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Medical Imaging and Interventions
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# Prediction of local tumor progression after radiofrequency ablation of hepatocellular carcinoma: retrospective registration of pre- and post-ablation imaging

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# Preface

In this master thesis, I present the work that I have done in the past 9 months, during my graduation internship at the department of radiology in the Leiden University Medical Center (LUMC). Here, I have worked within the sections of interventional radiology and nuclear medicine. The final product of the research that I have conducted is split into two articles. The first article is about the use of pre- and post-interventional imaging for the prediction of local tumor progression after radiofrequency ablation (RFA) in liver cancer. This article is currently under revision of the co-authors. The second article has been published last May in *'Nederlands Tijdschrift voor Oncologie'* (The Dutch Journal of Oncology). The English summary is included in this thesis, and the entire article in Dutch is added as appendix. In this article, I write about the clinical study that we perform in the LUMC, in which radioembolization is used as adjuvant treatment to RFA in early stage hepatocellular carcinoma (HCC) patients. The thesis starts with a general introduction in which the different concepts are introduced that form the foundation of the research. In a final general discussion, I review the work in the perspective of general trends within the field of research to treatment of unresectable HCC. The last pages of this document consist of the 'verantwoording', in which I write about my side projects, and reflect on my learning experience of my graduation internship. I hope this thesis will be as inspiring to you as the conduct of this research has been to me.

~ Pim Hendriks

# Table of Contents

# Introduction

# Introduction

Liver cancer is the second leading cancer-related cause of death worldwide, with 746.000 deaths in 2012 (1). Hepatocellular carcinoma (HCC) is a malignancy of the liver that accounts for 90% of all primary liver cancers (2). The incidence of HCC is rising fast, and an increase is expected of over 300% between 2008 and 2020 in Europe (2). HCC is categorized according to the Barcelona Clinic for Liver Cancer (BCLC) classification into 5 stages (0, A, B, C and D), as can be seen in Figure 1 (3). Only patients with stages 0 and A are candidates for a treatment with curative intent, such as resection, radiofrequency ablation (RFA), or transplantation. Recurrence rates after RFA are comparable to those after surgical resection in very early stage (0) HCC patients. As RFA treatment is less invasive, this is the preferred treatment in these patients. However, in early stage (A) HCC patients, the recurrence rates after RFA are higher than those after surgical resection. Therefore, surgical resection remains the treatment of choice in early stage HCC patients, although this treatment is associated with higher morbidity and mortality. Nevertheless, many patients are not eligible for surgery due to their underlying liver disease or comorbidity. These patients currently receive suboptimal treatment. In order to provide all early stage HCC patients with the best treatment option and to reduce invasiveness of the treatment, improvement of minimally invasive RFA treatment in early stage HCC patients is desired.

## HEPATOCELLULAR CARCINOMA

In 2016, 600 Dutch patients were diagnosed with HCC, which is a two-fold increase since 2005 (4). HCC accounts for 90% of all primary liver cancers. It occurs predominantly in patients with cirrhosis due to underlying liver diseases like hepatitis B or C virus (HBV or HCV), non-alcoholic steatohepatitis (NASH), or as a result of alcoholism (2). Due to the high incidence of HBV and HCV in Africa and South-East Asia, a high incidence is found in these regions. In Europe, approximately 60% of all HCCs can be attributed to chronic hepatitis C, 10-15% to hepatitis B, and 20% to alcohol-induced liver disease. In the Netherlands, the 1-year survival rate of patients diagnosed with HCC is about 40%, whereas the 5-year survival is only 15% (4).

HCC is usually discovered during routine surveillance in high-risk patients, or after onset of symptoms caused by an obstruction of the biliary system or liver vasculature. As the disease may remain unnoticed for a long time, many cases are detected in advanced disease stages. (2). Due to implementation of surveillance programs in high-risk populations, early HCC diagnosis is now feasible in 30-60% of all patients in developed countries (2). Diagnostics are performed by a multiple phase CT (unenhanced, arterial, portal-venous and late), or a contrast-enhanced MRI scan, whereas surveillance is performed bi-annually with use of ultrasound.

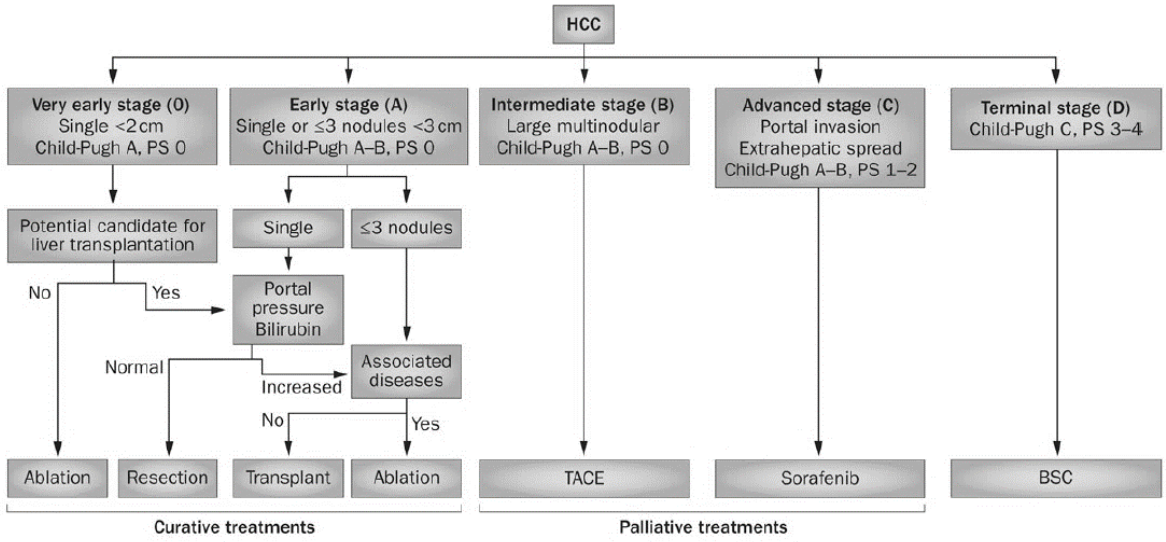


**Figure 1** The BCLC classifies HCC into five stages based on the extent of disease, Child-Pugh score, and ECOG performance status. Prognosis and treatment allocation is done based on this classification. In case of contraindicated treatment, the treatment option of the next stage should be considered. Abbreviations: BCLC = Barcelona Clinic Liver Cancer, BSC = best supportive care, ECOG = Eastern Cooperative Oncology Group, HCC = hepatocellular carcinoma, PS = performance status, TACE = transarterial chemoembolization (3).

HCC patients are categorized according to the BCLC classification system, as can be seen in figure 1. This system provides guidelines for treatment allocation according to pre-established prognostic variables, based on the tumor size, severity of liver disease, and the patients' general condition (3). The Child-Pugh score is a hepatic disease classification system that incorporates the clinical markers serum bilirubin, albumin and INR, ascites, and encephalopathy (5). The ECOG performance status is a classification system for general health status (6).

Patients with very early stage and early stage HCC are treated with curative intent. Besides (laparoscopic) surgical resection, ablation therapies and liver transplantations are executed in this context. A liver transplantation is only performed in patients who meet the Milan criteria (7), which implies that the patient may only have one lesion <5 cm, or up to 3 lesions <3 cm each. Moreover, according to these criteria no extrahepatic manifestation of the disease is allowed, nor vascular invasion.

## RADIOFREQUENCY ABLATION

Ablation therapies have been widely studied and used as a treatment of liver cancer lesions. Different underlying techniques have clinically been used, such as percutaneous alcohol injections, laser induced thermal therapy, radiofrequency ablation, cryoablation, and microwave ablation. All therapies share the ultimate goal to destroy cells locally at the tumor site, while their approaches differ. The most widely accepted ablation technique for liver cancer is radiofrequency ablation (RFA). At the tip of the RFA-needle, an alternating monopolar electric current causes extreme heating of local tissue, which leads to ionic cell agitation, and ultimately to cell necrosis. In very early stage HCC patients, RFA treatment has

been proven equally effective as surgical resection, with similar recurrence rates (8). However, in early stage HCC patients, RFA recurrence rates are higher. In the most recent Cochrane systematic review, hepatic resection shows better outcome than RFA with respect to local progression and overall survival, with a hazard ratio of 0.38 (95% CI: 0.17-0.84) and 0.56 (95% CI: 0.40-0.78) respectively (9). According to a systematic review by Tiong and Maddern, local recurrence rates after surgery were 0-10%, whereas recurrence rates of 7-24% were found following RFA (10). Despite the higher recurrence rates, the Cochrane review reports on evident advantages of RFA regarding complication rate (HR of 8.24 (2.12-31.95)) and length of hospital stay (HR 2.18 (1.97-2.39)) (9). Herein lies an incentive for improving RFA outcomes in order to be able to treat patients less invasively. Noteworthy in the study of Tiong and Maddern is that an intrahepatic recurrence rate of 28-68% was found after RFA, compared to 33-51% after resection (10). As these values are comparable, the key towards a better RFA outcome seems the reduction of local recurrences.

Different underlying causes for local recurrences after RFA can be identified. Firstly, the heat of RFA is generated at the tip of the needle. Heating peripheral tumor zones is based on the heat conduction properties of tissue. Due to insufficient heat conduction, peripheral parts of the tumor may not be heated enough for necrosis to occur, resulting in residual tumor mass. Even when complete necrosis of the tumor was reached, in 57% of patients viable satellite nodules were found in the direct proximity of the primary tumor in excised livers of patients who underwent transplantation subsequently to their RFA (11). These satellite nodules may later develop into recurrent tumor. Lastly, the RFA heat spread may be compromised if the tumor is situated near a blood vessel. During RFA, heat is conducted away through continuous blood flow, resulting in the heat-sink phenomenon (12). In patients whose tumor is located adjacent to a blood vessel, local tumor progression is found from insufficiently treated tumorous tissue, as the heat has continuously been drained during RFA. Treatment guidelines recommend to treat the tumor with an ablation zone of at least 0.5 cm for an ablation to be successful (13).

### CORRELATION TO TUMOR SIZE

Multiple cohort studies are executed to evaluate recurrence rates after RFA in HCC patients. In the evaluation of the outcome of these studies, a relation can be found between tumor size and local recurrence rate, as can be seen in Table 1. Local tumor recurrence rates of 14-39% were found in HCC patients with lesions >2.5 cm (14-16). Studies with a median tumor size <2,5 cm all revealed a local tumor recurrence rate of <10%, comparable to surgery (8, 17-19). Earlier described mechanisms may attribute to higher recurrence rates in larger tumors. Heat conduction errors are more likely to occur in larger tumors, more satellite nodules were found more near larger tumors, and larger tumors are more likely to border a blood vessel causing heat sink effect.

**Table 1** Lesion size versus local tumor progression rate in clinical studies

| *Author (reference)* | *Tumor inclusion* | *No. of patients* | *Median (mean) tumor size (cm)* | *Median (mean) follow-up (in months)* | *Local tumor recurrence rate (%)* |
|---|---|---|---|---|---|
| Livraghi et al (2008) (8) | HCC ≤2 cm | 218 | ND | 31.0 | 0.9 |
| Rossi et al (1996) (17) | Single HCC ≤3 cm | 41 | (2.2) | (22.6) | 5.0 |
| Li et al (2010) (18) | HCC | 117 | 2.4 | (21) | 9.4 |
| Lu et al (2005) (19) | HCC in transplant candidates | 52 | (2.5) | (12.7) | 5.8 |
| Lin et al (2004) (14) | HCC ≤4 cm | 52 | (2.9) | (24.5) | 14.0 |
| Morimoto et al (2010) (15) | Single HCC 3.1-5 cm | 18 | 3.7 | (32) | 39.0 |
| Hänsler et al (2007) (16) | HCC | 21 | (4.2) | ND | 21.0 |

## TECHNOLOGICAL BACKGROUND

Radiofrequency ablation has become a standard treatment in hepatic cancer therapy. Both in primary tumors, as in (colorectal) metastasis, heating by RFA is performed to reach local temperatures of >50 °C at the lesion site. Temperatures of 50-60 °C will induce cell coagulation, followed by death in a short matter of time, whereas temperatures >60 °C induce instantaneous cell death (20). After inserting the needle, a monopolar alternating electric current is induced through the tissue. Imperfect conduction properties of the tissue cause resistive heat to occur. This heating occurs at the direct proximity of the needle, as electric current chooses the shortest path. More peripheral tumor zones are heated due to the local tissue thermal conduction properties. RFA uses a frequency of around 480 kHz. Factors that influence its success rate therefore are electric conductivity, thermal conductivity and impedance (inversely proportional to electrical conductivity). These parameters may vary from one tissue type to another. As most HCC patients have underlying liver cirrhosis, conductivity and impedance is inhomogeneous in these livers. Moreover, heating influences the tissue parameters as well. As conductivity parameters strongly rely on water contact and cellular makeup, a large change in conductivity is expected at a temperature of 100 °C, at which water boils (20). Moreover, one should be aware of slight changes in conductivity and tissue properties at the onset of coagulation. Initially, tissue impedance slowly decreases with a temperature increase until approximately 80 °C. At higher temperatures, the impedance rises quickly (20).

An earlier listed major influence on heat conducting properties of the tissue is its perfusion. In highly perfused tissue, there is a higher chance for heat sinking to occur, as the veins tend to transfer heat from the ablation zone, especially in proximity of vessels >3mm (21). Figure 2 is an image from the article of C.L. Brace and shows an example of heat sink occurrence in liver tissue. Although this phenomenon has been widely described in literature, exact estimation of the occurrence and extent of the effect remains challenging. Technically, the heat-sink effect can be overcome by producing a larger heating zone, or increasing the thermal gradient to increase the net temperature at the vessel site. The effect of local compromised intrahepatic blood flow on the size and shape of the ablation area has been

investigated in several clinical studies (22-24). Although the effect seems positive, it increases the complexity of the procedure and may be difficult to perform for all vessels.

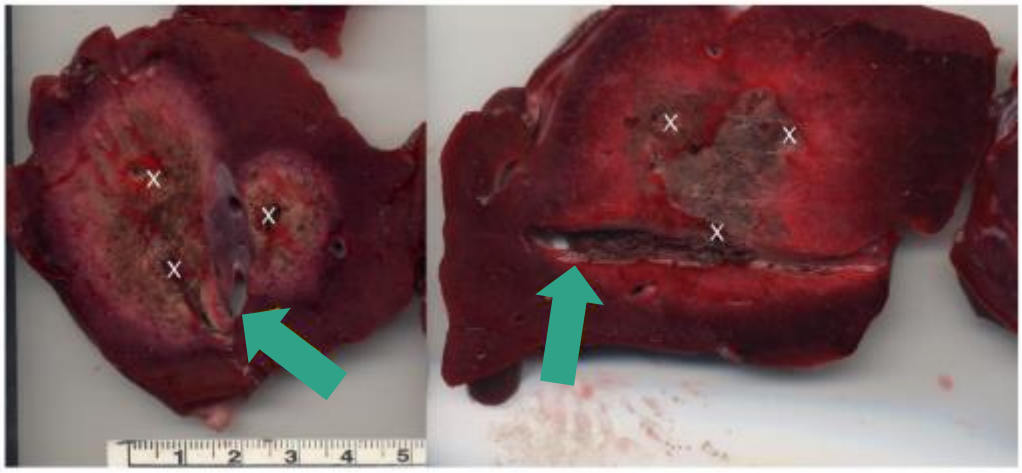

**Figure 2** Perivascular ablation with use of RFA (left) and microwave ablation (right). Applicator locations are marked with 'x' and the vessel is marked by the arrow. A heat sink zone is seen around the vessel in RFA ablation, whereas a more thorough ablation is reached with microwave ablation (20).

## MICROWAVE ABLATION

A common alternative to the use of RFA is microwave ablation (MWA). RFA makes use of radiofrequency waves, where MWA uses microwaves instead. Microwaves are closer to visible light on the electromagnetic spectrum and have a higher frequency, a shorter wavelength and a higher energy. Typically, MWA is performed using a frequency of about 2.45 GHz. MWA is a dielectric technique that induces oscillation of water molecules, as it utilizes the polarity of the water molecules (25). Tissues containing a high percentage of water have a high conductivity and are more suitable for MWA. Direct heating of MWA does not only occur in direct proximity to the needle, but also in the volume around it. Whereas RFA requires a direct conduction path, MWA also propagates better through materials of low conduction. In general, MWA reaches higher temperatures. RFA reaches an oscillating steady temperature state at about 90 °C. MWA reaches 100 °C within a minute, and increases until a steady state is reached at about 160 °C (20). The fast generation of heat in MWA in combination with a higher net temperature gradient is found to be the underlying cause for the heat sink effect to occur less when using MWA (26-28). Although technically MWA has great potency for faster, bigger and better ablation, practical results do not necessarily confirm this hypothesis, as highly variable results have been reported (28).

# RADIOEMBOLIZATION

External radiotherapy has been used in a wide variety of cancer types as single treatment or part of a multi-modality approach as (neo-)adjuvant therapy to surgery. In HCC care, radiotherapy is not administered, as the liver is prone to radiation induced liver disease (RILD), of which the onset is expedited by liver cirrhosis (29, 30). RILD symptoms may occur as soon as after administration of a total liver dose of 40 Gy, which is generally insufficient to accurately treat HCC (31). In radioembolization treatment, radioactive microspheres are administered trans-arterially to the tumor site. The microspheres are loaded with beta radiation-emitting Yttrium-90 or Holmium-166. One single microsphere has a very limited therapeutic effect, but a therapeutic dose can be reached as millions of spheres are administered. Radioembolization treatment makes use of the biological advantage that is found in hepatic tumor perfusion. Regular parenchyma is perfused for 80% by the portal vein and 20% by the hepatic artery, whereas tumors are typically perfused for 90% by the hepatic artery. A treatment release into the latter artery therefore results into a highly targeted administration of the treatment dose into the tumor and a low dose at the healthy liver parenchyma.

## CURRENT STATUS

Radioembolization is currently reimbursed in the Netherlands for the use in advanced HCC and as salvage therapy for unresectable colorectal liver metastases. In a large trial for HCC patients, radioembolization has been found to be as effective as Sorafenib, but less invasive (32). In colorectal liver metastasis patients, salvage therapy with radioembolization was found to increase average overall survival by almost 6 months (12.0 vs 6.3 months) in comparison to best supportive care (33).

## WORK-UP AND CLINICAL DOSIMETRY

Patients eligible for radioembolization all undergo a pre-treatment diagnostic procedure with Technetium-99m labeled macro albumin aggregate (99mTc-MAA). This procedure is similar to the actual treatment, but carried out with another agent. MAA is of similar size as the microspheres used during the actual treatment, and 99mTc is a pure gamma-emitting isotope, which makes diagnostic SPECT/CT feasible. The SPECT/CT images are principal for treatment planning and dosimetry. To ensure treatment safety, a low long-shunt resulting in a lung dose <30 Gy is required. Furthermore, no extrahepatic activity may be found, and the total dose on normal liver parenchyma should not exceed 50 Gy to ensure safe treatment. Between 50 Gy and 70 Gy, liver function should be taken into account in calculating treatment dose. In order to prevent extrahepatic uptake, coiling of aberrant intrahepatic arteries may be necessary during the MAA procedure. To effectuate a treatment effect, local dose of >120 Gy is desired locally in the lesion, as advised by manufacturers of Yttrium-90 microspheres. However, a recent study of Garin et al. found a threshold value of 205 Gy to obtain a better outcome (34). If higher dosing can be performed safely with respect to lung- and healthy liver dose, this should therefore be considered.

Radioembolization therapy is an intra-arterially administered internal radiation therapy. The procedure is executed in a collaboration between the departments of intervention radiology and nuclear medicine. Microspheres loaded with Yttrium-90 are mostly used for the treatment. The microspheres are made of resin (SIR-Spheres), or glass (TheraSphere). A new radioembolization product is produced by Quirem consisting of glass microspheres loaded with Holmium-166. Figure 3 shows a schematic representation of Holmium-166 decay and Yttrium-90 decay. As depicted, Yttrium-90 is an almost fully pure beta-radiation emitting isotope. The emitted energy per particle is 2.280 MeV and the half-life is about 64 hours. One of the main differences between 166Ho and 90Y is that 166Ho decay has two relevant decay cascades, and in 6.56% of all decays, a photon is emitted as well. Although Yttrium-90 only has a negligible fraction of gamma decay, SPECT can be used for post-therapeutic distribution monitoring. Enough bremsstrahlung is generated during the beta decay to provide for sufficient counts in the SPECT scan. However, for quantitative SPECT analysis of dose distribution, an exact number for bremsstrahlung fraction would be required. Technically, this can only be determined in homogeneous material, which is not the case for bremsstrahlung in the human body, especially in a cirrhotic liver. For this reason, Yttrium-90 is not suitable for post-interventional quantitative treatment distribution assessment. In Holmium-166 SPECT, two types of photons are to be found. Photons that are directly emitted in the decay cascade, and photons that are emitted as bremsstrahlung. By narrowing the sensitivity to the energy of photons from direct Holmium decay, a quantitative analysis is feasible with use of a multiplication factor derived from phantom studies (35).

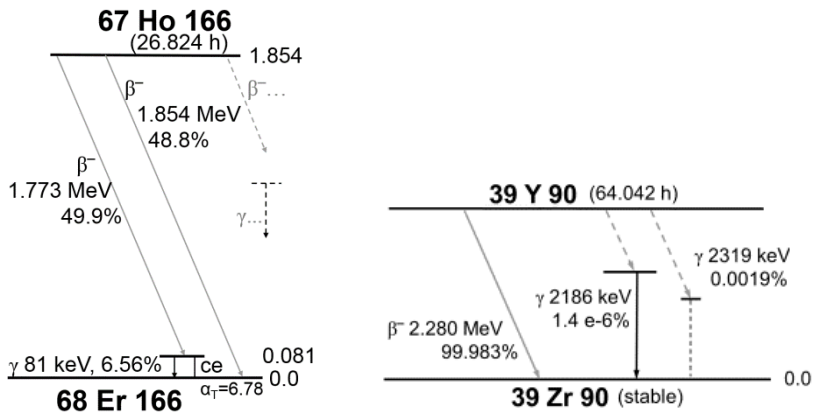


**Figure 3** Decay schemes of Holmium-166 into Eribium-166 and Yttrium-90 into Zirconium-90

On MRI, the effective transverse relaxation rate (R2*) is measured voxelwise by a sum of least-squares fitting algorithm (36). As the relaxivity of Ho-PLLA-MS is linear (r2*), a difference in relaxation rate (ΔR2*) is a measure for concentration of Ho-PLLA-MS. The concentration of Ho-PLLA-MS can be derived from the following equation, known that the relaxivity for Ho-PLLA-MS is found to be 180 s-1 mg-1 mL:

Combining the voxel size and the specific activity, an activity map in MBq can be generated. Lastly, the convolution with a SPECT-based dose point-kernel leads to a dose map in Gy. This dose point-kernel is derived from the Medical Internal Radiation Dose Pamphlet 7, with the assumption of a liver tissue density of 1.06 g/cm3 (37, 38). The entire workflow can be seen in figure 4 (36).

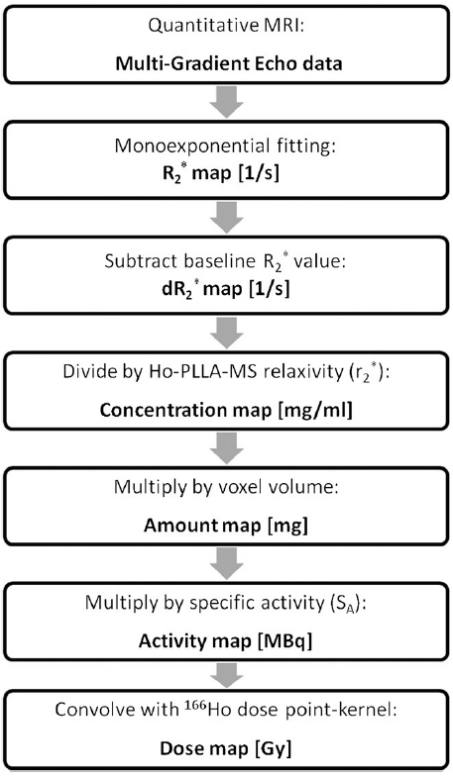


**Figure 4** Flowchart indicating the sequence of steps performed to derive three-dimensional voxel-based 166Ho radiation-absorbed dose distributions from magnetic resonance imaging (MRI) data. Ho-PLLA-MS = 166Ho-loaded poly (L-lactic acid) microsphere (36).

## RESEARCH OBJECTIVES

The long-term goal of this research in a broad sense is to improve RFA outcome in early-stage HCC patients. This research consists of two parts. In the first part, a quantitative ablation margin assessment is performed using non-rigid CT-CT co-registration. Besides, the interaction of RFA and radioembolization is investigated in a clinical trial.

### PART ONE: QUANTITATIVE ASSESSMENT OF ABLATION MARGINS

A first step within this research would be to investigate whether the role of pre- and post-interventional imaging could be improved in identifying these causes, and to aid in prediction of local tumor progression. CT-CT registration of the liver is challenging, as the liver is a deformable organ. In general, one Euclidean space has to be transformed to overlay two scans as good as possible, with a minor registration error. Algorithms are usually based on pixel intensity, contrast or unique shapes and structures (39). Due to the deformability of the liver, non-rigid registration algorithms are favorable.

Although many advances were made in optimizing these algorithms, significant errors have been reported of 7-9 mm on average (0.3-30 mm) (40). In a semi-automated registration method, it would be best to focus on local precise registration near the tumor to obtain an objective outcome.

After CT-CT registration, tumor and RFA zone delineation will be used for a quantitative assessment of the ablation zone. The main research question is whether local tumor progression can be predicted based on quantitative ablation zone assessment. In order to evaluate objectivity of the results, inter- and intra-observer variability are assessed. Inter-observer variability is the discrepancy between different assessments of a single scan by the same assessor, whereas inter-observer variability is the discrepancy between different assessments of a single scan by different assessors. Especially the latter is of importance. Margins of a minimum of 5 mm are generally accepted as predictors for the successfulness of an intervention (13). Therefore a high precision is required, and these parameters may help assessing the quality and precision of the observations (41-43).

## PART TWO: RADIOEMBOLIZATION AS ADJUVANT TREATMENT TO RFA

RFA is usually given as monotherapy. The combination of RFA and chemoembolization has been studied, but has not been proven to be of additional value (44). Radiation therapy is used extensively as (neo-)adjuvant therapy in cancer management. Moreover, radioembolization microspheres are smaller than the chemoembolization particles. Therefore, the treatment mechanism relies more on the radiation effect rather than the embolization. To evaluate a possible difference in outcome, a clinical phase I study to radioembolization as adjuvant therapy to RFA is started. In this phase I radiation dose-escalation study, the objective is to find the administration dose that results in an adequate treatment dose at the target area, consisting of the tumor with the surrounding margin.

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# Prediction of local tumor progression after radiofrequency ablation of hepatocellular carcinoma: retrospective registration of pre- and post-ablation imaging

# Prediction of local tumor progression after radiofrequency ablation of hepatocellular carcinoma: retrospective registration of pre- and post-ablation imaging

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Background: Radiofrequency ablation (RFA) is an established first line treatment for very early-stage HCC, and is used for unresectable early stage HCC. Post-ablation contrast-enhanced computed tomography (CECT) with qualitative ablation margin assessment is often used to determine technical success. Local tumor progression (LTP) rate of RFA for early-stage HCC is higher than after surgery. The objective of this study is to retrospectively use quantitative RFA margin assessment with use of non-rigid CT-CT co-registration, to assess whether there is a correlation of LTP and narrow margins.

Methods: Twenty-five patients that were treated with RFA for HCC between 2009 and 2014 were retrospectively included. Semi-automated co-registration of pre- and post-treatment CECT was done independently by two radiologists, using RTx Mirada Software. The tumor and ablation area were delineated, to identify the side and size of narrowest RFA margin. In addition, a qualitative assessment was performed independently by two other radiologists in which they determined whether the RFA was successful, the anatomical side of the narrowest margin, and its size. The outcome of the quantitative and qualitative margin assessments was compared with the occurrence of LTP, and inter-observer agreement was determined.

Results: The scans of 18/25 patients were technically feasible for CT-CT co-registration. Almost perfect inter-observer agreement was found for quantitative analysis with a $\kappa$ of 0.88 (SE: 0.116 and $p < 0.001$). The inter-observer agreement for qualitative RFA margin analysis was 0.640 (SE: 0.326 and $p = 0.004$). Based on quantitative analysis, the tumors of 12/18 patients were not fully ablated. LTP occurred in 8 (75.0%) of these patients. In 6/18 patients, the RFA area fully encompassed the tumor. None of these patients developed LTP.

Conclusion: Quantitative RFA margin assessment using non-rigid CT-CT co-registration is predictive for LTP in HCC patients ($p=0.013$).

Key words: *Radiofrequency ablation, ablation margins, hepatocellular carcinoma, local tumor progression, non-rigid co-registration*

# INTORUDUCTION

Radiofrequency ablation (RFA) has been recognized as first line treatment for very early stage hepatocellular carcinoma (HCC) (lesions ≤2 cm), and is used as treatment for unresectable early stage HCC (solitary lesion, or a maximum of 3 lesions ≤3 cm), according to the Barcelona Clinic for Liver Cancer (BCLC) staging system (1, 2). As a result of the implementation of surveillance in high-risk populations, diagnosis of BCLC very early- or early stage HCC is now feasible in up to 60% of all new HCC cases in developed countries (3). This makes RFA an increasingly used treatment modality. Recurrence rates for RFA in very early stage HCC patients are comparable to those after surgical treatment (1). However, higher recurrence rates are found in patients treated for larger HCC lesions (4-6).

After RFA treatment, two types of intrahepatic recurrences may occur. Local tumor progression is found in up to 50% of ablations (7), and is known to be associated with insufficient ablation margin, large tumor size, blood vessels in the direct proximity of the tumor, and adhesion of viable tumor cells to the RFA electrodes (8). Distant intrahepatic recurrence is related more to systemic parameters, such as the presence of vascular invasion, multifocal disease, elevated alpha-fetoprotein blood levels, and hepatitis C viral infection (9).

Unresectable early-stage HCC cannot be directly compared to similar resectable disease, as unresectable patients are associated with more advanced underlying liver disease, higher comorbidities, and more multifocal disease (1, 10). However, distant intrahepatic recurrence rates are comparable between the groups. Yet, overall recurrence, and LTP rates after RFA tend to be higher, and negatively associated with survival rates (4-6). To improve the results of RFA in early stage HCC, the reduction of LTP rates appears to be crucial.

No histological confirmation of total tumor necrosis after RFA is possible. In many centers, the current workflow involves qualitative assessment of RFA margins by scrolling through unregistered pre- and post-interventional images. Usually this is done only in two-dimensional axial images, which makes multi-directional assessment of margins difficult. Technical success is considered when treatment of the tumor was performed according to protocol and complete tumor coverage is confirmed on CECT either during, or immediately after the procedure with a sufficient ablation margin size (8). In general, an ablation margin of >5 mm, or ideally 10 mm, is recommended (8). Although some studies seem to support these rather arbitrary values, the evidence is limited and seems to be primarily derived from surgical standards. Liao et al. found in their study that intended ablation margins of 10 mm were associated to lower intrahepatic recurrence rates and longer recurrence free survival (11). In a study by Nakazawa et al, ablation margins of >5 mm were associated to low local tumor progression rates (12), and in another trial by Kim et al., a cut-off value for RFA margins of 3 mm was associated to 0% local tumor progression rate (13).

In this study, prediction of local tumor progression by a quantitative three-dimensional (3D-) margin assessment after non-rigid CT-CT registration of pre- and post-interventional imaging is performed for very early and unresectable early stage HCC patients that were treated with RFA.

# METHODOLOGY

Medical ethical approval was obtained for this retrospective cohort study. Informed consent was given for all RFA procedures but waived for the conduct of this study. Privacy was guaranteed as all

data were anonymized. The data were stored in an encrypted and secured database.

## PATIENTS

All patients that were consecutively treated with RFA for primary unresectable HCC between January 2009 and March 2014 (n=79) in our institution were included in this study. Of these 79 patients, 38 patients were excluded from the analysis due to multifocality (n=27), (laparoscopic) surgical approach (n=4) and combination treatment with TACE (n=7). Baseline patient characteristics of the remaining 41 patients who were treated for primary, unresectable, focal HCC are shown in Additional Table 1. Of the remaining 41 patients, 16 patients were assessed as unfeasible for analysis due to great difference in patient orientation and/ or liver morphology during the pre-ablation and post-ablation CT (n=11), or metal artifacts caused by in vivo ablation needles on the post-treatment CT (n=5). Baseline characteristics of the remaining 25 patients are shown in Table 1. Pre- and post-ablation multi-phase CECT scans with an arterial and portal-venous phase were available for all included patients.

## RFA PROCEDURE

The percutaneous RFA procedures were performed under general anesthesia, and with image guidance of ultrasound and/or CT. Based on tumor characteristics and availability, one of the single electrode RFA systems (3 cm exposed tip Cooltip (Medtronic minimally invasive products, Gosport Hamspire, United Kingdom) or StarBurst XL (AngioDynamics, Amsterdam, Netherlands)), or multiple electrode RFA system (3 or 4 cm exposed tip Cooltip with switch control system (Covidien, Gosport Hamspire, United Kingdom)) was used. The ablation time was set 12 minutes for single Cooltip electrode, and 16 minutes for the multiple

**TABLE 1** Patient characteristics of analyzed patients

| | | *n* | |
|---|---|---|---|
| **total** | | 25 | |
| **age** | mean (SD) | 62,1 | 11,8 |
| **sex** | male | 20 | 80,0% |
| | female | 5 | 20,0% |
| **Cirrhosis** | yes | 25 | 100,0% |
| | no | 0 | 0,0% |
| **Ascites** | yes | 7 | 28,0% |
| | no | 18 | 72,0% |
| **Etiology** | Hepatitis B | 2 | 8,0% |
| | Hepatitits C | 8 | 32,0% |
| | Alcohol abuse | 15 | 60,0% |
| | NASH | 2 | 8,0% |
| | Cryptogenal | 1 | 4,0% |
| **ECOG** | 0 | 24 | 96,0% |
| | 1 | 1 | 4,0% |
| | 2 | 0 | 0,0% |
| **Child-Pugh** | A | 12 | 48,0% |
| | B | 13 | 52,0% |
| **BCLC** | very early | 10 | 40,0% |
| | early | 15 | 60,0% |
| **lesion size** | median (range) | 20 | 12-45 |
| **year of RFA** | 2009-2011 | 10 | 31,3% |
| | 2012-2014 | 15 | 46,9% |

Cooltip electrodes. Temperature-based ablation was performed with the StarBurst XL electrode.

Immediately after ablation, a CECT of the liver was performed on a 16-slice spiral CT (Aquillion-16, Toshiba, Tokyo, Japan) with the settings: 120 kV, rotation 0.5 s, 16×1 mm scanning. Dose weight dependent Ultravist 370 contrast agent, or Xenetex 350 contrast agent was used with a 15 seconds and 75 seconds delay after bolus triggering for arterial and portal venous phase, retrospectively. RFA procedures were executed according to the vendor-based pre-defined settings for the delivery of the right amount of energy to obtaining an ablation area sufficient to encompass the tumor with a >5 mm margin.

At the time of the intervention, CECT scans were qualitatively evaluated for success by the interventional radiologist performing the procedure. The scans were checked for the RFA coagulated area to have fully encompassed the tumor, and the absence of residual tumor enhancement. Eye-balling visual assessment, with help of 2D measurements were used in this assessment.

## FOLLOW-UP

All patients were routinely invited to the outpatient clinic of the department of gastroenterology and hepatology, and underwent blood tests (including alpha fetoprotein), and CECT every three months after treatment. Upon discretion of the referring physician or interventional radiologist, multiphase MRI was used instead of CECT. Correlation was determined between the side of local tumor progression (within 18 months post-interventional) and the side of the minimal ablation margin. A comparison of patient characteristics between those with and without local progressive disease took place. Liver explants of patients that underwent an orthotopic liver transplantation (OLTx) were pathologically examined for local tumor progression. The liver was cut into sub-centimeter slices and examined in smaller slices more precisely in and near lesions.

## SCORING

CT-CT registration and delineation of the tumor volume and RFA necrosis were performed in Mirada RTx software (MIRADA, Oxford, UK). Two radiologists independently performed the CT-CT co-registration and delineation of the tumor and RFA margins, while being blinded for follow-up information. CT-CT co-registration was performed using a semi-automated deformable registration algorithm with manual alteration when necessary. These manual alterations were either done by rotation and translation of a scan, or with use of a landmark algorithm that interpolates a deformable transformation by manually selecting corresponding anatomical landmarks in both scans. The registration performance was graded on a 5-points skill with 1 being completely unreliable, and 5 a perfect registration. Patients with CT-CT registration performance of 1-3 were excluded from further analysis.

Delineation of the tumor volume and ablation area was done using a greyscale-based semi-automatic delineation tool with manual adjustments for accurate segmentation. In a fused-imaging view, RFA margins were quantitatively assessed by expanding the tumor's contour until line intersection with the delineated ablation area. In case the tumor was not located completely within the ablation area, negative margin size was determined in the same way by expanding the ablation area delineation. Besides the narrowest margin in millimeters (mm), the anatomical location of the narrowest margin was determined as well. In case the tumor exceeded the tumor ablation area, the anatomical location of the highest tumor excess was recorded. Inter-observer agreement was determined for the assessment of margin size (categorical: negative, 0 to 5 mm, or ≥5 mm).

Two other radiologists independently scored the original post-ablation scan, based on eye balling and 2D measurements, while being blinded for follow-up information. For all patients, it was reviewed whether the RFA margin size was 1: negative, 2: 0 to 5 mm, or 3: >5 mm. Moreover, the anatomical side of narrowest margin was recorded. Inter-observer agreement was determined for the assessment of margin size.

## STATISTICS

Inter-observer agreement was determined by using unweighted Cohen's Kappa statistics, estimating the agreement while taking into account the chance

of a similar observation. A κ of 0 meant that the agreement was the same as chance, whereas a κ of 1 meant perfect agreement (14). This statistical parameter was used for all inter-observer agreement measures in this study.

Continuous data were analyzed with the independent t-test and categorical data with the chi-square test. SPSS version 23.0 was used to perform the data analysis and a significance interval of 5% was used. Boxplots were created using GraphPad Prism 5 (GraphPad Software, San Diego, California, USA).

## RESULTS

In 18 patients, the co-registration of pre- and post-ablation scans were rated a 4 or 5, which was sufficient for further analysis. In 8 (44.4%) of these patients, LTP was found. Of these 8 patients, 5 recurrences (27.8%) were based on radiological imaging, and 3 (16.7%) were pathologically proven after OLTx. In 1 (5.6%) patient, distant intrahepatic recurrence was found. Out of the remaining 9 (50%) patients, 3 (16.7%) underwent OLTx within 1 year after RFA (average 9.3 months). Patient and lesion characteristics are presented in table 2.

### SCORING

The inter-observer agreement between the two radiologists for quantitative assessment with use of CT-CT co-registration and delineation was almost perfect, with a κ of 0.88 (SE: 0.116 and p <0.001). Also, the agreement on the minimal margin size was categorically evaluated (negative, 0 to 5 mm, or ≥5 mm). This resulted into the same κ of 0.88 (SE: 0.116 and p <0.001). Agreement was reached between the observers on whether the tumor was fully encompassed by the ablation area for the cases that they initially disagreed on. This was done by consensus reading that involved re-evaluation of the CT-CT co-registration and delineation.

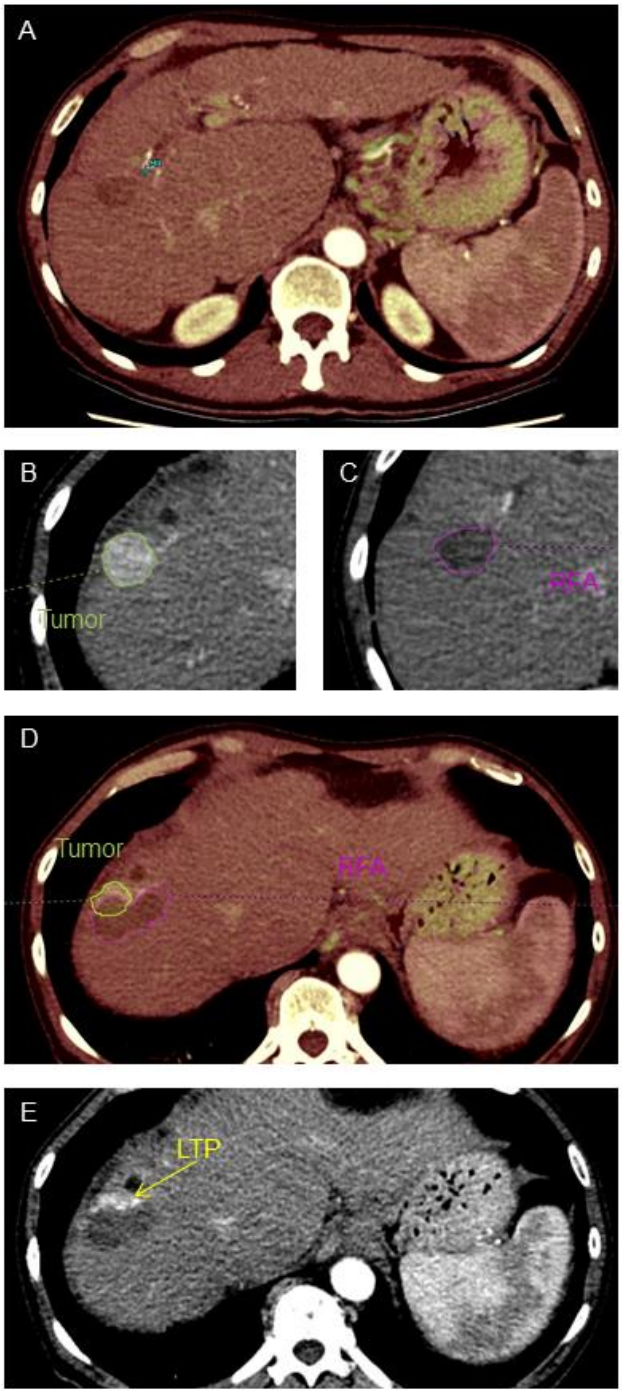


**Figure 1** Image analysis protocol. **A:** registration of pre-interventional and post-interventional CT scans. **B:** Semi-automatic delineation of tumor volume. **C:** Semi-automatic delineation of RFA area. **D:** Image fusion plane: margin analysis. **E:** Follow-up scan with local tumor progression.

**TABLE 2** Patient characteristics of patients technically feasible for quantitative analysis

| | | Total | | No LTP | | LTP | | |
|---|---|---|---|---|---|---|---|---|
| | | ***n*** | | n | | n | | *p-value* |
| **Total** | | **18** | | 10 | | 8 | | |
| **Age** | mean (SD) | **64.9** | **(9.0)** | 66.1 | (10.7) | 63.4 | (6.5) | *0.538* |
| **Sex** | male | **14** | **77.8%** | 7 | 70.0% | 7 | 87.5% | *0.375* |
| | female | **4** | **22.2%** | 3 | 30.0% | 1 | 12.5% | |
| **Cirrhosis presence** | yes | **18** | **100.0%** | 10 | 100.0% | 8 | 100.0% | |
| | no | **0** | **0.0%** | 0 | 0.0% | 0 | 0.0% | |
| **Ascites presence** | yes | **5** | **27.8%** | 3 | 30.0% | 2 | 25.0% | *0.814* |
| | no | **13** | **72.2%** | 7 | 70.0% | 6 | 75.0% | |
| **Etiology** | Hepatitis B | **0** | | 0 | | 0 | | |
| | Hepatitis C | **4** | | 2 | | 2 | | *0.800* |
| | Alcohol abuse | **5** | | 2 | | 3 | | *0.410* |
| | NASH | **2** | | 2 | | 0 | | *0.180* |
| | Cryptogenic | **1** | | 0 | | 1 | | *0.250* |
| **ECOG** | 0 | **17** | **94.4%** | 10 | 100.0% | 7 | 87.5% | *0.250* |
| | 1 | **1** | **5.6%** | 0 | 0.0% | 1 | 12.5% | |
| | 2 | **0** | **0.0%** | 0 | 0.0% | 0 | 0.0% | |
| **Child-Pugh** | A | **9** | **50.0%** | 5 | 50.0% | 4 | 50.0% | *1.000* |
| | B | **9** | **50.0%** | 5 | 50.0% | 4 | 50.0% | |
| | C | **0** | **0.0%** | 0 | 0.0% | 0 | 0.0% | |
| **BCLC** | very early | **6** | **33.3%** | 3 | 30.0% | 3 | 37.5% | *0.737* |
| | early | **12** | **66.7%** | 7 | 70.0% | 5 | 62.5% | |
| | intermediate | **0** | **0.0%** | 0 | 0.0% | 0 | 0.0% | |
| | advanced | **0** | **0.0%** | 0 | 0.0% | 0 | 0.0% | |
| **lesion size** | median (range) | | | | | | | |
| **OLTx <1 year** | yes | **6** | **33.3%** | 3 | 30.0% | 3 | 37.5% | *0.737* |
| | no | **12** | **66.7%** | 7 | 70.0% | 5 | 62.5% | |
| **Distant intrahepatic Recurrence** | yes | **1** | **5.6%** | 1 | 10.0% | 0 | 0.0% | *0.357* |
| | no | **17** | **94.4%** | 9 | 90.0% | 8 | 100.0% | |
| **RFA on Target Quantitative assessment** | yes | **6** | **33.3%** | 6 | 60.0% | 0 | 0.0% | *0.013* |
| | no | **12** | **66.7%** | 4 | 40.0% | 8 | 100.0% | |
| **RFA on Target Qualitative assessment** | yes | **16** | **88.9%** | 10 | 100.0% | 6 | 75.0% | *0.094* |
| | no | **2** | **11.1%** | 0 | | 2 | 25.0% | |
| **year of RFA** | 2009-2011 | **7** | **38.9%** | 2 | 20.0% | 5 | 62.5% | *0.066* |
| | 2012-2014 | **11** | **61.1%** | 8 | 80.0% | 3 | 37.5% | |

The inter-observer agreement of two radiologists who qualitatively assessed the ablation margins was moderate: 0.640 (SE: 0.326 and p= 0.004). Agreement on margin assessment was very poor for categorical evaluation (negative, 0 to 5 mm, or ≥5 mm) with a κ of 0.242 (SE of 0.267 and p= 0.164). Consensus was reached between the observers on whether the tumor was fully encompassed by the ablation area for the cases that they initially disagreed on, for further analysis.

## LOCAL TUMOR PROGRESSION RATE

Table 2 shows all patient and tumor characteristics of the cases that were technically feasible for quantitative analysis. Differences in patient and tumor characteristics were analyzed for patients who developed LTP (n=8) and patients who did not. OLTx was performed in 6/18 (33,3%) patients within 1 year of follow-up (average time until OLTx = 6,01 months). Of these patients, 3 developed LTP prior to their OLTx surgery. No relevant differences were found in patient and tumor characteristics between the groups. All patients who developed local tumor progression, did so at (one of) the anatomical side(s) with a negative ablation margin. An example of the entire work-up and occurrence of local recurrence at a negative ablation margin is shown in figure 1.

Based on the quantitative analysis, the RFA area fully encompassed the tumor in 6/18 (33.3%) of all patients with a mean margin of 0,91 mm (SD: 1,11 range: 0-3 mm). In none of these patients, LTP was found. Of all patients with negative ablation margins, 8 (66,7%) patients developed LTP, 3 (25,0%) patients underwent OLTx surgery with no viable tumor cells found in the specimen, and one (8,3%) patient did not develop LTP, nor underwent OLTx. All 8 patients who developed LTP were identified for having insufficient ablation margins, with a p-value of 0.013. The average minimal ablation margin was -6,38 mm (SD: 4,64). The quantitatively determined ablation margins were correlated to the occurrence of local tumor progression in figure 2. The mean ablation margin of patients who developed LTP was -8,44 mm (SD: 4,27), while patients who did not develop LTP showed an average ablation margin of -0,30 mm (SD: 2,00). The corresponding p-value from the T-test is 0.001.

Based on the qualitative analysis, 16 (88,9%) ablation areas fully encompassed the tumor, and in 2 (11,1%) the tumor exceeded the ablation margins in one or more directions. These patients both developed LTP.

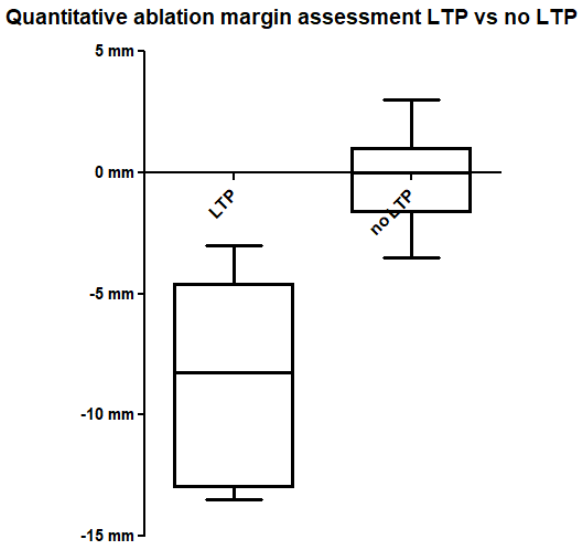


**Figure 2** Boxplot of quantitative ablation margin size for patients with and without local tumor progression.

## DISCUSSION

In this retrospective study, a quantitative assessment of tumor ablation margins was performed, using CT-CT co-registration in Mirada RTx software. The results show that quantitative assessment was predictive for the occurrence of LTP (p = 0.013).

Minimally invasive treatments are gaining more prominence in HCC treatment of both early as advanced stages. A disadvantage of these therapies is that no pathological confirmation of treatment success can be obtained. Thermal ablation treatment success is obtained when sufficient energy has been delivered to the tumor site to create an ablation area that fully encompasses the tumor with a margin of at least 0.5 or 1 cm, based on experimental data in health animal livers. However, these ablation margin sizes are rather arbitrary chosen, and the in-vivo- size and shape of the ablation is dependent on factors such as heat-sink, tumor heterogeneity, absence or presence of tumor capsule, and liver parenchyma fibrosis or cirrhosis. Post-ablation CECT is most commonly used to determine technical success after RFA, and ablation margins are often estimated by 2D-eyeballing and – measurements. The results of this study indicate that eyeballing assessment may result in an overestimation of the ablation margins assessment.

In this study, the tumors of 3/25 (12%) patients were considered to be incompletely ablated, based on retrospective qualitative assessment by two independent radiologists. All of these patients did develop LTP. Therefore, the qualitative re-assessment was highly specific, as they correctly

identified all patients who would not develop LTP. However, as only 3/10 (30%) of patients who reveal LTP within 18 months, were successfully identified in this way, sensitivity of the qualitative assessment was found to be very low: 30%.

The LTP-rate in this study is 44.4%, which is comparable to studies with a similar population. In a randomized study including 701 patients treated with RFA, the HEAT III study, tumor progression rates of 53.3% were found after treatment with RFA in a population with slightly more unfavorable patient and tumor characteristics (15).

In an earlier study presented by Kim et al. (13), 130 HCC tumors were quantitatively analyzed in a similar way as presented in this study. Their findings were similar to ours with respect to recurrence rate and the discrepancy between quantitative and qualitative analysis. They found a cut-off value of a minimum ablation margin of >3 mm. Wider margins excluded the occurrence of LTP in their study: 15/110 (13,6%) met this cut-off value, while the treatments were aimed at reaching an ablation margin of at least 5 mm. A difference to our approach was that they used a rigid registration method for CT-CT co-registration. Other studies that used non-rigid registration for better local image fusion, were performed in smaller cohorts lacking inter-observer agreement evaluation (16). The study of Kim et al., however also assessed the feasibility of non-rigid registration algorithms for RFA margin assessment. Their results are in line with the present study, showing similar inter-observer reproducibility factors (17).

The present study suggests that a full ablation with minimal ablation margins is sufficient to adequately treat HCC by RFA with low chance of LTP. However, as this study was performed based on post-ablation imaging, it does not take into account intra-therapeutic tumor shrinkage. In the follow-up scans after ablation, the RFA necrosis volume is known to be subject to shrinkage (18). Yet, tissue shrinkage has been reported also during ablation, and has further been studied in ex-vivo studies (19-21). A post therapeutic ablation margin of 0 mm may therefore mean that the initial tumor was actually completely treated with a few mm of surrounding tissue, as shrinkage may have occurred. However, tissue shrinkage is hard to monitor, as it seems to occur in an inhomogeneous and unpredictable way (19). If shrinkage indeed is hard to predict, the clinical value of positive ablation margins will remain arbitrary, as it does not necessarily quantify the actual distance between pre-treatment tumor boundaries and the surrounding zone defined as target treatment area. This does not mean that quantification of ablation margins is meaningless, as our study results show that this parameter is a significant predictor of LTP. However, a better understanding of in-vivo intra-therapeutic tissue volume changes is necessary to relate pre-treatment planning better to post-treatment images, for defining treatment success.

The main limitation of this study is its low sample size. Although the initial cohort consisted of 79 patients, the final number of patients within a uniform cohort that were technically feasible for quantitative analysis was low. We were strict in patient selection to be truly able to assess the performance of quantitative analysis of ablation margins in the RFA treatment of HCC. Unfeasible registrations were usually caused by big differences in morphology of the liver, mostly caused by different patient positioning.

The next step would be to prospectively use quantitative tumor ablation margin assessment to improve the treatment outcome in means of LTP. In order to create a feasible and robust workflow, a fast and non-rigid registration algorithm with intuitive segmentation tools would be necessary during the

treatment. Moreover, the technical feasibility rate must increase to achieve a more robust method, decreasing the amount of unfeasible registrations. To overcome the high drop-out rate, a pre-interventional CT in the same bed position as the RFA and with similar lung expansion is required. Under these circumstances, intra-procedural quantitative assessment of RFA margins could contribute to the reduction of LTP.

# CONCLUSION

Quantitative RFA margin assessment using non-rigid CT-CT co-registration is predictive for LTP in HCC patients. Qualitative analysis of RFA margins by eye-balling seems to overestimate ablation margins.

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**EXTRA TABLE 1** Patient characteristics of initial population

| | | *n* | |
|---|---|---|---|
| **total** | | 41 | |
| **age** | mean (SD) | 61,9 | 11,0 |
| **sex** | male | 31 | 75,6% |
| | female | 10 | 24,4% |
| **Cirrhosis presence** | yes | 41 | 100,0% |
| | no | 0 | 0,0% |
| **Ascites presence** | yes | 15 | 36,6% |
| | no | 26 | 63,4% |
| **Etiology** | Hepatitis B | 2 | 4,9% |
| | Hepatitits C | 12 | 29,3% |
| | Alcohol abuse | 24 | 58,5% |
| | NASH | 2 | 4,9% |
| | Cryptogenal | 2 | 4,9% |
| **ECOG** | 0 | 40 | 97,6% |
| | 1 | 1 | 2,4% |
| | 2 | 0 | 0,0% |
| **Child-Pugh** | A | 23 | 56,1% |
| | B | 15 | 36,6% |
| | C | 3 | 7,3% |
| **BCLC** | very early | 18 | 43,9% |
| | early | 23 | 56,1% |
| | intermediate | 0 | 0,0% |
| | advanced | 0 | 0,0% |
| **lesion size** | median (range) | 20 | 12-45 |
| **year of RFA** | 2009-2011 | 23 | 56,1% |
| | 2012-2014 | 18 | 43,9% |

# Holmium radioembolization as adjuvant treatment to radiofrequency ablation for early stage hepatocellular carcinoma: a dose-finding study (summary)

# Holmium radioembolization as adjuvant treatment to radiofrequency ablation for early stage hepatocellular carcinoma: a dose-finding study (summary)

In collaboration with L.F. de Geus-Oei, A.R. van Erkel, M.J. Coenraad, M.C. Burgmans

Hepatocellular carcinoma (HCC) is divided into five different stages according to the Barcelona Clinic for Liver Cancer (BCLC), of which 'very early' and 'early stage' HCC are treated with curative intent. Due to surveillance in high-risk populations, more patients are now diagnosed with BCLC 'early stage' HCC. Nevertheless, many of these patients are not operable due to their underlying liver cirrhosis, or other comorbidity. Thermal ablation is a good alternative for patients with irresectable 'very early' and 'early stage' HCC. Recurrence rates for thermal ablation in 'early stage' HCC patients (a solitary tumor  2 cm or a maximum of three tumors of maximally 3 cm each) are higher than after surgical resection. Radioembolization is a relatively new internal radiation treatment, which is used in advanced liver cancer. Radioactively loaded microspheres are administered trans-arterially into the liver and cause tumor necrosis through beta radiation. QuiremSpheres (Quirem Medical B.V.) microspheres are used in this study. These spheres are loaded with holmium-166 and have unique imaging features that allow quantitative dosimetry with the use of SPECT/CT and MRI. In this clinical study, the biodistribution of holmium-166 microspheres after radiofrequency ablation (RFA) will be analyzed to determine the optimal administrated dose when radioembolization is used as adjuvant treatment to RFA.

*This was the English abstract of an article published in Nederlands Tijdschrift voor Oncologie (The Dutch Journal of Oncology). The full article is in Dutch and can be found in Appendix I.*

# Discussion

# Discussion

HCC proves itself to be an aggressive tumor proliferative disease with high local and distant recurrence rates, and a bad prognosis. In the fight against this distinctive disease, radiofrequency ablation has proven potential to fulfill the role of a safe and effective treatment for very early stage HCC, with recurrence rates similar to surgery. Although RFA is currently considered the gold standard for treatment of early stage HCC, there is still much room for improvement. Primary objectives include the increase of RFA treatment predictability, and the ability of targeting radical, minimally invasive (combination) treatments. These treatments need to have a larger target area for better elimination of satellite lesions. Moreover prevention, early detection, and early treatment of intrahepatic distant metastases are among current challenges.

A major limitation of RFA is the lack of pathologic confirmation of treatment radicality. As a result, much research is conducted exploring different ways of assessing and obtaining treatment outcome. Fields that have obtained high interest in this research are the use of image guidance, needle positioning and better treatment planning, by using heat distribution models and real-time temperature mapping. On the clinical side, a research focus is within developing combination therapies for RFA, and the optimization of patient selection. In this thesis, the conduct of a retrospective study into image guided treatment confirmation, and a study design of a prospective clinical trial of a combination treatment have been discussed.

Liver tissue is rather inhomogeneous, as it contains many arterial and venous blood vessels, bile ducts and lymphatic vessels. The inhomogeneity of the tissue, the intrahepatic blood flow, and different types of heat conduct for different ablation techniques make it hard to accurately predict heat distribution during treatment (1). To make an accurate prediction of the heat sink effect, one should take into account the exact location of the lesion, central lesions are prone to the heat sink effect in a greater extent than peripheral lesions, due to the larger vessels centrally in the liver. Moreover, HCC often develops in a fatty, fibrotic and cirrhotic liver, which compromises regular heat. All of these factors increase the complexity of heat distribution modelling even further, making it challenging to create a robust treatment prediction model. Nevertheless, prediction models do support in identifying optimal needle positioning and treatment challenges in a pre-treatment setting (2). Real-time temperature mapping during RFA could contribute to validation in the execution of the planned treatment protocol. However, real-time temperature mapping techniques have not yet been validated for clinical use, and tend to bring additional uncertainties into the model (3).

This thesis focused on radiofrequency ablation, as this is still the most commonly used technique, and the preferred technique in the LUMC (4, 5). However, the technique has its drawbacks, such as a rather slow tissue heating, and occasionally an insufficient maximal treatment volume (6). The most commonly used alternative to RFA is MWA. This technique makes use of other frequencies on the electromagnetic spectrum. The microwaves cause direct hysteresis and can readily penetrate through a larger variety of tissue types (4, 7). Moreover, the use of MWA was associated with lower heat-sink effects in some

studies (8). However, in a recent meta-analysis, no superiority of MWA over RFA was demonstrated (9). A rather new ablation technique is called irreversible electroporation (IRE). This non-thermal therapy creates a pulsed direct current that induces cytotoxicity by altering the transmembrane potential of cells (10). This technique is highly adjustable by e.g. probe positioning and alternating the number of probes used. In small patient cohorts treated for small HCC lesions that were untreatable by other modalities, the results seem promising (11). A theoretical advantage of this technique is that no heat sink is likely to happen, as this technique is a non-thermal ablation technique. Another thermal ablation technique is cryoablation, which involves ice-ball formation. In a clinical study, the outcomes were found to be inferior to RFA (12). Percutaneous laser ablation (PLA) reaches initial complete response rates comparable to RFA, whereas the overall survival seems inferior to RFA (13). In conclusion, many thermal and non-thermal ablative techniques are applied and tested for HCC treatment. Out of these techniques, MWA and IRE seem to be most promising, in addition to RFA. Nevertheless, the main problem of ablative treatments is not resolved by changing the technique. Pathologic confirmation remains unavailable, and therefore all techniques will be dependent on treatment planning, image guidance, and radiological formation. Although MWA and IRE performance seems less influenced by tissue properties, superiority of these techniques is not yet proven.

Another attempt at treatment improvement is the use of needle guidance, or needle positioning systems. There are several techniques available, that for example make use of infra-red tracking (14) or robotic positioning (15, 16). These needle positioning systems use imaging-based RFA treatment planning. Positioning support aims at reducing the distance between probe and predefined target to a minimum before ablation. In terms of accuracy, some techniques perform well, as only a slight error between aim and actual position is found (14, 16). Clinically, no superiority has been proven for regular ablations. However, it enables easier targeting in small lesions, or in lesions that are hard to image with ultrasound. In such cases, it fulfills the role of ultrasound-CT or ultrasound-MRI registration for targeting (17). Moreover, assistance in needle positioning may shorten the duration of the procedure.

Multimodality treatment approach of a primary malignancy is part of standard care in multiple cancer types, by adding (neo-)adjuvant therapy to the golden standard. Besides the optimization of ablation techniques themselves, research on combination treatments has gained much interest over recent years. Especially the combination treatment of thermal ablation with trans-arterial chemoembolization (TACE) has been studied in multiple trials. In a meta-analysis comparing RFA plus TACE versus RFA only, no difference in efficacy was found in the entire population (18). Also, sorafenib has not been found to be of added value as adjuvant treatment (19).

Trans-arterial radioembolization (TARE) is currently principally used in more advanced HCC. Clinical trials with radioembolization have focused on its use in advanced stage HCC, in which no superiority was found over sorafenib (20, 21). Patients treated with TARE did however suffer less from side effects than those treated with sorafenib. In the meantime, TARE treatment planning has evolved, and advanced dosimetry has shown high potential for the use of TARE for more selective treatment targeting (22). By using TARE in more selective way, high dosing is feasible with fewer side-effects. In clinical perspective, TARE and TACE are often compared, as they are both trans-arterial minimally invasive

treatments. Nevertheless, their mechanisms of action differ. In radioembolization, beads are 5-10 times smaller (20-60 µm) and radiation is the hallmark of the therapy, rather than embolization. Besides, due to the 99mTc-MAA pre-therapeutic diagnostic procedure, advanced dosimetry could be used for predictability of effectiveness. From this starting point, the HORA EST HCC study has started by investigating whether the use of TARE in adjuvant setting affects the treatment behavior in such a way that this should be compensated for in the dosimetry. Ultimately, phase 2 and phase 3 will have to be performed to find out whether TARE is an effective adjuvant therapy to RFA, and whether it improves long term patient outcome.

The current status of HCC management is insufficient. Considering the incidence rates are rising rapidly, there is a strong need for better treatment options. The presented studies in this thesis contribute to the present trends in research. Despite the small sample size, a relation was found between ablation margins and local tumor progression. This gives an incentive for a study in which the use of quantitative ablation margin assessment is used in a prospective setting. The presented clinical trial contributes to the general trends within this field of research as well, as it is a novel combination treatment. Besides the use of radioembolization as adjuvant therapy, it is one of the initial studies to use radioembolization in early-stage HCC.

In the wide field of research towards better HCC management, no holy grail has been found yet, to radically improve the prospective for HCC patients. At this moment, it is more likely that the combination of the described research domains will incrementally result into better understanding of treatment mechanisms, and outcome. As imaging and image analysis play a key role in all of the developments described, it is likely that it will be the fundament for better treatment planning, prediction and analysis, to ultimately provide for a brighter future to HCC patients.

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# Appendix I

# Holmium radio-embolisatie als adjuvante therapie bij radiofrequente ablatie voor 'early-stage' hepatocellulair carcinoom: een studie naar de juiste dosis

## Holmium radioembolization as adjuvant treatment to radiofrequency ablation for early stage hepatocellular carcinoma: a dose-finding study

P. Hendriks, BSc[1], prof. dr. L.F. de Geus-Oei[2], dr. A.R. van Erkel[3], dr. M.J. Coenraad[4] en dr. M.C. Burgmans[3]

**SAMENVATTING**

Het hepatocellulair carcinoom (HCC) wordt volgens de 'Barcelona Clinic for Liver Cancer' (BCLC) ingedeeld in vijf stadia, waarvan de stadia 'very early' en 'early' in opzet curatief kunnen worden behandeld. Door surveillance in risicopopulaties worden steeds meer patiënten met BCLC 'early stage' gediagnostiseerd. Veel van hen blijken echter inoperabel vanwege onderliggende cirrose of andere comorbiditeit. Voor patiënten met niet-operabel 'very early stage' of 'early stage' HCC is thermale ablatie een goed alternatief. De recidiefpercentages na thermale ablatie zijn bij 'early stage' (enkele tumor ≥2 cm of maximaal drie tumoren van maximaal 3 cm elk) groter dan bij chirurgische resectie. Radio-embolisatie is een relatief nieuwe inwendige bestralingstherapie die vooralsnog enkel wordt gebruikt bij vergevorderde leverkanker. Hierbij worden radioactieve bolletjes transarterieel in de lever ingebracht. In de huidige studie wordt gebruikgemaakt van holmium-166-microsferen van Quirem Medical B.V. Deze microsferen hebben een therapeutische werking dankzij bètastraling en zijn tevens geschikt voor kwantitatieve dosimetrie met behulp van SPECT/CT en MRI. Holmium-166-microsferen zijn bij uitstek geschikt om biodistributie na radiofrequente ablatie (RFA) te bestuderen. Het doel van deze klinische studie is om de dosis te bepalen waarin radio-embolisatie als adjuvante therapie zou moeten worden toegevoegd aan RFA.

**(NED TIJDSCHR ONCOL 2018;15:106-9)**

**SUMMARY**

Hepatocellular carcinoma (HCC) is divided into five different stages according to the Barcelona Clinic for Liver Cancer (BCLC), of which 'very early' and 'early stage' HCC are treated with curative intent. Due to surveillance in high-risk populations, more patients are now diagnosed with BCLC 'early stage' HCC. Nevertheless, many of these patients are not operable due to their underlying liver cirrhosis, or other comorbidity. Thermal ablation is a good alternative for patients with irresectable 'very early' and 'early stage' HCC. Recurrence rates for thermal ablation in 'early stage' HCC patients (a solitary tumor ≥2 cm or a maximum of three tumors of maximally 3 cm each)

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**Trefwoorden:** adjuvante therapie, hepatocellulair carcinoom, radio-embolisatie, radiofrequente ablatie

**Keywords:** adjuvant therapy, hepatocellular carcinoma, radioembolization, radiofrequency ablation

are higher than after surgical resection. Radioembolization is a relatively new internal radiation treatment, which is used in advanced liver cancer. Radioactively loaded microspheres are administered trans-arterially into the liver and cause tumor necrosis through beta radiation. QuiremSpheres (Quirem Medical B.V.) microspheres are used in this study. These spheres are loaded with holmium-166 and have unique imaging features that allow quantitative dosimetry with the use of SPECT/CT and MRI. In this clinical study, the biodistribution of holmium-166 microspheres after radiofrequency ablation (RFA) will be analyzed to determine the optimal administrated dose when radioembolization is used as adjuvant treatment to RFA.

## INLEIDING

Leverkanker is de tweede kanker-gerelateerde doodsoorzaak wereldwijd.[1] 90% van alle leverkankers wordt veroorzaakt door het hepatocellulair carcinoom (HCC), en de incidentie van dit type kanker behoort tot de snelst stijgende.[2] Het HCC wordt volgens de 'Barcelona Clinic for Liver Cancer' (BCLC) gestadieerd in vijf categorieën op basis van prognostisch klinische en tumorspecifieke parameters.[3] Patiënten met BCLC 'very early' of 'early stage' leverkanker kunnen in opzet curatief worden behandeld. In het verleden kwam slechts 10% van de patiënten hiervoor in aanmerking. Dit percentage is echter gestegen na implementatie van screeningsprogramma's voor risicogroepen. Hierdoor is vroege opsporing van HCC nu mogelijk bij 30-60% van de patiënten.[2]

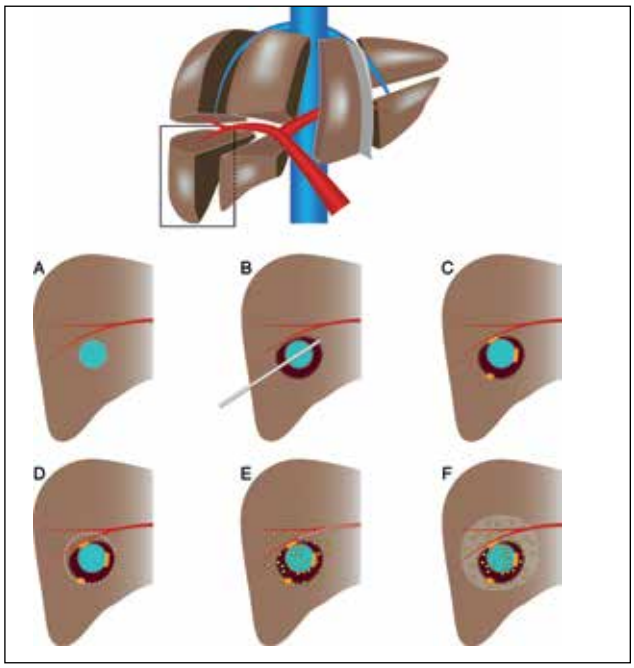
'Very early stage' HCC-tumoren zijn <2 cm en kunnen curatief worden behandeld. In de beschikbare klinische studies is er een vergelijkbaar recidiefpercentage gevonden na percutane ablatie en resectie, waarbij percutane ablatie minder invasief is gebleken.[4] In een recente update van het BCLC-stageringsschema wordt ablatie aangemerkt als therapie van eerste keuze voor 'very early stage'.[5]

Een solitaire tumor van ≥2 cm of maximaal drie tumoren van maximaal 3 cm elk worden geclassificeerd als BCLC 'early stage'. Bij tumoren van deze grootte wordt na percutane ablatie een hoger recidiefpercentage gezien dan bij chirurgische behandeling.[6,7] Een groot deel van patiënten met 'early stage' HCC is echter niet operabel vanwege comorbiditeit en/of onderliggende leverziekte zoals hepatitis of cirrose. Bij deze patienten wordt doorgaans percutane ablatie toegepast, ondanks het hogere recidiefpercentage dat hiermee gepaard gaat.

De meest voorkomende recidieven na percutane ablatie zijn op te delen in drie categorieën. Allereerst kan de warmtedistributie van de ablatie niet ver genoeg reiken, waardoor een residu achterblijft aan de rand van de tumor. De tweede categorie bestaat uit micrometastasen die zich in de directe nabijheid van de primaire tumor bevinden.[8] Na de ablatie kunnen deze uitgroeien tot een tumorrecidief. Als derde kan het 'heat-sink'-effect optreden indien de tumor in de nabijheid ligt van een bloedvat.[9] Gedurende de ablatie kan het langsstromende bloed zorgen voor afvloeiing van de gegenereerde hitte. Hierdoor kan een onbehandeld gebied achterblijven, direct grenzend aan het bloedvat, dat later mogelijk uitgroeit tot recidief. Deze drie categorieën zijn allemaal locoregionale oorzaken van tumorrecidieven die het verschil in recidiefpercentage tussen resectie en ablatie zouden kunnen verklaren.

Radio-embolisatie is een reeds gebruikte therapie die bij vergevorderde leverkanker in palliatieve setting wordt ingezet om tumorprogressie te vertragen. Bij deze behandeling wordt vanuit de lies een katheter opgevoerd tot in de leverarterie, waardoor radioactief geladen microsferen worden toegediend die vastlopen in de microvasculatuur van een tumor. Deze behandeling berust op het feit dat tumoren meer arterieel worden gevoed dan het overige leverparenchym. Hierdoor komen de radioactieve microsferen bij deze behandeling terecht in de tumor en ondervindt het niet-tumoreuze leverparenchym weinig radiatieschade. In eerder onderzoek naar uitwendige bestraling is gebleken dat de lever een lage tolerantie heeft voor straling.[10] De specifieke distributie van straling bij radio-embolisatie maakt het optreden van radiatie-geïnduceerde leverschade zeldzaam.[11] In deze studie wordt gebruikgemaakt van QuiremSpheres, microsferen geladen met holmium-166. Deze in het UMC Utrecht ontwikkelde sferen hebben een therapeutische werking dankzij de emissie van bètastraling en bieden ten opzichte van yttrium-90-microsferen unieke voordelen met betrekking tot de post-therapeutische beeldvorming. QuiremSpheres kunnen worden gevisualiseerd met SPECT/CT en MRI, en lenen zich op deze wijze bij uitstek voor studies naar biodistributie en dosimetrie.

Percutane ablatie resulteert in een gebied van necrose. Hierop ontstaat een necrose-geïnduceerde inflammatoire respons, die bij 78% van de post-therapeutische CT-scans zichtbaar is als een hyperemische zone.[12,13] Radiologisch is de hyperemie na een maand doorgaans niet meer zichtbaar.[13] Op cytologisch niveau blijkt dat zich in de buitenste transitiezone van de RFA-holte fibrotische cellen ophopen, wat gepaard gaat met een persisterende inflammatie die overgaat in chronische inflammatie.[14] Bij ablatie wordt doorgaans beoogd om de gehele tumor te coaguleren met een marge van ten minste 1 cm.




**FIGUUR 1.** Een stapsgewijze weergave van de radiofrequente ablatie (RFA) met adjuvante radio-embolisatie. **A.** In segment 6 bevindt zich een hepatocellulair carcinoom (HCC). **B.** RFA van de tumor. **C.** Potentiële locoregionale recidieven. **D.** Doelgebied van de behandeling. **E.** Toediening radio-embolisatie. **F.** Daadwerkelijk behandelgebied.

In de praktijk blijkt het, zeker bij grote tumoren, lastig om een dergelijke marge te bewerkstellingen. Vitale tumorcellen kunnen achterblijven in de rand van de ablatiezone. In dit onderzoek worden de eerste stappen gezet naar het toevoegen van radio-embolisatie als adjuvante therapie na radiofrequente ablatie bij HCC-patiënten. Bij het gebruik van radio-embolisatie bij vergevorderde leverkanker is er een gevalideerde methode van dosisbepaling om een therapeutische dosis van ten minste 120 Gy te bereiken in het doelgebied. Door de nieuwe toepassing van radio-embolisatie als adjuvante therapie na RFA wordt in deze eerste studie onderzocht welke dosis aan de patiënt moet worden toegediend om een therapeutische dosis te bereiken in de hyperemische, inflammatoire zone rondom de ablatieholte, te weten het doelgebied.

## DOEL VAN DE STUDIE

In deze studie wordt de dosis van adjuvante radio-embolisatie bij radiofrequente ablatie onderzocht. Bij een stralingsdosis van >120 Gy op weefsel wordt een therapeutisch effect verondersteld op basis van beschikbare dosis-responsstudies. Dit is het eerste onderzoek naar het gebruik van gecombineerde RFA en radio-embolisatie bij HCC-patiënten. Doordat de RFA potentieel van invloed is op de perfusie van de tumorloge, zal in dit fase 1-onderzoek worden onderzocht hoeveel dosis radioactiviteit moet worden toegediend om technisch succes te bereiken. Technisch succes is gedefinieerd als het bereiken van >120 Gy in het doelgebied. Het doelgebied bestaat uit de hyperemische zone rondom de necrotische RFA-holte.

## DOELGROEP

Deze studie wordt verricht bij 'early stage' HCC-patiënten met een enkele, irresectabele laesie van 2-5 cm, of met een maximum van drie laesies van ≤3 cm in één leverkwab. Enkel de patiënten die nog geen eerdere behandelingen voor leverkanker hebben ondergaan komen in aanmerking voor deelname. Exclusiecriteria zijn Child-Pugh-score >7, ECOG-score >2, bilirubine >2.000 $\mu$g/dl, >5× de bovengrens van normaalwaarde aspartaat-aminotransferase (ASAT), >5× de bovengrens van normaalwaarde alanine-aminotransferase (ALAT) en trombocyten <50×10$^9$/l.

## STUDIEOPZET

Het doel van deze studie is om de juiste dosering holmium-166 te vinden waarmee na RFA een therapeutisch effect in het doelgebied zal optreden, zonder te overdoseren. De holmium-166-microsferen worden via een microkatheter toegediend. Deze katheter wordt geplaatst in de arterie van het leversegment waarin de tumor zich bevindt. Na RFA zullen de microsferen zich niet gelijkmatig verdelen over het segment. Er ontstaat na RFA namelijk hyperemie in de zone rondom de ablatieholte. De microsferen zullen hierdoor preferentieel stromen naar de hyperemische zone, omdat deze tijdelijk beter doorbloed is dan het omliggende leverparenchym. De hyperemische zone neemt dus meer microsferen op dan het omringende normale leverparenchym, maar het is niet bekend hoe deze opnameverhouding precies is. Dat wordt in deze studie naar biodistributie onderzocht. *Figuur 1* geeft een stapsgewijze weergave van de RFA-procedure met adjuvante radio-embolisatie. Op de dag na RFA zal een $^{99m}$Tc-macroalbumine-aggregaat ($^{99m}$Tc-MAA)-SPECT/CT worden verricht als 'work-up' voor de radio-embolisatie. Een transarteriële injectie van $^{99m}$Tc-MAA met aansluitend SPECT/CT wordt standaard verricht voorafgaand aan radio-embolisatie om te voorspellen hoe de distributie van microsferen zal zijn. Het dient om 'shunting' naar de long uit te sluiten en om dosiscalculaties uit te voeren. De radio-embolisatie volgt in deze studie 5-10 dagen na de $^{99m}$Tc-MAA-injectie. Deze standaardtoediening is predictief voor de distributie van de radioactiviteit tijdens de werkelijke radio-embolisatie.

Een eerste cohort patiënten wordt behandeld met een lage dosis. Er wordt berekend welke dosis holmium-166 moet worden toegediend om het gehele leversegment met 60 Gy te bestralen. Bij de berekening wordt echter uitgegaan van een homogene distributie van de microsferen. In werkelijkheid

## AANWIJZINGEN VOOR DE PRAKTIJK

1. **Deze fase 1-studie onderzoekt een nieuwe combinatie van radiofrequente ablatie (RFA) met adjuvante holmium-166-radio-embolisatie.**
2. **Dit onderzoek is een dosisescalatiestudie naar de toedieningsdosis die zal resulteren in een therapeutisch effect ter plaatse van de hyperemische zone rondom de RFA-holte.**
3. **Primaire 'early stage' HCC-patiënten, die niet in aanmerking komen voor chirurgie, worden gescreend voor deelname.**
4. **Voor meer informatie of contact over eventuele deelname aan de studie kunt u contact opnemen met de eerste auteur: p.hendriks@lumc.nl.**

zullen de microsferen dus preferentieel naar de hyperemische zone stromen en kan het zijn dat de dosis op deze zone >120 Gy blijkt (en van het leverparenchym in dat segment daarmee <60 Gy). Een berekende dosis van 60 Gy op het gehele segment kan dus voldoende blijken voor een tumoricide dosis in de hyperemische zone. Als de dosis is gevonden waarbij 9 van de 10 patiënten de effectieve dosis van >120 Gy op de hyperemische zone krijgen, is het eindpunt van de studie bereikt. Er wordt gerekend met de doses 60 Gy, 90 Gy en 120 Gy in maximaal drie cohorten van maximaal 10 patiënten.

## TE VERWACHTEN RESULTATEN

De verwachting is dat er een gunstige dosisdistributie ten aanzien van het RFA-gebied zal zijn. Hierdoor zal de toedieningsdosis lager zijn dan 120 Gy holmium-166, berekend op het gehele segment. De invloed van RFA op de locoregionale perfusie is echter nog niet geheel duidelijk. Het verschil in recidiefpercentage tussen chirurgische resectie en percutane ablatie zal voor een groot deel toe te schrijven zijn aan locoregionale residuen en recidieven. Wanneer de adjuvante therapie in de juiste dosering op de juiste plaats komt, is er daarom een betere uitkomst voor de patiënt te verwachten ten opzichte van enkel RFA, resulterend in een lager recidiefpercentage.

## CONCLUSIE

Patiënten met 'early stage' HCC die niet in aanmerking komen voor resectie hebben een relatief hoge recidiefkans. Door de grootte van de laesie is het technisch lastig om een radicale ablatie uit te voeren van tumoren die in deze categorie vallen. Radio-embolisatie zou als adjuvante therapie bij RFA uitkomst kunnen bieden om locoregionaal beter te behandelen, leidend tot een reductie in het aantal recidieven. In de HORA EST HCC-studie wordt onderzocht in welke dosering radio-embolisatie het beste kan worden toegediend om een therapeutisch effect in de hyperemische zone rondom de RFA-holte te bewerkstellingen.

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