0.04$).

Conclusions: Durable local control can be achieved after RFA when tumors are carefully selected. Intraoperative parameters can aid the surgeon in the prediction of success.

Introduction

Radiofrequency ablation (RFA) has been shown to be a valuable therapy in patients with colorectal liver metastases who are not amenable to liver resection due to extensive disease or the inability to tolerate major hepatic resection [1-3]. Reported local recurrence rates after RFA range from 2% to 60 [4,5], leading to significant controversy regarding the efficacy and utility of this therapy for curative intent [6-9]. While differences in technical devices, approach, and surgeon experience may explain some of this variability, careful selection of the lesion for ablation likely plays an important role in durable success of RFA. In addition, the efficacy of RFA can be hampered by the inability to accurately target and monitor the ablation process intraoperatively. To determine whether an RFA has been technically successful, the surgeon often must rely on indirect parameters of success, such as the ultrasound visualization of probe position and deployment relative to the visualized tumor, achievement of target temperatures, and micro bubble distribution. Characterization of these intraoperative parameters is currently poorly defined. To address this question, we performed a prospective clinical trial to evaluate the value of several intraoperative ablation parameters, including the surgeon's intraoperative assessment of technical success, as well as the immediate postoperative radiologic assessment of completeness to predict local recurrence in a cohort of patients undergoing RFA of CRLM with long term follow-up. In addition, the correlation between preoperative patient and tumor characteristics and the probability of local recurrence after RFA was investigated.

Patients and Methods

Data collection

Between 2000 and 2009, 135 patients underwent open surgical ablation of colorectal liver metastases at the Johns Hopkins Hospital. Of these, the first 54 consecutive patients were included in a prospective observational study in order to evaluate collected intraoperative parameters and surgeon assessment of ablation confidence at predicting long-term success of RFA. In this trial, procedural parameters were captured, including (1) intended margin (based on planned tine deployment), (2) achievement of target temperatures during ablation (>100C), (3) encompassment of the tumor by micro bubble formation and (4) the need for multiple probe applications. In addition, an ablation score or A-status was determined by the surgeon immediately upon completion of the lesion ablation, scoring the confidence in which the surgeon felt adequate ablation occurred. This score was based on assessment of five factors: tumor echogenicity and conspicuity, accuracy of probe positioning and deployment, micro bubble distribution relative to the tumor, cool-down time, and overall confidence in the completeness of the ablation. Each lesion was scored on a 5-point scale and grouped by A-status: A0 (5 points, complete encompassment), A1 (4 points, close ablation margin), and A2 (≤ 3 points, incomplete or poor ablation). This study was approved by the Johns Hopkins Institutional Review Board.

In addition, a retrospective cohort study including all 135 patients with CRLM treated by open surgical ablation was performed to study the impact of patient related and tumor related variables on local recurrence.For each patient, the following data were collected: location and stage of primary tumor, preoperative CEA, disease-free interval between diagnosis of primary and metastatic disease, chemotherapy use, and tumor number. Tumor-associated parameters included lesion size and proximity to major vascular structures or the liver capsule. Major vessels were defined as > 3mm in diameter (hepatic or portal veins) and close proximity defined as the tumor edge within 5 mm of the structure.

Ablation Procedures

Indications for performing RFA in these patients were left to the discretion of the treating surgeon, based on the institutional policy to offer RFA in cases in which patients are not candidates for tumor resection. In all cases, RFA was performed during laparotomy under ultrasound guidance using a 4.0-8.0 MHz curvilinear transducer (Phillips ATL HDI 5000). In most procedures, RFA was performed using a RITA 1500X RF generator with a RITA XL or RITA XLie probe (Angiodynamics, Queensbury, NYC), based on the discretion of the surgeon. In three patients, a multiple parallel needle RFA applicator was used (Radionics, Burlington, MA). In all cases, the intent was curative, with a goal of achieving at least one centimeter ablation margin when possible. In some cases in which a single application did not create a sufficient zone of tissue ablation, the probe was repositioned to create a larger zone of tissue destruction when possible.

Postoperative Imaging and Determination of Local Recurrence

Radiologic assessment of completeness of ablation was performed by comparing the preoperative cross-sectional imaging study (CT or MRI, within 40 days of the procedure) with the postoperative imaging study, performed 4-7 days following the RFA procedure. All imaging was performed with contrast enhancement. Studies were reviewed by an experienced hepatobiliary radiologist on a lesion-by-lesion basis who was blinded to clinical and operative information. For patients included in the prospective trial, ablations were scored for technical success or completeness as defined by Goldberg et al [10] using the same 5-point scale that was used for the intraoperative score.

All patients were followed with serial contrast-enhanced CT or MRI every three months for two years, and then every 6 months. Follow-up studies were compared to immediate postoperative and prior studies for determination of local recurrence. Imaging studies were reviewed by both a central experienced radiologist and an independent observer, scoring recurrence on a lesion-by-lesion basis. Local recurrence was defined by the International Working Group on Image-guided Tumor Ablation as the appearance of tumor growth at the site of treatment or directly adjacent to it on any imaging modality during fo

Statistical analysis

All analyses were performed on a per-lesion basis. Tumors with inadequate follow-up (< 6 months) or incomplete post-operative imaging were excluded from the analysis. Data are presented as median and range for continuous variables and as proportions for categorical variables. Groups were compared using the Chi-square test for proportions and Mann-Whitney U test for continuous variables. The influence of clinical, pathological and radiological tumor characteristics on local recurrence was investigated using the non-parametric Kaplan-Meier method, log-rank test and Cox regression analysis. Hazard ratios and 95% confidence intervals were calculated. Data were clustered per patient to adjust for multiple tumors in the same patient. Differences in sensitivity and specificity between intraoperative and postoperative assessment of the success of ablation were calculated using the McNemar test. A p-value of < 0.05 was considered to be statistically significant. All statistical analysis was performed using Stata 12 (Statacorp, Collegetown, Tx).

Results

Patient and Tumor Characteristics

After excluding patients with inadequate follow-up from the analysis, 45 patients with 110 ablated metastases were left for analysis in the prospective study and 90 patients with 201 ablated CRLM were left for analysis in the retrospective cohort study (figure 3.2.1). Demographic and clinical characteristics of the 90 *patients* in the retrospective cohort are outlined in Table 3.2.1. Twenty-two patients (24%) were female and the median age was 60 years (range 23-84 year). The majority of patients had multiple tumors, with 42% of patients having three or more metastases. Sixty percent presented with synchronous metastases, 70% had node-positive primary tumors, and 73% received preoperative chemotherapy. In 16 patients (17%), RFA was the only surgical treatment, whereas in the remaining 74 patients (83%), RFA was combined with a liver resection, either major or minor. The median number of tumors treated with RFA was one, ranging from 1 tumor to 7 tumors.

Table 3.2.2 summarizes the characteristics of the ablated tumors on a *per lesion* basis for the retrospective cohort (n=201). 82% of the ablated tumors were ≤3 cm in diameter, and 36% were ≤ 1cm in size. Only 38% of tumors were within 5 mm

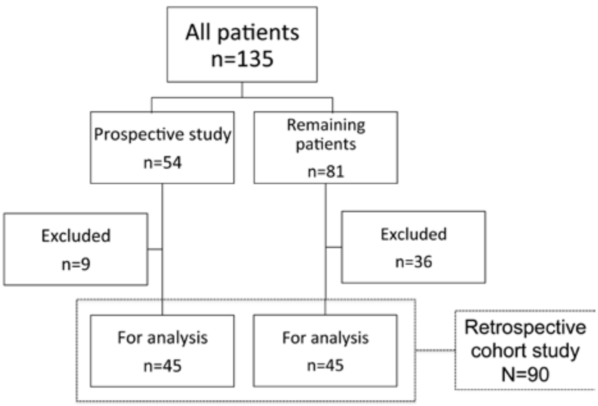

FIGURE 3.2.1: Flowchart of the study cohort.

TABLE 3.2.1: Clinicopathologic characteristics of 90 patients in the retrospective cohort treated with open RFA for CRLM in between 2000 and 2009 in the Johns Hopkins Hospital

Variable	Number (%)
Demographics	
Sex	
Male	68 (75.6)
Female	22 (24.4)
Median age (range)	60 (23-84)
Primary tumor characteristics	
N-stage	
N0	27 (30.3)
N1&2	62 (69.7)
Unknown	1
Metastases characteristics	
Disease-free interval	
Synchronous	54 (60.0)
Metachronous	36 (40.0)
Number of metastases diagnosed	
1	16 (17.8)
2-3	36 (40.0)
> 3	38 (42.2)
Pre-operative CEA	
> 100	7 (8.9)
< 100	72 (91.1)
Unknown	11
Treatment details	
Pre-operative chemotherapy	
No	24 (26.7)
Yes	60 (73.3)
Post-operative chemotherapy	
No	28 (31.1)
Yes	57 (63.3)
Unknown	5 (5.6)
Surgery	
Ablation only	16 (17.8)
Ablation + minor resection	24 (26.7)
Ablation + major resection	50 (55.5)
Median number of ablated lesions (range)	1 (1-7)

TABLE 3.2.2: Analysis of tumor related factors impacting on local recurrence rates in CRLM after open RFA

Retrospective study cohort (n=201 ablated tumors)						
Variable	n	1-year LR (%)	2-year LR (%)	Hazard Ratio	95% CI	p-value
Tumor diameter						
≤1 cm	72	11.2	22.2	1.0 (ref)		
>1 - 2 cm	58	14.2	24.7			
>2 -3 cm	34	21.5	29.0	2.25	1.27-3.99	
> 3 cm	37	57.8	78.1			
Size of major vessel †						
Not in proximity or < 3mm vessel	136	17.1	27.6	1.0(Ref)		
In proximity to 3-10mm vessel	52	29.0	35.5	2.04	1.21-3.44	0.008
In proximity to >10mm vessel	13	55.0	92.3			
Distance to closest major vessel ¥						
Major vessel more than > 5mm from tumor	125	13.3	22.4	1.0 Ref		
Major vessel 2-5mm from tumor	25	20.6	20.6	3.08	1.84-5.03	<0.001
Major vessel < 2mm from vessel	51	43.7	65.4			
Tumor diameter in tumors away from vessel *						
≤1 cm	50	9.9	9.9			
>1 - 2 cm	37	3.6	12.7	1.0 (ref)		
>2 -3 cm	20	10.0	20.6			
> 3 cm	18	47.9	68.3	7.66	3.61-16.2	<0.001
Location						
Parenchymal	176	22.7	35.5	1.0 (ref)		
Subcapsular	39	21.5	33.6	0.82	0.41-1.66	0.59
Pre-op chemotherapy						
No	57	26.3	37.8	1.0 (ref)		
Yes	140	21.8	29.3	0.75	0.43-1.33	0.336
Post-op chemotherapy						
No	74	31.6	41.7	1.0 (ref)		
Yes	104	18.0	29.85	0.55	0.33-0.90	0.018

† If within 5mm from tumor
¥ Vessel larger than 3mm
* away from vessel is defined as vascular structure with a diameter larger than 3 mm within 5mm from the closest tumor margin

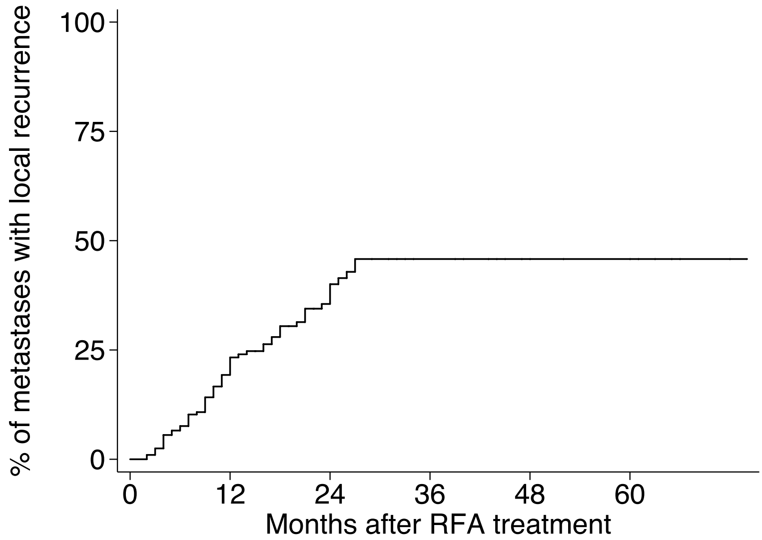

FIGURE 3.2.2: Kaplan Meier curve showing the rate of LR in 201 tumors included in this study

of a major vascular structure, most commonly the inferior vena cava or proximal hepatic vein, and 18% were near the liver capsule.

Per Lesion Analysis of Tumor Factors Associated with Local Recurrence

The median follow-up for the 201 CRLM in the retrospective cohort was 18 months (range 6-80 months). Recurrence was observed in 63 tumors (31.3%) during follow-up, Median time to local recurrence was 10.8 months following ablation, with more than 93% of local recurrences being diagnosed within 2 years (figure 3.2.2). Median time of follow-up of tumors without local recurrence was 18 months (range 6-74)

The prevalence of various tumor-related factors and their association with LR were analyzed based on the entire cohort of 201 ablated lesions in 90 patients (table 3.2.2) on a *per lesion* basis. In the univariate analysis, tumor size > 1cm was significantly associated with higher rates of local recurrence (HR 2.25 1.27-3.99; p=0.001) with an associated 2-year risk of LR of 22.2% for tumors ≤ 1cm and 42% for tumors > 1 cm. In addition, the risk of LR was associated with both the distance to the closest vascular structure (HR 3.08 95%CI 1.84-5.03;p<0.001) as well as the size of the closest vascular structure (HR2.04 95%CI 1.21-3.44; p=0.008). Local recurrence rates were significantly decreased in tumors that were both small and not in close proximity to major vessels: Among tumors ≤ 1cm in diameter and more than 5 mm from a major vessel, the 2-year LR rate was only 9.9%. When tumor size was ≤ 3 cm and tumors were >5mm away from vessels, LR was observed in 13.8% of tumors after 2 years of follow-up. At last, the administration of postoperative chemotherapy was associated with a lower rate of local recurrence during follow-up (HR 0.55 95%CI 0.33-0.90; p=0.018).

Intraoperative Parameters of Ablation and Association with Local Recurrence

For the 110 tumors included in the prospective study median follow-up was 24 months (range 6-80). During follow-up, 34 tumors had a local recurrence after ablation (30.9%). Adequate target temperatures, micro bubble encompassment, and sufficient intended margin were achieved in more than 80% of ablations. Multiple RFA applications were only performed in 6% of cases. With regard to the A-status as determined by surgeon score, an A0 was accomplished in the majority of cases (76%), whereas A1 and A2 ablations were performed in only 18% and 5%, respectively.

As in the retrospective cohort, tumor size and the proximity of vascular structures were significantly associated with risk of LR following RFA (table 3.2.3). In addition, procedural parameters were also independently associated with LR. Specifically, when target temperatures were not achieved, or micro bubble encompassment failed to be established despite all attempts, LR was observed in 85% and 100% respectively. Conversely, when target temperatures were reached and IOUS showed good encompassment of the tumor by the ablation zone, no local recurrence was observed in three-fourths of lesions after two years. In particular, the overall surgeon assessment of ablation completeness as measured by the A-status was also strongly associated with the prediction of LR during follow-up. Among the 84 ablated metastases scored as A0, the 1-year and 2-year risk of LR were 11.4% and 28.7% respectively. In contrast, 1-year and 2-year LR were 41.3% and 64.7% in the 20 A1 ablations (HR 2.69; p=0.031) and 66% and 100% in the 6 ablations scored as A2 (HR 6.82; p<0.001).

TABLE 3.2.3: Analysis of procedure related factors impacting on local recurrence rates in CRLM after open RFA

Prospective study cohort (n=110 ablated metastases)						
Variable	n	1-year LR (%)	2-year LR (%)	Hazard Ratio	95% CI	p-value
Target temperature reached						
Yes	94	12.4	32.7	1.0 (ref)		
No	13	76.9	-	7.88	3.75-16.6	<0.001
No data	3					
Outgassing encompassment on IOUS						
Yes	102	16.9	33.7	1.0 (ref)		
No	8	62.5	100	5.39	2.63-11.0	<0.001
Intentional Margin						
≤ 1 cm	20	20.1	43.2	1.0 (ref)		
> 1 cm	90	20.7	58.7	0.69	0.36-1.35	0.288
Multiple ablation applications						
Yes	7	71.4	85.7	4.06	1.81-9.10	0.001
No	103	16.4	34.4	1.0 (ref)		
A-status						
A0	84	11.4	28.7	1.0 (Ref)		
A1	20	41.3	64.7	2.69	1.11-6.60	0.031
A2	6	66.6	100	6.82	3.76-12.36	<0.001

Finally, we sought to determine the ability of the A-status to refine the risk of LR among small lesions (≤3cm) not in close proximity to major vascular structures. Specifically, among 62 tumors ≤ 3 cm and not in proximity to major vessels, A0 status (n=55) was associated with a 9% risk of LR compared to 46.4% LR among A1 ablations (n=7) with this size and location after 2-years of follow-up (HR 3.95 95% CI 1.06-14.7; p=0.04).

Postoperative Radiologic Assessment of Technical Success and Recurrence

Radiologic review of the immediate postoperative cross-sectional images was performed in 105 lesions in the observational study group (5 lesions were not adequately visualized on pre-operative imaging). Among these, technical success or complete ablation (5 points) was reported in 89% whereas only 12 ablations were scored as incomplete (1-4 points). LR was observed during follow-up in 28% of those scored as technically successful compared to 58% LR among those scored as less successful.

The accuracy of the operative assessment was compared to postoperative radiologic assessment with regard the recognition of an inadequate ablation. Specifically, the sensitivity (44.4%) and positive predictive value (61.5%) of the A-status to predict an inadequate ablation resulting in LR were slightly better when compared to the sensitivity (21.2%) and positive predictive value (58.3%) of the postoperative radiological assessment (p=0.09). However, the specificity for detecting an inadequate ablation was equal for both studies, with a specificity of 86.8% for the intraoperative A-status and a specificity of 93.1% for the postoperative assessment of a poor ablation and recurrence, corresponding to a negative predictive value of 78.6% and 72.0% respectively (p=0.43)

Discussion

Surgical ablative approaches have expanded the options for local curative-intent therapies for patients with hepatic colorectal metastases [1,11]. In the present study we evaluated tumor-related as well as intraoperative factors associated with local recurrence after operative RFA in long-term follow-up. This study focused on a per-lesion analysis with an attempt to identify parameters that may identify those patients who might derive the greatest benefit from ablative therapy and be offered a curative-intent approach potentially comparable to extirpative therapy. In addition, through a prospective observational trial to assess intraoperative parameters of successful ablation, we define a level of predictable efficacy during a procedure analogous to operative histological analysis of pathologic margin status for liver resection.

Confirming most other reported series, we found local recurrence following ablation to be associated with specific tumor-related factors of tumor size and proximity to major vascular structures. Specifically, we found that tumors that were ≤ 3-cm and not in proximity to major vasculature had a 2-year LR rate of 13.8%; if these tumors were ≤1-cm in size, the 2-year local recurrence rate was only 9.9%. In our experience, 36% of tumors were ≤ 1-cm and most tumors (82%) were ≤3 cm in diameter. While tumors abutting major vascular structures can be safely ablated provided the major bile ducts are not treated, the presence of vascular heat sinks is associated with a higher local recurrence rate [12]. In our series, 37% of ablated tumors were close proximity to major vessels, specifically the major hepatic veins or inferior vena cava. As with other reports, we found major vascular proximity to be associated with higher risk of local recurrence.

The correlation of local recurrence with tumor size is well established in the literature [6,7,9,11,13,14]. Mulier et al reported results from a meta analysis of RFA of various tumor types which identified tumor size as the only independent tumor related predictor of local recurrence[7]. Similarly, Kingham et al, found tumor size > 1-cm to be associated with higher local recurrence [13]. As with this current study, Van Duijnhoven et al identified both tumor size and vascular proximity to be associated with risk of local recurrence [14].

Unlike previous reports, this study attempted to characterize and incorporate intraoperative parameters into the determination of risk for local recurrence. Specifically, we conducted a prospective observational study of procedural parameters of ablation in order to further refine factors associated with long-term efficacy of ablation for CRLM. Indeed, factors such as achievement of target temperatures and tumor encompassment by the ablation zone were highly predictive of LR. Importantly, operative assessment of ablation confidence through an A-status score, based on a compilation of procedure-related factors (tumor conspicuity on IOUS, probe targeting and positioning, micro bubble distribution, and temperatures) were strongly predictive of long-term local recurrence. In point of fact, the operative assessment outperformed the postoperative radiologic determination of technical success regarding predictability of local recurrence. Determination of the A-status could be utilized much like histological frozen section analysis (A0: "margin negative" ablation, A1" "close margin ablation, and A2: untreated residual disease) to alter the intraoperative procedure when possible. In addition, stratification based on ablation confidence may allow for improved comparisons between reported series.

While predictive, the A-status as defined in this study may be too lenient. 76% of ablative procedures were scored as A0. Although A1 or A2 ablations were associated with very high rates of local recurrence, over 20% of ablations scored as an A0 developed local recurrence as well. This suggests that even in those cases where intraoperative imaging and monitoring indicate an adequate ablation, viable tumor cells might persist in the ablation zone. On the other hand, when intraoperative imaging and monitoring suggest a poor ablation, a local recurrence during follow-up is highly probable. Hence, when a tumor is scored as an A2 ablation, this could be classified as a technically unsuccessful ablation conforming to the standardization of terminology and reporting criteria by Goldberg et al.10

The intraoperative assessment of A-status may provide a more refined determination of LR risk in tumors with more feasible characteristics. Tumors ≤ 3cm away from vascular structures with an A0 ablation recurred in 9% of cases after 2-years of follow-up. Therefore, much as the R-status is predictive of local recurrence following liver resection [15], determination of A-status can be used as a surrogate for post-ablation margin status in these tumors.

The findings from this study can provide useful information when guiding the clinical care of patients undergoing tumor ablation of colorectal liver metastases. First, when and intraoperative assessment indicate a suboptimal ablation and a high local recurrence risk is anticipated, therapeutic management may be altered. For example, ablation delivery may be altered during the procedure if possible. Application of additional RFA zones can be considered or an alternative ablative technology employed (e.g. microwave or irreversible electroporation). In addition, utilization of additional regional approaches such as hepatic artery infusion chemotherapy might be considered [13,16]. Second, we found that when careful tumor selection and operative assessment is undertaken, durable efficacy of RFA in many cases is demonstrated, with local recurrence observed in approximately 9%. This may offer the option of considering RFA in selected cases in which the disease is otherwise resectable. Third, long-term follow-up and careful high-quality serial imaging is required to monitor these post-ablation lesions as local recurrence can occur beyond 12-24 months following therapy. Studies with shorter follow-up may be underestimating the risk. Finally, we identified the importance of intra-procedural assessment at determining efficacy. Yet, current assessment remains problematic, based on limited ability to perform and determine accurate probe placement and monitoring of therapy. This study illustrates the importance of developing more sophisticated methods to assist and evaluate accurate probe placement as well as to monitor the effects of tumor ablation in real-time [17]. Ongoing research to develop and test new technologies are awaiting refinement and validation, including ablation simulation tools, robotic delivery systems, and improved ultrasound monitoring technologies such as elastography and contrast-enhancement [18-20].

Operative assessment and scoring as performed in this study has several limitations. First, the intraoperative A-status determination may in part be influenced by tumor-related factors such as size and proximity to major vascular structures, and therefore not independent. Indeed, larger tumors and tumors close to vascular structures were more likely to have a poor A-score and high risk operative features such as failure to reach target temperatures despite maximum attempts to meet the manufacturers recommendations for treatment. Yet, the A-status was still able to discriminate good from poor ablations, even in cases where the tumor size and location may have predicted favorable results. Second, the intraoperative A-status can only be determined at the time of the procedure, and cannot be used as a preoperative factor to select tumors best suitable for ablation beforehand. However, much like the use of intraoperative histological assessment with frozen-section, determination of A-status can be useful in patient management. Finally, the intraoperative assessment of the A-status as described in this study may be criticized for being subjective. In order to minimize subjectivity, the prospective part of this study was performed by a single surgeon. However, it might be difficult to precisely quantify, semi-quantitative objective parameters were incorporated into the score, including tumor echogenicity, probe position, micro bubble distribution, and tissue temperatures.

In conclusion, the probability local recurrence after operative RFA is highly dependent on tumor-related factors of size and vascular proximity. In addition, intraoperative assessment and determination of an ablation score provides an important additional measure of ablation efficacy.

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Adapted From: Safety and Efficacy of Radiofrequency Ablation for Hepatic Adenomas

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3.3

Radiofrequency Ablation and Hepatic Adenomas

Abstract

Purpose: To investigate the safety and efficacy of radiofrequency ablation (RFA) for the treatment of hepatocellular adenomas (HCA).

Methods: From 2000-2009, 170 patients with HCA were referred to our tertiary hepato-biliary center. Medical records of 18 patients treated with RFA were retrospectively analyzed.

Results: All patients were female and the majority had a history of hormonal contraceptive use. Ten patients (56%) had multiple HCA, with a median number of 2 lesions (range 1-12) per patient. Median size of HCA at the time of RFA was 3.0 cm (range 0.8-7.3). A total of 45 HCA were ablated in 32 sessions (open n=4, percutaneous n=28). RFA was complete after the first session in 26 HCA (57.8%), and the majority of patients underwent multiple RFA sessions to fully ablate all HCA. Two patients developed major complications.

Conclusions: RFA can be effectively utilized in the treatment of HCA. However, multiple sessions are often required and signs of residual adenoma might persist in some patients despite repetitive treatment. RFA might be especially beneficial for patients not amenable for surgery or those that would require major hepatic resection otherwise.

Introduction

Hepatocellular adenoma (HCA) is an uncommon benign tumor of the liver, which is most often diagnosed in young women in the second or third decade of life [1]. While the development of HCA has traditionally been associated with long term use of hormonal oral contraceptives, an association with pregnancy has also been reported, where existing or new-found HCA tend to grow under the influence of elevated serum estrogen levels [2].

Although benign in itself, HCA have a potential for malignant degeneration or spontaneous rupture with potentially life threatening hemorrhage. Therefore, treatment is indicated in selected patients [3,4]. Historically, surgical resection has been the treatment of choice for the management of HCA, however sometimes these young and otherwise healthy patients would require large hepatic resections for centrally located or bilaterally distributed HCA, with associated morbidity and costs [5-7]. Several authors have therefore described the successful application of minimal invasive strategies such as transarterial embolization and radiofrequency ablation (RFA), although no series larger than 10 patients have yet been reported [8-13]. We here describe our experience with RFA for the treatment of HCA in 18 patients. IRB approval is not required in our institution for retrospective studies.

Patients and methods

Between 2000 and 2009 a total of 170 consecutive patients with the diagnosis of HCA were referred to Erasmus Medical Center in Rotterdam, the Netherlands. Sixty-two patients (36%) underwent invasive treatment and in 18 patients (11%) this treatment involved RFA of one or more HCA, either open or percutaneous. Furthermore, in 39 patients (23%) surgical resection of one or more HCA was the treatment of choice. Another 5 patients (3%) underwent transarterial embolization of their HCA. Medical records of 18 patients treated with RFA were retrospectively analyzed.

Patient characteristics

The median age of the 18 patients undergoing RFA for one or more HCA was 29.5 years (range; 21-37) and all patients were female. The median BMI was 26 (range;

18-44) and all but one patient had a history of hormonal oral contraceptives use. None of the patients had a history of viral hepatitis or liver cirrhosis. In the majority of patients, HCA were asymptomatic and were detected incidentally on imaging studies obtained for other purposes. Two patients presented with acute bleeding from previously unknown HCA (patient 6 and 10), one of them requiring transarterial embolization to control bleeding 2 years prior to final RFA treatment. RFA treatment of the residual HCA tissue was initiated in these two patients since both of these patients had an active pregnancy wish. As such, treatment of HCA was felt to be indicated because of an active pregnancy wish in 13 patients with proven hormone sensitive HCA (72%), growth of HCA lesions in two patients (12%) of which one during pregnancy, HCA exceeding 5 cm in size in one patient (6%), and a lifelong dependence of hormone substitution in two patients (12%).

Diagnosis and work-up

Initial treatment was usually conservative, consisting of discontinuing oral contraceptives for at least six months and radiological follow-up with CT or MRI every 3-6 months to monitor growth or regression of HCA. Patients were amenable for invasive treatment either when lesions remained larger than 5 cm or did not stop growing after oral contraceptives cessation, when malignancy could not be excluded or when patients expressed a desire for pregnancy in the presence of hormone sensitive HCA larger than 3 cm. In addition, RFA was the treatment of choice for those patients that were not amenable for surgery due to the location or number of HCA or if resection was considered potentially harmful due to insufficient volume of the future liver remnant (< 25% of total liver volume). If uncertainty existed regarding the diagnosis based on radiological examinations, surgical resection was the treatment of choice and RFA was not considered.

Diagnositic imaging included a triphasic CT-scan in all patients and dynamic four phase contrast enhanced MRI scan in 17 patients. In none of the patients included in this study diagnostic histological biopsies were obtained.

Procedures

RFA was performed using a 480 kHz RF Cool-tip RF system with a single 3cm needle (if < 3 cm) or three clustered 3 cm needles (for larger lesions) (Radionics, Burlington, MA) or a 250 W RITA RF generator with Starburst RF-ablator (Angiodynamics, Queensbury, NY). Procedures were carried out under general anesthesia by an experienced interventional radiologist (P.P. > 100 cases, T.L. > 50 cases). For the open approach, a right sub costal incision was made to gain access to the abdominal cavity and the liver was mobilized for optimal exposure. In each case, RF electrodes were introduced into the lesion under contrast enhanced CT or US guidance. Lesions were ablated for 5-15 minutes after target temperatures were reached (100° C) until the ablation zone was felt to encompass the entire tumor based on ultrasound and CT-images .Ablation of approximately 0.5 cm of healthy liver tissue surrounding the lesion was attempted in all cases. In the majority of cases, efficacy of treatment and the presence of complications (e.g. pneumothorax) was estimated using contrast enhanced CT obtained directly after ablation. Due to unavailability of intraoperative CT scanning, open ablations were performed under US guidance.

Assessment of response and follow-up

All patients underwent tri-phasic contrast enhanced CT-scanning at 4-6 weeks after RFA procedures to asses for any residual disease (figure 3.3.1). When no signs of residual disease were found, follow-up scans were made every 3-6 months for the first year after RFA treatment.

Statistical analysis

Data are expressed as median (range) and mean (standard deviation) for continuous variables and proportions (%) for binary variables. Differences between groups were investigated using the students t-test for continuous variables and the chi-square test for binary variables. All statistical analysis were performed using SPSS 16.0 (Chicago, IL).

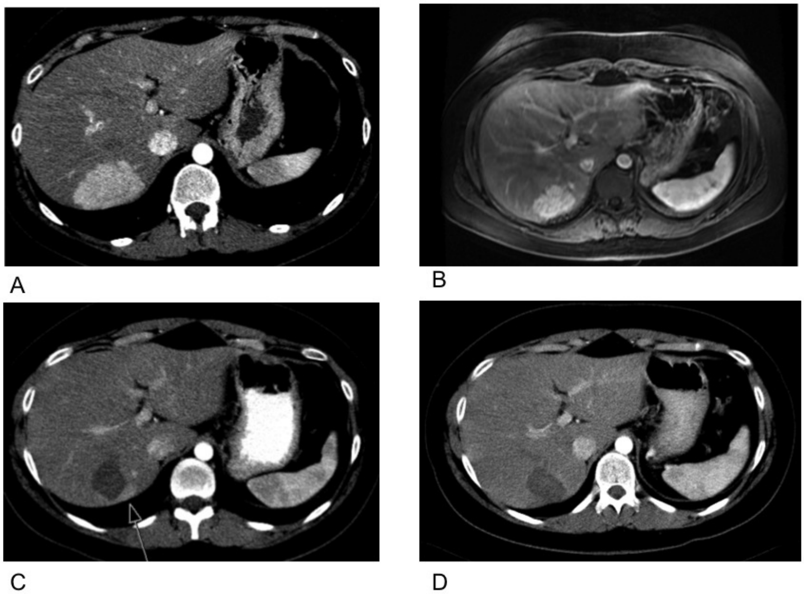

Figure 3.3.1:
(A) Arterial Phase CT-image of a large HCA (large closed arrow) in the posterior segment of the liver in a 22 year old woman.
(B) Contrast Enhanced MR Image of the same HCA (large closed arrow) 8 months after stopping oral contraceptives use.
(C) Arterial phase CT-image of HCA after incomplete percutaneous RFA. Hyperattenuating residual HCA tissue (small open arrow) is observed at the posterior border of the hypoattenuating RFA zone.
(D) Arterial phase CT-image of HCA after successful repeat percutaneous ablation. No residual HCA tissue is observed (small closed arrow).

Results

A total of 76 HCA were diagnosed in the 18 patients included in this study, ranging from 1.0 cm to 14 cm in size at the time of diagnosis. The median number of HCA per patient was 2 (range; 1-12). Multiple HCA (2-9 nodules) were found in 7 patients and liver adenomatosis (≥ 10 nodules) was present in 3 cases. After various times of conservative management including oral contraceptives cessation and serial imaging, the median diameter of the largest HCA at the time of treatment was 3.8 cm (range; 1.5-7.3) (mean±SD; 3.9 ± 1.2 cm).

TABLE 1: Clinical characteristics and treatment outcome of 18 women with HCA and treated with radiofrequency ablation. Abbreviations: BMI, Body mass index; RFA, radiofrequency ablation; HCA, hepatocellular adenoma; OC, oral contraceptives; CVA, cerebrovascular accident.

ID	Age	BMI	Number diagnosed	Number ablated	Size largest (mm)	Indication	Treatment	Complications	Outcome
1	32	26	1	1	38	Pregnancy wish	1 x Percutaneous RFA	-	No evidence of disease
2	36	22	12	9	73	Pregnancy wish	1x Open RFA + resection 1x Percutaneous RFA	Hematoma in abdominal wall, no intervention	No evidence of disease
3	34	18	1	1	30	Hormone dependency	2x Percutaneous RFA	-	Minimal residual HCA
4	31	23	1	1	44	Pregnancy wish	3x Percutaneous RFA	-	No evidence of disease
5	32	27	10	9	61	Size > 5cm	1x Open RFA + resection 3x Percutaneous RFA	Bleed from right hepatic artery, no intervention	Minimal residual HCA
6	23	24	1	1	38	Pregnancy wish	2x Percutaneous RFA	-	Minimal residual HCA
7	23	29	1	1	42	Pregnancy wish	2x Percutaneous RFA	-	No evidence of disease
8	21	20	1	1	32	Hormone Dependency	2x Percutaneous RFA	-	No evidence of disease
9	26	20	6	2	35	Pregnancy wish	2x Percutaneous RFA	Bleed from liver parenchyma, no intervention	No evidence of disease
10	22	23	1	1	15	Pregnancy wish	Percutaneous RFA	-	No evidence of disease
11	30	32	2	2	40	Pregnancy wish	1x Percutaneous RFA	-	No evidence of disease
12	37	29	7	2	35	Pregnancy wish	2x Percutaneous RFA	-	Minimal residual HCA

TABLE 1: Clinical characteristics and treatment outcome of 18 women with HCA and treated with radiofrequency ablation. Abbreviations: BMI, Body mass index; RFA, radiofrequency ablation; HCA, hepatocellular adenoma; OC, oral contraceptives; CVA, cerebrovascular accident. (continued)

ID	Age	BMI	Number diagnosed	Number ablated	Size largest (mm)	Indication	Treatment	Complications	Outcome
13	27	41	1	1	36	Pregnancy wish	Percutaneous RFA	-	No evidence of disease
14	32	44	9	5	45	Pregnancy wish	3x Percutaneous RFA	-	1 untreated HCA
15	33	26	4	3	36	Pregnancy wish	1x Percutaneous RFA	-	No evidence of disease
16	28	35	2	1	28	Pregnancy wish	1x Percutaneous RFA	Liver abcess, percutaneous drainage	No FU available
17	25	33	6	3	37	Growth	1x Open RFA + resection	-	Minimal residual HCA
18	29	24	10	1	43	Growth during pregnancy	1x Percutaneous RFA	CVA, no intervention	Minimal residual HCA

Treatment

Table 3.1.1 shows the clinical and treatment related characteristics of the 18 patients with HCA that were treated with RFA. In these 18 patients a total of 45 HCA lesions were ablated in 32 RFA sessions. The median number of HCA ablated per patient was 1 (range; 1-9) (mean±SD 2.8 ± 3.2) with a median size of 3 cm (range; 0.8-7.3) (mean±SD; 2.9 ± 1.2 cm). The median number of RFA sessions per patient was 2 (range; 1-4). While the majority of patients were only treated with percutaneous RFA, an open approach was applied in 3 patients combining RFA with resection of one or more HCA (n=5). One of these patients underwent additional percutaneous treatment of two HCA after incomplete ablation of these lesions. Furthermore, in one patient with liver adenomatosis (≥ 10 nodules) located in both lobes of the liver, a staged procedure was attempted with complete resection and ablation of all left sided lesions during a first stage as well as right portal vein embolization to obtain adequate volume of the left lobe to allow for a later right hepatectomy. However, during the second stage of the operation it was felt that the quality of the left lobe was insufficient to withstand a right hepatectomy and it was chosen instead to ablate all HCA in the right liver lobe 2 months after right portal vein embolization. In addition, another patient with two HCA, one lesion was treated using percutaneous RFA and another HCA was treated using percutaneous ethanol injection, since this lesion was not considered to be eligible for RFA due to its proximity to the gallbladder in segment 5. Finally, a total of 25 small HCA were left untreated in 6 patients with multiple adenomas (all < 1.5 cm in diameter).

Outcome

Eight patients (44.4%) required one session of RFA, seven patients (38.9%) required two sessions of RFA and two patients (11.1%) required more than 2 RFA sessions in order to obtain a satisfactory treatment of all HCA (no need for further treatment). In one patient (5.5%) with extensive adenomatosis (> 9 HCA), four dominant lesions were successfully treated in two percutaneous RFA sessions, however one lesion (4.5 cm) in the left lateral segment was situated superior to the stomach and could not be reached percutaneously during both sessions.

Despite a clinically satisfactory result, minimal residual disease (< 1 cm) was observed around one ablated HCA after one or more subsequent sessions of

RFA in five patients. However, no further treatment was offered and rather it was chosen to follow these patients radiologically. Three of these patients also had several small HCA lesions (< 1cm) in the remainder of the liver that we choose not to treat.

When analyzed per lesion, 26 of 45 HCA (57.8%) were successfully ablated at the first attempt and nine (20.0%) HCA were successfully ablated in a second session. Three HCA required 3 (n=2) or 4 (n=1) RFA sessions to achieve complete ablation (figure 3.3.2). Seven HCA (in five patients) still have evidence of minimal residual HCA tissue adjacent to the ablation zone at the time of this study. No difference was observed when comparing the mean size of HCA with residual disease after one RFA session (2.9 cm) with those HCA that showed a complete radiological response after one session (3.1 cm, *students t-test*; p=0.73). However, open RFA was significantly more often successful at the first attempt (15/19 HCA; 79%) compared to percutaneous ablation of HCA (11/26 HCA; 31% p=0.013). The median follow-up of all lesions was 440 days (range 41-1351).

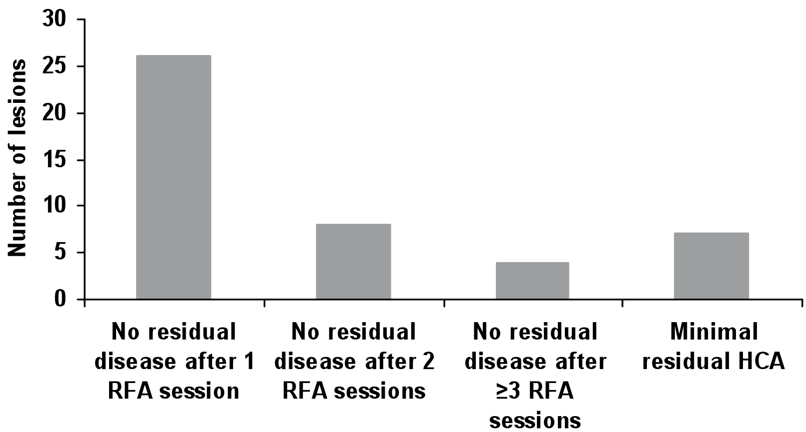

FIGURE 3.3.2: Barchart showing the rate of success after ablation of HCA per lesion. The y-axis shows the number of lesions that were ablated in one, two, three or more sessions respectively as indicated on the x-axis.

Complications

There were two major procedure-related complications (SIR D). One patient undergoing open RFA of two lesions as well as a right posterior segmentectomy for a third HCA developed cerebral ischemia with concomitant temporary paresis of the left arm due to severe intra-operative bleeding. However, complete neurological recovery was observed three months after surgery. Further cardiologic work-up revealed a persistent foramen ovale which has likely contributed to the development of this serious complication. Another patient that underwent concomittant percutaneous ethanol injection of one HCA developed a hepatic abscess at the site of ethanol injection requiring prolonged percutaneous drainage. Minor complications (SIR A) included minor bleeding in three patients requiring no further intervention[14].

Discussion

The diagnosis of HCA in young women often raises dilemmas for the treating physician. A conservative approach consisting of discontinuation of oral contraceptives, radiological follow up and a negative advise towards pregnancy would be preferable for many patients. However, the (albeit small) risk of life-threatening hemorrhage or malignant degeneration as well as the need for life-long radiological follow-up or a desire to have children has often led to a preference for active treatment. In patients with centrally located HCA or multiple HCA in both lobes of the liver, a large liver resection can be required in order to obtain complete resection of all lesions, something that is rather avoided in this young and otherwise healthy population. In addition, hepatic adenomatosis is frequently associated with moderate to severe steatosis, precluding major hepatic resection [15].

Previously, we reported on the cost-effectiveness of various treatment strategies for HCA, showing that RFA was the least expensive with the most gained quality-adjusted life years [16], however no data were provided on the efficacy of RFA for HCA. Limited data from other centers shows that with the availability of minimal invasive techniques such as RFA, HCA can be treated with low morbidity and high satisfaction [9-13], however these data were largely anecdotal. One larger series of ten patients described the successful application of RFA for the management of one or two HCA. All patients tolerated the procedure well and no local failures were reported.

Our current data suggest that RFA of HCA can lead to complete destruction of all viable HCA tissue with no signs of residual or recurrent HCA on follow-up imaging in some patients, thereby "saving" these patients from hepatic resection. Nonetheless, residual HCA tissue adjacent to the zone of ablation was often observed on follow-up imaging, resulting in repeat RFA sessions in many of our patients. In selected patients, we choose not to treat this residual HCA tissue, especially when other small sub centimeter HCA were also present. We hypothesize that a small amount of remaining HCA tissue will behave similar to small HCA; Despite that some growth might occur, the chance of reaching dimensions that pose a risk for rupture remains unlikely [17]. The fact that none of these residual lesions has shown growth or other changes visible on CT or MRI during follow up supports this strategy.

Clearly our local technical failure is relatively high in our series with many HCA requiring multiple sessions of RFA treatment. This is somewhat surprising given the efficacy of RFA that has been reported in the treatment for HCC, which are similar to HCA in many ways [18]. However, the absence of liver cirrhosis and a capsule around the tumor might limit the efficacy of RFA when applied to HCA as compared to RFA for HCC. Also, more than half of the treated HCA in this study were larger than 3 cm, which could have contributed to the failure of this technique. Furthermore, several other factors might have contributed to failure of RFA in these patients, including difficulties with 3-D probe-positioning as well as decreased target visualization as a result of steatosis and patients' central adiposity. The fact that open ablation resulted in more complete ablations at the first attempt supports this, since targeting is often easier in an open setting. Also, the inability to accurately monitor the effect of thermal ablation in these lesions might have partly contributed to the high local failure rate [19]. In all patients with residual HCA tissue on follow-up scans there was discordance between the immediate post-procedural CT-scan (showing a complete ablation) and the 4-6 week follow-up scan (demonstrating residual HCA tissue). Also, in several cases ultrasound did hardly show any tissue specific changes resulting from ablation, making it particularly difficult to assess the success of this treatment in real time.

Supposedly, these limitations can be addressed in the future with the availability of new ablation methods such as microwave ablation, more sophisticated image guidance platforms and novel techniques to monitor thermal ablation such as magnetic resonance thermography or ultrasound elasticity imaging [20,21]

In the currently presented cohort of patients, the diagnosis of HCA was made exclusively based on radiological studies, including contrast enhanced MR imaging and multi-phasic CT imaging. On both modalities, HCA typically show early homogeneous arterial enhancement without signs of washout or capsular enhancement and are usually well differentiated from HCC or FNH lesions [22]. Tissue biopsy was not performed routinely given the high specificity of cross sectional imaging and the associated risk of tumor seeding and sampling error. Rather, resection was the treatment of choice for those lesions that raised concern for malignancy.

Which patients should undergo surgical treatment for HCA and which patients should not remains a topic largely open for debate. Numerous data show that the risk of bleeding and malignant degeneration is particularly elevated in large or growing HCA. A recent large retrospective multicentre study including 124 patients showed that malignant degeneration and bleeding only occurred in patients with very large HCA (> 8 cm and >7 respectively) and most would agree that HCA in this size range require surgical intervention in most cases [4]. Moreover, a recent systematic review incorporating all reports on malignant degeneration in HCA showed an overall risk of 4.2% of malignant degeneration in HCA, of which only 4% occurred in lesions smaller than 5 cm [23]. One should however acknowledge a selection bias based on the fact that these reports only include patients that underwent surgical intervention and do not include those patients that have been managed conservatively.

Taking these data into consideration, it might be controversial to treat lesions below the threshold of 5 cm. However, in our opinion a special group of patients comprises the young woman with one or more HCA below the 5 cm threshold and an active pregnancy wish. When these HCA have shown to be hormone sensitive (e.g. have shrunk after cessation of oral contraceptives) one might anticipate these HCA to grow during pregnancy. Given the increased risk of bleeding and its associated morbidity and mortality in growing adenomas as well as the possibility of these lesions reaching a size out of reach of local ablative therapies and thus requiring liver resection, we have often considered early treatment of these relatively small lesions. [17]

This study might offer some guidance to the clinician to define the position of RFA in the treatment of HCA. Satisfactory results were obtained in most patients and the post-procedural course was mild in the majority of patients. We suggest that RFA could be applied selectively for the treatment of HCA, especially in patients who would require large liver resections otherwise or in whom resection is not possible due to bilateral spread of HCA. However, even percutaneous RFA can be associated with severe complications (although both patients with major complications also underwent other concomitant procedures) and many patients required multiple sessions of RFA, increasing the costs and use of resources significantly for these patients. Therefore, future research should focus on the comparison of different treatment modalities for the treatment of HCA including laparoscopic liver resection, alternative ablative therapies and trans-arterial embolization.

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Adapted From: Development of Hepatic Pseudotumors for Image Guided Interventional and Surgical Research in a Porcine Model.

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3.4

Experimental Model for Radiofrequency Ablation Research

Abstract

Purpose: Real-time image guidance and navigation have become increasingly important in an era of minimally invasive interventional and surgical procedures in the liver. In order to develop, test and implement tools for real time image guidance, we sought to create an in-vivo tumor mimic with realistic imaging and treatment capabilities.

Methods: Hepatic pseudotumors were created by injecting 1-2 ml of Kromopan Alginate (a hydrocolloid) directly into the liver parenchyma in 8 live pigs and 2 dog cadavers. Tumors were imaged using B-mode ultrasound, ultrasound elasticity imaging, multi-detector row CT, CT-Fluoroscopy and MRI to assess for imaging capabilities. Procedures performed using the alginate pseudotumors included radiofrequency ablation (RFA) and robotic needle guidance.

Results: Twenty-four hepatic pseudotumors were created ranging from 10 to 28 mm in size at an average depth of 6 mm. Average time of preparation and insertion was 3 minutes. All tumors were palpable under the surface of the liver and were easily visible on B-mode ultrasound, ultrasound elasticity imaging, CT-imaging and MR-imaging. Tumors were successfully "treated" using RFA and gross examination of the liver showed good encompassment of the tumor by the zone of thermal coagulation. Also, the pseudotumors allowed for easy introduction of various types of needles, including RFA probes and experimental steerable needles.

Conclusions: Alginate pseudotumors are easily imagable and allow for different procedures to be performed. This model can be used for various research purposes.

Introduction

Recently minimal invasive therapies such as local tumor ablation have emerged in the treatment of primary and metastatic liver tumors. Whether these techniques are applied percutaneous or via an open or laparoscopic surgical approach, the development and testing of advanced image guidance and navigation systems are paramount for adequate tumor targeting and monitoring of the treatment effect[1,2]. However, such research is hampered by the lack of tumor-bearing models or practical surrogates for large animal studies. Historically, pseudotumor models can be difficult to develop and impractical for studies related to image-guided therapies of the liver and other solid organs due to time of preparation or viscoelastic properties [3,4]. Ideally, a tumor model should include the following important features: (1) easy to create and implant, (2) biocompatible, safe, and durable; (3) capable to be imaged using a variety of modalities (e.g. US, CT, MR); (4) visible on macroscopic visualization, and (5) comparable in texture and elasticity to real tumor tissue (for biopsy, etc). We therefore sought to create realistic solid hepatic pseudotumors in a live porcine model as well as in a cadaveric canine model that would include all characteristics as mentioned above. To create these pseudotumors, we used Kromopan Alginate, a hydrocolloid. This material was previously described to create renal tumor mimics by Eun et al[5]. Among its many advantages are the low costs ($15 per 450 grams), short preparation time (3 minutes) and realistic visco-elastic properties.

Methods

Pseudotumor creation

Pseudotumors were created by injecting a Kromopan Alginate (Lascod, Italy) mixture in the liver. The mixture was prepared by adding cold water to the Kromopan powder and stirring thoroughly for 30 seconds. During solidification, the mixture turns from purple to pink after 30 seconds, indicating it is properly mixed. After 2 minutes the mixture turns white which indicates it is no longer malleable and has reached a semi-solid state.

The optimal concentration of Kromopan Alginate (Lascod, Italy) was assessed by injecting different concentrations of the mixture (0.1 gr/ml to 0.5gr/ml) in ex-vivo porcine livers. Eventually, a concentration of 0.3gr/ml proved to result in a mixture which was easily injected and formed firm palpable tumors in the liver parenchyma.

Using this concentration, Alginate pseudotumors were created in 8 live 27-31 kg female pigs (n=20) and two 23-27 kg canine cadavers (n=4). All study related procedures were approved by the institutional Animal Care and Use Committee. Pigs were intubated and placed under general anesthesia consisting of intravenous propofol. Anesthesia was maintained using inhalational agents. Pseudotumors were injected during laparotomy in eight animals (pigs n=6, dogs n=2) and percutaneous in 2 animals (pigs n=2). During open injection of the pseudotumors, access to the abdomen was obtained via a 10 cm midline incision that allowed for adequate exposition of the liver. The site of implantation was determined by ultrasound, at least 5 mm away from large vessels and bile ducts and at a location with adequate thickness of the surrounding liver. After mixing, the alginate was loaded in a 5 ml syringe and 1-2 cc of the mixture was slowly injected in the liver through a 4 cm 18 G needle. For percutaneous injection of pseudotumors a 12 cm 16 G spinal needle was inserted in the liver using an abdominal ultrasound probe equipped with a biopsy needle guide. Tumors were injected in a similar fashion as in the open approach. To assess for intra-abdominal leakage of alginate after percutaneous injection, the abdomen was opened.after the procedure was completed.

Imaging of pseudotumors

Imaging capabilities of the pseudotumors were investigated using ultrasound, CT and MR imaging. B-mode ultrasound images were collected immediately (< 30 minutes) after creation of the pseudotumors using either the Aloka ProSound alfa 7 console equipped with a convex array 3.75-10.0 MHz fingertip probe or a multi-frequency convex array abdominal probe (Aloka, Wallingfort, CT), or the Ultrasonix RP US console equipped with a 38mm 5-12 MHz linear array probe or 60 mm 5-14 MHz linear array probe (Ultrasonix, Richmond, BC, Canada). Transcutaneous B-mode US images were collected 4 days and 7 days after implantation to evaluate the evolution of the ultrasonographic appearance over time. Ultrasound elasticity images were collected in six animals prior to and after RF ablation of the pseudotumors to investigate the elastic properties of treated and untreated tumor mimics.

In three animals, a triphasic CT scan was obtained directly, four days and seven days (n=2) after implantation of the pseudotumors, using a 320 row Toshiba Aquillon CT-scanner (Toshiba Medical Systems, Tustin, CA). After obtaining a non-enhanced scan, sequential series were performed 30 and 70 seconds after intravenous injection of 30 ml of a non-iodine contrast agent (Isovue #370). In one animal, a contrast enhanced MRI scan was obtained on the day of implantation using a 1.5 T Siemens Espree scanner (Siemens Medical Solutions, Malvern, PA).

Radiofrequency ablation of pseudotumors

We then investigated the distribution of radiofrequency generated heat and current through the pseudotumors and tissue. Several large (> 3cm) pseudotumors were created in ex-vivo livers and ablated for 15 minutes using a 3 cm monopolar Radionics probe with the active part of the probe completely buried in the alginate tumor. Livers were serially sectioned and the zone of ablation around the pseudotumors was measured.

To investigate the behaviour of these pseudotumors during in-vivo ablation, pseudotumors were ablated using a RITA multi-tine RFA (Angiodynamics, Queensbury, NY) in seven swine. The RFA needle was placed in the pseudotumor under ultrasound guidance, deployed and pseudotumors were ablated for 5 minutes after target temperatures were reached. In two dog cadavers, pseudotumors were targetted with steerable needles.as part of a different experimental protocol.

Results

Ex-vivo and in-vivo pseudotumor creation

Both open injection into the liver parenchyma as well as percutaneous injection resulted in easily palpable pseudotumors ranging in size from 10 to 28 mm (mean size±SD; 16±4.9mm) at an average dept of 5.8±7.8mm. Injections with direct exposure of the liver through a midline laparotomy were successful in every instance. Percutaneous injection failed in two instances, where the alginate was inadvertently injected in between two overlying lobes of the liver.

Biocompatability and Durability

Examination of the liver tissue around three pseudotumors that were explanted 7 days after implantation showed no gross abnormalities. Microscopic examination of hematoxylin-eosin coloured slides of the liver surrounding these tumors revealed a minimal rim of pressure necrosis (<1mm) and some reactive inflammation (< 1 mm). Around one tumor, alginate was observed to have extravasated in small venous structures around the tumor.

One of the animals scheduled to survive for 7 days after implantation developed a severe bowel obstruction 4 days after implantation forcing us to sacrifice the animal earlier than scheduled due to severe discomfort. Upon re-exploration of the abdomen, multiple bowel adhesions were found with macroscopic evidence of small remnants of alginate in between loops, suggesting a role of the alginate in the formation of these adhesions.

Imaging Capabilities of Pseudotumors

Ultrasound

Specific imaging characteristics of the pseudotumors are outlined in table 3.4.1. On B-mode ultrasound, the alginate pseudotumors showed as highly conspicuous hyperechoic lesions directly after injection (figure 3.4.1a). The echogenicity of these lesions changed from hyperechoic to slightly hypoechoic within several hours after injection. Pseudotumors remained slightly hypoechoic but well demarcated from the surrounding liver up to seven days after creation (figure 3.4.1b). On ultrasound elasticity imaging, pseudotumors had an obvious increased stiffness compared to the surrounding liver, which shows as a zone with increased intensity (dark) on the ultrasound elasticity images (figure 3.4.1c).

Computed Tomography

On contrast enhanced CT images, pseudotumors showed as conspicuous hyperintense lesions compared to the surrounding liver parenchyma in the unenhanced and early arterial phase (figure 3.4.2a). In the portal venous phase

TABLE 3.4.1: Imaging characteristics of alginate pseudotumors

Imaging modality	Characteristics
Ultrasound	• Hyperechoic directly after implantation • Iso - hypoechoic several hours after implantation • Increased stiffness on ultrasound elasticity imaging
Computed tomograpy	• Hyperintense on unenhanced and arterial phase • Isointense to slightly hyperintense on portal venous and delayed phase
Magnetic resonance imaging	• Isointense on T-1 • Hypointense on T-2 • Hypointense with subtle rim enhancement on contrast enhanced scans

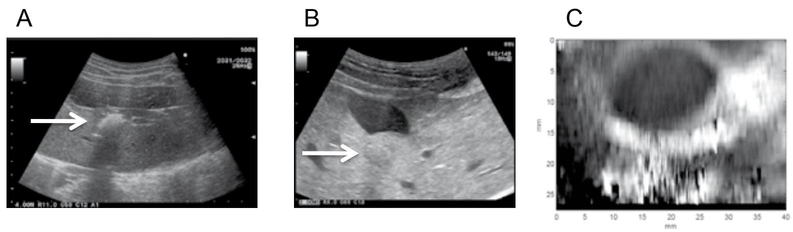

FIGURE 3.4.1: (A) B-mode ultrasound image of freshly implanted pseudotumor, directly post-implantation (B) Bmode ultrasound image of established pseudotumor, day 7 (C) Ultrasound elasticity image of pseudotumor.

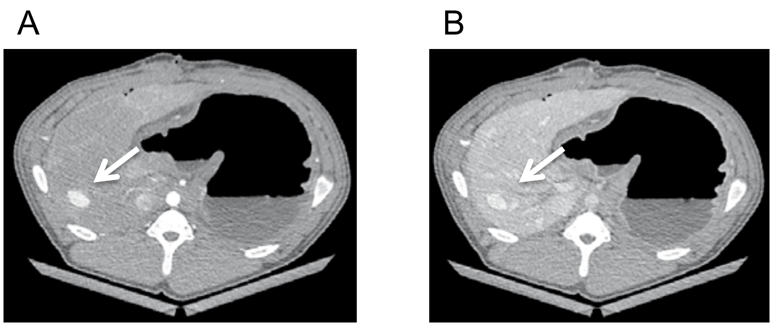

Figure 3.4.2: (A) Arterial phase CT-scan of pseudotumor, directly after implantation. (B) Delayed phase CT-scan of pseudotumor, day 0.

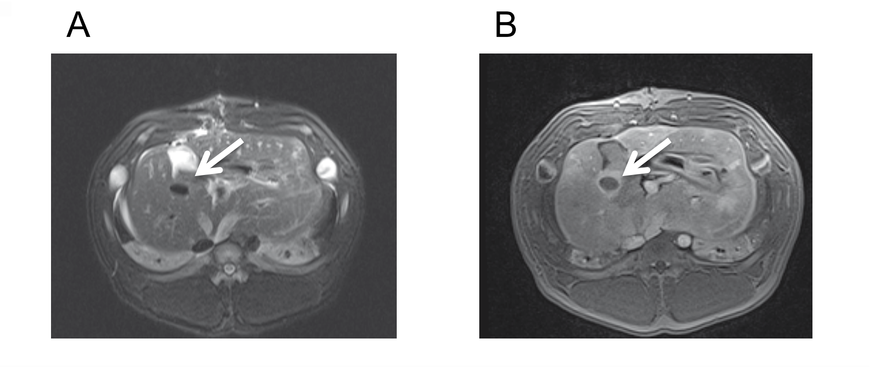

Figure 3.4.3: (A) Unenhanced T-2 weighted MR image of pseudo tumor directly after implantation. (B) T-2 weighted MR image of pseudotumor after intravenous injection of Gadolinium.

and delayed phase pseudotumors took on a more isointense appearance on CT imaging but remained well visible in between the more intense venous structures (figure 3.4.2b).

Magnetic Resonance Imaging

On MR imaging, pseudotumors were somewhat isointense and difficult to visualize on T-1 weighted images. On T-2 weighted images, pseudotumors were easily identified as hypointense focal lesions with a hyperintense rim surrounding the tumor, likely as a result of edema (figure 3.4.3a). After injection of intravenous gadolinium, pseudotumors became more hypointense compared to the surrounding liver parenchyma, especially in the portal venous and delayed phases (figure 3.4.3b).

Radiofrequency Ablation Capability of Pseudotumors

During ex-vivo experiments peudotumors were easily penetrated using the radionics RFA-probe. After ablation, a conspicuous zone of gross tissue coagulation and necrosis was observed around the pseudotumors, even when the active part of the probe was completely buried in the tumor. During various in-vivo experiments, alginate pseudotumors were easily targeted using intra-operative ultrasound. While palpable and much stiffer than regular liver tissue, a RITA multitine RFA probe was easily introduced in these tumors and tines were

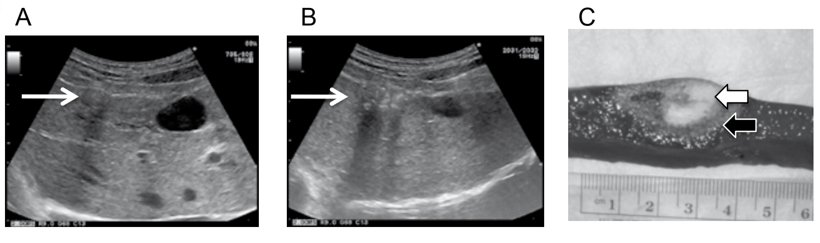

Figure 3.4.4: (A) B-mode ultrasound image of pseudotumor directly prior to ablation. (B) B-mode ultrasound image of pseudotumor directly after ablation. (C) Gross specimen after RFA of pseudotumor (White arrow: pseudotumor. Black arrow: coagulative necrosis around tumor as a result of RFA treatment).

deployed without experiencing any obstruction. During and after ablation, typical microbubble formation and outgassing was witnessed around the pseudotumors on B-mode ultrasound (figure 3.4.4a and 3.4.4b). After gross sectioning of ablated pseudotumors, a zone of thermocoagulation was observed around the ablated pseudotumors (figure 3.4.4c). Four pseudotumors created in dog cadavers where targeted using a robot with bend tip needles capable of following a curved track through tissue. In these experiments too, pseudotumors allowed for easy introduction of various types of needles.

Discussion

In the current study, we describe a method for the creation of an in-vivo model for hepatic tumor mimics using Kromopan alginate, a biocompatible hydrocolloid. We were able to create multiple pseudotumors ex-vivo and in-vivo by injecting this substance directly into the parenchyma of the liver, thereby creating a versatile and highly usable tumor model for image guided surgery research.

Previously, Scott et al. described a mixture of 3% agarose, 3% cellulose, 7% glycerol and 0.05% methylene blue to create tumor mimics in the liver of several pigs[6]. This model has since been used and adapted by many others, and we too have used this model in the past[3,4,7,8]. However, several disadvantages were noted, including long preparation times, expensive components and the need for injection at high temperatures (>65º C) possibly resulting in thermal injury to the surrounding liver. Moreover, this model produces tumors with a consistency equal to that of the surrounding liver, a characteristic that was considered undesirable in our research on ultrasound elasticity imaging and RFA, where we used the relative stiffness of the background liver, the tumor and coagulated liver to differentiate ablated from unablated tissue [9,10]. A prior report on the use of Kromopan Alginate for the creation of solid renal pseudotumors as well as our results as described above show that this specific mixture could offer a viable alternative to the agar model with several advantages, including its visibility on many different imaging modalities[5].

One of the favorable characteristics of the current model is its ease of use. There were only very few failures while injecting the mixture and hardly any preparation time was needed before injection of the alginate, as opposed to earlier described models based on Agarose mixtures. Moreover, while created in an open fashion in the majority of cases, our experience with percutaneous injection of the tumors shows that these tumors could as well be created with less morbidity and discomfort for the animal in case of survival studies via a less invasive approach.

Two animals were survived for 7 days after implantation without any complications and histopathological examination showed no evidence of massive inflammation or necrosis, suggesting that alginate can be used safely to create biocompatible and durable pseudotumors. One adverse event was noted, with one of the animals developing a significant bowel obstruction as a result of multiple adhesions. Since this might be partly caused by the presence of alginate in between bowel loops. spillage is now prevented by changing gloves in between mixing and injecting and covering abdominal content with sterile towels.

One of the more relevant features of the current model is its capability to be visualized on various different imaging modalities, including ultrasound, computed tomography and magnetic resonance imaging. On ultrasound, the alginate appeared as markedly hyperechoic tumors directly after implantation and became slightly hypoechoic within hours after implantation, likely as a result of ongoing polymerization of the mixture and dissipation of any air following injection. On CT and MR images, tumors were visible on both the unenhanced scans as well as on the arterial and venous phases.. These characteristics make this a highly useful model for studies on image registration.. Moreover, research can now be done using these tumors for (percutaneous) tumor targeting with different or combined imaging modalities.

Another desirable characteristic of the model is its specific visco-elastic properties. While free hand palpation and ultrasound elasticity imaging showed a relatively increased stiffness of the tumors when compared to the surrounding liver (data not shown), we did not experience difficulties in needle placement or needle biopsy of these pseudotumors. Moreover, on macroscopic appearance, the tumor was easily discernable from the surrounding liver, which could be an important feature when assessing the technical success of biopsy guidance systems. Finally, these pseudotumors were capable of being thermally ablated, without melting, burning, or impeding direct or conductive heat.

One limitation of the model is the relatively small size of the created tumors. However, the size limitation was largely due to the porcine liver size and not the property of methodology. The maximum capable lesion size using this injection technique was not explored in this study. The assessment of appearance on MRI was also only conducted on one sample. While we do not expect significant variation in MRI appearance, larger sample size would more clearly define imaging characteristics with this modality.

In conclusion, the described model can be a valuable tool for further studies on image guidance and navigation systems for minimally invasive and surgical procedures to the liver. The relatively simple and minimally invasive method for lesion creation, biocompatibility, robust imaging characteristics, elastic properties, and integrity retention with ablation make this model highly valuable to address various questions in tumor diagnostic and therapeutic investigations.

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3.5

Ultrasound Elasticity Imaging and Radiofrequency Ablation

Abstract

Background: Thermal ablation is an accepted therapy for selected hepatic malignancies. However, the reliability of thermal ablation is limited by the inability to accurately monitor and confirm completeness of tumor destruction in real-time. We investigated the ability of ultrasound elasticity imaging (USEI) to monitor thermal ablation.

Objectives: Capitalizing on the known increased stiffness that occurs with protein denaturation and dehydration during thermal therapy, we sought to investigate the feasibility and accuracy of USEI for monitoring of liver tumor ablation.

Methods: A model for hepatic tumors was developed and elasticity images of liver ablation were acquired in in-vivo animal studies, comparing the elasticity images to gross specimens. A clinical pilot study was conducted using USEI in nine patients undergoing open radiofrequency ablation for hepatic malignancies. The size and shape of thermal lesions on USEI were compared to B-mode ultrasound and post-ablation CT.

Results: In both in-vivo animal studies and in the clinical trial, the boundary of thermal lesions was significantly more conspicuous on USEI when compared to B-mode imaging. Animal studies demonstrated good correlation between the diameter of ablated lesions on USEI and the gross specimen (r=0.81). Moreover, high-quality strain images were generated in real-time during therapy. In patients undergoing tumor ablation, good size correlation was observed between USEI and postoperative CT (r=0.80).

Conclusions: USEI can be a valuable tool for the accurate monitoring and real-time verification of successful thermal ablation of liver tumors.

Introduction

Interstitial tumor ablation is currently considered a viable alternative to the resectional therapy, with similar outcome in some patient groups [1]. Unlike liver resection however, tumor ablation is limited by the ability to precisely monitor and document complete margin-negative tumor destruction using current technology. This limitation likely contributes to high local recurrence rates sometimes observed [2]. Currently, the intraoperative assessment of the succes of thermal ablation is largely based on indirect information such as target temperatures and empiric knowledge such as the predicted size of the ablation zone. Also, B-mode ultrasound is often used to target and guide hepatic thermal ablation, including radiofrequency ablation and microwave ablation. While this modality is widely available, including in operative suites, and easily allows for real-time imaging during targeting and treatment, visualization of the zone of ablation is difficult using standard B-mode imaging technology. Tissue heating can result in outgassing and micro bubble formation in the targeted region but this feature cannot readily distinguish whether the thermal zone has fully encompassed the tumor boundaries. Various studies have shown that there is only limited correlation between the zone of ablated tissue on the B-mode US imaging when compared to the gross specimen [3,4]. Post-ablation cross-sectional imaging with either CT or MRI has been found to more reliably determine completeness of tumor destruction [5,6]. Yet, post-procedural, non-real-time assessment limits the ability to adjust or modify the therapy based during the procedure.

Advances in US imaging technology afford the opportunity to improve the capability of US to monitor thermal ablation. Specifically, ultrasound elasticity imaging (USEI) has emerged as a potentially useful augmentation to conventional B-mode ultrasound imaging. USEI relies on two important observations: (1) different tissues may have significant differences in their mechanical properties and (2) the information encoded in the coherent scattering (i.e. speckle) may be sufficient to calculate these differences following a mechanical stimulus or compression [7]. During thermal ablation, protein denaturation and tissue dehydration increase the relative stiffness of the tissue with increasing ablation temperatures [8]. Application of USEI to achieve real-time visualization of the boundary between ablated and unablated tissue may offer the opportunity to improve the efficacy and safety of thermal ablation of liver tumors. While this novel technique is still investigational and its clinical applicability has yet to be established, we sought to investigate the ability of this novel technique to visualize the zone of thermal ablation in-vivo in order to guide future work. Therefore, the objectives of this study were to (1) investigate the accuracy of free-hand intraoperative USEI to visualize the zone of thermal ablation in an in-vivo tumor model using off-line reconstructed elasticity images and (2) to evaluate USEI in a clinical pilot study in order to determine its capability to visualize the ablation zone in patients undergoing radiofrequency ablation (RFA) of liver tumors, both with off-line reconstructed images as well as in real time.

Methods

In-vivo Animal Study: Tumor Ablation Model

In order to determine the accuracy of USEI for visualization of liver ablation compared to the actual specimen examination, a model of in vivo pseudotumor therapy in a porcine model was utilized. The study was approved by the institutional Animal Care and Use Committee. Specifically, six pseudotumors were created in livers of three 70-80 lb female pigs, a model which has been previously described for other purposes [9]. Using a midline laparotomy, pseudotumors were created through the intrahepatic injection of 1-2 cc of dental alginate mixture (Kromopran, Lascot, Florence, Italy) under US guidance. This technique reliably results in spherical lesions within the liver parenchyma measuring approximately 1.0-1.5 cm in diameter which are visible both on US and CT. Moreover, these targets are of a consistency that needle-based ablation probes easily penetrate through and into the surrounding liver. These pseudotumors can be thermally ablated without comprise of lesion integrity or effect on thermal conduction, and upon liver sectioning, the pseudotumors are easily visible as white material within the visualized ablated region. US and Contrast-enhanced CT imaging was performed in all animals prior to ablation in order to confirm adequacy of pseudotumor size and location.

Ablation of these targets were performed either on the same day (n=1) or 4 days (n=2) after insertion using a RITA-XL multi-tine RFA probe (Angiodynamics, Queensbury, NY) Model 1500X generator. Different time points were chosen to assess for the elastographic utility of established pseudotumors vs. newly created pseudotumors. Probe placement within each pseudotumor was performed using B-mode US applied directly to the liver surface.

In-vivo Animal Study: Image Collection and Analysis

B-mode and USEI images were collected using a Siemens Antares US scanner with a 4 cm 7.27 MHz linear array probe (Siemens Medical Solutions USA, Inc. Ultrasound Division, Issaquah, WA) or a Ultrasonics US Scanner (Sonix Medcorp), both equipped with an ultrasound research interface to access raw US data. Conventional B-mode and raw US data were collected in both transverse and sagittal planes directly before and after ablation. Tissue strain was generated by gently compressing and decompressing the region of interest with the US-probe while applied on the liver surface, usually with <1 cm excursion. After collection of all imaging data, animals were sacrificed and livers were harvested. The ablated pseudotumors where serially sectioned transversely, measured and photographed. For this part of the study, elasticity images were generated off-line using previously described algorithms [10]. In short, consecutive compressed and uncompressed ultrasound images are automatically compared and processed to provide a map of local displacement measures which can then be used to determine the elastic properties of the tissue. These local elastic properties can then be used to generate a user interpretable image. Pre- and post-ablation diameter measurements from real-time and off-line USEI were compared to B-mode US, CT, and gross tissue measurements at the greatest transverse plane.

Clinical Study

US elasticity images were collected in nine patients undergoing RFA for hepatic malignancies between March 2008 and May 2010 at the Johns Hopkins Hospital. Institutional Review Board approval was obtained and all participants provided informed consent for in the study. Five patients had metastatic colorectal cancer, two patients had neuroendocrine liver metastases, one patient had metastatic lung cancer and one patient had a recurrent HCC lesion in the liver . Ablation was performed using a RITA-XL RFA probe and Model 1500X RF generator (Angiodynamics, Queensbury, NY). Tumor sizes ranged from 7–19 mm and all ablations were performed with curative intent with a planned diameter 2-cm larger than the tumor. Adequate targeting, array deployment, and target temperatures were achieved in all cases. Ultrasound image data (raw US and B-mode data) were captured and recorded during intraoperative US imaging as conducted standard. US images were acquired before and after RFA therapy using a Siemens Antares US scanner (Siemens Medical Solutions USA, Inc. Ultrasound Division, Issaquah, WA) with URI in order to access raw US data. The US probe was a 7.27 MHz Siemens VF 10-5 linear array. In four patients, raw data were collected for off-line reconstruction of USEI as described above [11,12]; in five patients, real-time elasticity images were obtained using the onboard elasticity imaging module on the Siemens Antares ultrasound machine, which allows for high resolution real time elasticity images that are projected over the B-mode ultrasound image. Elasticity images were captured during manual cycled intermittent compression of the region of interest using the hand-held US probe, with typical excursion of < 1-cm. All US images were collected in a transversal plane, to allow for comparison with postoperative CT images. All patients underwent contrast enhanced CT-scanning of the liver 3-4 days post-ablation as done as part of our routine. Ablation lesion diameter by CT was determined based on the size of the non-enhancing hypodense coagulation region [13]. Maximum lesion diameter measured using USEI, B-mode US, and CT was compared.

Statistical analysis

All statistical analysis was performed using SPSS 17.0 (Spss inc. Chicago, Ill.). Mean tumor diameters and their respective standard deviations were calculated and the existence of a linear correlation between the diameters of ablated lesions on different imaging modalities was investigated by calculating the Pearson product moment correlation coefficient.

Results

Animal Studies

All six alginate pseudotumors were readily visible on conventional B-mode US and CT imaging as well-demarcated spheroid masses ranging in size from 9-19 mm (mean 13.6 mm) as measured on CT-images. On US, the lesions were hyperechoic lesions whereas on CT, lesions were attenuated compared to the surrounding liver (figure 3.5.1a and 3.5.1b). The pseudotumors were found to be somewhat

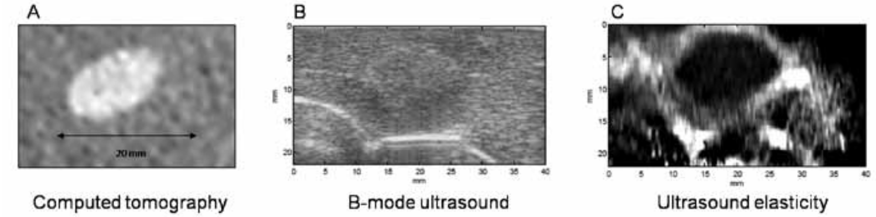

FIGURE 3.5.1 : Pre-ablation imaging of in-vivo porcine pseudotumors. (A) CT scan, (B) B-mode ultrasound and (C) ultrasound elasticity image.

stiffer than the hepatic parenchyma, reflected as conspicuous darker mass on USEI (figure 3.5.1c). The size and shape of the alginate tumors correlated with high precision between CT and USEI (r=0.87; p<0.001).

Following ablation of the pseudotumors, the zone of thermal coagulation around the pseudotumor was only poorly visualized by conventional B-mode US, primarily characterized by patchy regions of hyperechoic and hypoechoic changes associated with gas formation and edema (Figure 3.5.2a). Within 20-30 seconds, the dissipation of the gas resulted in almost complete failure to observe any difference between pre- and post-ablation appearance on B-mode US. In contrast, the zone of thermal ablation was highly visible on USEI (Figure 3.5.2b). Moreover, the imaging characteristics of the ablation zone on USEI remained unchanged, likely due to the irreversibility of the elasticity properties of ablated tissue. On these images, the zone of thermal ablation around the pseudotumors was characterized by increased tissue stiffness, exceeding that of the pseudotumor

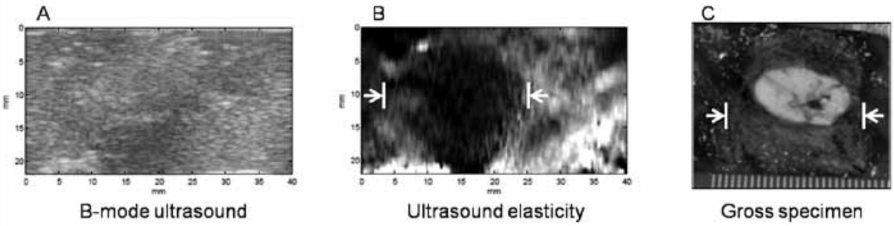

FIGURE 3.5.2 : Post-ablation imaging of in-vivo porcine pseudotumors. (A) B-mode ultrasound, (B) Ultrasound elasticity image and (C) sectioned specimen.

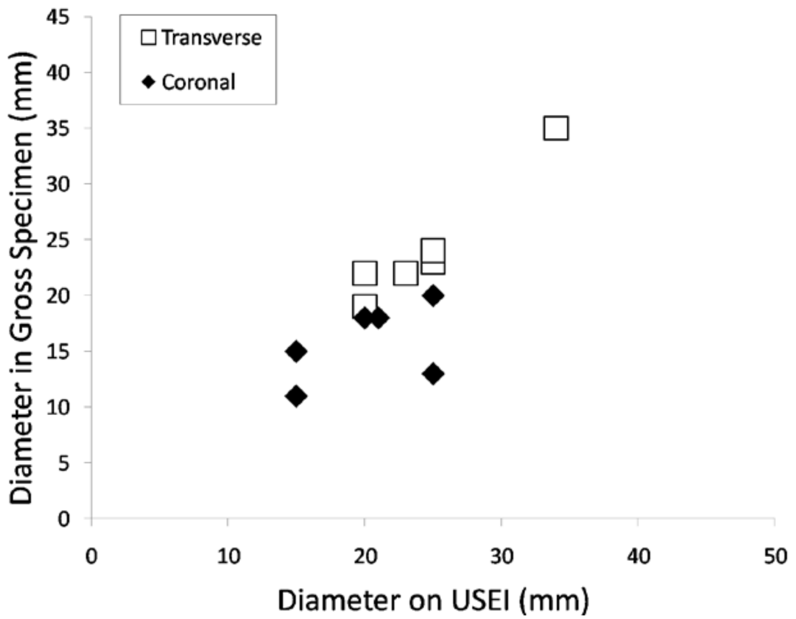

FIGURE 3.5.3: Scatterplot demonstrating the correlation between pseudotumor diameter as measured by elasticity imaging and in the gross specimen (transverse and coronal measurements).

itself (24 vs. 14 mm in mean transverse diameter). Once the ablation region exceeded the tumor volume, the pseudotumor no longer could be visualized within the ablation zone. Importantly, there was a good correlation between the diameter of the ablated pseudotumors on elasticity images when compared to the gross specimen ($r=0.81$; $p<0.001$) (figure 3.5.3).

Clinical Study

Successful elasticity images were obtained after RFA in all nine patients using free-hand palpation. Figures 3.5.4 and 3.5.5 provide examples from two patients, comparing post-ablation (a) B-mode US, (b) real-time USEI, and (c) postoperative CT images. As shown, the region of tissue ablation is poorly visualized on B-mode US compared to real-time USEI. In one patient, multiple overlapping ablations were performed that eventually outsized the size of the ultrasound probe, precluding meaningful correlation with the postoperative CT scan. Among the

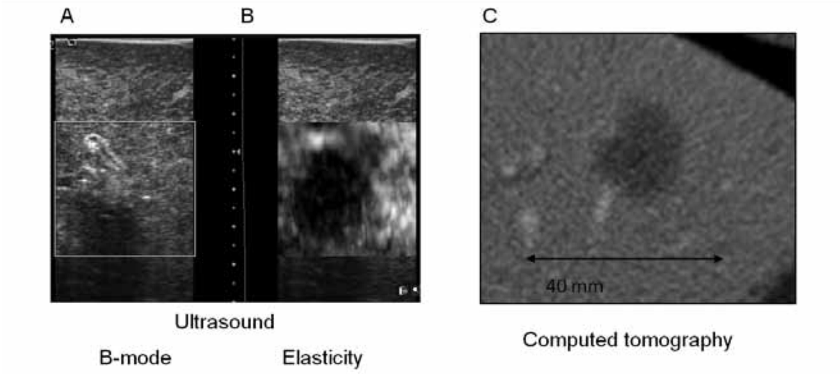

FIGURE 3.5.4: Post-ablation imaging in a patient with a recurrent hepatocellular carcinoma in segment 3 of the liver after undergoing radiofrequency ablation. (A) Real-time B-mode ultrasound, (B) real-time elasticity image and (C) post operative CT scan (day 3).

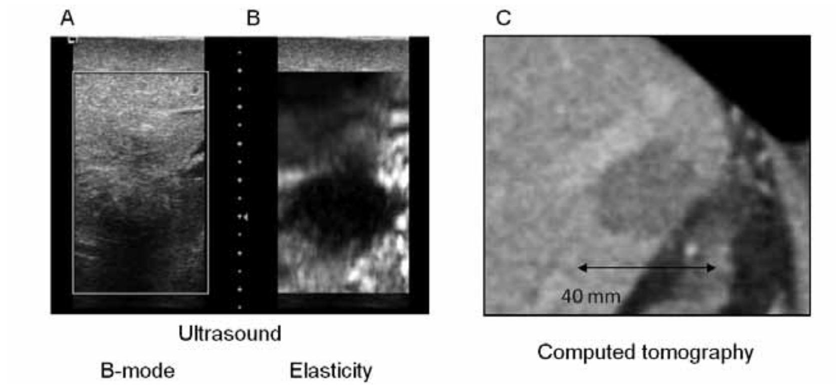

FIGURE 3.5.5: Post ablation imaging in a patient with a colorectal liver metastasis in the dome of the liver after undergoing radiofrequency ablation. (A) Real-time B-mode ultrasound, (B) real-time elasticity image and (C) post operative CT scan (day 3).

remaining eight patients, ablation diameter correlated well between USEI and that of postoperative CT ($r=0.80$, $p<0.001$) (figure 3.5.6).

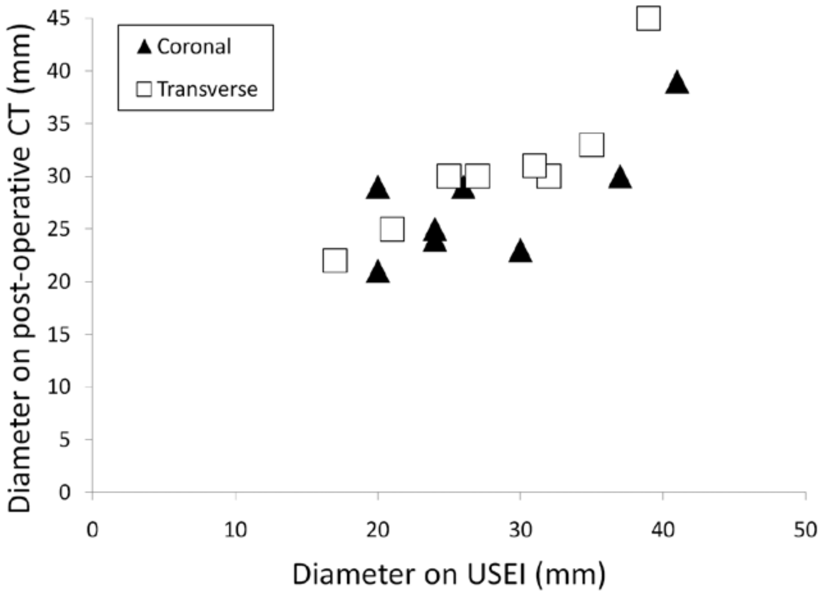

FIGURE 3.5.6: Scatterplot demonstrating the correlation between diameter of the ablation zone in eight patients as measured by elasticity imaging and on the post-operative CT scan (transverse and coronal measurements).

Discussion

Thermal ablation therapies have emerged as a widely available treatment modality for primary and secondary liver cancer over the last decade. However, its efficacy is severely limited by several factors, including the inability to adequately monitor the ablative process and document the margins of ablation. In this study, we aimed to determine if advanced ultrasound technology in the form of elasticity imaging might offer an opportunity to image and delineate the boundary of thermal ablation in more detail and eventually in real-time, potentially allowing for confirmation and adjustment of the ablative process.

Our animal studies demonstrated that USEI can provide improved delineation between thermally ablated tissue and non-ablated liver tissue with a good correlation between the size of the elasticity images and the actual ablation size and shape. Using an uncomplicated and reproducible porcine pseudotumor model, we found this novel imaging system capable of visualizing an ablation margin around an existing lesion.

In this study, we found that it was often difficult to visualize the zone of ablation on conventional US imaging. While B-mode ultrasonography is perhaps the most commonly used method to guide and monitor the thermal ablation of liver tumors, its ability to distinguish ablated tissue from non-ablated tissue has been proven inadequate to monitor and adequately confirm ablation success [3,4,14]. The formation of gas and micro bubbles cause frequent over- or underestimation of the actual ablation size, leading at times to excessively large or too small ablation volumes. CT-imaging and MRI can offer a more accurate estimation of size of thermally ablated lesions, but are less feasible for real-time ablation monitoring due to unavailability in most operating suites, radiation exposure and higher costs.

The findings from this limited clinical trial reinforce the potential of ultrasound elasticity imaging for application during thermal ablation therapy. In this early study of elasticity imaging, free-hand compression using the hand-held US probe and 2-D image rendering was used. Yet, even with such a basic system, accurate and useful imaging information was obtained, potentially allowing early introduction of this cost-effective advanced US capability into the clinical arena. Controlled tissue compression systems and 3-D imaging as well as image segmentation could further refine elasticity image capability.

Determination of adequacy (or inadequacy) of an ablation application during the procedure would allow for the ability to control the volume of tissue destruction in a more precise fashion, potentially resulting in better outcomes and improved patient safety. Specifically, repositioning of the ablation probe, adjustment of the ablation time, or change in the extent of probe deployment can be done based on monitoring information. Future ablation systems which allow volume shaping and spatially variable power delivery might be particularly useful when determination of the ablation boundary can be visualized at the time of therapy.

Others investigators have examined application of ultrasound elasticity imaging as a method to monitor thermal ablation. While most reports are limited to *ex-vivo* and *in-vivo* animal studies [15-17], Fahey et al. recently reported on their initial experience of ultrasound elasticity imaging in five patients undergoing hepatic RFA [18]. In this study, tissue strain (compression) was generated by sending acoustic waves through the tissue to induce tissue movement rather than by direct tissue compression. While this approach is different from ours, their results confirm the feasibility and potential of ultrasound based elastography to image margins of thermal tumor ablation.

An interest has expanded in recent years regarding the application of elasticity imaging in a variety of clinical settings. In addition to several reports on its application for monitor thermal ablation, ultrasound elasticity imaging has also been described for the characterization of tumors in breast, thyroid and liver [19-21]. This increasing interest is reflected by the fact that many commercially available ultrasound systems are already equipped with on-board real-time elasticity imaging capability similar to that used in our clinical study. While these elasticity imaging modules allow for fast and accurate generation of ultrasound elasticity images, utilized algorithms (eg. cross correlation) are highly susceptible to noise and distortion due out-of-plane motion and non-linear movement during tissue compression[12]. As such, current clinical systems are limited in the quality and usefulness of real-time elastography imaging acquisition, particularly when the region of interest is in a difficult position that cannot be easily reached with the ultrasound probe and axial tissue compression is difficult to achieve (e.g. posterior segments of the liver). These limitations can be addressed using more sophisticated algorithms which account for noise and distortion (e.g. dynamic programming). These experimental systems come at a higher computational price, currently limiting real-time imaging capability. However, efforts are being made by our group to develop rapid, high resolution real-time elasticity imaging using these more robust algorithms [10,12]. Other potential improvements in the technique include the application of real time 3D elastography and segmentation of the images.

While this study aimed at investigating this novel technique to monitor radiofrequency ablation in an open surgical situation, its applicability is not necessarily limited to this approach. Specifically, elasticity imaging using probe compression can be achieved using the laparoscopic IOUS probe on the surface of the liver. Even percutaneous ablation can be visualized this way using transabdominal compression or respiratory motion of the liver. In addition, we anticipate the potential application of elasticity imaging with other thermal ablation devices in addition to RFA. In particular, microwave ablation, while perhaps somewhat more conspicuous on B-mode US than RFA, the ability to precisely delineate the thermal ablation zone in relation to the tumor periphery using elastography could prove exceedingly useful.

One limitation of this study is the small sample size, and correlation coefficients should therefore be cautiously interpreted. In addition, variability in live animal and clinical imaging datasets without precise image registration limited the ability to be exact regarding the plane in which the various imaging measurements were compared. Yet, correlation was found to be well within acceptable clinical parameters. Furthermore, false estimation of the tumor morphology (eg microsatellitosis) might render the imaged margin of ablation less accurate. Therefore, additional studies are warranted to further validate the usefulness of this approach.

In conclusion, ultrasound elasticity imaging may provide a safe and effective method for improving monitoring of thermal ablation, potentially significantly impacting on how patients with solid tumors of the liver and other sites may be treated in the future. Such improvements in real-time imaging capability during tumor ablation may help to further improve outcomes and safety in patients undergoing these therapies.

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4

Preoperative Imaging and Outcome

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4.1

CT-based Assessment of Sarcopenia and Complications

Abstract

Background: As indications for liver resection expand, objective measures to assess risk of perioperative morbidity are needed. We investigated the impact of sarcopenia on patients undergoing liver resection for colorectal liver metastasis(CRLM).

Methods: Sarcopenia was assessed in 259 patients undergoing liver resection for CRLM by measuring total psoas area(TPA) on CT-scans. The impact of sarcopenia was assessed after controlling for clinicopathological factors using multivariate modeling.

Results: Median patient age was 58 years and most patients(60%) were male. Forty-one(16%) patients had sarcopenia(TPA$\leq$ 500 mm^2/m^2). Post-operatively, 60 patients had a complication for an overall morbidity of 23%; 26 patients(10%) had a major complication(Clavien grade$\geq$ 3). Presence of sarcopenia was strongly associated with increased risk of major post-operative complication(OR 3.33;p=0.008). Patients with sarcopenia had longer hospital stays(6.6 vs. 5.4 days;p=0.03) and a higher chance of extended ICU stay(> 2 days;p=0.004). On multivariate analysis, sarcopenia remained independently associated with increased risk of post-operative complications(OR 3.12;p=0.02). Sarcopenia was not significantly associated with recurrence-free(HR=1.07) or overall(HR=1.05) survival(both P>0.05).

Conclusions: Sarcopenia impacts short-, but not long-term outcomes following resection of CRLM. While patients with sarcopenia are at an increased risk of post-operative morbidity and longer hospital stay, long-term survival is not impacted by the presence of sarcopenia.

Introduction

Advances in multimodality treatment of metastatic colorectal cancer has increased survival with complete resection of colorectal liver metastasis (CRLM) now being associated with a 5-year survival of 50-55%.[1-4] With the introduction of more effective chemotherapy regimens and widening criteria for surgical therapy, the number of patients with CRLM eligible for surgery has also expanded.[5] Advances in operative techniques and perioperative care have minimized mortality with contemporary series reporting a mortality of less than 5%.[4,6-9] Post-operative morbidity, however, remains a concern with some studies reporting complication rates up to 20-50%.[4,7,10-13] Post-operative morbidity can lead to prolonged hospital stays, increased need for resource expenditure, greater medical costs, and possibly even worse long-term survival.[14,15] Identification of those patients at greatest risk for developing perioperative complications is therefore critical.

Unfortunately, data to stratify patients with regard to risk of perioperative morbidity remain ill-defined. The American Society of Anesthesiologist (ASA) classification, Eastern Cooperative Oncology Group (ECOG) performance status, and body mass index (BMI) – among others – have been utilized with varying degrees of success, but often fail to identify correctly those patients at highest risk of perioperative complications. Frailty, a measure thought to estimate the patient's physiologic reserves, has recently been proposed as a more robust predictor of post-operative complications.[16] Assessments of frailty have been criticized, however, for relying on scales that depend on subjective evaluations of weakness, exhaustion, and physical activity. As such, some investigators have proposed using sarcopenia – depletion of muscle mass assessed by computed tomography (CT) – as a potentially better predictor of outcome. In general, sarcopenia is assessed by measuring the surface area of skeletal muscle at the level of the third lumbar vertebrae by measuring the total area of skeletal muscle in this particular cross section or by measuring the surface area of the psoas muscle. Previous data have suggested that sarcopenia was associated with worse outcomes among patients being treated with chemotherapy for breast, pancreatic, prostate, and renal cell carcinoma.[17-21] In addition, Englesbe et al. reported that sarcopenia was predictive of mortality among patients undergoing liver transplantation.[22]

We herein sought to investigate the incidence of sarcopenia and sarcopenic obesity in patients undergoing liver resection for CRLM. Moreover, we investigated the impact of sarcopenia on both short- and long-term outcome among patients undergoing hepatic resection for CRLM. We hypothesize that sarcopenia and sarcopenic obesity may be associated with increased morbidity in patients undergoing hepatic resection for CRLM.

Methods

Patients and data collection

Between January 2000 and December 2009, 389 patients who underwent curative intent surgery for CRLM were identified from the Johns Hopkins Hospital liver database. Perioperative abdominal CT-scans (i.e. within 30 days of surgery) were available for re-review for 259 patients who, in turn, represent the study cohort. Clinical and pathological data were collected including information on demographics, primary tumor stage, extent of metastatic disease, operative details, as well as length of intensive care unit (ICU) and overall hospital stay. Data on perioperative mortality and morbidity were also collected. Specifically, complications were scored by the Clavien-Dindo classification with major complications being defined as Clavien grade ≥ 3.[23] The study was approved by the Johns Hopkins Institutional Review Board.

Image analysis

Sarcopenia was assessed by measuring the cross-sectional area of the right and left psoas muscles (Total psoas muscle area = TPA) by three trained observers (IK, PP, MV). TPA was measured at the level of L3 on the first image where both vertebral spinae were clearly visible. Measurements were performed in a semi-automated fashion by manually outlining the borders of both psoas muscles and setting the threshold between -30 and 110 Hounsfield Units (HU). This allowed for automatic calculation of the total surface area of skeletal muscle in the outlined area that excluded both vasculature and, more importantly, areas with fatty infiltration based on the respective HU. All images were analyzed using the Ultravisual software package (Merge Emageon, Birmingham, AL). The measured psoas area was then normalized for height, as is conventional for other body composition measures (TPA mm / m^2).

Statistical analyses

Data are provided as mean and standard deviation (SD) for continuous variables and proportions for binary variables. The impact of sarcopenia was evaluated both as a continuous and a categorical variable. To obtain the categorical cut-off value for sarcopenia, optimum stratification was assessed through a series of sensitivity analyses that defined 500 mm^2/m^2 as the most relevant cut-off value. As such, sarcopenia was defined as TPA of ≤ 500 mm^2/m^2. Sarcopenic obesity was defined as the presence of sarcopenia in patients with a BMI ≥ 30.

The impact of sarcopenia and sarcopenic obesity on postoperative morbidity and mortality was examined using univariate and multivariate analyses. Multivariate logistic regression models included other potential predictors of outcome such as sex, BMI and extent of liver resection. Recurrence-free and overall survival were evaluated using the non-parametric Kaplan Meier method. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using Stata 10.0 (StataCorp, College station, Texas).

Results

Clinical, Operative, and Sarcopenia Characteristics

The clinical and pathologic characteristics of the 259 patients included in the study are outlined in Table 4.1.1. Most patients had metastatic disease from a primary colon tumor (n=191; 73%), while 68 (27%) patients had a primary rectal lesion. The majority of patients had T3/T4 primary colorectal tumors (n=218; 84%) and associated lymph node metastasis (n=176;70%). Regarding the extent of CRLM, the median number of treated hepatic metastasis per patient was 2 (range, 1 to 15) and the median size of the largest lesion was 2.6 cm (range, 0.3 to 10.0 cm). At the time of surgery, surgical treatment was resection only (n=198; 76%) or resection plus RFA (n=61; 24%). No patient underwent RFA alone. A major hepatic resection (> 3 segments) was undertaken in 121 patients (47%). Specifically, the extent of hepatic resection was less than a hemihepatectomy in 7 (6%) patients, a

TABLE 4.1.1: Clinicopathological Characteristics of 259 patients included in the study.

Characteristic	Number (%)
Demographics	
Age, mean ± SD	58 ± 12
Gender, n (%)	
Male	155 (60%)
Female	104 (40%)
Anthropomorphic Characteristics	
BMI (kg / m2)	
< 30	191 (74%)
≥ 30	68 (26%)
TPA cm2 / m2	
≥ 500 mm2 / m2 (sarcopenic)	41 (17%)
> 500 mm2 / m2	217 (83%)
Primary Tumor Characteristics	
Node status, n (%)	
Positive	176 (84%)
Negative	74 (16%)
Missing	9
Primary Location, n (%)	
Colon	191(74%)
Rectum	68 (27%)
CRLM Characteristics	
Size, n (%)	
< 3cm	132 (54%)
≥ 3cm	113 (46%)
Number, n (%)	
≤ 3	196 (87%)
> 3	62 (13%)
Interval, n (%)	
Synchronous	155 (60%)
Metachronous	104 (40%)
Hepatic Surgery Operative Details	
Extent of liver resection	
Minor resection (< 3 segments)	138 (53.3)
Major resection (≥ 3 segments)	121 (46.7)
RFA	
Resection only	198 (76%)
Resection + RFA	61 (24%)

Abbreviations: SD, standard deviation; BMI, body mass index; CRLM, colorectal liver metastasis; RFA, radiofrequency ablation

TABLE 4.1.2: Comparison of demographic, clinical and surgical characteristics of sarcopenic versus non-sarcopenic patients.

Characteristic	Sarcopenia n=41 (15.8%)	No Sarcopenia n=218 (83.2%)	p-value
Gender			
Male	8 (19.5%)	147 (67.4%)	<0.001
Female	33 (80.5%)	33 (32.6%)	
Mean Age (SD)	59 (1.4)	58 (1.2)	0.68
BMI			
< 30	36 (87.8)	155 (71.1)	0.026
≥30	5 (12.2)	63 (28.9)	
Primary Tumor Nodal Status			
Positive	28 (68.3)	148 (67.9)	0.85
Negative	11 (26.8)	63 (28.9)	
No data	2 (4.9)	7 (3.3)	
Timing of CRLM Diagnosis			
Synchronous	23 (56.1)	132 (60.6)	0.61
Metachronous	18 (43.9)	86 (39.4)	
Mean Size of Largest CRLM, cm (SD)	2.8 (1.5)	3.1 (1.9)	0.26
Mean Number of CRLM Treated (SD)	2.7 (2.4)	2.8 (2.5)	0.75
Extent of Liver Resection			
Minor	22 (53.7)	116 (53.2)	0.99
Major	19 (46.3)	102 (46.8)	
RFA			
Yes	8 (29.5)	53 (24.3)	0.51
No	33 (80.5)	165 (75.7)	

Abbreviations: SD, standard deviation; BMI, body mass index; CRLM, colorectal liver metastasis; RFA, radiofrequency ablation

hemihepatectomy in 84 (69%) patients, and an extended hemihepatectomy in 30 (25%) patients.

Among the 259 patients evaluated, the mean TPA was 2070 ± 690 mm^2; after normalizing for patient height, mean TPA/m^2 was 700 ± 200 mm^2/m^2. Overall, 41 patients (17%) had sarcopenia defined as TPA/m^2 ≤ 500 mm^2/m^2. Sarcopenia was more prevalent among women (32%) compared with men (5%) (Figure 4.1.1). While sarcopenia was observed across a wide range of BMIs, sarcopenia was less frequently observed among obese patients with a BMI ≥ 30 kg/m^2 (Figure 4.1.2). Of the 68 patients who had a BMI ≥ 30 kg/m^2 only 5 (2%) also had sarcopenia and therefore were characterized as having sarcopenic obesity. Other demographic and clinicopathological factors were similar among sarcopenic patients versus non-sarcopenic patients (Table 4.1.2).

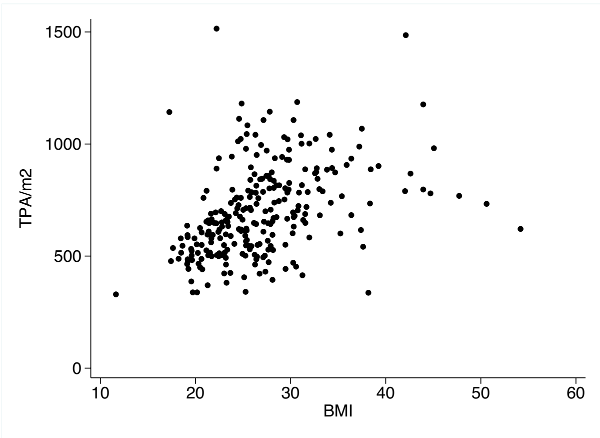

FIGURE 4.1.2: Scatterplot showing the distribution of TPA/m2 according to body mass index (BMI).

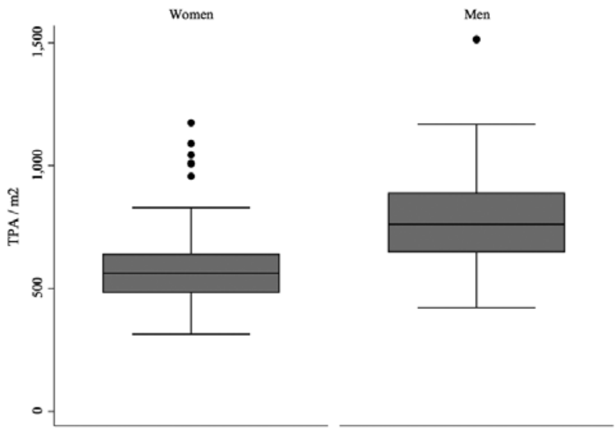

FIGURE 4.1.1: Boxplot showing the distribution of TPA/m2 stratified by gender.

Postoperative complications: influence of sarcopenia, obesity and sarcopenic obesity

Of the 259 patients who underwent surgery for CRLM, 60 patients developed a complication for a perioperative morbidity rate of 23%. Morbidity following surgery was mostly associated with liver-related complications such as post-operative bleeding (n=6), liver insufficiency (n=4), liver abscess (n=2), biloma (n=2), sepsis (n=1), and portal vein thrombosis (n=1). Other complications included symptomatic pleural effusion requiring drainage (n=6), pneumonia (n=2), and hematologic (n=3), cardiovascular (n=1), pulmonary (n=2), gastrointestinal (n=2), or other / non-specified (n=6). Of the 60 complications, 26 (43%) were major in nature (Clavien grade ≥ 3). Sarcopenia was associated wtih overall morbidity risk (OR 2.22; p=0.02), as well as the risk of experiencing a major complication in the perioperative period. Specifically, mean TPA / m^2 was significantly lower among patients who post-operatively developed Clavien grade ≥ 3 complications compared with those patients who did not (618 ± 212 mm^2/m^2 vs. 712 ± 197 mm^2/m^2, respectively; p=0.02). In fact, patients with sarcopenia had over a three-fold increased risk of developing major Clavien grade ≥ 3 complications following liver resection (Clavien grade ≥ 3 complications: non-sarcopenia 8% vs. sarcopenia, 22%, OR 3.44; p=0.008). On univariate analysis, other factors, including sex, age, BMI and extent of liver resection were not associated with risk of increased morbidity (all p>0.05). In a multivariate logistic regression model that accounted for these other variables, sarcopenia remained independently associated with risk of major Clavien grade ≥ 3 complications (OR 3.12, 95% CI 1.14-8.49; p=0.02) (Table 3.4.1). Patients with sarcopenic obesity had an even more pronounced risk of Clavien grade ≥ 3 complications compared with patients who did not have sarcopenia (sarcopenic obesity, 40% vs. non-sarcopenia 8%, p=0.02). Only 2 patients died following surgery for a perioperative mortality rate of 0.8%. Of the 2 patients who died, one had sarcopenia.

Median ICU and overall length of hospital stay was 1 days (range: 0-28 days) and 5 days (range: 2-41 days), respectively. Patients who had sarcopenia were more likely to have a prolonged ICU stay (i.e. > 2 days) (15%) versus patients who did not have sarcopenia (4%) (p=0.004). Similarly, patients who had sarcopenia were more likely to have a longer overall hospital stay (mean 6.6 ± 6.1 days) versus patients who did not have sarcopenia (mean 5.4 ± 3.2 days) (p=0.03). Patients

TABLE 4.1.3: Results of logistic regression analysis on the correlation between post-operative complications and sarcopenia (TPA mm2 / m2 ≤ 500).

	Univariate			Multivariate		
	OR	**95% CI**	**p-value**	**OR**	**95%CI**	**p-value**
Age (per 1 year increase)	1.00	0.97- 1.04	0.85	1.00	0.97-1.04	0.79
Gender (male)	0.53	0.24-1.22	0.14	0.78	0.31-1.97	0.60
BMI (≥ 30)	1.04	0.42- 2.59	0.94	1.34	0.52-3.46	0.78
Resection (major)	1.37	0.61- 3.10	0.44	1.40	0.60-3.23	0.43
Sarcopenia (present)	3.33	1.36-8.09	0.008	3.12	1.14-8.49	0.02

with sarcopenic obesity were at an even higher risk of a prolonged ICU (20%) and overall hospital stay (12.6 ± 16.1 days) (p<0.001).

Long-term outcome: does sarcopenia have an impact?

For the entire cohort, disease-free survival at 1-, 3- and 5-years was 65%, 28%, and 26%, respectively. The median disease-free recurrence was 18 months. The presence of sarcopenia was not associated with the risk of recurrence. Specifically, the 5-year recurrence-free survival was 23% versus 27% for sarcopenic and non-sarcopenic patients, respectively (p=0.78) (Figure 4.1.3a). To further explore any potential impact of TPA on recurrence, disease-free survival was analyzed by stratifying TPA into quartiles; TPA, however, remained not predictive of disease-free survival (Figure 4.1.3b). Furthermore, no difference in recurrence-free survival was noted among patients with sarcopenic obesity when compared with the rest of the cohort (p=0.26). The median overall survival for the entire cohort was 46 months with a corresponding 5-year survival rate of 40%. Similar to disease-free survival, sarcopenia was not associated with long-term overall survival (Figure 4.1.4). While patients with sarcopenic obesity tended to have a shorter median survival (30 months) compared with the rest of the cohort (46 months), this did not reach statistical significance (p=0.05).

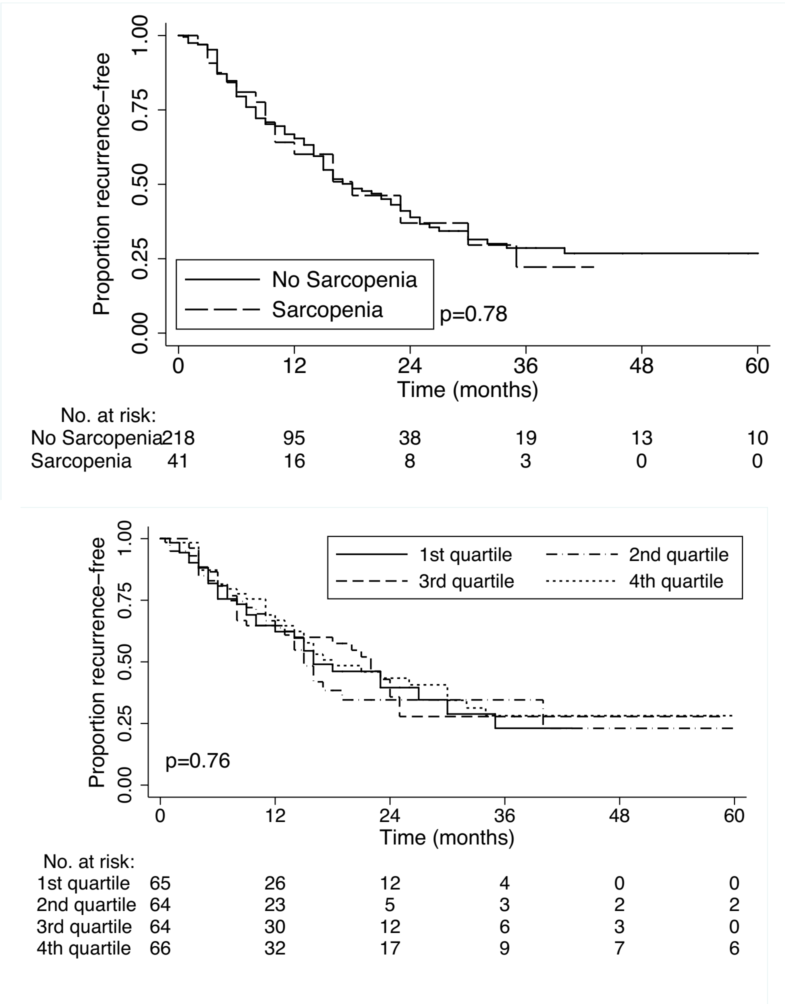

FIGURE 4.1.3: Recurrence free-survival stratified by (a) sarcopenia (TPA/m2 ≤ 500 vs. TPA/m2 > 500) and (b) TPA/m2 divided into quartiles.

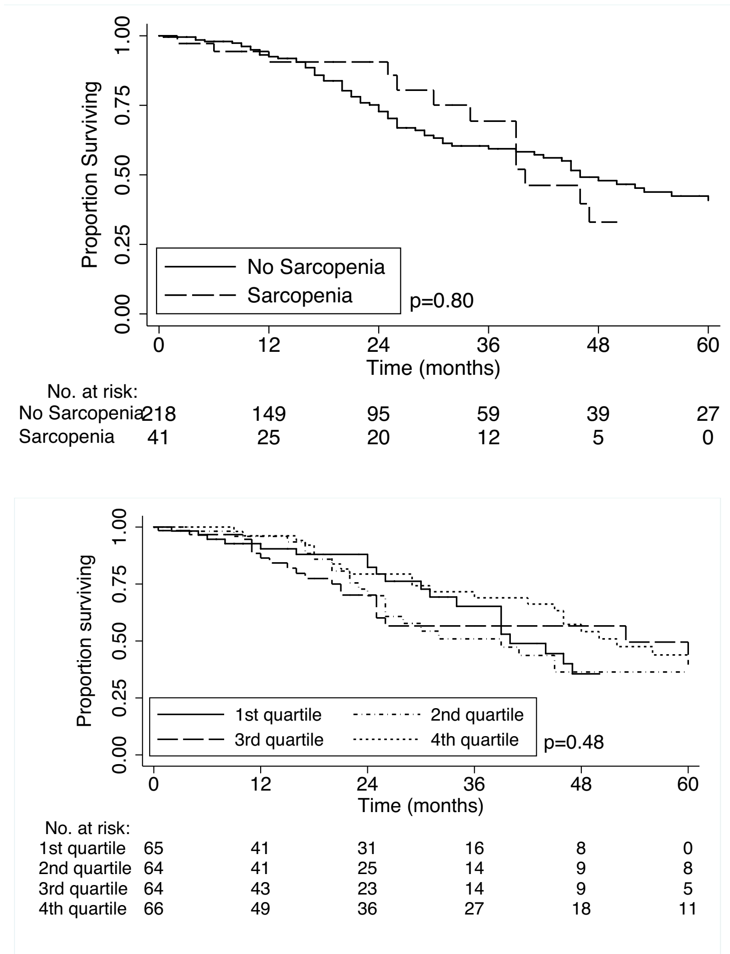

FIGURE 4.1.4: Overall survival stratified by (a) sarcopenia (TPA/m2 ≤ 500 vs. TPA/m2 > 500) and (b) TPA/m2 divide into quartiles.

Discussion

Despite the expansion in the utilization of liver surgery, the mortality associated with hepatic resection has dramatically decreased to less than 5% over the last few decades. In fact, perioperative mortality is less than 5% even following extended hepatic resection,[8] two-stage hepatectomy[24] and resection combined with radiofrequency ablation.[25,26] The morbidity associated with liver surgery, however, remains problematic with a perioperative complication rate up to 50% in some series.[4,7,10-13] While perhaps nonlethal, serious complications occur in a subset of patients, which can adversely impact patient quality of life, as well as increase hospital utilization and costs. There is a need, therefore, to identify patients at highest risk for perioperative complications following liver surgery for CRLM. The current study examines a large cohort of patients who underwent hepatic resection for CRLM to identify a possible predictor of perioperative morbidity. The data demonstrated that the presence of sarcopenia was strongly associated with increased risk of major post-operative complications. Moreover, patients with sarcopenia had both a higher chance of an extended ICU stay and a longer overall hospital stay. These data have important implications as they strongly suggest that measurements of sarcopenia, which can be easily obtained from preoperative cross-sectional imaging, may help identify those patients at highest risk of perioperative complications following resection of CRLM.

The ability to stratify patients at risk for perioperative complications following CRLM has several benefits. Risk prediction of perioperative morbidity can help provide prognostic information to the patient, guide treatment strategies, and can allowing for more accurate comparisons of outcome among various studies / hospitals.[27] Most previous reports, however, have focused exclusively on post-operative mortality, with few studies examining factors that might be predictive of morbidity.[16,27] Breitenstein et al. proposed a preoperative score to predict morbidity after liver resection that included ASA grade, alanine aminotransferase (AST), extent of liver resection, and extrahepatic procedure. The authors ascribed a point system to these factors and noted that patients who had an increased score were at higher risk of Clavien grade ≥ 3 complications.[27] This scoring system can be criticized, however, for relying too heavily on factors that may not be applicable to many patients undergoing liver resection for CRLM. For example, many patients undergoing liver resection for CRLM have a normal AST and do not undergo a simultaneous extrahepatic procedure. As such, a scale that utilizes these two factors may not be applicable to many patients undergoing CRLM surgery. In addition, the score proposed by Breitenstein and colleagues relies heavily on procedure related factors (e.g. extent of resection, extrahepatic procedure) and predominately incorporates patient-level factors only through the use of ASA grade. The ASA score has, however, been criticized as a subjective estimate of organ disease and likelihood of survival.[16,28] As such, some investigators have suggested more global assessments of physiological reserve such as frailty.[16,29,30]

In fact, Makary et al. reported that frailty independently predicted postoperative complications among a cohort of patients who underwent a wide variety of elective operations at a university hospital.[16] While the use of frailty allows for a more global assessment of the patient, it similarly can be criticized as being potentially subjective and susceptible to recall bias. Specifically, frailty scores depend on measurement of factors such as "exhaustion" and "physical activity," which are assessed by questioning the patient.[16] One well-described component of frailty that can be measured more objectively, however, is muscle loss / sarcopenia.[31-33] While sarcopenia can be associated with the aging process, it has also been shown to be accelerated with malnutrition and in chronic medical illness.[33-35] As such, sarcopenia may be an attractive objective measurement of frailty and overall physiologic reserve that might help predict risk of perioperative morbidity.

In the current study, while perioperative mortality was rare, morbidity occurred in up to one-quarter of patients undergoing surgery for CRLM. Many complications were liver-related including bleeding, liver insufficiency, and biloma. A full one-third of complications were, however, non-liver related, including cardiac and pulmonary morbidities – among others. Even more noteworthy was our finding that of the 60 complications noted, nearly one-half (43%) were major in nature. While factors such as sex, age, BMI and extent of liver resection were not associated with morbidity, the presence of sarcopenia was strongly associated with the risk of serious complications following surgery. In fact, patients who experienced a serious complication had a mean TPA mm^2 / m^2 about 100 mm^2 / m^2 lower than those patients who did not. This translated into over a three-fold (OR 3.44; p=0.008) increased risk of developing a major Clavien grade ≥ 3 complication among patients with sarcopenia. As such, our data strongly suggest that the finding of muscle loss (sarcopenia) on cross-sectional imaging may be a valuable preoperative characteristic to stratify a patient's risk of perioperative morbidity.

The impact of sarcopenia on long-term outcome remains ill-defined. Previous data have suggested that sarcopenia was associated with worse outcomes among patients being treated with chemotherapy for a range of malignancies.[17-21] In addition, Englesbe et al. reported that sarcopenia was predictive of long-term mortality among patients undergoing liver transplantation.[22] Specifically, in this latter study, the authors noted that when stratified into quartiles based on psoas area, 1-year survival ranged from 50% for the quartile with the smallest psoas area to 87% for the quartile with the largest. Survival at 3 years among these groups was 26% and 77%, respectively.[22] In contrast, in the current study, we did not find an association between the presence of sarcopenia and long-term outcome. In fact, when we stratified psoas area into quartiles, there was no discernible difference in either disease-free or overall survival (Figures 4.1.3 and 4.1.4). These data serve to emphasize the impact of sarcopenia on long-term outcomes may be a function of the underlying patient populations being studied. Whereas the impact of sarcopenia may be more pronounced on a patient's ability to tolerate an acute stress such as surgery, the role that sarcopenia has to play in long-term outcome is probably more complicated and may involve a complex interplay between overall general health status and the natural history of the patient's specific disease process.

The current study had several limitations. Because our study was based on a retrospective analysis of our hepatobiliary database, we were not able to capture data on other frailty parameters such as grip strength, walking speed, or levels of exhaustion.[16] Such data would have allowed us to examine the impact of sarcopenia relative to other parameters of frailty. In addition, despite being a major hepatobiliary center, the number of patients included in the study was somewhat limited. This was due in part to the limited availability of radiology scans for re-review, which required exclusion of 130 patients who had imaging performed at outside institutions.

In conclusion, while mortality following hepatic resection of CRLM was a rare phenomenon (0.8%), up to one-quarter of patients experienced a perioperative complication following surgery. Among those patients who had a complication, almost one-half had a serious complication. Sarcopenia, a measurement of muscle mass that can easily be obtained from cross-sectional imaging, accurately predicted those patients at highest risk of serious complications. In addition, the presence of sarcopenia was associated with both a prolonged ICU and overall hospital stay. As such, assessment of sarcopenia may provide an easy preoperative tool to help identify those patients at highest risk of perioperative morbidity following liver surgery for CRLM.

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Adapted From: Body composition and outcome in patients undergoing resection of colorectal liver metastases

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4.2

CT-based Assessment of Sarcopenia and Survival

Abstract

Background Recent evidence suggests that a depletion of skeletal muscle mass (sarcopenia) and an increased amount of intra-abdominal fat (central obesity) influence cancer statistics. This study investigated the impact of sarcopenia and central obesity on survival in patients undergoing liver resection for colorectal liver metastases (CLM).

Methods Diagnostic imaging from patients who had hepatic resection for CLM in one centre between 2001 and 2009, and who had assessable perioperative computed tomograms, was analysed retrospectively. Total cross-sectional areas of skeletal muscle and intra-abdominal fat, and their influence on outcome, were analysed.

Results Of the 196 patients who were included in this study 38 patients (19.4%) were classified as sarcopenic. Five year disease-free (15% versus 28,5%; $p = 0.002$) and overall (20% versus 49,9%; $p < 0.001$) survival rates were lower for sarcopenic patients at a median follow-up of 29 months (range, 1–97). Sarcopenia was an independent predictor of worse recurrence-free (HR 1.88; $p = 0.002$) and overall survival (HR 2.53; $p < 0.001$). Central obesity was associated with an increased risk of recurrence in men ($p = 0.032$), but not in women ($p = 0.712$).

Conclusions Sarcopenia has a negative impact on cancer outcomes following resection of CLM.

Background

Complete resection or ablation of colorectal liver metastases (CLM) offers the best option for definitive cure [1-4]. Historically, clinicopathological factors have been incorporated into scores to stratify patients according to predicted outcomes. These scores do not consider whether a poor general condition of the patient (either pre-existent or cancer related) may predict worse outcomes. Current measurements of performance and overall condition such as the American Society of Anaesthesiologists (ASA) score, weight loss and body mass index (BMI) are inadequate [5,6].

Computed tomography (CT) based measurements of body composition such as an increased amount of intra-abdominal fat (central obesity) and depletion of skeletal muscle mass (sarcopenia) can be predictors of cancer survival [7-11]. Until now, no such data are available for patients undergoing liver resection for CLM. Therefore, the aim of the current study was to investigate the influence of the quantity of subcutaneous and intra-abdominal fat as well as the quantity of skeletal muscle mass on survival following CLM resection.

Methods

Patients

Within the Erasmus MC, a digital database was prospectively maintained including all patients who underwent hepatic surgery for CLM containing data with regard to the primary tumour, hepatic metastases, surgical and chemotherapeutic treatment, recurrence and survival. Only patients with peri-operative abdominal CT-scans available for review (no more than two months prior to surgery or one month after surgery) were included. Patients with only peri-operative MRI-scans available were excluded.

Pre-operative work-up and chemotherapy

All patients in the current study underwent pre-operative CT or MRI scanning in the Erasmus MC or in the referring hospital and were presented to a multidisciplinary liver board, including a hepatobiliary surgeon, medical oncologist, hepatologist, pathologist, radiologist and radiation oncologist.

Indications for neoadjuvant chemotherapy included borderline resectability, bilobar disease or >3 metastases. Response to chemotherapy was assessed by CT or MRI scanning after 2 or 3 cycles. Administration of chemotherapy was stopped/ended in case of partial response or when initially unresectable metastases became resectable, and no more than 6 cycles of chemotherapy were administered. Resectability was defined as the ability to leave at least two consecutive liver segments in place with intact arterial, venous and biliary in- and outflow representing at least 25% of the total liver volume. RFA was applied for those lesions that could not be resected due to their location or spread.

Post-operative follow-up

Follow-up for disease recurrence was performed routinely every three months in the first year after surgery and every 6 months for the five years thereafter. Follow-up consisted of serum CEA levels and thoracic and abdominal CT-scans; Colonoscopy was performed 2-3 year after resection of the primary tumor or when indicated.. No adjuvant chemotherapy was administered after liver resection.

Assessment of adipose and skeletal muscle tissue

The quantity of intra-abdominal fat and skeletal muscle mass was determined using standard diagnostic CT-scans. For this purpose, a newly developed software application was used based on the MeVisLab version 2.2.1 (MeVis Medical Solutions AG, Bremen, Germany) software package. Cross-sectional areas (cm^2) of different tissue compartments were measured at the caudal end of the third lumbar vertebra based on their specific differences in attenuation (Hounsfield Units; HU). This was done by roughly manually outlining these compartments and segmenting the tissue of interest based on HU thresholds (-30 HU to + 150 HU for skeletal muscle and -190 HU to -30 HU for adipose tissue [12,13]). The total cross sectional area of the segmented tissue was then automatically calculated. Intra-colonic content initially marked as adipose tissue was manually corrected. The obtained body-mass indices were then normalized for stature (cm^2/m^2).

Statistical Analysis

Continuous data are presented as mean ± standard deviation or median (range) as appropriate. Categorical data are presented as proportions. Differences between groups were investigated using the student *t*-test for continuous variables and the χ^2 test for categorical variables. To investigate the cut-off values for the cross sectional area's of skeletal muscle mass and adipose tissue at which the difference in survival was most significant, sex-specific cut-off values were determined using optimum stratification to find the most significant p-value by use of log-rank statistics. This method has been previously described in literature as a method to solve the threshold value of the continuous covariate at which, based on log-rank statistics, patients of two categories (e.g. sarcopenic and non-sarcopenic) were best separated with respect to time to event outcome (e.g. mortality) [8]. Overall and disease-free survival were calculated and compared by the non-parametric Kaplan-Meier method and log-rank test. To investigate the correlation between sarcopenia, central obesity and survival, univariable and multivariable Cox regression analyses were performed and hazard ratio's (HR) and 95% confidence intervals were calculated. The following variables were included in the univariable analysis: Sarcopenia, age, gender, diabetes, BMI, ASA-score, primary tumour localization, synchronous staging, tumour number, tumour size, CEA, neo-adjuvant systemic therapy, and RFA. All variables were checked for interaction and confounding and were included in the multivariable model when significant. Introducing a time varying predictor variable in the model as well as calculating Schoefeld residuals indicated that the assumption of proportionality was met for this model. All statistical analyses were performed using SPSS 17.0 (SPSS. Inc, Chicago, IL), and Stata 11 (Statacorp, collegetown, TX). A p-value < 0.05 was considered to be statistically significant.

Results

Patient characteristics

One-hundred and ninety-six patients qualified for the current study.
The clinicopathological features of these patients can be found in table 4.2.1.
All patients were treated between 2001 and 2009. Systemic therapies (administered to 91 patients) were mostly oxaliplatin-containing combination regimens (86%). A major hepatic resection (three segments or more) was performed in 63 (32%) patients, whereas a segmentectomy of two or less segments and non-anatomic resections were in 133 (68%) of the patients.

TABLE 4.2.1 – Demographic and clinicopathological characteristics of the 196 patients included in the study

Sex (male to female ratio)		120 (61.2) : 76 (38.8)
Median Age, Years (range)		64.5 (31 – 86)
Primary tumour		
Location	Colon	116 (59.2)
	Rectum	80 (40.8)
T-Stage†	T1	0 (0)
	T2	25 (13.2)
	T3	148 (78.3)
	T4	16 (8.5)
	No data	7
N-Stage†	N0	80 (41.9)
	N1	77 (40.3)
	N2	34 (17.8)
	No data	5
Metastases	Synchronous	93 (47.4)
	Metachronous	103 (52.6)
Disease-free interval	< 12 months	129 (65.8)
	≥ 12 months	67 (34.2)
No. of metastases	≤ 3 tumours	147 (75.0)
	> 3 tumours	49 (25.0)
Maximum tumour size	< 5 cm	144 (73.5)
	≥ 5 cm	52 (26.5)
ASA physical status score	ASA 1	46 (23.5)
	ASA 2	122 (62.2)
	ASA 3	28 (14.3)

Values in parentheses are percentages unless indicated otherwise. † Missing data for some patients.
Abbreviations: ASA American Society of Anaesthesiologists

Radiofrequency Ablation (RFA) was applied in 39 (20%) of the patients, of which 5 received open RFA alone.

Measurements of body composition

A wide range of body compositions was found in the analysis of the CT-images (Fig 1). Intra-abdominal fat ranged from 7.0 cm^2/m^2 to 171.6 cm^2/m^2 with a mean of 58.8 cm^2/m^2. For skeletal muscle mass the range was found to be smaller, with a

FIGURE 4.2.1 – A CT-Image showing highlighted areas of subcutaneous (green) and intra-abdominal (yellow) fat and skeletal muscle mass (red).

minimum of 31.5 cm^2/m^2 and a maximum of 75.9 cm^2/m^2, with a mean of 50.4 cm^2/m^2 for skeletal muscle mass.

Skeletal muscle mass and survival

Sex-specific cut-off values for skeletal muscle mass associated with overall mortality obtained by means of optimum stratification were 41.10 cm^2/m^2 for female patients and 43.75 cm^2/m^2 for male patients. By these definitions, 38 patients (19%) were found to be sarcopenic. Demographic and clinical characteristics are compared between these groups in Table 4.2.2. Sarcopenia significantly correlated with the female gender (Figure 4.2.2), low BMI and a lower quantity of intra-abdominal fat (Table 4.2.2). No difference in subcutaneous fat was found between the sarcopenic and non-sarcopenic population. No statistical differences were found between the two groups with regard to known risk factors and pre-operative systemic therapy.

In total, 126 (64%) patients had a disease recurrence after a median follow-up of 29 months (range, 1–97). The median disease-free survival was 11.8 months with 1-, 3- and 5-year disease-free survival rates of 49.3, 32.9 and 25.7 respectively.

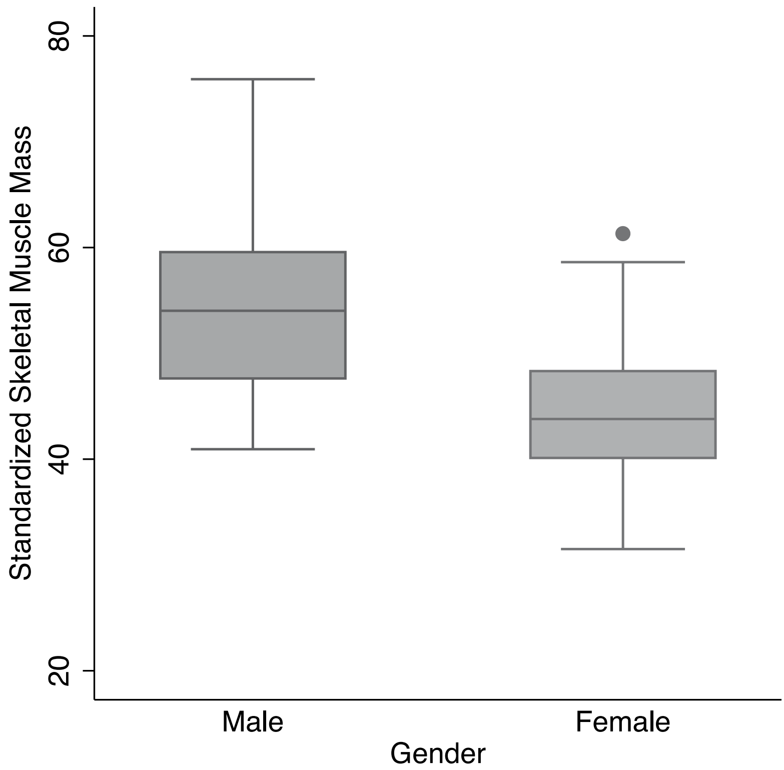

FIGURE 4.2.2 – Box-and-whisker plot showing the correlation between gender and total cross-sectional area (CSA) of skeletal muscle mass. Male patients had a median skeletal muscle mass of 54.02cm2/m2 (40.94 – 75.91), whereas female patients were found to have a significantly lower median skeletal muscle mass of 43.79cm2/m2 (31.50 – 61.32) ($p < 0.001$).

Eighty-four (42,9%) patients died during follow-up, with a median overall survival of 50.3 months and corresponding 1-, 3- and 5- year survival rates of 94%, 58% and 43% respectively.

Patients with sarcopenia had a significantly shorter median disease-free survival than patients without sarcopenia (8.7 versus 15.1 months; $P = 0.002$); 1-, 3- and 5-year disease-free survival rates for patients with sarcopenia were 31%, 20% and 15% versus 54.0%, 36.3% and 28.5% respectively for those without sarcopenia

TABLE 4.2.2 – Comparison of demographic and clinical characteristics among sarcopaenic and non-sarcopaenic patients using the cut-off values obtained by means of optimum stratification; 41.10cm2/m2 for female patients and 43.75cm2/m2 for male patients.

		Sarcopaenic N=38 (19.4%)	Non-sarcopaenic N=158 (81.6%)	p
Median Age, Years (range)		65.50 (47 – 84)	65.00 (31 – 86)	0.229
Sex	Male	11 (28.9)	109 (69.0)	< 0.001
	Female	27 (71.1)	49 (31.0)	
Mean BMI		23.66 ± 3.01	26.66 ± 3.53	< 0.001
Primary Tumour Site	Colon	21 (55.3)	95 (60.1)	0.587
	Rectum	17 (44.7)	63 (39.9)	
Primary Tumour Node	Negative	14 (37.8)	66 (42.9)	0.711
	Positive	23 (62.2)	88 (57.1)	
	No data	1	4	
Disease-free interval	< 12 months	27 (71.1)	102 (64.6)	0.554
	≥ 12 months	11 (28.9)	56 (35.4)	
Detection	Synchronous	21 (55.3)	72 (45.6)	0.366
	Metachronous	17 (44.7%)	86 (54.4)	
CEA	< 200	30 (78.9)	133 (84.2)	0.471
	≥ 200	8 (21.1)	25 (15.8)	
Max. Tumour Size	< 5cm	26 (68.4)	118 (74.6)	0.421
	≥ 5cm	12 (31.6)	40 (25.4)	
No. of Tumours	≤ 3	29 (76.3)	118 (74.6)	1.000
	> 3	9 (23.7)	40 (25.4)	
Pre-operative	Yes	18 (47.4)	73 (46.2)	1.000
chemotherapy	No	20 (52.6)	85 (53.8)	
ASA	1	9 (23.7)	37 (23.4)	0.755
	2	25 (65.8)	97 (61.4)	
	3	4 (10.5)	24 (15.2)	
Mean Intra-abdominal Adipose Tissue		42.23 ± 23.89	62.77 ± 30.92	< 0.001
Mean Subcutaneous Adipose Tissue		61.54 ± 22.65	57.29 ± 23.35	0.387

Abbreviations: CEA Carcinoembryonic Antigen, ASA American Society of Anaesthesiologists, BMI Body-Mass Index

(P = 0.002) (Fig. 4.2.3). Similarly, median overall survival was worse in patients with sarcopenia (23.8 months versus 59.8 months in those without sarcopenia; P = 0.001), with corresponding 1-, 3- and 5-year survival rates of 84%, 34% and

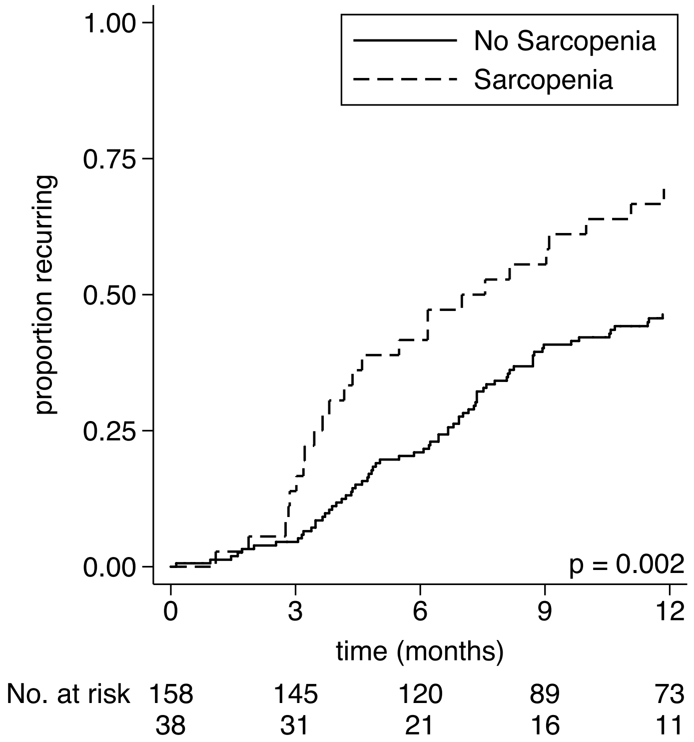

FIGURE 4.2.3 – Disease-Free survival in the sarcopaenic vs non-sarcopaenic population.

20% compared with 96.2%, 64.6% and 49.9% respectively for patients without sarcopenia ($P < 0.001$) (Fig. 4.2.4). Moreover, when adjusting for well known risk-factors, sarcopenia was found to be an independent predictor of worse disease-free (HR 1.88 95% CI 1.25 – 2.82; $p = 0.002$) and overall survival (HR 2.53 95% CI 1.60 – 4.01; $p < 0.001$)(table 4.2.3 and 4.2.4). To investigate whether the impact of sarcopenia might be different for patients who did and who did not receive pre-operative chemotherapy, recipients of chemotherapy were compared to patients who did not receive systemic therapy. In both groups, sarcopenia was found to have a negative impact on overall survival (HR 2.56 95% CI 1.27 - 5.18; $p = 0.009$ and HR 2.44 95% CI 1.32 - 4.48; $p = 0.004$ respectively). Also, sarcopenia negatively impacted on disease-free survival in both groups, although this did not reach statistical significance in the chemotherapy group, likely as a result of

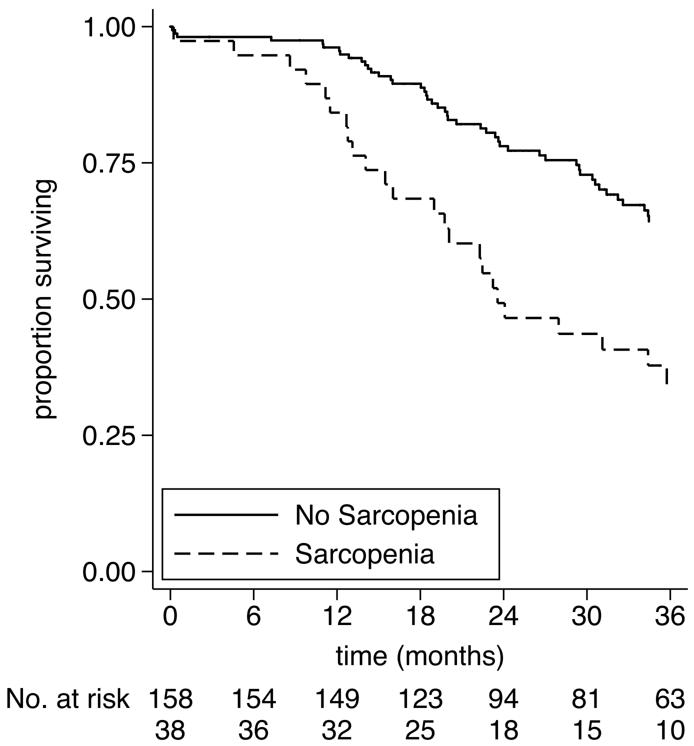

FIGURE 4.2.4 – Overall survival in the sarcopaenic vs non-sarcopaenic population

smaller numbers (HR 1.72 95% CI 0.96 - 3.08; p = 0.070 and HR 1.95 CI 1.11 - 3.43; p = 0.021 respectively).

Central obesity and survival

Optimum stratification did not detect any correlation between central obesity and survival. Also, no statistical significant correlation between intra-abdominal fat, disease-free and overall survival was found when the total cross sectional area of intra-abdominal fat was treated as a continuous variable (table 4.2.3 and 4.2.4). However, subgroup analysis showed a significant impact of intra-abdominal fat on disease-free survival in male patients, using a cut-off of 94.00cm^2/m^2 obtained by means of optimum stratification (log-rank p = 0.032) which for purpose of this study we defined as central obesity. By this definition, 20 (16.7 per cent) of the 120 men in the study had central obesity, and their median disease-free survival

TABLE 4.2.3 – Impact of muscle mass and other clinical characteristics on disease-free survival

	Univariable Analysis			Multivariable Analysis		
	HR	95% CI	p	HR	95% CI	p
Sarcopaenia †	1.880	1.252 – 2.822	0.002	1.957	1.290 – 2.969	0.002
Age	1.003	0.986 – 1.021	0.694			
Gender (female)	1.195	0.837 – 1.706	0.328			
BMI ≥ 25	1.076	0.750 – 1.543	0.692			
Diabetes	0.821	0.503 – 1.337	0.428			
Primary tumour site (colon)	0.861	0.604 – 1.225	0.405			
Synchronous*	1.345	0.948 – 1.909	0.096			
Tumour No. (> 3) †	1.975	1.341 – 2.907	0.001	1.750	1.088 – 2.815	0.021
Tumour size (≥ 5cm)	1.047	0.709 – 1.546	0.818			
CEA (≥ 200) †	1.739	1.125 – 2.689	0.013	1.749	1.117 – 2.739	0.015
Pre-operative chemotherapy	1.302	0.918 – 1.848	0.139			
Radio-frequency ablation †	1.843	1.225 – 2.772	0.003			NS
ASA physical status (≥ ASA 3)	0.875	0.537 – 1.425	0.591			
Resection margin (≤ 1mm) †	1.629	1.101 – 2.410	0.015			NS
Visceral adipose tissue CSA	1.001	0.994 – 1.007	0.829			

Abbreviations: BMI Body-Mass Index, ASA American Society of Anaesthesiologists, CEA Carcinoembryonic Antigen, CSA Cross-Sectional Area
† Included in the multivariable analysis
* Synchronous indicates detection of hepatic metastases within three months of diagnosis of the primary tumour.

was significantly worse than that of non-obese men (9.8 versus 18.0 months respectively; P = 0.032). Corresponding 1-, 3- and 5-year disease-free survival rates were 38, 19 and 0 per cent, compared with 56.8, 39.9 and 28.0 per cent respectively for non-obese men (P = 0.032) (Fig. 4.2.5). Overall survival was not affected by central obesity in men (P = 0.837). For women, no association was found for central obesity and overall survival (p = 0.566).

TABLE 4.2.4 – Impact of muscle mass and other clinical characteristics on overall survival

	Univariable Analysis			Multivariable Analysis		
	HR	95% CI	p	HR	95% CI	p
Sarcopaenia †	2.531	1.596 – 4.012	< 0.001	2.685	1.671 – 4.315	< 0.001
Age	1.006	0.984 – 1.028	0.621			
Gender (female)	1.264	0.820 – 1.948	0.289			
BMI ≥ 25	0.832	0.539 – 1.282	0.404			
Diabetes	0.742	0.402 – 1.368	0.339			
Primary tumour site (colon)	0.770	0.501 – 1.183	0.232			
Synchronous*	1.111	0.723 – 1.707	0.632			
Tumour No. (> 3) †	1.652	1.036 – 2.633	0.035			NS
Tumour size (≥ 5cm)	1.015	0.628 – 1.640	0.952			
CEA (≥ 200)	1.155	0.639 – 2.091	0.633			
Pre-operative chemotherapy	0.904	0.586 – 1.396	0.694			
Radio-frequency ablation †	1.711	1.020 – 2.870	0.042			NS
ASA physical status (≥ ASA 3)	0.620	0.320 – 1.201	0.156			
Resection margin (≤ 1mm) †	1.795	1.135 – 2.840	0.012	1.853	1.154 – 2.972	0.011
Visceral adipose tissue CSA	0.996	0.989 – 1.004	0.348			

Abbreviations: BMI Body-Mass Index, ASA American Society of Anaesthesiologists, CEA Carcinoembryonic Antigen, CSA Cross-Sectional Area
† Included in the multivariable analysis
* Synchronous indicates detection of hepatic metastases within three months of diagnosis of the primary tumour.

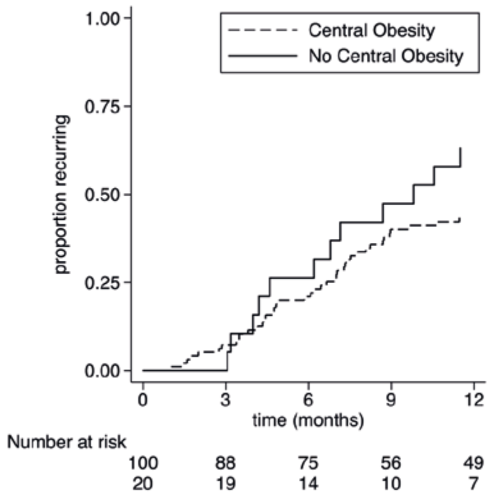

FIGURE 4.2.5 – Disease-Free survival in the obese vs non-obese subpopulation of male patients.

Discussion

A wide variety of body composition can be found in patients with CLM, with nineteen percent of patients being sarcopenic in the present study. Sarcopenia was a strong indicator of a worse prognostic outcome for disease-free survival and overall survival. The occurrence and effect of sarcopenia was independent of physical status (ASA classification) and tumor related factors known to impact on survival after liver resection for CLM[14]. These findings emphasize the importance of the assessment and potential treatment of sarcopenia in cancer patients scheduled for curative intent surgery.

Sarcopenia may reflect increased metabolic activity of a more aggressive tumour biology leading to systemic inflammation, causing muscle wasting[15]. Sarcopenia should not be confused with "clinical cachexia" or simple weight loss, since many sarcopenic patients have a normal or elevated BMI [15-17]. Sarcopenia can therefore only be uncovered by precise quantification of skeletal muscle mass. CT analysis has been shown to be a widely available and highly precise method for the detection of sarcopenia[13].

Patients with sarcopenia did not have a worse physical status according to the ASA classification when compared with non-sarcopenic patients. Also, the ASA score did not predict long term outcome. While the ASA score gives some estimation of organ disease and functional status, it has been criticized for being subjective and imprecise[18]. Frailty has been reported to allow for a more global assessment of a patients health status and physiological reserve. However, most frailty scores include measurements of weakness and physical activity assessed by patient questionnaires and can therefore be potentially subjective and susceptible to bias as well. Sarcopenia has been described as a more robust measure of frailty and might therefore give a more objective assessment of a patient's functional reserve than currently used scoring systems.

Sarcopenia was an independent predictor of worse disease-free and overall survival. Prado et al showed an impaired survival in sarcopenic obese patients with gastrointestinal and respiratory tract malignancies[8]. Other studies have confirmed the impact of sarcopenia on survival in patients with pancreatic cancer and patients undergoing liver transplantation[9,10]. The correlation between the amount of intra-abdominal fat and long term outcome was less defined in the current study. Others have found reduced disease-free survival in patients with colorectal cancer where there were high visceral / subcutaneous fat ratios[7]. Intra-abdominal adiposity has also been associated with an increased incidence of malignant and pre-malignant tumours of the gastro-intestinal tract[19]. The exact mechanisms by which sarcopenia and central obesity affect survival in cancer patients have yet to be unravelled, although both conditions have been described to both impact on the risk of cancer development as well as short- and long term post operative outcomes [7-9,11,18,19].

Several limitations apply to the current study. First, patients were excluded from analysis due to unavailability of CT scans. This has undoubtedly caused a selection bias with regard to pre-operative chemotherapy, disease status and post-operative morbidity. Second, the inclusion of post-operative CT-scans created a potential bias since patients with post-operative CT-scans might have suffered from more complications with a negative impact on long term outcome. Regardless, long-term

outcome was similar for patients with a preoperative or postoperative CT scan, and no difference in the incidence of sarcopenia was found for the two groups (data not shown). The cut-off values for sarcopenia may not be directly applicable to another population set and should be further validated.

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5

Summary, Conclusions and Future Directions

5.1

Summary and General Discussion

Intraoperative Ultrasound

In addition to providing detailed visualisation of the anatomy of the liver during surgery and guidance for needle biopsy or ablation, IOUS has long been considered important for improved intrahepatic staging and lesion detection in patients undergoing surgery for colorectal liver metastases [1-3]. Recent literature has challenged the role of IOUS for detecting otherwise occult intrahepatic disease when modern, high quality cross-sectional (MDCT, MRI) and functional (5-FDG PET) imaging is used[4]. Moreover, the impact of hepatic steatosis and pre-operative chemotherapy on the appearance of CRLM on IOUS and their impact on the usefulness of IOUS have not been previously investigated. These concerns were specifically addressed in part one of this thesis. In **chapter 2.1**, it was found that intra-operative ultrasound allows for the detection of additional metastatic lesions at the time of surgery in 10% of patients undergoing curative intent surgery for colorectal liver metastases, despite modern high quality pre-operative imaging. All 213 patients included in the study underwent contrast enhanced CT scanning and the use of pre-operative 5FDG-PET scanning or MR imaging did not impact on the rate of additional metastases found with IOUS. The probability of finding additional lesions was about five times higher (OR 5.45; $p<0.001$) in patients with multiple (≥4) liver metastases on pre-operative imaging when compared to patients with a single metastasis detected pre-operatively. Moreover, the chance of finding occult metastases not detected on pre-operative imaging was markedly decreased (OR 0.18; $p=0.007$) when primarily visualized metastases had an isoechoic appearance on intraoperative ultrasound when compared to patients with more conspicuous hypoechoic or hyperechoic tumors. At last, patients with isoechoic metastases had a higher chance of early intrahepatic recurrence (OR 3.24; $p=0.03$), suggesting the presence of small occult metastases during surgery not picked up by IOUS due to their isoechoic appearance. Based on these results, it is recommended that IOUS should be an important part of the standard of care for patients undergoing surgery for colorectal liver metastases, especially in those patients with multiple metastases on pre-operative imaging and more conspicuous hypoechoic tumors on ultrasound.

Chapter 2.2 describes the correlation between hepatic steatosis and tumor echogenicity in 126 patients with 191 CRLM. By performing a *per lesion* analysis, it was found that the probability of encountering hypoechoic tumors was 60%

in patients with mild steatosis and over 90% in patients with moderate or severe steatosis, compared to a 33% chance of finding hypoechoic tumors in patients without steatosis ($p<0.001$). This correlation was independent of the aetiology of steatosis (pre-existent or chemotherapy associated). In addition, of the nine metastases included in this analysis that had disappeared on pre-operative imaging as a result of preoperative chemotherapy, eight metastases (89%) were hypoechoic and the majority was found in a steatotic liver. This suggests that the presence of steatosis increases the chance of finding these tumors despite their disappearance from pre-operative imaging. These findings are important for several reasons. First, when a grossly steatotic liver is encountered during surgery, one should anticipate on finding hypoechoic tumors on ultrasonography. In addition, besides decreasing the size of metastases and thus making them harder to visualize on pre-operative imaging [5], chemotherapy can result in significant hepatic steatosis [6-8], supposedly further decreasing the sensitivity of pre-operative imaging studies for the detection of liver metastases [9,10]. Conversely, our data suggests that steatosis might increase the visibility of small liver metastases on IOUS by making them more conspicuous, re-enforcing the use of IOUS after chemotherapy. This hypothesis was recently confirmed by Ferrero et al, who investigated the value of IOUS in patients with disappearing colorectal liver metastases after chemotherapy[11]. In this study, the presence of steatosis was independently associated with the intraoperative detection of metastases that had disappeared on pre-operative imaging (OR 9.96; $p=0.016$).

The influence of pre-operative chemotherapy on the yield of IOUS to detect CRLM was further analyzed in **Chapter 2.3**. In this study, the incidence, intra-operative detection rate and fate of disappearing colorectal liver metastases (DLM) after chemotherapy were investigated in 168 patients undergoing liver resection. In almost a quarter of these patients (23.8%), one or more metastases were found to disappear during chemotherapy. DLM were often small prior to chemotherapy (median size 1 cm) and the likelihood of disappearance increased with the number of cycles of pre-operative chemotherapy. Due to the high probability (31% to 83%) of viable tumor cells in these DLM [5,12], the current paradigm is to completely resect or ablate these tumors when possible. However, DLM can be difficult to find intraoperatively. In our study, these disappearing liver metastases (DLM) were found using intra-operative ultrasound and palpation of the liver in

45% of patients and 55% of all lesions. In the study by Benoist et al. only 30% of DLM were detected intra-operatively. Auer et al. recently reported an even lower intra-operative detection rate of 11%[5,13]. These variable results are likely due to differences in pre-operative imaging strategies, the duration of pre-operative chemotherapy and the thoroughness of the IOUS protocol.

In the current study, a true pathological response or durable remission was observed in 45% of metastases that had disappeared on pre-operative imaging studies, which is in accordance with other studies on this subject. Not surprisingly, the median time to intrahepatic recurrence was worse for those patients in whom DLM were not found during surgery and were left untreated (11 months) when compared to patients in whom all original disease sites were surgically treated (20 months; p=0.04). However, repeat curative intent surgery was often possible (either resection or RFA) and the median overall survival of these patients did not significantly differ from those patients in whom all original disease sites were surgically treated (65 months vs. 54 months respectively; p=0.66). These results are consistent with other series reporting on the survival of patients with DLM. Elias reported a 94% 3-year overall survival in 16 patients with DLM left in place[14]. Other series confirm that approximately two thirds of patients with DLM are still alive 5 years after surgical treatment[15]. Thus, several recommendations can be made based on this study. First, the number of cycles of pre-operative chemotherapy should be limited to avoid the disappearance of metastases, especially if small lesions are present. When metastases are found to disappear, IOUS is an essential part of the intra-operative assessment to aid in the detection and eradication of all sites of disease, but also to allow for parenchymal sparing procedures by saving a patient from a "blind" hemi-hepatectomy due to the unknown location of DLM. At last, in those circumstances in which all sites cannot be identified and when incorporation of undetected original sites in a resection is not safe or possible, leaving them behind can be considered in selected cases. However, these untreated DLM have a high risk of in-situ recurrence and therefore it is advised that one must only consider surgical therapy for those patients in whom all original sites can be treated, either at the time of initial surgery or when a recurrence occurs.

Local Ablative Therapies and Imaging

Part two of this thesis describes the utility of radiofrequency ablation (RFA) in patients with different types of liver tumors and the way radiological studies can be used by the liver surgeon to minimize local recurrence.

Chapter 3.1 reports on the results after combined resection and ablation of CRLM in a large multi-institutional cohort of 125 patients. As shown in this study, surgical resection combined with ablation can be a valuable strategy in patients with extensive disease confined to the liver. While rates of recurrence were high (67% of patients recurred during follow-up) and overall survival was worse when compared to patients treated with surgical resection only (median 35 vs. 51 months), overall survival was significantly better when compared to patients that underwent a histological biopsy only (20 months; $p<0.001$). In addition, with an overall surgical mortality rate of 1.6%, the addition of RFA to surgical resection seems to add little risk to the procedure as long as enough viable remnant liver is preserved. These results compare well to other studies describing resection combined with ablation for the treatment of CRLM. In a study by Abdallah et al. patients treated with resection and ablation had a median overall survival of about 30 months and any site recurrence was observed in 63% of these patients[16]. Gleisner et al. reported similar results[17]. In both studies, the use of RFA in addition to resection was associated with higher rates of intra-hepatic recurrence and worse overall survival when compared to patients that were treated with resection only, a phenomenon also observed in the current study. Caution should be kept however, when comparing surgical resection alone to surgical resection combined with ablation, since groups might be very different. In the current study, baseline characteristics associated with poor prognosis such as synchronicity of primary and metastatic disease and a high tumor number were more common in patients treated with resection and ablation when compared with patients treated with resection only. Similar findings were described by Gleisner et al, who used propensity indices to show that meaningful comparison between both groups with regard to overall survival is severely hampered by differences in base-line characteristics. Regardless, the rate of intrahepatic recurrence has steadily been described to be relatively high after resection combined with surgical ablation. In the current study, the number of lesions that were ablated, but not the total number of tumors treated was associated with an increased rate of recurrence.

These data suggest that the use of RFA is the main contribution to recurrence in these patients. Based on these results, it is recommended that RFA can be safely combined with liver resection in patients with extensive liver metastases. However, due to high rates of intra-hepatic recurrence and potentially worse overall survival when compared to resection only, this strategy should be reserved for patients that would not be surgical candidates otherwise.

In **chapter 3.2**, tumor-related as well as intraoperative factors associated with local recurrence after operative RFA were evaluated. Both tumor size as well as the proximity of major vascular structures were found to significantly impact on local recurrence rates. These data confirm the importance of these parameters when selecting tumors for RFA treatment[18,19]. In addition, a prospective observational study of procedural parameters of ablation was performed in order to further refine factors associated with long-term efficacy of ablation for CRLM. Indeed, factors such as achievement of target temperatures and tumor encompassment by the ablation zone were highly predictive of LR. Importantly, operative assessment of ablation confidence through an A-status score, based on a compilation of procedure-related factors (tumor conspicuity on IOUS, probe targeting and positioning, micro bubble distribution, and temperatures) were strongly predictive of long-term LR. Incorporation of tumor size and location with the operative assessment of A-status provided an even more refined determination of LR risk. Small tumors away from vascular structures had an excellent local control rate when an A0 ablation was achieved (9% 2-year LR rate). Therefore, much as the R-status is predictive of local recurrence following liver resection [20], determination of A-status can be used as a surrogate for post-ablation margin status.

The findings from this study can provide useful information when guiding the clinical care of patients undergoing tumor ablation of colorectal liver metastases. First, when and intraoperative assessment indicate a suboptimal ablation and a high local recurrence risk is anticipated, therapeutic management may be altered. Second, it was found that when careful tumor selection and operative assessment is undertaken, durable efficacy of RFA in many cases is demonstrated, with local recurrence observed in approximately 9%. Finally, we identified the importance of intra-procedural assessment at assessing efficacy. Yet, current assessment remains problematic, based on limited ability to perform and determine accurate probe placement and monitoring of therapy [21-23]. This study illustrates the importance of developing more sophisticated methods to assist and evaluate accurate probe placement as well as to monitor the effects of tumor ablation in real-time, perhaps by using contrast enhanced ultrasonography or ultrasound elasticity imaging[24].

In **chapter 3.3**, the safety and efficacy of RFA to treat hepatocellular adenoma's (HCA) were investigated. HCA are benign tumors in itself but spontaneous rupture and malignant transformation have been described in growing or large (>5 cm) HCA[25]. This has resulted an aggressive surgical approach towards these lesions in many patients over the past years [26-28]. Since HCA are essentially benign and occur mostly in young and otherwise healthy patients, treatment modalities other than (major) liver resection are urgently needed to save these patients from the morbidity and mortality associated with this procedure. As such, we studied the use of RFA for the treatment of HCA. In this study, RFA was employed in 18 patients and a total of 45 HCA's were treated in 32 sessions. Most patients (56%) required multiple sessions to be ridden of all HCAs and 58% of HCA's showed signs of residual disease on follow-up CT-scanning 6-8 weeks after ablation, indicating a high number of technical failures according to the definitions of the ISIR[29]. While the clinical relevance of a small amount of residual adenoma with regard to its capability for growth or rupture can be debated, these results underline the need for improved image guidance during RF ablation: In all cases, RFA was considered to be technically successful on direct post-procedural ultrasound or CT. Based on the results of this study, it is recommended that RFA is only employed when surgical resection of HCAs is not deemed possible (eg in patients with adenomatosis) and when a conservative approach can not be defended. Moreover, novel data suggests that the treatment of hormone sensitive HCA in young women with a pregnancy wish might not be indicated in all cases [30] and that the biological behavior of HCA might be depending of it's the histological subtype rather than its size or growth pattern[31]. Therefore, future research should focus on sharpening the indications for treatment of these tumors and explore other minimally invasive modalities for the treatment of HCA such as transarterial embolisation[32].

Chapter 3.4 describes the development of a tumor bearing large animal model for liver tumor ablation. Kromopan alginate (a hydrocolloid) was successfully used to create a total of 24 hepatic pseudotumors in eight live pigs and two dog cadavers. As shown in the reported experiments, these pseudotumors were easy to create and implant with little associated costs. Once implanted, these tumors maintained their viscoelastic and radiologic capabilities for several days without endangering the health of the host animal, suggesting good biocompatibility. The created pseudotumors were easily visualized by a variety of imaging modalities (such as ultrasonography, computed tomography and magnetic resonance imaging) and visible on macroscopic visualization. Moreover, these tumors were very similar to real tumor tissue in terms of viscoelastic properties and behavior during RFA, making them highly suitable for experimental research concerning the guidance and monitoring of local ablative therapies. This model has therefore several advantages over the use of previously described models for agar based artificial liver tumors, which are more expensive and time consuming to prepare and lack the viscoelastic properties of kromopan alginate [33].

In **chapter 3.5**, this pseudotumor model was utilized to study a novel device for ablation monitoring**.** In this study, the ability of ultrasound elasticity imaging to visualize tissue specific stiffness was used to depict the zone of thermal coagulation around a liver tumor after RFA. Hence, the stiffness of tissue increases significantly when thermally coagulated which results in a decreased compressibility of this tissue. Ultrasound elasticity imaging can capture these changes in elasticity by measuring the relative displacement of ultrasound pixels when tissue is compressed by an external stimulus, in this case free-hand palpation with the ultrasound probe. Both in-vivo animal experiments as well as several clinical experiments showed that USEI was capable of depicting the ablation zone more accurately then conventional B-mode ultrasonography.
In the animal experiments, the size of thermal coagulation around six ablated pseudotumors was clearly depicted by USEI and correlated well with the actual size of the ablation upon gross macroscopic evaluation of the harvested liver (r=0.81). In addition, clinical experiments showed a good correlation between the size of the ablation on ultrasound elasticity imaging and the size of ablation on post-operative CT-scanning. Most importantly, USEI images often provided a clearer image of the ablated tissue than conventional B-mode ultrasound images did. While these results are promising, several drawbacks were noted during the study as well. First, obtaining good quality USEI images proved to be somewhat

difficult using free-hand registration, mainly due to "out of plane" movement of the ultrasound probe during free hand palpation. This issue can be addressed in several ways. One way would be to apply a different external stimulus for tissue compression, such as sound waves or movement of the RFA needle[34,35]. Another option would be to use a robotic arm for free hand palpation with the ultrasound probe attached for steadier image acquisition. A third and more sophisticated way to increase image quality would be to electromagnetically track the ultrasound probe and use only those images for elasticity reconstruction that have been acquired in the right plane. This approach has recently been investigated and significantly increased image quality in both experimental as clinical experiments [Pezhman et al, in press]. A limitation of this study is that we were not able to exactly align the elastography images with the gross pathology or CT-images, which had to be done by hand. This has undoubtly introduced some bias and makes it very hard to make any assumptions with regard to the exact way in which USEI depicts the zone of tissue necrosis (eg underestimation or overestimation). This has to be validated first before USEI can be safely employed in a clinical setting. Regardless, ultrasound elasticity imaging is a promising technique for real time ablation monitoring.

Pre-operative Imaging, Body Composition and Outcome

In part three of this thesis, it was investigated how CT-based measurements of body composition can aid the surgeon to identify patients at high risk of post-operative morbidity and mortality and worse long term outcome. In **chapter 4.1**, the impact of a relative depletion of skeletal muscle mass (sarcopenia) on short-term outcomes after liver resection was investigated. The presence of sarcopenia was assessed by measuring the total area of psoas muscle (TPA) on a single CT-scan slice at the level of L4, similar to the method described by Engelsbe et al. After adjusting for patient height, sarcopenia was defined as a TPA of less than 500 mm/m^2 using optimal stratification. This method solves the threshold value of the continuous co-variable at which patients in two categories (such as sarcopenic and non-sarcopenic) are best separated with respect to the main outcome variable (in this case major postoperative complications).

By this definition, 41 (16%) of the 256 patients included in the study were found to be sarcopenic. Sarcopenia was significantly more often observed in women and associated with a lower BMI. More important, sarcopenia was associated with a higher probability of post-operative morbidity (odds ratio (OR) 3.33; P= 0.008), longer hospital stays (6.6 vs. 5.4 days; P= 0.03) and an increased need for longer ICU admissions (>2 days) (15% vs. 4%; P= 0.004) when compared to patients without sarcopenia. In this study, sarcopenia was not associated with worse long term outcome, since recurrence-free and overall survival were not significantly different for patients with sarcopenia and non- sarcopenic patients (p>0.05).

Chapter 4.2 describes the presence and impact of sarcopenia and visceral obesity on long-term survival in a different cohort of patients with CRLM consisting of 196 patients. In this study, the presence of sarcopenia was assessed by measuring the total area of skeletal muscle mass at the level of the fourth lumbar vertebra (cm^2). After normalizing for height, sex-specific cut-off values at which the survival difference between two groups was most pronounced were determined using optimal stratification to find the most significant *P* value by means of log rank statistics. By this method, sarcopenia was defined as a total cross sectional area of muscle mass per meter squared of less than 41.1 cm2/m2 for women and less than 43.75cm2/m2 for men. In this study too, sarcopenia was associated more often in women and was associated with a lower BMI although sarcopenia was also observed in patients with a normal or even elevated BMI. In this study, sarcopenia was associated with significantly worse long term outcome. Five-year disease-free (15 per cent versus 28·5 per cent in patients without sarcopenia; p = 0·002) and overall (20 per cent versus 49·9 per cent respectively; P < 0·001) survival rates were lower for patients with sarcopenia at a median follow-up of 29 (range 1-97) months. Sarcopenia was an independent predictor of worse recurrence-free (hazard ratio (HR) 1·88, 95 per cent confidence interval 1·25 to 2·82; P = 0·002) and overall (HR 2·53, 1·60 to 4·01; P < 0·001) survival. In addition, central obesity (as defined as a total cross sectional area of more than 94cm2/m2) was associated with an increased risk of recurrence in men (p = 0·032), but not in women (p = 0·712).

Based on these studies, measurement of sarcopenia can offer important information to the practicing surgeon. While a poor physical status as a result of cancer cachexia is traditionally assessed by "eyeballing" the patient and relying on parameters such as weight loss and BMI, these data show that significant cancer or age related muscle wasting can be present despite a normal or even elevated BMI. This can now easily be objectified using already present diagnostic CT-scans. Others have confirmed the importance of sarcopenia and its detrimental effect on outcome in patients with malignancies in the gastrointestinal tract[36], pancreatic carcinoma[37], melanoma[38], patients undergoing liver transplantation[39] and aortic repair for aneurysms[40].

The results with regard to long term outcome may seem conflicting between these two studies, although it is very likely that sarcopenia influences both short term outcome as well as long term outcome after oncologic surgery. Sarcopenia can result from aging and inactivity due to multiple comorbidities, but can also be part of cancer-induced cachexia, which has been associated with poor long-term outcomes in patients with a variety of malignancies. Both versions seem to be associated with different pathways and might influence outcome in their own way[41,42]. Sarcopenia as a result of aging and coexisting conditions might alter the ability to withstand major surgical trauma and result in more adverse events and longer hospital stay in sarcopenic patients. Conversely, when sarcopenia is part of cancer cachexia as a result of systemic inflammation, anorexia and increased metabolism as a direct result of malignant disease, long-term outcome might be affected more dramatically.

Differences in long-term outcome between the two studies might be explained by various factors. First, the way in which sarcopenia was assessed was markedly different for both studies; In chapter 4.1 the total psoas area was measured at the level of the fourth lumbar vertebra where as in chapter eleven the total cross sectional area of muscle mass at the level of L3 was used. While these measurements are certainly correlated, they are not interchangeable and one might be more suitable to detect subtle changes in body composition than the other. Second, in chapter 4.1, cut-off values were not determined for each gender separately whereas they were in chapter 4.2. As other studies have shown, body composition is gender specific and its effect on long-term outcome might be different for men and women. Moreover, cut-off values were determined based on a single outcome variable, which was different for both studies (postoperative morbidity vs. long term results). It might very well be that the level at which a depletion of muscle mass dictates outcome is different for long term outcome and short-term outcome. At last, these studies were performed in two different cohorts from two different continents where distributions of body composition might be different. These questions should be addressed in future research to further refine and establish cut-off values for sarcopenia.

Both studies suggest that sarcopenia can be used as a preoperative parameter to identify patients at greatest risk for post-operative morbidity and poor prognosis. For now, these data can be used by the practicing surgeon in several ways. First, the definition of sarcopenia (or cancer cachexia) based on CT images provides a much better estimation of the true extend to which a patients suffers from this phenomenon, since sarcopenia was present even in patients with a normal BMI. Second, identifying the sarcopenic patient might guide the surgeon towards less invasive treatment modalities to reduce the risk of serious adverse events in the postoperative phase.

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5.2

Conclusions

Chapter 2.1: Even with the use of modern cross-sectional preoperative imaging, IOUS detects additional liver metastases in 10% of patients. The sensitivity of IOUS for detecting occult CRLM is highly dependent on the number and echogenicity of detected tumors

Chapter 2.2: The echogenicity of CRLM is significantly affected by the presence of liver steatosis, with decreased echogenicity and increased conspicuity of lesions despite overall poorer image quality. These findings reinforce the usefulness of intraoperative ultrasonography in identifying additional CRLM in patients undergoing surgical therapy, even in those with fatty liver tissue.

Chapter 2.3: Disappearing liver metastases (DLM) are frequently observed in patients undergoing preoperative chemotherapy for CRLM. Half of these can be detected during surgery. Survival is comparable in patients with untreated DLM, in spite of high intrahepatic recurrence rates seen in these patients. Therefore, aggressive surgical therapy should be considered in patients with marked response to chemotherapy, even when all DLM sites cannot be identified

Chapter 3.1: Combined resection and ablation is associated with long-term-survival in a subset of patients; however, recurrence is common. The number of lesions ablated increases risk of intrahepatic recurrence but does not impact overall survival.

Chapter 3.2: Durable local control can be achieved after RFA when tumors are carefully selected. Intraoperative parameters can aid the surgeon in the prediction of local recurrence.

Chapter 3.3: RF ablation can be used effectively in the treatment of HCA. However, multiple sessions are often required, and signs of residual adenoma might persist in some patients despite repetitive treatment. RF ablation might be especially beneficial in cases not amenable to surgery or in patients who would require major hepatic resection otherwise.

Chapter 3.4: Alginate pseudotumors can easily be imaged and allow for different procedures to be performed. This model can be used for various research purposes.

Chapter 3.5: USEI can be a valuable tool for the accurate monitoring and real-time verification of successful thermal ablation of liver tumours

Chapter 4.1: Patients with sarcopenia are at an increased risk of post-operative morbidity and longer hospital stay after resection of CRLM.

Chapter 4.2: Sarcopenia has a negative impact on cancer outcomes following resection of CRLM. Patients with sarcopenia have worse disease-free and overall survival.

5.3

Nederlandse Samenvatting

Summary (in Dutch)

Peroperatieve Echografie

Levertumoren kunnen worden onderscheiden in primaire en secundaire vormen. Het primaire hepatocellulair carcinoom is een van de meest voorkomende vormen van kanker wereldwijd, vooral door de hoge incidentie van virale hepatitis. Behandeling van de levertumor door een lokale resectie of een levertransplantatie kan de overleving zeer positief beïnvloeden, met een 5-jaars overleving van 70%. We spreken van secundaire levertumoren wanneer tumoren die zich elders ontwikkelen uitzaaien uit naar de lever (metastasering), zoals bijvoorbeeld tumoren uit de dikke darm en het rectum (colorectaal carcinoom). Indien deze levermetastasen samen met de tumor in de darm verwijderd kunnen worden door het deel van de lever waar deze metastasen zich bevinden te verwijderen (een leverresectie), is er een reële kans op genezing; ongeveer 40-50% van de patiënten met colorectale levermetastasen is 5 jaar na de operatie nog in leven.

Voor een geslaagde operatie is het van essentieel belang dat alle tumoren of metastasen verwijderd worden door de leverchirurg. Deze worden voor de operatie (preoperatief) aangetoond met behulp van computed tomography (CT-scans) en magnetische resonantie imaging (MRI). In het geval van colorectale levermetastasen is het echter vaak zo dat kleinere metastasen preoperatief niet gezien worden. Het gebruik van directe echografie van de lever tijdens de operatie (peroperatieve echografie) helpt vaak om deze "occulte" metastasen alsnog op te sporen en te verwijderen[1-3]. In een aantal recent verschenen studies is de rol van de peroperatieve echografie echter ter discussie gesteld, omdat preoperatieve onderzoeken zoals CT-scans en MRI scans de laatste jaren steeds beter worden[4]. Daarnaast heeft het toegenomen gebruik van chemotherapie voorafgaand aan leverresecties en het toenemend overgewicht in de westerse populatie de incidentie van leververvetting (steatosis hepatis) fors verhoogd. Dit heeft wellicht een negatieve invloed op de waarde van de peroperatieve echo. Deze vraagstukken werden behandeld in het eerste deel van dit proefschrift.

In **hoofdstuk 2.1** werd gevonden dat peroperatieve echografie bij ongeveer 10% van de patiënten die een leverresectie ondergaan voor colorectale levermetastasen occulte tumoren detecteerde die preoperatief nog niet waren aangetoond, ondanks moderne preoperatieve beeldvorming. Alle 213 patiënten in de studie ondergingen een preoperatieve CT-scan van de lever met intraveneus contrast. De frequentie waarmee occulte metastasen werden aangetoond door peroperatieve echografie was niet verschillend voor patiënten die preoperatief wel of geen MRI of PET scan hadden ondergaan. Vooral bij patiënten met meerdere levermetastasen en patiënten met goed zichtbare hyperechogene of hypoechogene tumoren was de kans om met de echo nog één of meerdere voorheen niet gedetecteerde tumoren te vinden in de lever fors verhoogd. Analoog hieraan was de kans op een vroeg intrahepatisch recidief toegenomen bij patiënten met slecht zichtbare isoechogene tumoren, mogelijk omdat kleine isoechogene metastasen tijdens de operatie nog niet werden gezien. Op basis van deze resultaten is het routinematig gebruik van de echo tijdens leverresecties voor colorectale levermetastasen sterk aanbevolen, zeker bij patiënten met meerdere levermetastasen en patiënten waarbij de bekende metastasen een hypo- of hyperechogeen voorkomen hebben.

Hoofdstuk 2.2 beschrijft de relatie tussen de mate van leververvetting (steatose) en de echogeniciteit van colorectale levermetastasen op een peroperatief echogram. Hiervoor werden peroperatief verkregen echobeelden vergeleken met het microscopische aspect van de metastasen en de omliggende lever na resectie. In deze studie werd gevonden dat vooral de mate van leversteatose van invloed was op de echogeniciteit van colorectale levermetastasen; patiënten met een ernstige vervetting van de lever hadden bijna allemaal (>90%) hypoechogene tumoren terwijl dat niet het geval was bij patiënten met een normale lever (<40%). Daarnaast bleken tumoren die door preoperatieve chemotherapie niet meer zichtbaar waren op de preoperatieve CT- of MRI scans, soms nog wel zichtbaar te zijn op de echo. Dit was vooral het geval als de omliggende lever steatotisch was. Dit is belangrijk om verschillende redenen. Allereerst kan de chirurg op basis van deze kennis tijdens de operatie op zoek gaan naar hypoechogene tumoren indien de lever er op het eerste gezicht steatotisch uitziet. Daarnaast laat deze studie zien dat chemotherapie er weliswaar voor zorgt dat tumoren kleiner worden en daardoor lastiger te detecteren tijdens een operatie, maar dat dezelfde chemotherapie leververvetting kan veroorzaken hetgeen tumoren beter zichtbaar maakt met behulp van een echo tijdens de operatie [5 6-8 9,10]. Deze resultaten werden recent bevestigd door Ferrero et al, die de waarde van peroperatieve echografie onderzocht in patiënten met colorectale levermetastasen die door preoperatieve chemotherapie geheel verdwenen waren op de preoperatieve scans. In deze studie werd gevonden dat de aanwezigheid van leververvetting een gunstige invloed had op de mate waarin men met behulp van de peroperatieve echo in staat was deze verdwenen metastasen weer op te sporen en uiteindelijk te behandelen[11].

Een toenemend aantal patiënten met colorectale levermetastasen krijgt chemotherapie voorafgaand aan een eventuele leverresectie. Indien deze goed aanslaat, gebeurt het soms dat één of meerdere metastasen niet langer zichtbaar zijn op preoperatieve scans. Het is bekend dat deze tumoren –alhoewel niet langer zichtbaar- zeker niet altijd verdwenen zijn en dat de kans op nieuwe uitgroei aanzienlijk is indien deze niet behandeld worden. Hoe peroperatieve echografie kan helpen bij het opsporen van deze "verdwijnende" metastasen werd beschreven in **hoofdstuk 2.3.** Tevens werd onderzocht in hoeveel procent van de verdwenen tumoren ook daadwerkelijk geen levende kankercellen meer aanwezig zijn. Al laatste werd uitgezocht of er in geselecteerde patiënten kan worden overwogen om deze "verdwenen" tumoren niet te behandelen.

Het bleek dat chemotherapie resulteerde in het verdwijnen van een of meer metastasen bij bijna een kwart van de patiënten. Dit werd met name gezien bij patiënten met kleine metastasen en patiënten die meerdere cycli chemotherapie hadden ontvangen. In 45% van de patiënten met verdwenen metastasen was de chirurg tijdens de operatie in staat om deze tumoren alsnog te vinden met behulp van de peroperatieve echo en het nauwkeurig inspecteren van de lever. Dit is meer dan voorgaande studies hebben laten zien, en suggereert een essentiële rol voor de peroperatieve echografie bij patiënten die preoperatief chemotherapie hebben ontvangen.[5,12,13].

Daarna werd onderzocht hoeveel procent van deze verdwenen metastasen daadwerkelijk geen levende kanker cellen meer bevatten. Indien preoperatief verdwenen tumoren tijdens de operatie alsnog teruggevonden werden, bleek er bij microscopisch onderzoek in ongeveer 60% van de tumoren nog sprake van levensvatbare kankercellen. Indien verdwenen tumoren tijdens de operatie niet teruggevonden werden, gebeurde het soms dat deze niet verwijderd werden. Tijdens follow-up kwamen deze in 46% van de gevallen terug. Tot slotte werd de overleving van patiënten waarbij alle bekende tumoren werden verwijderd tijdens de operatie vergeleken met patiënten waarbij een of meerdere verdwenen tumoren niet meer gevonden werden tijdens de operatie. Alhoewel patiënten in deze laatste categorie vaker een recidief in de lever ontwikkelden dan patiënten in de andere groep, kon dit in bijna alle gevallen opnieuw behandeld worden en was de lange termijn overleving niet verschillend voor beide groepen. Op basis van deze studie kunnen een aantal aanbevelingen worden gedaan. Allereerst is het belangrijk om terughoudend te zijn met chemotherapie voorafgaand aan een leverresectie als in opzet curatieve resectie mogelijk is; kleine tumoren zijn soms niet langer zichtbaar na meerdere cycli van chemotherapie en worden niet altijd teruggevonden tijdens de operatie. Wanneer onbehandeld, geven deze een verhoogde kans op het ontwikkelen van een recidief. Indien het echter zo is dat het achterlaten van een verdwenen metastase de enige mogelijkheid is om een "curatieve" leverresectie uit te voeren, kan dit overwogen worden indien deze metastase in een tweede instantie (bij recidief) alsnog behandeld kan worden.

Locaal ablatieve therapie en beeldvorming

Helaas komt slechts een klein deel (ongeveer 20%) van de patiënten met een maligne levertumor in aanmerking voor een leverresectie. Bij veel patiënten is de ziekte al in een te ver gevorderd stadium of laat de algehele conditie van de patiënt een grote operatie niet toe. Wanneer tumoren beperkt zijn tot de lever kan het gebruik van lokaal ablatieve therapieën uitkomst bieden. Dit kan als op zichzelf staande therapie worden toegepast, maar kan ook worden gecombineerd met een leverresectie. Op dit moment is radiofrequente ablatie (RFA) de meest toegepaste vorm van locaal ablatieve therapie voor lever tumoren. Hierbij wordt de punt van een lange naald in de levertumor ingebracht welke door gebruik van een hoogfrequente alternerende stroom zorgt voor lokale weefselverhitting, hetgeen uiteindelijk resulteert in coagulatie en necrose van het omliggende weefsel (figuur 2). Dit kan zowel tijdens een operatie worden gedaan (open of laparoscopische RFA) als via een klein gaatje in de huid (percutane RFA). Hoewel RFA een veel toegepaste behandeling voor levertumoren is, blijkt de behandeling in veel gevallen onvolledig te zijn en is de recidiefkans na RFA hoger dan na een leverresectie. Dit komt onder andere door een hoge moeilijkheidsgraad van het positioneren van de naald en het gebrek aan methoden om het effect van de RFA behandeling goed te monitoren op de operatiekamer. Deel twee van dit proefschrift onderzoekt de toepassing van RFA voor verschillende soorten levertumoren en focust in het bijzonder op de manier waarop peroperatieve beeldvorming kan worden gebruikt en ontwikkeld om de recidiefkans te verlagen. **Hoofdstuk 3.1** beschrijft de het gebruik van RFA gecombineerd met een leverresectie bij 125 patiënten met meerdere colorectale levermetastasen, welke zonder het gebruik van RFA niet in aanmerking waren gekomen voor een curatieve behandeling. Hoewel de recidiefkans en kans om te overlijden als gevolg van de ziekte relatief hoog waren wanneer deze groep patiënten werd vergeleken met patiënten die met alleen een leverresectie werden behandeld (mediane overleving 35 vs 51 maanden), was de prognose van deze patiënten nog altijd stukken beter dan die van patiënten die geen behandeling ondergingen (20 maanden; $p<0.001$). Daarnaast bleek RFA gecombineerd met een leverresectie een relatief veilige ingreep, met een postoperatieve mortaliteit van 1.6%. Deze resultaten bevestigen wat in andere studies ook gevonden werd. In een studie van Abdallah et al. hadden patiënten een mediane overleving van 30 maanden na een gecombineerde behandeling met RFA en leverresectie. Gleisner et al. beschreven soortgelijke resultaten[14]. In beide studies hadden patiënten die met een leverresectie en RFA werden behandeld een slechtere prognose dan patiënten die met alleen een leverresectie werden behandeld. Wanneer deze twee patiëntengroepen echter worden vergeleken, blijkt de resectie en RFA groep al een slechtere baseline prognose te hebben; Patiënten die met RFA en resectie behandeld werden hadden vaker synchrone metastasen en hadden meer tumoren dan patiënten die met alleen een resectie behandeld konden worden. Het is echter niet onwaarschijnlijk dat het gebruik van RFA *an sich* al geresulteerd heeft in een hogere recidiefkans en slechtere overleving; hoe meer tumoren met RFA behandeld werden in deze studie, hoe groter de kans op het ontwikkelen van een recidief. RFA is kan daarom succesvol ingezet worden bij de behandeling van patiënten met meerdere bilaterale levermetastasen, maar dient voorbehoudend te worden voor de behandeling van patiënten waarbij een leverresectie alleen geen uitkomst biedt.

In **hoofdstuk 3.2** werden factoren bijdragend aan de recidiefkans na RFA van colorectale levermetastasen nader onderzocht. Hierbij werden zowel tumor gerelateerde factoren (diameter, locatie in de lever) als intraoperatieve RFA parameters (doeltemperatuur, echografisch beeld) geëvalueerd. Zoals verwacht hadden tumoren met een grotere diameter en tumoren dicht bij grote bloedvaten in de lever een verhoogde kans op een lokaal recidief na RFA behandeling[15,16]. Tevens bleken intraoperatieve parameters, zoals het onvermogen om target temperatures te bereiken tijdens RFA en de afwezigheid van een goede "encompassment" op de peroperatieve echografie zeer voorspellend te zijn voor het ontwikkelen van een lokaal recidief. Daarnaast werd de waarde van het oordeel van de behandelend chirurg over de effectiviteit van de RFA behandeling onderzocht, welke werd gebaseerd op verschillende procedure gerelateerde factoren (zichtbaarheid en echogeniciteit van de tumor, positionering van de naald, vorming van microbubbels rond de tumor tijdens RFA, doeltemperaturen). Dit oordeel werd uitgedrukt als een zogenaamde A-status (A0; geslaagde RFA procedure, A1; suboptimale RFA procedure, A2; waarschijnlijk onvolledige RFA procedure) en bleek zeer voorspellend voor het optreden van een lokaal recidief bij een A1 of A2 ablatie. Een A0 ablatie resulteerde in in een fors aantal van de tumoren nog steeds in een lokaal recidief. Anderzijds, wanneer de intraoperatieve A-status werd gecombineerd met tumor diameter en locatie, kon langdurig succes in 91% van de tumoren worden voorspeld.

Deze resultaten zijn bruikbaar voor de clinicus op verschillende manieren. Wanneer intraoperatieve parameters een onvolledige RFA behandeling suggereren, kan de behandeling van de patiënt worden aangepast, bijvoorbeeld door het plannen van een tweede operatieve behandeling of het toedienen van postoperatieve chemotherapie. Daarnaast onderstrepen deze resultaten het belang van zorgvuldige tumor selectie voor RFA behandeling. Tumoren groter dan 3 centimeter of dichtbij grote bloedvaten hebben een hoge recidiefkans en dienen bij voorkeur niet met RFA behandeld te worden. Als laatste illustreert deze studie het belang van de ontwikkeling van betere technieken om de RFA naald te positioneren en de behandeling te monitoren[17-20].

In **hoofdstuk 3.3** werden de veiligheid en effectiviteit van RFA om hepatocellulaire adenomen (HCA's) te behandelen geëvalueerd. Hoewel dit in essentie goedaardige levertumoren zijn, kunnen deze tumoren ruptureren en maligne ontaarden[21]. Om deze reden wordt er regelmatig besloten tot het behandelen van deze tumoren middels het uitvoeren van een leverresectie [22-24]. Aangezien leveradenomen vooral voorkomen bij jonge vrouwen en vaak verspreid zijn door de lever, zijn alternatieve behandelwijzen nodig om deze patiëntencategorie een grote, potentieel gevaarlijke leverresectie te besparen. RFA zou hier uitkomst kunnen bieden. In deze studie werd RFA toegepast bij 18 vrouwelijke patiënten met een of meerdere leveradenomen. In totaal werden 45 HCA's behandeld in 32 RFA sessies. Bij de meeste patiënten waren meerdere sessies noodzakelijk om voldoende behandelresultaat te verkrijgen en in 58% van de adenomen leek er na een eerste sessie nog sprake te zijn van resterend adenoomweefsel op de follow-up scans, waardoor vele patiënten een tweede of soms derde RFA sessie moesten ondergaan. Hoewel de klinische relevantie van een kleine hoeveelheid restadenoom waarschijnlijk laag is, onderstrepen deze resultaten de noodzaak om betere beeldgeleiding te hebben tijdens RFA; in alle gevallen lieten de directe controle CT-scans of echografie een complete behandeling van het HCA zien, terwijl follow-up scans in veel gevallen nog vitaal HCA weefsel lieten zien. Het is daarom aan te raden om RFA alleen te gebruiken bij de behandeling van HCA's indien een conservatieve behandeling niet gepast is en een chirurgische resectie van de tumoren niet mogelijk is op basis van de ligging of verspreiding door de lever. Daarnaast dient toekomstig onderzoek zich te focussen op andere minimaal invasieve behandelingsmodaliteiten (zoals transarteriële embolisatie) en het verscherpen van de indicaties voor de behandeling van HCA's.

Hoofdstuk 3.4 beschrijft de ontwikkeling van een nieuw tumormodel in grote proefdieren om preklinisch onderzoek naar lokaal ablatieve therapieën te faciliteren. In deze studie werden 24 pseudotumoren gecreëerd in de lever van 8 levende varkens en 2 honden kadavers. De tumoren waren eenvoudig te implanteren en konden zonder problemen enkele dagen in situ gehouden worden in de levers van levende varkens zonder hun viscoelastische en radiologische kenmerken te verliezen. De gecreëerde tumoren waren eenvoudig te visualiseren met behulp van echografie, CT-scans en MRI scans en konden ook bij macroscopisch onderzoek van de post-mortem verwijderde levers eenvoudig worden geïdentificeerd. Daarnaast waren deze tumoren gelijk aan echte levertumoren met betrekking tot hun gedrag bij RFA behandeling, waardoor deze tumoren zeer geschikt zijn voor onderzoek naar beeldvorming tijdens RFA behandelingen. Dit model heeft als grote voordeel boven eerder beschreven tumormodellen op basis van Agarosegel dat ze eenvoudig te implanteren en goedkoop is en niet haar viscoelastische eigenschappen verliest tijdens RFA behandeling[25].

In **hoofdstuk 3.5** werd dit pseudotumor model gebruikt om een nieuwe methode voor het monitoren van RFA behandeling te testen. In deze studie werd onderzocht of het mogelijk is om de relatieve elasticiteit (of stevigheid) van weefsel te visualiseren met behulp van ultrasound elasticity imaging (USEI). Omdat geablateerd weefsel minder elastisch is dan niet geablateerd weefsel (rauw vs gebakken), kan er op deze manier onderscheid gemaakt worden tussen behandeld en onbehandelde tumor. USEI kan deze verschillen in beeld brengen door beelden van gecomprimeerd weefsel te vergelijken met beelden van niet gecomprimeerd weefsel; indien de pixels bij compressie veel verschuiving laten zien, is de elasticiteit van het weefsel verhoogd (figuur 3). Indien de pixels bij compressie weinig verschuiving laten zien, is de elasticiteit van het weefsel laag. Door het weefsel met de ultrasound probe te comprimeren ("palperen"), en de gecomprimeerde en niet gecomprimeerde beelden door een computer met elkaar te vergelijken, kan een zogenaamde elasticity map worden uitgerekend welke op zijn beurt weer kan worden omgezet in een klinisch interpretabel beeld.

In de huidige studie bleek uit zowel dierexperimenteel onderzoek als uit klinische experimenten dat USEI in staat was om de verschillen tussen met RFA behandeld weefsel en onbehandeld weefsel goed te visualiseren. In de dierexperimenten werd de zone van gecoaguleerd weefsel rondom zes geablateerde pseudotumoren duidelijk in beeld gebracht door USEI en de afmetingen van de zone van verhoogde elasticiteit op de USEI correleerde goed met de zone van weefselnecrose tijdens macroscopisch onderzoek van de lever. Deze resultaten werden bevestigd in acht klinische experimenten, waar de afmeting van de geablateerde zone op de elasticity images werd vergeleken met de afmeting van de ablatie zone op CT beelden. Hoewel deze resultaten toekomstig onderzoek naar het gebruik van USEI ondersteunen, kan er op basis van deze resultaten nog niet gezegd worden of USEI beter presteert dan conventionele beeldvorming. Tevens zal bevestigd moeten worden dat de zone van verhoogde elasticiteit op USEI ook daadwerkelijk correleert met de zone van adequaat gecoaguleerd weefsel. Daarnaast dient toekomstig onderzoek zich te focussen op het verbeteren van de "freehand palpation" techniek.

Preoperative Beeldvorming, Spiermassa en Uitkomsten na Leverchirurgie.

In deel drie van dit proefschrift werd onderzocht hoe preoperatieve beeldvorming kan worden gebruikt om patiënten te selecteren met een hoog risico op postoperatieve complicaties en slechte lange termijn uitkomsten. Verschillende auteurs hebben beschreven dat het verlies van spiermassa (sarcopenie) een ongunstig effect heeft op korte en lange termijnuitkomsten in kankerpatienten[26-29]. Sarcopenie kan zowel een gevolg zijn van ouderdom en comorbiditeit, maar ook onderdeel zijn van het kanker cachexie syndroom. Cachexie wordt gekenmerkt door significant gewichtsverlies bij kanker patiënten waarbij met name spierverval een belangrijke rol speelt en is geassocieerd met een slechte prognose. Een hoge of normale BMI sluit aanzienlijk spierverval echter niet uit en kan significant spierverval maskeren. Het gebruik van abdominale CT-scans om de hoeveelheid spiermassa van een patient te meten kan helpen om patiënten at risk te selecteren. In **hoofdstuk 4.1** wordt de invloed van een relatieve depletie van spiermassa (sarcopenie) op de korte termijn uitkomsten na leverresecties voor colorectale levermetastasen in een cohort van Amerikaanse patiënten onderzocht. Sarcopenie werd geobjectiveerd door het meten van de totale oppervlakte van de psoas spier op abdominale CT-scans op het niveau van de 4e rugwervel, zoals eerder werd beschreven door Engelsbe et al[29]. Zestien procent van de patiënten bleek sarcopeen te zijn en sarcopenie was geassocieerd met een verhoogde kans op postoperatieve complicaties (OR 3.33; p=0.008), langere ziekenhuisopnames (6.6 vs. 5.4 dagen; p=0.03) en langer verblijfsduur op de intensive care (>2 dagen)(15% vs 4%; p=0.004) wanneer vergeleken met patiënten zonder sarcopenie. In deze studie werd er geen correlatie aangetoond tussen lange termijn uitkomsten en sarcopenie.

Hoofdstuk 4.2 beschrijft de invloed van sarcopenie op de lange termijnuitkomsten na resectie van colorectale levermetastasen in een Nederlandse populatie. In deze studie werden abdominale CT-scans gebruikt om sarcopene patiënten te identificeren, alhoewel in deze studie de totale oppervlakte van skeletspier op het niveau van de 3e ruggenwervel werd gemeten, zoals beschreven door Prado et al. In deze studie werd sarcopenie gedefinieerd als een totale oppervlakte van skeletspier van minder dan 41.1 cm2/m2 voor vrouwen en minder dan 43.75cm2/m2 voor mannelijke patiënten. Sarcopenie was geassocieerd met een significant slechtere vijf jaars recidief vrije (15% vs 28.5% resp; p<0.001) en vijf jaars totale overleving (20 vs 49.9% resp; p<0.001) na resectie van colorectale levermetastasen wanneer vergeleken met niet sarcopene patiënten. Daarnaast leken mannelijke patiënten met een toegenomen intra-abdominale vetmassa een slechtere recidiefvrije overleving te hebben dan mannen met een normale abdominale vetmassa.

Op basis van deze studies heeft de aanwezigheid van sarcopenie belangrijke implicaties voor de behandeling van patiënten met colorectale levermetastasen. Hoewel een slechte fysieke status momenteel vaak herkend wordt door bevindingen tijdens de anamnese en het lichamelijk onderzoek (co-morbiditeit, gewichtsverlies, lage BMI), laten deze studies zien dat een aanzienlijk spierverval aanwezig kan zijn in patiënten met een ogenschijnlijk normale of zelfs hoge BMI. Het gebruik van abdominale CT scans, die vaak al gemaakt zijn voor diagnostische doeleinden, kan helpen om deze patiënten te identificeren. Indien ernstig spierverval wordt gediagnosticeerd, kan overwogen worden om een minder ingrijpende behandeling aan de patiënt aan te bieden, zoals RFA in plaats van een grote lever resectie om aanzienlijke morbiditeit te voorkomen. Alvorens sarcopenie echter een vaste plaats in behandelalgoritmes kan verkrijgen, dienen deze resultaten gevalideerd te worden in andere populaties.

Hoewel de resultaten met betrekking tot de invloed van sarcopenie op de lange termijnuitkomsten na lever resectie voor colorectale levermetastasen met elkaar conflicteren, is het zeer waarschijnlijk dat sarcopenie zowel de korte als de lange termijn uitkomsten na oncologische operaties negatief beïnvloedt. Sarcopenie als gevolg van ouderdom en inactiviteit bij multipele comorbiditeiten resulteert waarschijnlijk in een verminderde tolerantie voor chirurgische stress en moet gezien worden in het kader van een algeheel verminderde fysieke status van de patiënt. Deze vorm van sarcopenie heeft waarschijnlijk vooral een negatieve uitkomst op de korte termijn resultaten na lever resecties. Indien sarcopenie echter onderdeel is van het cachectisch syndroom in voorheen relatief jonge en gezonde kankerpatienten, zal met name de lange termijnuitkomst beïnvloed worden. Op basis van de huidige metingen kan echter geen onderscheid gemaakt worden tussen beide vormen van sarcopenie en het is goed mogelijk dat beide vormen tevens in gecombineerde vorm kunnen bestaan. Toekomstig onderzoek zal deze hypothesen daarom moeten bevestigen. Discrepanties tussen de huidige twee studies kunnen waarschijnlijk worden verklaard door verschillen in methodologie, waarbij de cut-off points voor sarcopenie werden gekozen op basis van de primaire uitkomstmaat en de twee populaties mogelijk zeer verschillend waren met betrekking tot lichaamssamenstelling.

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5.4

Future Directions

Pre-operative planning and fusion imaging in liver Surgery

Novel software applications can aid the surgeon in the pre-operative planning phase by providing a more detailed 3D view of the hepatic vascular anatomy and the positions of the tumor herein through (figure 5.4.1)[1,2]. Moreover, parenchymal areas and their corresponding feeding and draining vessels can be calculated and dissection planes can be suggested, allowing for more precise dissection based on functional areas rather than anatomical segments. Several manufacturers have attempted yet to incorporate these segmented images in the operating theatre by registering them to the patient by liver scanning, point based registration or CT-Ultrasound based registration [2-4](figure 5.4.2). The major problem to overcome here is the rigid nature of the CT-images compared to the highly deformable and moving liver, resulting in varying registration errors and thus inaccuracy of the images. Regardless, these techniques offer promising perspectives for complex liver resections (extended left hepatectomy or central resections), patients with disappearing metastases and more parenchyma sparing approaches.

Navigation and Needle Steering in Liver Surgery

While freehand navigation of a needle towards a target in the liver can yield good results with regard to biopsy, ablation or implantation of brachytherapy seeds, it requires many years of training, excellent spatial skills to convert single planar 2D

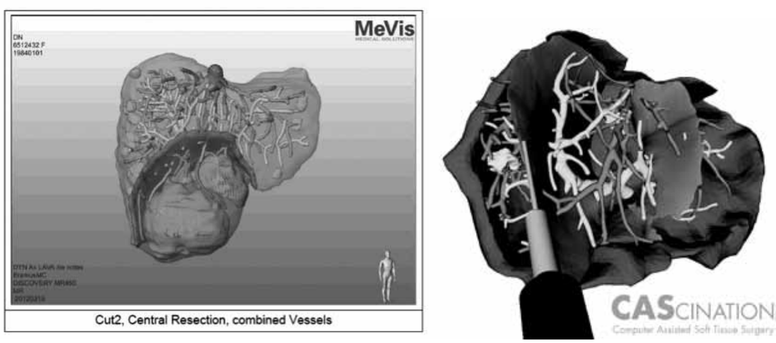

FIGURE 5.4.1 Examples of pre-operative planning before liver resection using 3-D reconstruction (left) and intra-operative registration of segmented CT-images and surgical instruments (right).

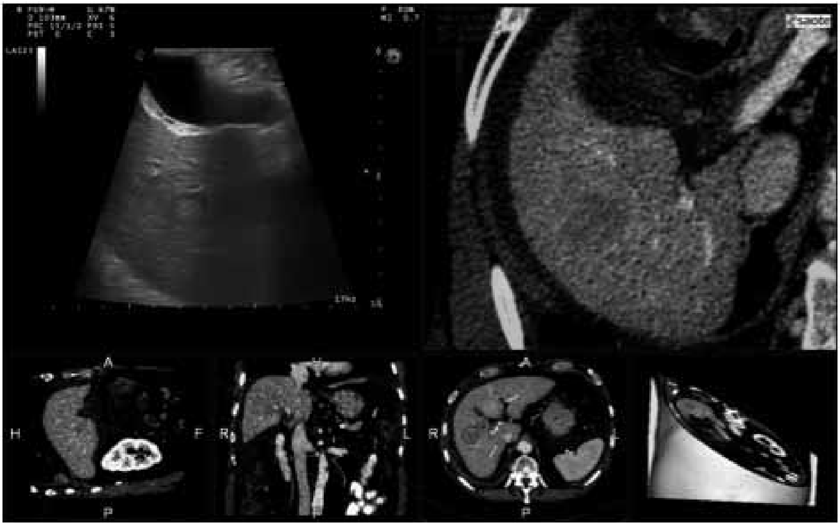

FIGURE 5.4.2 Registration of pre-operative CT-images and intraoperative ultrasound.

ultrasound or CT information to a 3D situation and is therefore prone to human error. Several have attempted to aid the surgeon or interventionalist in this task by incorporating 3D navigation in the workup. For instance, a 3D representation of the needle and its target on ultrasound can greatly enhance the ease of free-hand needle navigation and has been incorporated several commercially (yet unestablished) available devices to be used for the guidance of thermal ablation needles (esaote, cascination)[5,6]. A group from Germany suggested the use of stereotaxy for ablation of large tumors resulting in impressive results with regard to local recurrence[7]. In addition, robot assisted needle navigation might further decrease the human error in lesion targeting by either steering a straight needle towards a target based on 3D ultrasound [8] or using robotic steerable needles to reach a target that would be difficult to reach otherwise (Majevicz et al, in press).

Novel Devices for Tissue Ablation

While radiofrequency ablation is perhaps the most established method for thermal ablation of liver tumors, several newer methods (in varying stages of development) are knocking on the door to replace this method. First, microwave ablation (MWA) has been shown to be able to create larger ablations in less time when compared to RFA and is supposed to suffer from the heat sink effect caused by close vasculature to a lesser extent. While several studies have proven MWA to be safe and efficacious, no data is available yet comparing MWA to RFA[9,10]. Another and much newer method to locally destroy tissue in a minimal invasive way is called *irreversible* electroporation. This technique was derived from laboratory work where *reversible* electroporation is used to temporarily increase the permeabity of the cell membrane of an individual cell or group of cells by exposing these cells to a rapid series of high voltage shocks. This then allows for certain large molecules to enter the cell (for instance chemotherapy, DNA)[11]. In irreversible electroporation the same thing is done, however for a much longer time period (seconds), causing an irreversible permeability of the cell membrane resulting in apoptosis through a cascade of cellular mechanisms[12]. The benefit of this method over RF ablation is that is causes no heat and leaves other tissues (eg vascular or biliary) undamaged. It could therefore be very suitable for the treatment of tumors located centrally in the liver, too close to the central bile ducts[13]. Otherwise, IRE has been used to treat unresectable adenocarcinomas of the pancreas with surprisingly good results[14].

Better Monitoring of Thermal Ablation

As shown in this thesis, current methods for real time monitoring of open RF ablation are not able to discern a good from a bad ablation, especially in larger ablations. Multiple papers have shown that MRI based assessment of the zone of tissue destruction is the most accurate, but this is unavailable in most operating theatres. The same applies to CT-based monitoring. Thus, with regard to the monitoring of open RF ablation, ultrasound is likely to remain the cornerstone in the real time assessment of the treatment effects of RFA. While conventional B-mode ultrasound might not be the best method for this, ultrasound elasticity imaging could greatly enhance the potential of ultrasound for this task. We have already shown that USEI is able to pick up the zone of ablated tissue, but several refinements can still be made. First, due to out of plane motion during free-hand palpation, the signal to noise ratio can be low. This can be further improved by electromagnetic tracking of the device, which allows for automatic correction of this out of plane motion and generates better images (Pezhman et al, in press).

Diagnosis and Treatment of Sarcopenia in Cancer Patients

While many have described the importance of sarcopenia with regard to short and long term outcomes in cancer patients, as many methods are available to measure sarcopenia. While CT-scanning might be sensitive to detect a depletion of muscle mass and might be cost-effective since many patients will have diagnostic CT-scans available, different methods have been described to assess for the amount of muscle mass (psoas muscle density, total cross sectional area of muscle mass on different levels, volumetry of muscle mass in certain area). Future research should focus on comparing these measurements as well as defining an international standard.

In addition, while the knowledge of the detrimental value of sarcopenia is valuable, it is hardly incorporated in clinical practice at this moment. Identifying those patients with sarcopenia and adjusting surgical or medical treatment according to these findings, as well as offering these patients an intervention aimed at stopping or even reversing the process of muscle wasting could potentially improve the way we care for these patients. Before this is possible, underlying mechanisms leading to sarcopenia will have to be identified. While decreased food intake due to alimentary tract dysfunction or obstruction and an altered metabolic state might be partially responsible for the process of progressive muscle wasting, the most important factor contributing to the development of sarcopenia is the so-called systemic inflammatory response mediated by cytokines such as TNF and IL-1. Further knowledge, derived from clinical observational studies and experimental animal work might shed some more light on the way malignant tumors interact with their host and result in muscle wasting and ultimately death [15].

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6

Appendices

List of publications

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List of Publications

Journal Articles

1. Foroughi P, Carnegie DA, **van Vledder MG**, Kang HJ, Choti MA, Hager GD, Boctor EM. *A Freehand Ultrasound Elastography System with Tracking for In-vivo Applications*. Ultrasound Med Biol. 2012 (in press)
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1. Rivaz H., **van Vledder MG**, Choti MA, Hager G, Boctor E. “Liver ablation guidance: discriminating ablation tumor from the cancer tumor with ultrasound elastography”, SPIE Med. Imag., 2010 7629131-7629138

Curriculum Vitae

Mark van Vledder werd op 6 januari 1984 geboren in Dordrecht. In 2002 behaalde hij zijn VWO diploma op het Johan de Witt Gymnasium in Dordrecht. Hierna studeerde hij geneeskunde aan de Erasmus Universiteit in Rotterdam. In 2008 ging Mark voor zijn afstudeeronderzoek naar het Johns Hopkins Hospital in Baltimore (VS), waar hij onder leiding van Michael A Choti zijn onderzoek naar peroperatieve beeldvorming bij leverchirugie opzette. In 2009 behaalde hij zijn artsexamen *cum laude* en ging hij terug naar Baltimore waar hij gedurende een jaar als research fellow werkzaam was en verder werkte aan het eerder gestarte onderzoek. In oktober 2010 begon Mark als ANIOS in het Erasmus MC te Rotterdam (Opleider; Prof. Dr. JNM IJzermans) en later in het IJsselland ziekenhuis te Capelle a/d IJssel (Dr. I Dawson). Op 1 juli 2011 is Mark met zijn opleiding tot algemeen chirurg begonnen in het Erasmus MC welke hij vanaf 2013 zal voortzetten in het IJselland ziekenhuis.

Dankwoord

En dan nu het meest gelezen deel van het proefschrift! Graag zou ik de volgende mensen willen bedanken voor hun hulp en interesse bij het tot stand komen van dit boekwerk; jullie vakinhoudelijke of juist totaal niet vakinhoudelijke ondersteuning was onmisbaar!

Dear Prof.dr. Choti, there is no way even beginning to express how grateful I am for the opportunities you have given me by inviting me to be your research fellow. Your enthusiasm for novelties and the combination of clinical work and research have been truly inspiring for me as a young researcher. While our weekly Saturday morning meetings have become less frequent Sunday afternoon Skype sessions, I look forward to all our future conversations. I hope you get to enjoy the Dutch in their own country for a couple of days this year; it is a pleasure and an honor to have you here. Dear Mike, thank you for everything!

Geachte Prof. Dr. IJzermans, beste Jan, vijf jaar na onze eerste ontmoeting is daar dan eindelijk het boek! Onder uw hoede werd wat begon als student-onderzoeker in Rotterdam uiteindelijk een promotietraject in Baltimore en een opleidingsplaats om chirurg te worden in Rotterdam. Uw nieuwsgierigheid en brede interessegebied hebben altijd zeer aanstekelijk gewerkt en mede bijgedragen aan mijn vorming als arts-onderzoeker. Bedankt voor de vrijheid om mijn onderzoek naar eigen inzicht in te delen en de kritische noot op de momenten dat daar behoefte aan was, ik kijk uit naar onze toekomstige werkzaamheden!

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Dear Tim Pawlik, Michael Torbenson, Peter Peng, Luis Fernando Moreira, Sanjay Munireddy, Emad Boctor, Philipp Stolka, Ann Maijevicz and Pezhman Foroughi. Whether it was to review pathology slides, harvest and fry a liver, get my statistics and tables right for a presentation or to experiment with electronically engineered contraptions I will probably never understand, my time in Baltimore would not have been nearly as productive and fun as it would have been without you! Thanks for all the good times in the lab, the office, but also for the laughs and drinks outside the hospital! I hope we will all meet again sometime and I wish you good luck with your careers!

Shoshanna, Margie, Maria and Michelle, thanks for all your help with everything, even though the pile of paperwork on your own desks was sometimes higher than the dome of the hospital itself. You always made an effort to make my time as enjoyable as possible. Carola, Anneke en Conny, ook jullie mogen hier niet ontbreken. Dank voor alle afspraken tussendoor, printjes, handtekeningen en mental support! Jullie zijn toppers!

Assistenten, chirurgen en onderzoekers uit het Erasmus MC en IJselland ziekenhuis. Dat ik een groot deel van mijn vrije tijd met jullie op de racefiets, in de kroeg of op het terras wil doorbrengen naast een toch al lange werkweek met elkaar zegt al genoeg; ik zou me geen betere mensen om me heen kunnen wensen, jullie zijn super!!

Mechteld, wat een toptijd hadden wij in Baltimore! Met veel plezier denk ik terug aan de Natti Boh's, de Pizza's en mooie congresbezoeken. Je boek is af en je bent in opleiding, het ga je goed daar in het Maastrichtse!

Guus! Bro! Wat een mooi toeval dat wij elkaar op de stoep van the Stafford tegen het lijf liepen, het bleek het begin van een inmiddels al 2,5 jaar durende transatlantische vriendschap te zijn. Burger night, "werkoverleg" in de Daily Grind, slechte films en af een toe een stukje klimmen, het was legendarisch. Vanaf nu plannen we ons volgende biertje op Nederlandse bodem!

Laura! Wat goed dat we nog steeds af en toe in de kroeg kunnen hangen! Dank voor je eeuwige belangstelling voor mijn bezigheden, ook al zou je er zelf niet aan moeten denken. Veel geluk met je werk als huisarts en je gezin en wie weet worden we wel buren…!

Puck, Marten, Pieter, Rolien, Simon, huisgenoten van de Graaf Florisstraat en aanverwanten! De doos met goede en slechte verhalen uit mijn eerste thuis in Rotterdam zal wel nooit leeg raken, wat alleen maar aangeeft hoe onwijs hard we daar gelachen hebben! Inmiddels zijn we (bijna) allemaal wat ruimer behuisd en is er hier en daar al sprake van gezinsuitbreiding, maar wat mij betreft doen we er de komende jaren nog een heleboel verhalen bij!

Maud, het spannende van een avontuur is dat je nooit weet waar het eindigt, en met jou naar Amerika verhuizen was misschien wel ons grootste avontuur ooit. Hoewel de eindbestemming anders was dan verwacht, hoop ik nooit een dag van onze fantastische tijd te vergeten! Ik wens je al het geluk in de wereld!

Neven en nichten! Wat gezellig dat we nog steeds met elkaar kunnen lachen! Na twee bruiloften mogen jullie nu een avondje op mijn kosten feesten!

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Lieve Mieke, wat geweldig dat je mijn paranimf wilt zijn, zelfs na 38 weken zwangerschap! Je bent de beste huisgenote, studiegenote en vriendin geweest die ik me maar wensen kan! Ik heb jouw filosofisch-holistische kijk op het leven en ons werk altijd zeer kunnen waarderen (hoewel ik er soms iets anders in sta…). Dat ik nog maar veel van je mag leren!

Gerard, mijn beste! Dat jij hier vandaag achter me staat maakt deze dag per definitie tot een succes. Je bent een inspirerend mens en een geweldige vriend. Er is geen beginnen aan om al onze mooie (en minder mooie) ervaringen van de afgelopen jaren te benoemen, maar een ding is zeker: Ik had ze met niemand anders willen beleven dan met jou! Chapeau ouwe brombeer!

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PhD Portfolio

PhD Student:	Mark G. van Vledder
Erasmus MC Department:	Surgery
PhD period:	September 2009 – May 2012
Research groups:	- Johns Hopkins Hospital, Department of Surgery, Liver Clinical Research Center - Johns Hopkins University, School of Engineering, Medical Ultrasound Imaging and Intervention Collaboration - Erasmus Medical Center, Department of Surgery, Division of Hepatobiliary and Transplant Surgery
Supervisors:	• Prof.dr. J.N.M. IJzermans, Erasmus University Rotterdam • Prof.dr. M.A. Choti, Johns Hopkins University

PhD Training	Year	Workload (ECTS)
General courses		
Ultrasonography of the Liver	2009	1
Biostatistics in Public Health	2010-2011	11
Workshops		
Effective Abstract Writing	2009	0.1
Pubmed Searching	2009	0.1
Computer Assisted Liver Surgery	2012	0.5
Presentations		
National Conferences	2009	1
International Conferences	2009	2
International Conferences	2010	9
National Conferences	2012	3
Teaching		
Lecturing		
Lecturing (ICU nurses)	2012	1
Tutoring		
First Aid Examinations	2012	0.2
Supervising		
Supervising Masters Thesis	2011	5