

Fluid-modulated Radiofrequency Ablation - An Ex-vivo Study in Porcine Liver

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vorgelegt von
Halina Müller
aus
Berlin-Spandau

Berichter: Herr Professor
Dr. med. Andreas Horst Mahnken

Herr Universitätsprofessor
Dr. med. Dipl.-Ing. Thomas Schmitz-Rode

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1. INTRODUCTION

The importance of minimally invasive, image guided techniques including percutaneous ablative therapies in cancer treatment has grown tremendously in the past decades. Used at first in a mainly palliative approach, techniques have improved in the past years to now include curative treatment of smaller tumours. These techniques hold several potential benefits compared to surgical resection and systemic chemotherapy. They impose less strain on the patient than traditional cancer treatments, which often include radical surgery and lead to reduced intra- and postoperative morbidity and mortality [1].

Furthermore, these techniques can be performed on an outpatient basis, leading to lower procedural costs than established surgical methods. Therefore minimally invasive techniques are an option for so-called non surgical candidates, whose reduced state of health does not make them eligible for radical open tumour resection. This can be in the form of palliative treatment, as well as a curative option for patients with smaller tumours, as an alternative to open or laparoscopic surgery [2].

The purpose of minimally invasive techniques is to kill tumour cells in situ, either to prevent further tumour growth and to reduce the tumour burden as in palliative treatment, or as curative treatment to destroy the whole tumour. With all these techniques the destructive agent is applied directly to the tumour, preventing a systemic exposure to toxic substances. Several methods have been established in the line of minimal invasive treatment. One method is the percutaneous injection of tissue toxic chemicals such as ethanol or acetic acid, directly into the tumour (percutaneous ethanol injection PEI) [3]. Another way is to introduce toxic or embolising agents into the blood vessels of the tumour through various catheter-based techniques (transcatheter arterial chemoembolisation TACE) [4]. A third method is the direct intratumoural delivery of lethal energy doses into the affected tissue. Examples of thermotherapy are hypothermic methods such as cryotherapy and hyperthermal methods which can be achieved through laser energy, microwave energy, or through radiofrequency [5].

The main fields of application for percutaneous ablative therapies are hepatic and renal malignancies, but also malignancies in the lung, breast and bone. Important and frequent hepatic malignancies are the hepatocellular carcinoma (HCC) and metastases which originate mostly from cancer of the colon or from breast cancer.

This study focuses on radiofrequency ablation (RFA) as a percutaneous method for local tumour ablation. The experiments carried out in this study aimed at improving the ablation process by enhancing thermal and electric tissue conductivity by introducing conductive fluids into the targeted tissue. Research and studies mostly focussed on RFA as a method to treat hepatic malignancies, which is why this study will concern itself mainly with HCC and hepatic metastases and their possible therapies.

1.1 Hepatocellular carcinoma

Hepatocellular carcinoma (HCC), is the most common malignant tumour worldwide, and makes up 90% of all primary hepatic malignancies [6]. Throughout the world, there are more than 300,000 new cases of HCC per year. Europe and the United States have the lowest incidence of HCC with approximately 3 new cases per 100,000 persons per year. The incidence in Africa and Asia, especially Southeast Asia, is much higher with approximately 50-150 new cases of HCC per 100,000 per year [7] (see Figure 1). Men fall sick with HCC more often than women with a ratio of 4-6:1 respectively.

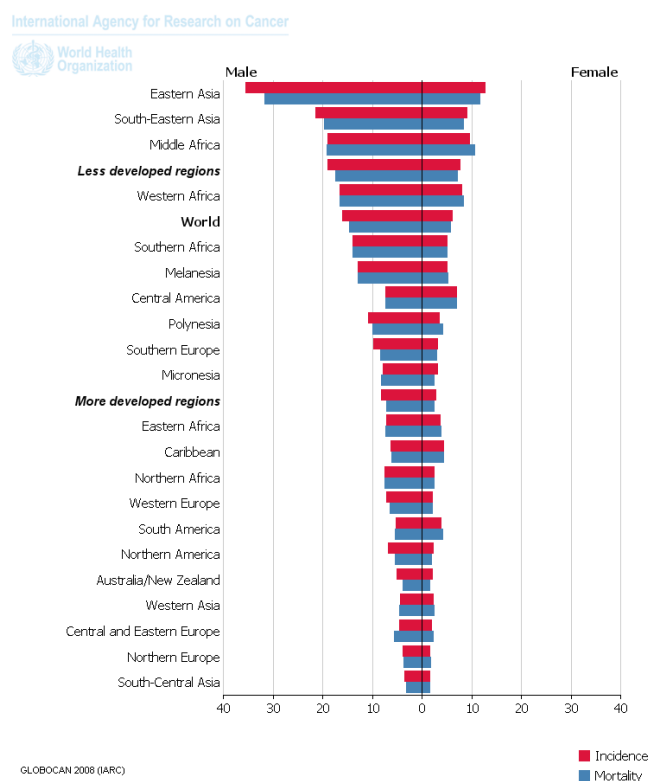


Figure 1: World wide incidence of HCC according to GLOBOCAN 2008 (IARC): Liver Cancer Incidence and Mortality World Wide in 2008 [8]

1.1.1 Aetiology and epidemiology of HCC

The aetiologies of HCC are well defined. The major factor contributing to the development of HCC is infection with the hepatitis B virus (HBV). Studies have shown that the risk of patients positive for the hepatitis B surface antigen (HBs-antigen) of developing HCC is 100 times higher than that of HBs-antigen negative patients [9]. Integration of the virus DNA into the genome of the host cell seems to be an initial trigger of HCC, together with other promoters such as inflammation and chemical co-carcinogens [10]. The earlier the time of infection with hepatitis B virus, the greater the risk of developing HCC. This is especially important in the case of neonatal infections with HBV. Another hepatic viral infection, which also provokes the emergence of HCC, is hepatitis C.

Aflatoxine, a toxin produced by the fungus *aspergillus flavus* is also able to provoke HCC, by inducing mutations in the p53 tumour suppressor gene [7]. This is especially a problem in tropical countries of the third world, where *aspergillus flavus* often contaminates whole harvests of wheat and peanuts. Other factors which can evoke HCC are alcohol abuse, abuse of steroids or anabolics, metabolic liver diseases such as hemochromatosis, α 1 antitrypsin deficiency and hereditary tyrosinaemia. Elevated body mass, especially in men [11], and diabetes mellitus [12] can also contribute to the emergence of HCC.

All aetiological agents share a common pathway through which the development of HCC is evoked. The aetiological agent causes an inflammation of the liver, which leads to an immune response followed by a regenerative process. The result is an increased cell turnover which, together with the oxidative DNA damage through inflammation and immune response, favours malignant transformation of hepatocytes. This may happen in the form of genetic alterations such as the inactivation of tumour suppressor genes, as in the case of aflatoxine which inactivates p53 [13, 14, 15]. Other possibilities are the activation of oncogenes, overall genomic instability, caused by DNA mismatch repair defects and impaired chromosomal segregation or the over- expression of growth and angiogenic factors, as well as telomerase activation [16, 17].

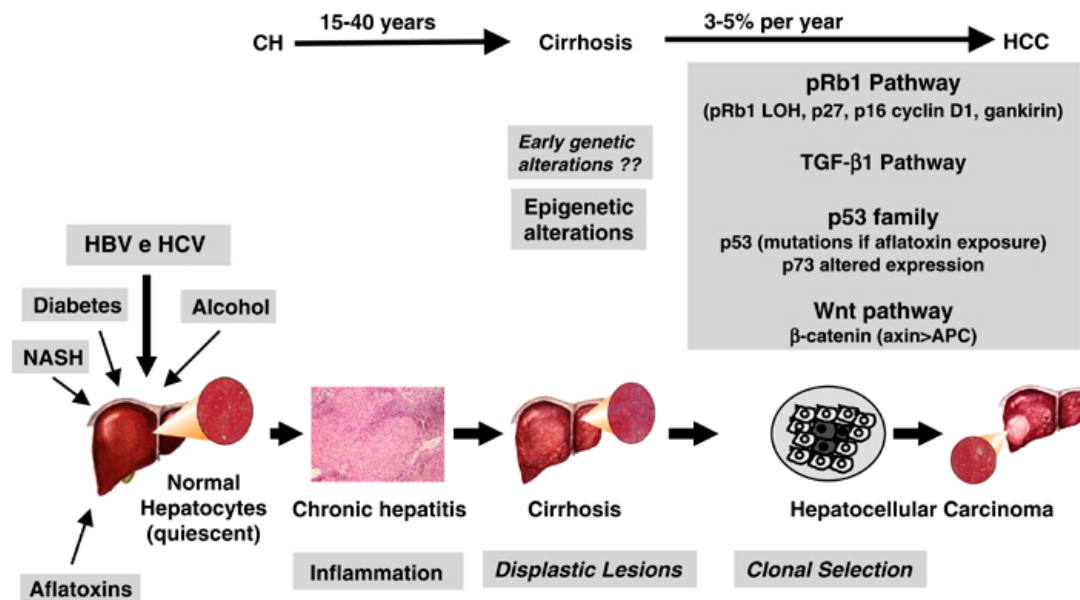


Figure 2: Pathway of pathogenesis of liver cancer according to M Levrero: Viral hepatitis and liver cancer: the case of hepatitis C [18]

HCC accounts for 70-85% of all primary liver cancers. This makes it the most common primary liver cancer worldwide [19]. The major clinical risk factor for developing HCC is liver cirrhosis. HCC develops in 70 to 90% of all cases with cirrhotic livers [7]. The International Agency for Research on Cancer maintains the GLOBOCAN database, which provides up to date estimates on the incidence and mortality of major cancers worldwide. According to GLOBOCAN it was estimated that in 2008, 85% of all liver cancer cases occurred in developing countries. Men fall sick with liver cancer more often than women, the male: female ratio in 2008 being 2.4 [8]. Even though the incidence of HCC is higher in African and South East Asian countries, due to higher infections rates with hepatitis B and also due to the higher contaminations rates with aflatoxin [20], the incidence of HCC caused by liver cirrhosis is higher in western industrialised countries. In Europe and the US the average age of patients developing HCC is above 40 years [21], whereas in Africa and Asia the majority of patients with HCC are younger. Elevated body mass, diabetes mellitus and alcohol abuse play a larger role in western countries, leading to the development of fatty liver and consequently liver cirrhosis, which in turn ends in hepatocellular carcinoma (see Figure 2).

The risk of developing HCC depends on the duration, activity and aetiology of the underlying disease. The most commonly used diagnostic methods are ultrasound, contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI). HCC is very difficult to diagnose without the use of imaging techniques as it has no specific symptoms, although it can be associated with an elevation of α -fetoprotein. At

the time of diagnosis, prognosis is often bad, with a life expectancy of less than 3 years. This can be explained by its high tendency to invade the liver blood vessels, which leads to seeding into the systemic blood circulation and to intra- and extra-hepatic metastases.

The aggressiveness of HCC makes it necessary to develop improved imaging techniques to discover it at an early stage and calls for improved curative therapies that put less strain on the patient than open radical surgery.

1.1.2 Treatment of HCC

There are five main categories of therapeutic options to treat HCC. Surgical resection and liver transplantation are therapies with a curative intention. Tumour resection is the treatment of choice in patients without liver cirrhosis and with a low rate of life threatening complications. This is the case in only 5% of HCC patients in western countries and in 40% of HCC patients in Sub- Sahara Africa and Asia [7]. In patients with concomitant liver cirrhosis, strict selection is required to avoid complications such as post-operative liver failure [22]. Factors indicating a high risk of postoperative liver failure, such as bilirubin and albumin concentration, platelet count, indocyanine green clearance [12, 23] and elevated serum concentrations of 7s collagen [12], need to be considered before tumour resection.

Survival of patients with HCC can be predicted by different classification systems, with common criteria such as tumour characteristics, functional status and liver function. In Europe the most widely used classification is the Barcelona Clinic Liver Cancer (BCLC) classification system [24], which focuses on the performance status, the tumour stage and the Child-Pugh score system (see table 1).

The Child Pugh score system is used to determine the severity of liver cirrhosis. Points are awarded according to the levels of serum-bilirubin, serum-albumin, INR, the amount of ascites determined per sonogram and the stage of hepatic encephalopathy which the patient appears to be in. According to the number of points achieved, the patient is then classified either as Child A (5-6 points) with a 1-year survival rate of 97.1%, Child B (7-9 points) with a 1-year survival rate of 82.1% or Child C (10-15 points) with a 1-year survival rate of 57.5% [25].

The BCLC classification system consists of 4 stages; early, intermediate, advanced and end-stage, as shown in table1 below and helps select the best therapeutic option for each candidate, for example radical resection for patients in the early stage, with only a solitary tumour [24].

Staging	Performance status	Tumour stage	Child-Pugh
(A) Early	0	Single <5 cm, 3 nodes <3 cm	A & B
(B) Intermediate	0	Large/multinodular	A & B
(C) Advanced	1–2	Vascular invasion extrahepatic spread	A & B
(D) End-stage	3–4	Any of the above	C

Table 1: Barcelona Clinic Liver Cancer Staging Classification of patients with hepatocellular carcinoma [26].

Even after successful tumour resection, the recurrence rate of HCC in patients with liver cirrhosis is very high. Local recurrence, as well as de novo tumours occur in about 70% of all patients within the first five years [7].

In September 2000 the European Association for the Study of the Liver (EASL) organized a conference on the clinical management of HCC in Barcelona. A result of this conference was a diagnostic algorithm designed to assist the clinical diagnosis of HCC. Patients with liver cirrhosis eligible for curative therapy should undergo an ultrasound and a control of serum alpha-fetoprotein (AFP) levels every 6 months [27].

If no nodule is detected in the ultrasound and AFP levels remain normal, surveillance ultrasound and AFP levels are carried out every 6 months. An increase in AFP levels without a detection of nodules in the ultrasound should be followed by a spiral CT scan. If nodules smaller than 1 cm in size are detected, ultrasound is to be repeated every 3 months. An increase in size means the lesion is most likely a HCC and further diagnostic measures such as CT, MRI or Angiography or, in the cases of lesions smaller than 2 cm, fine needle biopsies (FNAB), should be taken [27].

If liver nodules larger than 2 cm are detected further non-invasive diagnostic measures (restricted only to cirrhotics) should be taken. According to the conclusions of the Barcelona 2000 EASL conference, two coincident imaging techniques, such as US, spiral CT, MRI and angiography, both showing focal lesions larger than 2 cm in size with arterial hypervascularization are considered sufficient to diagnose an HCC. Alternatively one imaging technique showing a lesion larger than 2 cm with arterial hypervascularization in combination with raised AFP levels higher than 400 ng/ml, are also considered sufficient to diagnose an HCC. [27]

Liver transplantation is in principle the optimal therapeutic choice for HCC. It enables the removal of the tumour and in most cases, the removal of the underlying disease. However, the shortage of available donors and the stage of progression of the disease are undermining factors.

At present, the Milan criteria for liver transplantation require patients with HCC to have not more than one lesion with a maximum diameter of 5 cm, or not more than three lesions with a diameter of less than 3 cm each. Under these criteria the 5 year survival rate has been raised to 70% and more, with a tumour recurrence rate of less than 15% [28, 29]. Due to the fast progression of the disease, it is vitally important to shorten the waiting time for a donor organ to less than 6 months [7]. Due to a shortage in donors, this is often difficult to achieve. The longer the waiting time for transplantation, the higher the drop-out rate of patients, because as HCC progresses, it becomes more difficult to fulfil the transplantation criteria. In 2009 Facciuto et al carried out a study concerned with the survival rates of 157 patients within and outside the Milan criteria, after having undergone liver resection or orthotopic liver transplantation (OLT). After a median waiting time of 4 months for OLT, 21 patients (20%) dropped out due to tumour progression. Furthermore, the analysis showed cumulative drop out probabilities after 6, 12 and 24 months on the waiting list to be 34%, 57% and 86% respectively for patients outwith the Milan criteria. The cumulative drop out probabilities for patients within the Milan criteria after 6, 12 and 24 months were 2%, 12% and 58% respectively [30].

It has therefore become necessary to bridge the time to transplantation by inhibiting tumour progression and reducing tumour size. This can be done through locoregional therapies such as transarterial chemoembolisation (TACE) or radiofrequency ablation (RFA). The use of these methods to downstage tumours, prior to OLT, has shown to

improve the post-transplantation outcome and enables patients to better fulfil the transplantation criteria [31]. Yao et al. carried out a prospective study analysing the long term outcome of HCC down-staging in 61 patients. Downstaging was successful in 71.5% of these patients, and 57.4% could subsequently undergo OLT. In a 25-month follow up, none of these patients experienced a tumour progression [32].

Percutaneous interventions work best for small HCCs [33, 34, 35]. Transarterial interventions such as transarterial embolisation or chemoembolisation, are the most widely used treatments for unresectable HCCs that may not be effectively treated through percutaneous interventions [36, 37, 38, 39]. Whereas percutaneous interventions are potentially curative, transarterial interventions usually result only in palliation and lead to improved survival.

To reduce the tumour burden as much as possible and to achieve the best possible outcome, transplantation centres often combine several locoregional therapies. The common combinations are TACE and RFA, TACE and PEI or TACE and liver resection [40]. In a study carried out by Xiand-Jie et al, the combination of TACE and RFA showed a better 3-year overall survival than the use of just one method alone (92% vs 64%) [41].

Radiotherapy and drugs make up the fourth therapeutic option for HCCs. Radiotherapy plays only a minor role in the treatment of primary HCC. Several therapeutic techniques such as intrarterial injection of 131-iodine labelled lipiodol [42] or high dose proton beam radiotherapy and Yttrium-90 microsphere treatment have been considered [43, 44, 45], but further clinical evaluation is still needed. Most chemotherapeutic agents such as tamoxifen [46], octreotide [47] and interferon [36] have not shown to be effective during randomised controlled clinical trials [48, 46, 49].

Recently, the tyrosine kinase inhibitor sorafenib has been approved as the first line therapy of patients with advanced HCC. It suppresses tumour cell proliferation and tumour angiogenesis [50] and can therefore be applied to HCC with a highly vascularised tumour [51]. Results in animal studies on the effect of sorafenib adjuvant to RFA show that sorafenib promotes necrosis and leads to larger coagulation volumes [52].

There are several other substances which may prove to be effective in the future. However, none can yet be recommended outside clinical trials and clinical evaluation is still pending.

Finally, the fifth therapeutic option for HCCs contains gene and immune therapies based on suicide, cytokine and antiangiogenic genes of hepatocytes and DNA vaccination with tumour specific genes [53] and/or oncolytic viruses [54]. The purpose here would be to immunise liver cells against tumour cells and to allow the liver to fight HCCs through immune response. These options are still experimental and have not yet been fully explored.

At present the clinical situation depends on early detection of HCC through imaging techniques with an option of early curative treatment through open surgery or percutaneous interventions. Primary prevention focuses on acquired liver diseases and their early treatment and on preventing the liver disease from progressing into liver cirrhosis. Secondary prevention focuses on the prevention of local recurrence or de novo tumour development after successful surgical resection [7].

1.2.0 Hepatic metastases

The second important indications for radiofrequency ablation are hepatic metastases. These often originate from colorectal cancer or from breast cancer as the primary tumour.

The liver is a vital organ; two of its major functions are detoxification and synthesis of vital proteins such as albumin and coagulation factors, for example fibrinogen (see Figure 3). As it is situated between the portal-venous and the systemic blood circuit, it is the main localisation for metastases of colorectal cancer [55].

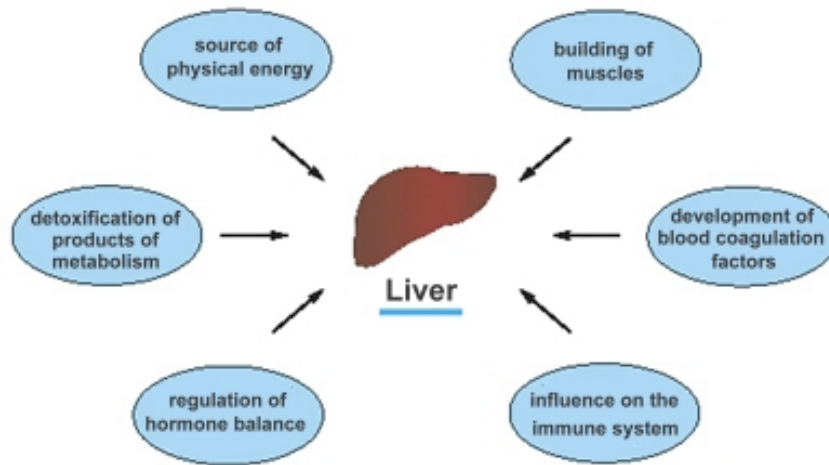


Figure 3: Diagram showing vital functions of the liver according to www.klinikum.uni-heidelberg.de/Liver [56].

1.2.1 Hepatic metastases of colorectal cancer

The colorectal carcinoma has the second highest incidence in Europe, after bronchial cancer in men and breast cancer in women. In 90% of all cases, the carcinoma occurs in patients over 50 years of age [57], but earlier occurrences have also been observed. There are several aetiologies out of which colorectal cancer can evolve. Genetic factors causing a disposition towards colorectal carcinoma are familial adenomatous polyposis (FAP), which causes a carcinoma to develop in nearly 100% of all patients, hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome and a family history of colorectal cancer [58].

Further risk factors, contributing to the development of colorectal cancer are: a diet which is low in fibre and high in fat and red meat, smoking and alcohol, and an age above 40 years [57].

Patients suffering from inflammatory bowel disease, such as ulcerative colitis, also have a higher risk of developing colorectal cancer, as well as patients who suffer from colorectal adenoma [57].

The colorectal carcinoma is an adeno-carcinoma, which develops out of a dysplasia of the intestine's epithelial lining. In 90% this dysplasia occurs in the form of an adenoma, which then develops into a carcinoma during a time span of up to 10 years. This is also known as the adenoma/dysplasia-carcinoma sequence (see Figure 4) and is caused by several genetic changes, such as mutation or loss of the APC-tumour suppression gene, the K-RAS-oncogene or the DCC-tumour suppression gene [59].

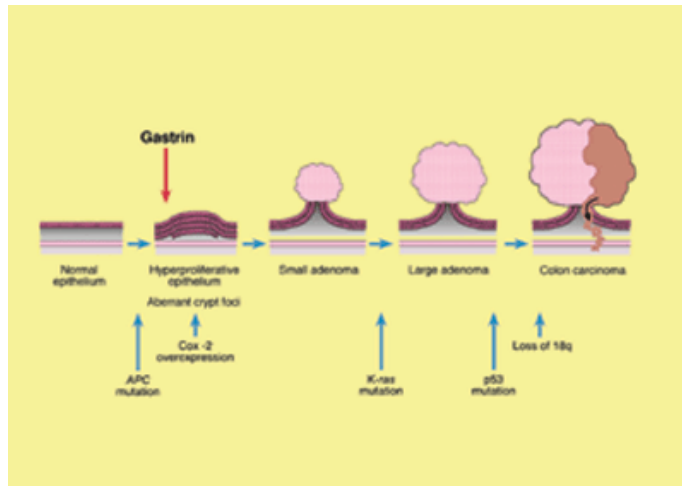


Figure 4: Diagram showing adenoma/dysplasia-carcinoma sequence according to Takhar A.S., Eremin O., Watson S.A.: The role of gastrin in colorectal carcinogenesis [60].

There are several classification systems which can be used in order to stage colorectal carcinoma. The most common staging system is the TNM-classification (tumour, nodes, metastasis), where T shows the degree of invasion into the intestinal wall, N shows the degree of lymph nodes involved and M shows the degree of metastases. The UICC (Union International Contre le Cancer) classification is derived from the TNM classification and is divided into stages 0-IV, a higher number indicating a more advanced form of the cancer with a poorer prognosis.

The oldest staging system is the Dukes classification, with stages A to D. Stage A means that the tumour is confined within the intestinal wall, in stage B it has invaded the intestinal wall, stage C refers to regional infiltration of lymph nodes and D to metastases [61].

Treatment can be curative in early stages of the cancer, through radical en-bloc resection of that part of the intestine which contains the tumour. In advanced stages, T1-3, N1-2, M0 or T4, surgery can be preceded by neo-adjuvant chemotherapy using 5-Fluoruracil. Patients with colorectal cancer in Dukes stage C, have shown to benefit from adjuvant chemotherapy after surgery, using 5-Fluoruracil and folic acid [62].

The colorectal carcinoma spreads through the paraaortal, pelvic and inguinal lymph nodes or via the blood vessels into the lung and liver. At the point of diagnosis, 20% of the patients have developed distant metastases, for example in the liver [63]. These may go unnoticed by the patient, but can also cause jaundice through bile duct

obstruction and abdominal pain, by putting pressure on the liver capsule. Hepatic metastases can either be removed surgically, with curative intention when there are only few or single lesions, or ablated through minimally invasive methods such as RFA [64]. In a retrospective study on patients having undergone CT-guided RFA, Mahnken et al showed RFA of liver metastases to be an effective therapeutic option for patients who are not susceptible for surgery. Complete ablation could be observed in 86.7% of all lesions. The median survival after RFA was 46 months with a calculated 1-, 3- and 5-year survival of 89%, 66.2% and 41.8% respectively [65].

1.2.2 Hepatic metastases of breast cancer

Breast cancer is a malignant tumour which originates from the epithelial cells of the mammary gland and lactiferous ducts. It makes up about 25% of malignancies occurring in women and is the most common cause of death in women in the western world between 35 and 55 years of age. The yearly incidence of breast cancer is 80:100,000; this means that every 8th woman is at risk of developing breast cancer at some point of her life [66]. Breast cancer rates worldwide differ according to ethnicity and socioeconomic class of the population. Social groups with higher income and higher levels of education are at a higher risk to develop breast cancer. Women belonging to white ethnic groups show a higher tendency to develop breast cancer than women belonging to non white ethnic groups [67]. According to the SEER registries from 1998-2002, white women in the US have an annual age standardised rate of 189/100,000 for women older than 25 years. Another example representing the breast cancer rate in Europe is the annual age standardized rate of 152/100,000 in Scottish women older than 25 years [66]. By comparison, age standardized rates for breast cancer in women older than 25 years in India account to 52/100,000 and in China to 67/100,000 as well as 45/100,000 in Korea [66]. An explanation for this is the varying exposure to risk factors, which promote breast cancer. Women of higher socio-economic groups tend to be older at first birth, present a lower parity, are older at entering menopause and are more likely to use menopausal hormone therapy, than women of lower socio-economic groups [68].

The exact aetiology of breast cancer is still unknown; however there are a few risk factors which are believed to positively affect its development. To date, two major genes have been identified to play an active role in breast cancer. Women carrying the

breast cancer susceptibility gene 1 (BRCA1) or breast cancer susceptibility gene 2 (BRCA2) have shown to have a life time risk of developing breast cancer between 60 and 85% [69]. However, these genes account only to 2-3% of all breast cancers [69]. Another risk factor is long term hormone substitution using ovulation inhibitors. The Nurses Health Study showed that hormone substitution lasting for more than five years increases the risk for breast cancer by a factor of 1.29 [70]. Other factors are an early menarche and late menopause, resulting in a longer exposure to oestrogen, breast cancer in the close family, regular consumption of more than 20 g of alcohol per day, late primigravida, as well as a diet rich in meat and fat [71]. The highest risk for developing breast cancer occurs in those patients who have already had breast cancer in the contralateral breast, the risk is here increased 2-6 fold [72].

Breast cancer is most commonly found in the upper, outer quadrant of the breast. 5-25% of all cases show a multicentric growth and 2-11% of all cases show a secondary carcinoma in the contralateral breast [72]. Dissemination already occurs at an early stage of tumour growth and can be both lymphogenic and haematogenous. Lymphogenic metastasis occurs into the regional lymph nodes of the axilla, whereas haematogenous metastasis is most commonly found in the skeletal system, lung, liver and brain.

The preferred surgical approach to breast cancer is a mastectomy or lumpectomy followed by adjuvant radiation or chemotherapy. Adjuvant radiation after lumpectomy has shown to lower the risk of a local recurrence from 30% to 5%. Substances used in chemotherapy of breast cancer are, for example, taxanes, gemcitabine, vinorelbine and capecitabine [73]. They are administered in form of polychemotherapy and are especially effective in premenopausal patients who are most often hormone receptor negative. Postmenopausal patients often have hormone receptor positive cancer and are therefore eligible for an endocrine adjuvant therapy using tamoxifen. An option to tamoxifen is the use of aromatase inhibitors such as anastrozole and letrozole, which inhibit the production of oestrogen [74].

Of patients with metastatic breast cancer, 15% have liver metastases [75]. Of these patients about one third has only the hepatic lesion and no other site of metastasis. Studies have shown that the resection of solitary hepatic metastases improves survival as it reduces the tumour burden of the patient and makes them more susceptible for adjuvant chemo- or hormone therapy [76]. Hepatic resection can either be in the form of

surgical hepatectomy or using RFA. Lawes et al conducted a study which showed the 30 month survival after RFA to be comparable to that after hepatectomy [77]. The patient group eligible for hepatectomy and RFA is similar as these techniques are only applicable to patients who have the disease confined to the liver or liver metastases in addition to a stable extrahepatic disease. As RFA is simple to perform, has comparatively few complications and can be carried out on an out-patient basis, it is an acceptable alternative treatment to hepatectomy.

2. MINIMALLY INVASIVE TECHNIQUES IN TUMOUR THERAPY

2.1 Percutaneous ethanol injection

Percutaneous ethanol injection (PEI) is used as an alternative to sectional liver resection in HCC, when the tumour is a single, localized mass. Under ultrasound or CT guidance, the liver is punctured through the skin and pure alcohol, for example ethanol, is injected into the tumour (see Figure 5). Ethanol is hyperosmotic and acts by drawing water out of the cells, causing denaturation of protein structures, which leads to death of the tumour cells. In addition it also causes vascular thrombosis in small vessels and reduces blood supply to the tumour and causes ischemia. In a similar way acetic acid can be used instead of ethanol. Several injections may be necessary to completely destroy the tumour. This procedure can be carried out under local anaesthetic and on an outpatient basis [78].

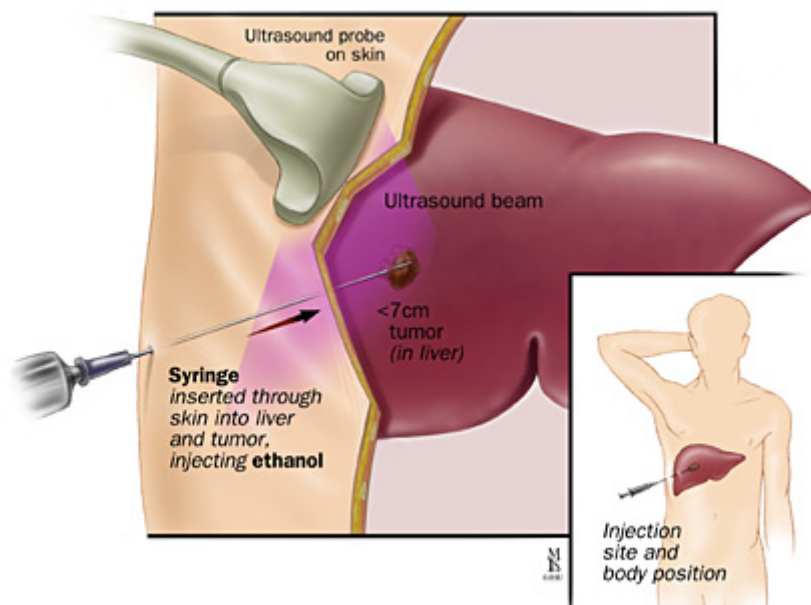


Figure 5: PEI according to www.hopkinsmedicine.org [79]. Please note that the indication of PEI for liver tumours <7 cm is now obsolete, as the indication is now limited to tumours <3cm [80].

Indications for PEI are small HCCs which are not larger than 3 cm in greatest diameter. Eligibility criteria also include no more than three lesions and no portal or segmental invasion adjacent to the tumour [80]. Larger lesions may be treated but require multiple needle insertion and it is not certain whether the ethanol diffuses evenly through larger

tumour masses, leaving the risk of remaining with intact tumour tissue. Hepatic metastases have shown to have a higher tendency to fibrosis than HCCs, causing a certain resistance to alcohol diffusion on repeated ethanol injections [81]. HCCs often have a pseudocapsule or fibrous capsule [82] which enables the alcohol to distribute homogeneously within the targeted area.

The most common adverse effects of PEI are fever, pain and a feeling of alcohol intoxication. Serious complications can occur from the alcohol's tendency to track along blood vessels adjacent to the targeted region, thus reaching healthy liver tissue or healthy neighbouring organs [81].

In the 1990's the use of PEI showed promising survival rates which were equivalent to that of surgical resection [81], but in the terms of cost effectiveness and duration as well as physical strain imposed on the patient, RFA has shown to be a more effective treatment strategy [80]. However in more recent studies, surgery is still proposed as the gold standard for small hepatic tumours and for patients with few comorbidities and a good functional liver reserve [83].

2.2 Catheter based techniques

Catheter based techniques are based on the principle of targeted delivery of chemotherapeutics and embolic agents directly into the tumour, using the fact that liver tumours are preferentially supplied through the hepatic artery, in contrast to normal liver parenchyma.

Transcatheter arterial embolisation (TAE) involves gaining percutaneous access to the hepatic artery, usually by introducing a catheter into the right femoral artery and threading it through the abdominal aorta, through the celiac trunk and through the common hepatic artery into the proper hepatic artery [84]. After an angiogram has been performed to identify the smaller branches of the proper hepatic artery which supply the tumour [84], smaller catheters are threaded into these.

During TAE the blood vessels supplying a tumour are selectively embolised by injecting embolising agents through the catheter into the vessel (see Figure 6). This then leads to a disruption of blood flow and induces tumour ischemia. The most commonly used embolising agents are lipiodol, gelfoam, or degradable microspheres, for example

gelatine-galactose particles, which cause a temporary occlusion of the targeted blood vessel.

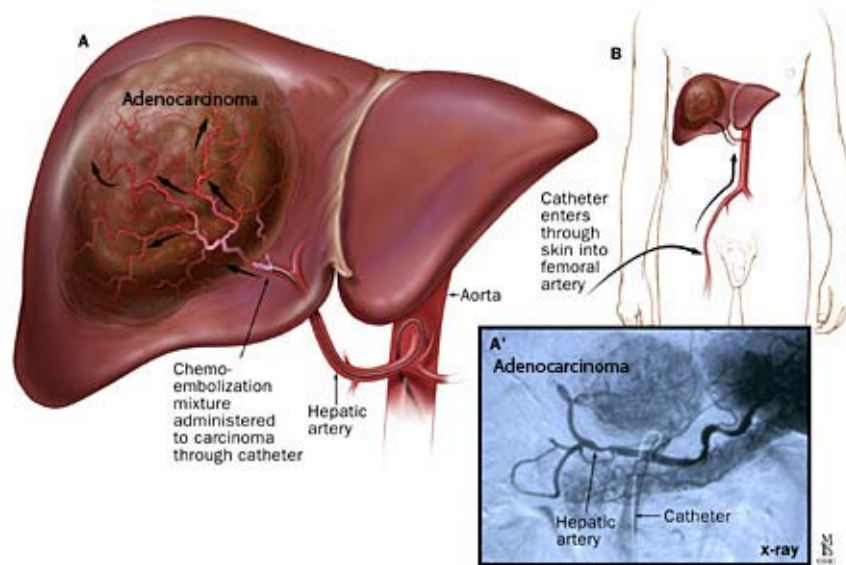


Figure 6: TAE and TACE according to www.hopkinsmedicine.org [85].

Transcatheter arterial chemoembolization (TACE) was first introduced in 1980 [2] and is based on the procedure used in TAE. A chemotherapeutic agent, the most widely used is doxorubicin [2], is injected through the catheter directly into the artery which feeds the tumour. Usually lipiodol [2], an iodinated ester derived from poppy-seed oil, is added for emulsification of the drugs. Tumour tissue in the liver is known to selectively retain lipiodol making it an ideal drug carrier and tumour seeking agent.

At the end of the procedure some degree of embolization of the tumour feeding arteries is performed. Here a variety of embolic agents can be used, for example polyvinyl particles [2], which reduce arterial blood flow into the liver by blocking the vessels. However, it is necessary to retain a certain minimum blood flow to allow TACE to be repeated [86, 87]. Also, there are some considerations concerning the possible upregulation of several molecular factors such as vascular endothelial growth factor VEGF to be made. Hypoxia, as caused by a sudden disruption of the blood flow, may well induce such an upregulation of VEGF or hypoxia-inducible factor-1, which in turn stimulate tumour growth, metabolism and invasion [88, 89].

The benefit of TACE is that it allows precise delivery of chemotherapeutic agents into the tumour mass, followed by selected embolisation of arterial vessels which supply the

tumour [90, 91]. This reduction of blood flow is particularly important as it causes direct ischemia within the tumour, enlarging the tumour destruction. Furthermore, due to reduced blood flow within the tumour, drug concentration within the mass is enhanced and systemic toxicity is reduced [2], because the drugs will remain mainly within the tumour bed.

The most common undesired side effect of TACE is known as postembolisation syndrome and occurs in 3.8 to 10% of all procedures [2]. It consists of pain in the right upper quadrant of the abdomen, where the liver is situated, nausea and vomiting, persistent fever and elevation of liver enzymes. This postembolisation syndrome is usually transient and subsides after seven to ten days. If the syndrome occurs, it is usually treated medically according to the symptoms. Other side effects can usually be accredited to the toxicity of doxorubicin and include alopecia, renal dysfunction, and cardiac toxicity [2].

TACE is used extensively as palliative treatment of hepatocellular carcinoma, but it is also used as bridge to OLT [92, 93, 94], where the entire diseased liver is replaced by a healthy donor organ. The goal is to slow down tumour progression and to extend the life expectancy of patients until a suitable donor liver is available. An advantage of TACE is that treatment can be repeated until the desired effect has taken place.

Recent studies have focused on improving drug delivery to the tumour tissue [36], with the aim of sparing healthy liver tissue surrounding the tumour while allowing maximum concentration of chemotherapeutics within the tumour itself. Drug loaded carriers, consisting of polyvinyl-alcohol-based microspheres, loaded with doxorubicin have entered phase I and II of clinical trials in Europe, United States and Japan [95]. Results in unresectable hepatocellular carcinoma so far appear to be promising, showing reduced adverse effects and better tumour response in comparison to conventional TACE [95, 96]. These microspheres are only 100 to 300 μm in diameter [2] and are able to lodge distally in small tumour blood vessels and slowly elute the doxorubicin, leading to increased tumour response. However, these trials will first need to be completed and final results need yet to be evaluated.

2.3 Thermal tumour ablation

Another approach to percutaneous ablative therapy is the use of high thermal energy doses which are applied directly into the tumour, known as thermal tumour ablation. This can either be achieved by applying cold to the tumour, literally freezing it, or by applying heat.

Cryoablation uses extreme cold to kill tumour tissue. During this procedure, hollow needles, or cryoprobes, are inserted into the tumour and are perfused with thermally conductive gases or fluids. These thermally conductive fluids or gases are cooled down in a peripheral freezing unit. As they circulate through the cryoprobe, the probe's tip cools down and removes heat from the adjacent tissue. This leads to several effects in the affected tissue. The tissue freezes, causing ice crystals to form within the cells, which disrupts cell structure and cell metabolism [97, 98]. The cold also induces blood coagulation, thus blocking the blood vessels supplying the tumour, which also leads to cell death through ischemia [98]. In addition, the cooling of the tissue itself induces apoptosis or programmed cell death, ending in tumour destruction [97].

Cryoablation is used mainly for solid malignancies such as prostate cancer or malignancies found in the kidneys. However, it is also applied for solid malignancies in the lung, breast and liver [99].

Other percutaneous thermal ablation therapies use heat to destroy tumour tissue. Laser induced thermotherapy (LITT) works with high energy laser radiation which is delivered through optical fibres straight into the targeted lesion. The light which is emitted by the laser generator is very similar to the input signal in terms of wavelength, phase and polarisation. This means that laser light is coherent, collimated and monochromatic. A laser is composed of an active laser medium and a resonant optical cavity. In the active laser medium, atoms are charged either chemically, optically or electrically. Laser light is generated when electrons fall from a higher to a lower energy level. The energy lost during this process is emitted as light. The resonant optical cavity contains a coherent beam of light between reflective surfaces more than once before being emitted from the output aperture [100]. LITT is generally performed using optical radiation in the range of infrared light using a wavelength range from 700 to 2000 nm.

The distribution of light in tissue is characterised by three processes: Absorption, scattering and bending. Photons are absorbed on an atomic level in different depth of the tissue and are converted into intra- and inter-molecular energy. This in turn leads to a rise in temperature in the tissue of up to 150 ° C [2], causing a coagulative necrosis. Lasers used in LITT are Neodymium:Yttrium-Aluminum-Garnite (Nd:YAG, with a wavelength of 1064 nm) [2] as well as visible gas lasers like Argon with a wavelength of 514 nm. Laser induced thermotherapy can be monitored in real time under MRI, allowing more precise placement of the optical fibres and a more accurate assessment of the actual thermal damage. Real time MRI temperature monitoring also enables observation of temperature development within the targeted tissue area [101].

Transmission of laser energy into the tissue is achieved by using laser fibres or optical fibres. Quartz fibres have proven to be the most effective for the transmission of laser light during LITT as they are heat resistant and flexible. Different designs in applications exist; among them are tipped fibres, ring mode applicators and zebra applicators [101]. Bare tipped fibres have been replaced by cooled diffuser tip applicators. Diffusion means that laser light is emitted circumferentially into the adjacent tissue, cooling means that the applicator contains a cooling medium to avoid tissue charring directly adjacent to the tip [102]. Charring or carbonization of tissue reduces the conductive qualities of the tissue and limits energy appliance. Applicators with a closed circuit for the cooling medium have a larger diameter than applicators which are open at the tip, allowing the cooling medium to evaporate into the tissue.

LITT is used for the treatment of recurrent or unresectable liver tumours, but also for the treatment of lung tumours or any other tumour found in the soft tissue such as the abdomen, the head or neck or the retroperitoneum [102].

Microwave coagulation therapy (MCT) is a form of thermoablation which uses microwave energy to destroy the tumour. Microwaves are electromagnetic waves with a frequency of 915 MHz to 2.45 GHz [103]. It is the most recent development in the field of thermoablation and is used mainly in China and Japan. A thin microwave antenna is placed into the lesion under imaging-guidance, for example using CT. Microwave energy is then emitted into the tissue through the non-insulated tip of the antenna. The electromagnetic waves agitate water molecules in the cells into rotation, which produces friction and therefore heat. This eventually leads to cell death and coagulation necrosis

of the tumour tissue. The coagulation necrosis has the form of a column or of a sphere, depending on the type of antennae used and the type of energy applied. MCT achieves higher intratumoural temperatures and faster ablation times than RFA, but leads to smaller coagulated areas [2].

So far indications for MCT are small hepatic tumours which are not larger than 2 cm in diameter [104], as well as bone sarcomas [105].

2.4 Radiofrequency ablation (RFA)

This study focuses on methods to improve RFA. Therefore the effects of radiofrequency waves on tissue, its clinical importance and the different methods of applying RFA will be described in detail.

RFA is a form of thermotherapy using a high frequency alternating current to create heat induced coagulation necrosis. The term radiofrequency alludes to the frequency of the electromagnetic waves used, ranging from 10^4 to 3×10^{12} Hz. Clinically a frequency of 350 to 500 kHz is used. This frequency is high enough (>20 kHz) to stimulate molecules and cause frictional molecular heating and low enough (<20 MHz) to be confined to a specific area without causing mass radiation, neuromuscular reactions or electrolysis. Radiofrequency waves are also non ionizing, in contrast to waves of a much higher frequency such as x-rays, making them safe to use [106].

The mechanism of RFA is basically a closed loop circuit with the individual components being placed in series. A needle electrode is inserted into the tissue, under US, CT or MRI guidance [107] and an alternating current flows from a generator through the electrode and via the ions of the tissue towards a dispersive electrode or grounding pad. The needle electrode and the dispersive electrode are active whereas the patient acts as a resistor. The high frequency range of the alternating current creates an electrical field between the active and dispersive electrode, or between the two active electrodes in bipolar systems, which oscillates with radiofrequency (see Figure 7).

The constantly changing direction of the electrical field in combination with the relatively high resistance of the tissue, results in ionic oscillatory agitation as ions in the tissue try to follow the current. Ionic agitation creates friction and thus heat, which can be tightly controlled through modulation of the energy deposited into the tissue. The use

of a grounding pad or a dispersive electrode decreases local current density and electric resistance in the tissue, confining heat production to the vicinity of the electrode [108, 106].

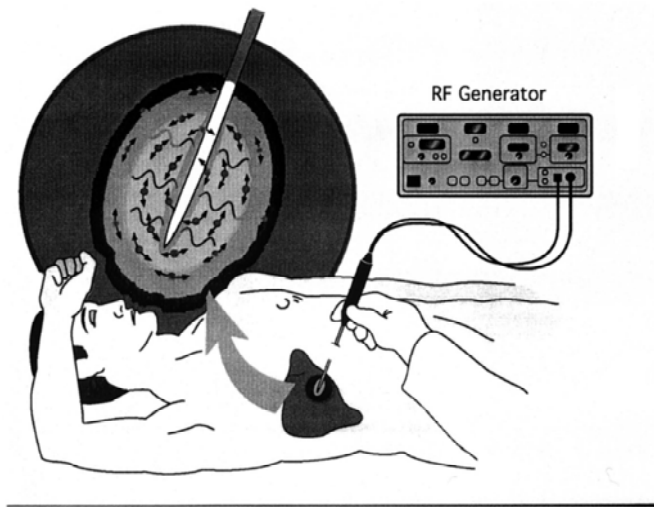


Figure 7: RFA performed on a patient according to Goldberg et al.: Image-guided Radiofrequency Tumour Ablation: Challenges and Opportunities—Part I [109].

2.5 What happens to the tissue during RFA?

Thermal injury begins at 42 ° C [110], by inducing cell degenerations similar to apoptosis or programmed cell death. At this temperature, cells also become more susceptible to chemotherapy or radiation. An increase in temperature reduces the time to reach cell death. Exposure to 45° C for several hours already produces irreversible cell damage [111, 108]. Temperatures between 55 ° C and 60 ° C shorten the time until irreversible cell damage is achieved down to 6 - 10 min [108]. At temperatures greater than 60 ° C instantaneous protein denaturation occurs, especially in the DNA's acid-histone complex and mitochondrial and cytosolic enzymes [112]. However, temperatures greater than 100° C have shown to cause tissue boiling, vaporisation and thus carbonization and charring [106, 108]. This leads to a massive rise in impedance in the tissue and works as an insulator, preventing further application of energy and thus limiting thermoablation. Therefore essential objectives of RFA are the achievement and maintenance of temperatures between 60 and 100 ° C, throughout the ablation session and the ablation volume for at least 4 to 10 min [113, 114]. Clinical data suggest longer heating times of 10 to 30 min due to slower thermal conduction in in-vivo tissue [108].

Temperature in the tissue is inversely proportional to the distance from the electrode, ($T \sim 1/r^4$, T being the temperature, r the radius around the electrode) [115]. Destructive

thermal heat is only effective within a volume with a maximum diameter of 2.2 to 2.4 cm around a single needle-like electrode. Its delivery into the tissue is therefore quite precise and controlled, making thermal damage to tissue further away from the electrode rather unlikely.

Heat efficacy is defined through the amount of heat produced and the amount of heat lost. The relationship between these factors has been characterised as “bio heat equation” [116]. Heat production is defined by the intensity and duration of the energy deposit into the tissue. Heat loss, with regard to the electrode, is measured by heat conduction or diffusion away from the tip. The main heat loss occurs through heat convection away from the ablation area, by blood circulation [108].

The pathophysiological processes of thermal coagulation necrosis induced by RFA differ from those in usual coagulation necrosis. Necrosis is a pathological cellular or tissue death in a living organism, irrespective of cause. It is defined by the sum of morphologic changes indicative of cell death, caused by the progressive degradative action of enzymes. This results in membrane dysfunction and subsequent internal release of various enzymes, leading to degradation of cellular structures. This is microscopically observable as nuclear karyorrhexis due to membrane and chromatin fragmentation and as cytoplasmatic eosinophilia due to break down of cytosolic enzymes and RNA. The entire process takes hours to days followed by a few weeks of tissue repair [106].

Under temperatures of 60 to 100 ° C, RFA produces an area of immediate coagulation in the tissue directly adjacent to the electrode and in the periphery of the lesion. The processes of usual coagulation necrosis do not occur. Cell structures and enzymes undergo an instantaneous thermal fixation, an effect equivalent to that of formalin [106]. Microscopically, tissue structures and cytosolic details appear well preserved and cannot be identified as cell death. This is known as the “ghost phenomenon” or “thermal fixation” [117, 118, 119, 120]. Therefore it is also possible to perform biopsies for patho - histological investigations after RFA has already taken place.

A decrease in microvascular tumour perfusion during RFA and a shutdown of vascular perfusion after RFA have positive effects on the ablation of tumours, because the heat induces endothelial swelling and microvascular embolization [117, 118, 119].

Experimental studies also indicate that heat plays a role in activating tumour-specific T-lymphocytes, creating immunity against cancer cells [121, 122].

Major complications during RFA are liver abscess, tumoural cell seeding, haemotorax, gastric bleeding, haemoperitoneum, liver infarction and cutaneous burns [123]. Rossaint et al observed that RFA under intravenous anaesthesia produces inflammatory and endocrine activity similar to systemic inflammatory response syndrome (SIRS). This could promote the development of sepsis or multiorgan failure in especially fragile patients [124].

2.6 Different application modes of RFA

Experiments with RFA in liver tissue started in 1990 [125, 126], using a monopolar electrode and a grounding pad. A radiofrequency electrode is typically an insulated metal shaft with a non-insulated, exposed and conductive tip, allowing direct electrical contact with the targeted tissue volume.

Monopolar electrodes require a ground pad or dispersive electrode which is usually attached to the patient's thigh. Electricity travels from the active electrode placed in the tumour, through the patient's tissue, to the grounding pad. This method was originally geared towards neurosurgical and cardiac ablation as they induce small but precise foci of tissue destruction [127, 128]. The first attempts to use RFA for the ablation of hepatic tumours were disappointing, because rapid rise in impedance due to carbonization, allowed only small lesions with a diameter of 0.6 to 1.7 cm to form [125, 126, 129]. Experiments increasing the length of the probes resulted in longer, sausage shaped coagulation volumes [129]. However, most hepatic tumours are spherical, making this modification of the needle electrodes unsuitable. This led to the development of different electrode modifications, with the aim of enlarging the lesions.

Multiprobe arrays were initially achieved by repeatedly inserting multiple RF electrodes into the tissue [130]. This process showed to be time-consuming and difficult to employ in a clinical setting, because multiple overlapping treatments have to be applied precisely to destroy the tumour in all three dimensions [131].

This led to the development of freestanding arrays [132], which reduced the duration of therapy and led to increased coagulation volumes. In a clinical setting, however, it was still difficult to place all probes precisely [132].

The development of umbrella shaped electrodes with retractable multiple hooked array or tines (expandable electrodes), helped to overcome this limitation (see Figure 8). They consist of multiple, curved, stiff wires which can be extracted from a single 14 or 16 gauge cannula [109]. Once the needle has been placed correctly into the tissue, the electrodes are released from the probe. At first, multiprobe arrays consisted of 4 to 6 hooks, making multiple applications necessary to achieve a sufficiently large necrosis [109]. At present multiple expandable electrodes contain up to 12 hooks, creating lesions which are clover leaf or daisy shaped, but fuse to spherical coagulations as heating continues. The effect created in the tissue by expandable electrodes is explained by the Faraday cage effect. Coagulation starts around the individual tips and then forms tubes. These then first fuse centrally, forming a clover leaf structure, and then in the periphery, forming more or less a sphere [106].

Experiments carried out by Mahnken et al in 2002, using the LeVeen umbrella shaped electrode, achieved a maximum coagulation volume of 9.2 x 8 x 10.5 cm [133].



Figure 8: Picture showing the tip of an expandable electrode (LeVeen, Boston Scientific) according to Dodd II G.D et al.: Minimally Invasive Treatment of Malignant Hepatic Tumours: At the Threshold of a Major Breakthrough [131].

Another modification to achieve larger coagulation volumes is the development of bipolar electrodes. At the beginning a second electrode needle was inserted parallel to the first, enabling the alternating current to flow between them [106]. Heat was not only generated around the individual needle but also in the space between the two electrodes

[109, 134]. This led to more homogenous heating, creating larger, elliptic zones of ablation. Difficulties arose in placing the two electrodes at an exact parallel distance percutaneously into the liver, which is one reason why this method was soon abandoned [130]. Celon first developed a probe where both electrodes are integrated into one needle (see Figure 9). This eliminated the need for a grounding pad and the risk of burns [109]. It also made it possible to use RFA on patients with a pacemaker, as the current-flow through the patient is limited to the vicinity of the electrode.

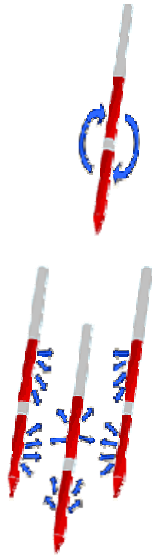


Figure 9: Diagram showing current flow in a bipolar electrode according to www.celon.de [135].

Internally cooled electrodes possess two lumina within the applicator [136, 137], allowing closed perfusion with a chilled perfusate, cooling the tip down. [106]. This leads to a heat sink effect, removing heat closest to the electrode, thereby reducing the risk of charring directly adjacent to the active electrode tip [109]. The warmed perfusate which has passed the tip is then removed to an external deposit [106]. In two ex-vivo livers studies energy deposit in tissue and achieved necrosis was significantly greater with cooling than without [136].

Cluster arrays, for example the cool tip cluster electrode from Radionics [138], are three closely spaced, internally cooled electrodes which offer the potential of large volume ablation. A sensor at the tip of the electrodes allows continuous measurement of temperature and impedance. Goldberg et al. demonstrated that cluster arrays spaced 1 cm apart produce larger coagulation volumes than arrays with electrodes placed 2 cm apart [132]. Apparently, at this distance apart, electrodes create a single RF field of a

significantly larger diameter, than could be created by overlapping fields of individual 18 gauge probes.

In a second series of experiments, electrodes were placed 0.5 cm apart, into specimen of ex-vivo liver, in-vivo liver and into specimen of muscle-tissue and energy was applied for 5 to 60 minutes. In ex-vivo liver, the cluster array achieved a coagulation diameter of 4.7 cm after 15 min, 6.2 cm after 40 min and 7.0 cm after 40 min. By comparison, a single cooled electrode achieved a diameter of only 2.7 cm after 45 minutes of heating [132]. During clinical practice, however, cluster arrays are difficult to insert intercostally and it is also harder to visualise all three electrodes at the same time, increasing the risk for injury to the patient [139].

Wet electrodes consist of a hollow metal shaft with one or more holes at the tip, through which isotonic or hypertonic saline solution is infused into the tissue [140, 141]. The success of wet electrodes is explained through hydration; the liquid fills the gap between tissue and electrode instead of gas or vapour [142], which acts as an insulant. Hydration and increased ion concentration improve electrical and thermal conductivity, the liquid decreases heating at the active tip and higher ion concentration spreads the current density over a larger area [106].

Increased thermal and electrical conductivity flatten the temperature curve around the electrode, allowing higher energy input to a larger area, without the risk of tissue boiling [143]. The decreased risk of tissue boiling is also explained by the higher boiling point of saline solution [117]. Experiments have shown that the slow infusion of 1-2 ml/min for a limited time causes liquids to stay in a concentrated small area around the electrode [144]. As the liquid is heated, it forms a liquid or “virtual” electrode with a much larger diameter and therefore a higher coagulation potential than the original metal electrode. Higher infusion rates cause saline solution to extend irregularly further into the tissue and to leak along the electrode track [145]. This has led to unexpected damage to distant structures. Both slow and fast infusion show irregular shapes and a tendency to spread along vascular axes, causing perivascular coagulation.

Wet electrodes have to be differentiated from saline enhanced RFA. In this procedure, electrode and injection needle are not incorporated into one probe. Saline solution is injected as a bolus before RFA begins or during RFA, usually via a separate needle.

However, experiments have shown that the saline is diffused or absorbed rapidly, beyond the targeted tissue, especially in well-perfused organs [106]. A minimum pre-injection combined with slow simultaneous infusion of hypertonic solution is therefore being considered as indispensable for improved RFA efficacy [117].

2.6 Examples of commercially available RFA electrodes

Each electrode modification requires individual optimisation of its energy delivery paradigm. Commercially available electrodes (see Table 2) come each with its own generator and control mode.

Company	Electrode type	Control mode
Covidien (Valleylab)	Internally cooled electrode	Impedance control
Angiodynamics (RITA Medical Systems)	Expandable-wet electrode	Temperature control or power input control
Boston Scientific (Radiotherapeutics)	Expandable, umbrella electrode	Impedance control
Integra life sciences (Berchtold)	Wet electrode	Impedance control or temperature control
Celon	Internally cooled, bipolar	Resistance controlled automatic power

Table 2: Characteristics of some commercially available RFA electrodes

Covidien (Valleylab), formerly known as Radionics, uses an internally cooled electrode, connected to a generator and a perfusion system [146]. The energy delivery period is predetermined, usually at 12 to 15 minutes and internal needle cooling is started with chilled saline solution 1 minute before RFA commences [106]. During the ablation process, tissue impedance is monitored. If impedance increases 10 Ω above baseline value, power is automatically shut off for 15 seconds and then automatically restarted [147]. This pause allows the dissipation of gas around the tip of the probe, which would otherwise act as an insulator [106].

Angiodynamics (RITA Medical Systems) produces a RITA generator and perfusion system for the RITA expandable-wet Star Burst Xli electrode (see Figure 10) [106, 146]. This system can be programmed to two energy delivery applications. During temperature control mode, the targeted temperature is set beforehand and power input is adjusted automatically to reach and hold that temperature [148]. The power control mode allows power input to be fixed at a chosen level until the desired temperature is reached; the temperature sensors are situated at the tip of each prong [146].

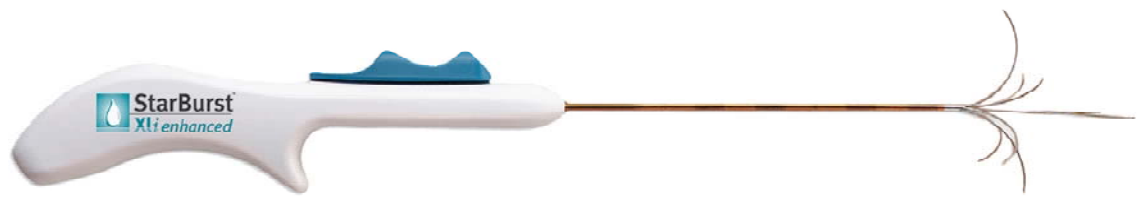


Figure 10: Expandable wet StarBurst Xli electrode by RITA Medical Systems according to <http://www.angiodynamics.com> [149]

Boston Scientific (Radiotherapeutics) uses the RF 3000 generator and an expandable umbrella electrode with a diameter of 2-5 cm [106, 150]. Power output is set manually and increased stepwise during fixed intervals [151]. Sudden increase in impedance causes the power to shut off, followed by a short pause to allow gas dissipation. Then a second ablation cycle sets in at a lower power level. After the second power shut off, treatment is stopped [146, 106].

The company Integra life sciences (formerly known as Berchtold), produces a wet electrode connected to a generator and a perfusion system [106]. Power level and treatment duration are chosen according to a treatment algorithm [151]. The power level is adjusted by two different power control modes. In impedance control mode, power output is interrupted automatically for a few seconds until tissue impedance returns to normal. In temperature control mode, power output is stopped when a predetermined temperature threshold is reached [152]. Perfusion of the wet electrode is started 1 minute before RFA, and perfusion speed is controlled automatically based on power input and impedance. When impedance becomes too high, an extra bolus of saline is injected to disperse gas build-up [146].

During this study, the CelonPowerSystem, produced by Celon, was used. In this system a bipolar, internally cooled electrode is connected to a resistance controlled energy generator and cooled continuously through a triple peristaltic pump [153].

2.7 Factors influencing RFA

The success of RFA is measured in terms of lesion size and relies on various factors, with the aim of ablating the entire lesion, including a 5-10 mm safety zone.

It is important to reach temperature ranges of 60 ° C to 100° C in order to ensure cell death, but also to prevent charring of tissue [106]. Another factor is the duration of heat exposure, which in turn depends on the current density and intensity, the length of the electrode's active tip, tissue conductivity and the duration and mode of RFA application.

RFA also depends on the perfusion of the affected organ. RFA has shown to be less effective in highly perfused tissue [146]. Large vessels, mainly hepatic veins, lead to the so-called heat-sink effect. Blood circulation prevents a homogeneous distribution of heat, causing heat loss, and leading to the formation of smaller lesions than expected, also known as type 1 distortion [106,151]. Therefore, the ablation volume could be increased by the temporary occlusion of these vessels, for example by balloon occlusion of a hepatic vein or through temporary occlusion of the portal vein in the Pringle manoeuvre [146, 154, 106].

In HCCs within cirrhotic livers, the “oven effect” can be observed [155]. The heat is trapped within the (pseudo) encapsulated area of the tumour, leading to much larger ablation volumes than expected for the specific needle design. Finally tissue density and electrolyte concentration, affecting electrical conductivity and impedance, should be taken into consideration.

Despite continuing modifications of RFA technology, the attainable size of the coagulation area is still limited to a diameter greater of about 5 cm [7]. As a 5-10 mm safety zone around the tumour has to be observed in order to ensure curative treatment, RFA is therefore limited to small tumours and metastases. This creates the need for improved RFA methods and technology in order to achieve a more volumetric energy deposition.

According to evidence-based practice guidelines on the management of HCC, proposed by the EASL and the American Association for the Study of the Liver Disease (AASLD), RFA is proposed as a non-surgical treatment option for early stage HCC in Patients with Child A or B liver disease, a solitary HCC nodule or no more than three nodules each <3cm in size [27]. The guidelines accept RFA and PEI as a safe and effective alternative to treat early stage HCC in patients who are not eligible for surgical resection due to high co-morbidity.

RFA as well as TACE are also accepted treatment options to downsize HCC nodules in order to meet the Milan transplantation criteria [156]. In accordance with the EASL guidelines, RFA is also used to bridge the time to transplantation, by reducing the tumour burden and inhibiting tumour progression [157].

Several studies have investigated ways to increase lesion sizes. One point of interest has been to improve electrical tissue conductivity by injecting conductive fluids before or during RFA. Solazzo et al. investigated the relationship between background conductivity and RF-heating, reaching the conclusion that an increase in NaCl concentration in the tissue positively affects heat production [158]. Lee et al. conducted two studies, one on RFA enhancement by acetic-acid-hypertonic saline solution instillation, and another on hypertonic saline solution instillation before bipolar RFA [159, 141]. Both studies showed that injecting a solution containing ions improved electric and thermal conductivity in tissues and produced larger lesions.

In 2006 Bruners et al investigated 16 fluids and their effects on RFA in a study on fluid modulated RFA [160]. The study concluded that ionic fluids enhance RFA, whereas non-ionic fluids, such as glucose solution or non ionic contrast agents, generate a protective effect during RFA.

3.0 AIM AND OBJECTIVES OF THE STUDY (INCL. HYPOTHESIS)

Despite the continuous development and improvement of RFA techniques, the size of the achievable coagulation necrosis remains the limiting factor. The success of RFA is also hampered by the rapid achievement of high temperatures within the targeted coagulation area, leading to carbonisation and reducing the conductive properties of the tissue. This limits indications for RFA to small HCCs and small hepatic metastases <3 cm in size [157]. As most hepatic malignancies are discovered at a rather late stage, a great number of tumours are not eligible for RFA at the point of diagnosis. Improved RFA would therefore widen the treatment options for many HCC patients.

The objective of this study is to develop a method to increase the output of energy into the tissue and thus achieve larger coagulation volumes. Several studies have already been conducted to investigate the influence of saline solution and acetic acid on tissue conductivity and RFA, and saline solution has shown to improve electrical and thermal conductivity, leading to larger coagulation volumes. This leads to the question whether fluids with a higher conductivity than saline solution would produce even better results. The hypothesis of this study was that fluids with a high conductivity would also improve tissue conductivity after injection. The higher the conductivity of the fluid injected, the higher the conductivity of the tissue should become. The increased ion concentration in the tissue should allow larger energy deposition, as heat is conducted further away from the electrode, leading to larger ablation volumes. As the heat is conducted further away from the active tip, high temperatures immediately adjacent to the electrode should be avoided and charring should be reduced.

The fluids used in this study had already been tested on their ability to conduct heat in a previous study. Bruners et al. [160] investigated in how far fluids with different conductive qualities enhance the radiofrequency ablation process. 16 fluids were investigated by heating three samples of 20 ml each with an internally cooled needle electrode. The application of energy was stopped when the fluid's temperature had reached 80 °C or when heating time exceeded 30 minutes. Five fluids achieved a temperature of 80°C; these were hydroxyethyl starch (HAES), Iopamidol (Solutrast 300®), Ioxithalaminacid (Telebrix®), Iotrolan (Isovist®) and Gadopentate dimeglumine (Magnevist®). The remaining fluids (40% Glucose, Ringer-Solution, 95%

Ethanol, 50% acetic acid, Sodium Amidotrizoate/ Meglumine amidotrizoate (Urografin 45%®), Gadobutrol (Gadovist®) and 0.9% NaCl) could not be heated to a temperature of 80°C. The results of this study indicated that HAES, Telebrix® and Magnevist® could be used to improve radiofrequency ablation, whereas glucose solution and non-ionic contrast agents such as Gadovist® showed high electrical resistance and could be used to protect surrounding organs during the ablation process.

The aim was therefore to inject fluids which had been tested on their conductivity beforehand into ex-vivo porcine liver prior to bipolar RFA and to investigate how these fluids affected the ablation process and the volume of the necrosis achieved. Not only should the size of coagulation necrosis be evaluated, but also the temperature of the tissue surrounding the active tip should be recorded, to evaluate not only the outcome of RFA but also the generation of heat and its distribution within the tissue.

4.0 MATERIALS AND METHOD

4.1 Radiofrequency ablation system

The RF-System (CelonLabPower, Celon AG Medical Instruments, Teltow, Germany) used for this study, is a bipolar/multipolar ablation system (see Figure 11), which can be used for percutaneous RFA but also during open surgery. The power control unit (CelonLabPower) is a generator which operates at a frequency of 470 +/- 10 kHz and delivers a variable power output between 2 and 250 Watt. A triple peristaltic pump (CelonAquaflow III) can be used to cool applicators with an active tip of 20 mm or more with chilled saline perfusate, at a pump rate of 30 ml/min. The entire system is mounted upon the system carrier (CelonMobile service trolley).



Figure 11: Celon LabPower with Celon Aquaflow III©Celon AG Medical Instruments, according to www.celon.de [161].

One to six applicators can be connected to the system. The CelonProSurge applicator is a bipolar RFA-applicator and has a rigid probe, with a sharp trocar tip (see Figure 12). The applicator is cooled by a closed flow of liquid through its shaft, kept up by the power system's triple peristaltic pump. The two electrodes are arranged closely together at the non-insulated tip of the applicator and current flows exclusively between them. This applicator was chosen for this study as it no longer needs a grounding pad, in

contrast to monopolar electrodes and because in a clinical setting it is used for the ablation of larger tumours such as hepatic tumours.



Figure 12: CelonProSurge applicator© Celon AG Medical Instruments, according to www.celon.de [161].

When only one applicator is connected, the system works in a bipolar mode and the current flows only between the two electrodes incorporated into the tip of the applicator. If two to three applicators are connected to the generator, the multipolar mode is activated. This means that the current flows between all electrode pairs, one after the other. When four to six applicators are connected to the generator, each one functions as a single electrode.

The power control unit is equipped with a Resistance Controlled Automatic Power (RCAP) control. For a generator output > 10 Watt, the resistance controlled automatic power mode can be used. The system's microprocessor evaluates the resistance of the tissue, determining its maximum power uptake and automatically adjusts the power unit accordingly. When resistance increases up to 900Ω or more, the power unit is switched off automatically. This helps to avoid early tissue desiccation and carbonization. The power control unit is operated through a foot switch, which needs to be pressed down in order for energy application to occur. During the RFA process, the system continuously monitors applied energy, tissue resistance and application time. This data can be recorded on a computer, connected to the system through a serial computer interface or USB interface, provided the CelonPower Monitor software has been installed (see Figure 13).

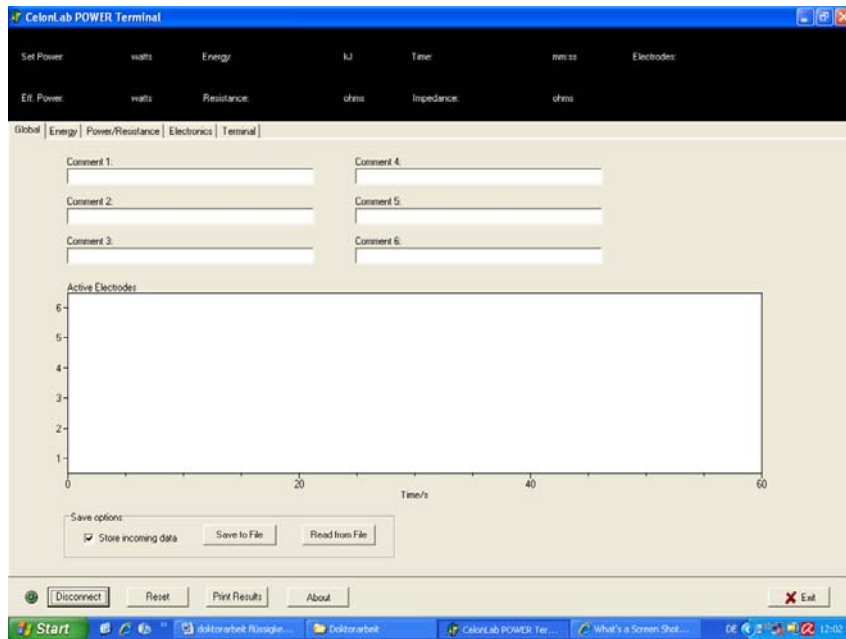


Figure 13: Screenshot of the CelonPower Monitor software

4.2 Fluids

Based on the results of an ex-vivo study on heating characteristics of different fluids [160], the following fluids were chosen with regard to their conductivity:

1. Isotonic saline solution containing 0.9 % NaCl (DeltaSelect, Pfullingen, Germany)
2. Gadopentate dimeglumine (Magnevist®, Bayer Schering Pharma, Berlin, Germany): an injectable contrast medium for MRI. Each ml of Magnevist® contains 469.01 mg of gadopentate dimeglumine (the N-methylglucamine salt of the gadolinium complex of dethyletriamine pentaacetic acid), 0.99 mg of meglumine, 0.40 mg of diethylenetriamine pentaacidic acid and water. Magnevist® has an osmolality of 1,960 mOsmol/kg water, which is 6.9 times that of plasma with 285 mOsmol/kg water. It is therefore a hypertonic solution.
3. Ioxithalamine acid (Telebrix Gastro®, Guerbet, Sulzbach, Germany): a water soluble, ionic x-ray contrast medium with a high osmolality. It contains ioxthalamate, a tri-iodinated benzoic acid.

4. 10% hydroxyethyl starch solution (HAES, Fresenius Kabi, Bad Homburg, Germany): This solution is composed of 10% hydroxyethyl starch, dissolved in a sodium chloride solution. It is used for the treatment and prophylaxis of volume deficiency, for example hypovolaemia or shock. 1 L of this solution contains poly(0-2-hydroxyethyl)starch, either 60.00 or 100.00 g, with an average molecular weight of 200.000 Dalton. Further ingredients are 9 g of Sodium chloride, Sodium hydroxide, Hydrochloric acid and water. The solution has a pH value of 3.5 - 6.0 and an osmolarity of 308 mosm/L.
5. Glucose solution containing 5% glucose (DeltaSelect, Pfullingen, Germany)
6. 95% Ethanol (B. Braun Melsungen, Melsungen, Germany): Ethanol had to be diluted with isotonic NaCl solution in a ratio of 1:5, because it has a relatively high resistance and could not be heated in a concentrated form, as demonstrated by Bruners et al. [160]
7. Distilled water (DeltaSelect, Pfullingen, Germany)

As a reference standard, one set of experiments was carried out using native liver specimens, which had not been injected with any liquid.

4.3 Experimental set-up

Bipolar RFA was performed on freshly excised porcine liver samples, using the CelonLabPower RF-System described above. During the experiments an internally cooled, needle shaped applicator with a shaft length of 150 mm, an active tip length of 20 mm and a shaft diameter of gauge 15 or 1.8 mm (Celon ProSurge T20) was used. The electrode was powered with a maximum of 15 Watts, according to recommendations of the manufacturer.

The freshly excised porcine liver was cut into blocks approximately 5 x 5 cm in size, taking care not to use parts of the liver that contained large blood vessels. The liver specimens were then put in a plastic bag containing isotonic saline solution and warmed to a temperature of 37 °C using a water bath (see Figure 14). Before RFA was started, 1 ml of the fluid to be tested was injected centrally into the liver tissue, using a 5 ml

single-use syringe (B. Braun Melsungen AG, D-34209 Melsungen) and a 21G hypothermic needle (Henke Sass Wolf GmbH, D-78532 Tuttlingen, Germany).

Afterwards the applicator was inserted along the puncture track, horizontally into the tissue, about 3 cm deep. Using a stencil, two channels at a distance of 5 mm and 10 mm from the applicator, were punctured into the liver parallel to the applicator shaft, using a catheter needle with an outer diameter of 1.2 mm (Spinocan® 1,30 x 75- G 18 x 3", B. Braun Melsungen AG, D-34209 Melsungen, Germany). Two fiberoptic thermocouples (SFF-2m, Luxtron Corporation, Santa Clara, CA, USA) were then inserted into the preformed channels to a length of 2 cm, at a fixed distance of 0.5 cm between applicator and probe 1, and probe 1 and probe 2 respectively (see Figure 15).

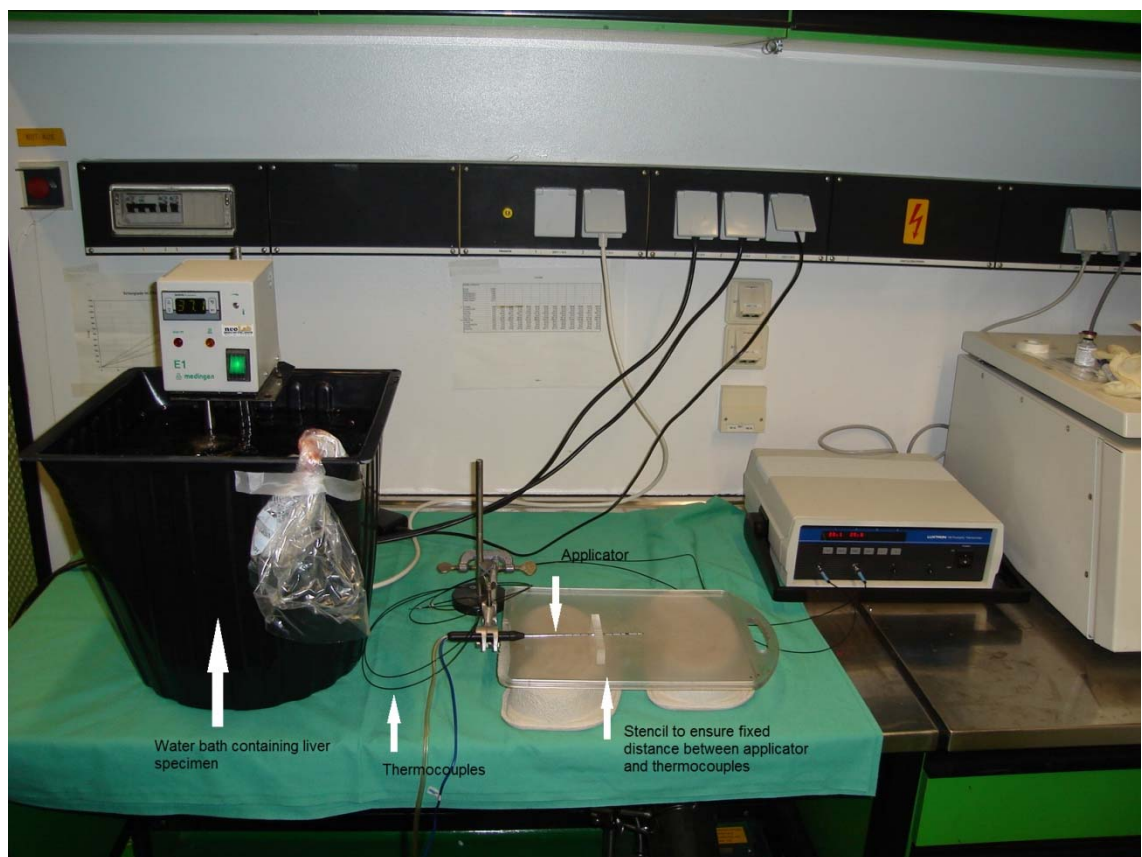


Figure 14: Experimental setup

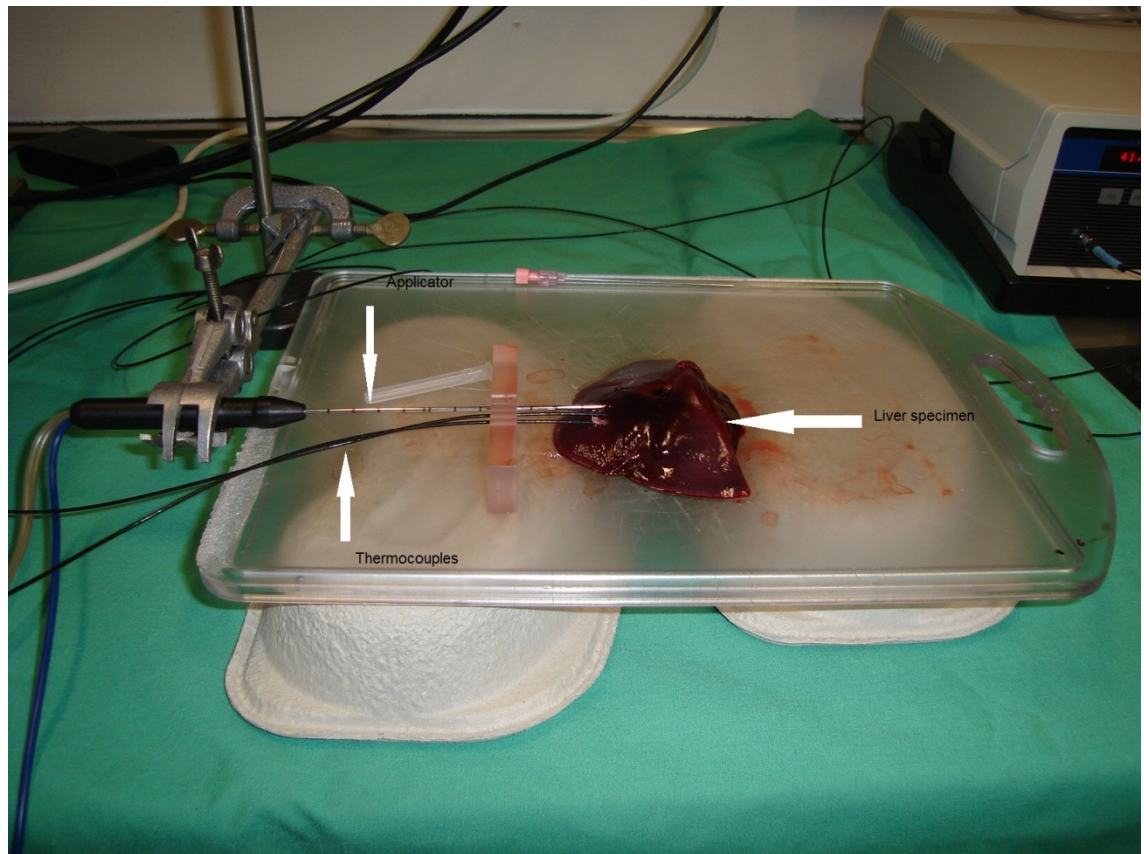


Figure 15: Placement of applicator and fibreoptic thermocouples into liver specimen

In accordance with the manufacturer's recommendations for the use of an electrode with a 20 mm active tip, the generator was set to a maximum output of 15 Watt (according to a personal communication with Dr. Thomas Stein, head scientist at Celon). The applicator was connected to the peristaltic pump and perfused with tap water at room temperature.

The generator was then activated via the foot switch and the temperature reported by the two fiberoptic temperature probes, placed parallel into the sample at 5mm and 10 mm from the electrode, was recorded every 15 seconds.

During the ablation process energy input [kJ] and time of energy appliance [sec] were recorded using the CelonPower Monitor software. These two parameters are important for the analysis of the ablation process, as the coagulation volume depends on the amount of energy deposited into the tissue and the time the tissue was exposed to that energy [129]. The software also recorded the tissue's resistance and impedance.

RF energy was applied until a rise in impedance $> 900 \Omega$ occurred, at which point the power control unit shut off automatically. After a pause of 1 minute, a second ablation cycle was performed until another rise in impedance occurred, marking the end of the

experiment. During these two ablation cycles the applicator was not repositioned. For each liquid a series of five experiments was carried out, as well as a series of five experiments without the injection of any liquid, serving as a reference standard.

Four applicators of the same make and model were used alternatingly, assuming that the energy output of all four probes should be identical and would therefore not affect the results.

After each experiment the applicator was cleaned and checked for any outer damage. Furthermore free circulation of fluid within the applicator was ensured. After each set of experiments the applicator was perfused with distilled water to prevent any precipitations within the shaft, which would obstruct circulation with the perfusate.

4.4 Lesion size measurement

After the ablation process liver specimens were dissected along the length of the applicator and the lesion size assessed macroscopically, using a ruler. A further cut was made in an axis perpendicular to that of the insertion, measuring the depth of the lesion created. Previous studies have shown that in ex-vivo specimen the tan to charcoal grey area of the RFA induced lesions indicate tissue which has undergone coagulation necrosis [106] (see Figures 18 to 20). The charcoal grey area immediately next to the electrode contains dessicated and carbonized tissue, whereas the tan area adjacent to it contains coagulated tissue [106].

The diameter of the lesion was measured along the electrode insertion axis (x) and in two planes perpendicular to that axis (y, z). The volume of the lesion was calculated by approximating the lesion to the shape of a sphere, using the following formula as published by Lee et al. [162]:

$$\pi (x \times y \times z) / 6$$

Liver specimens which contained larger vessels near the ablation zone were excluded from the macroscopic evaluation, because large vessels are known to distort the ablation volume of perfused tissue.

4.5 Statistical analysis

From the data the arithmetic mean and standard deviation for coagulation size, ablation time and applied energy was calculated. In order to evaluate the efficiency of each fluid, an efficiency index was calculated by dividing the coagulation volume achieved by the duration of each ablation procedure (cm^3/sec).

A two sample t-test was carried out to analyse statistically significant differences between the means of coagulation volume, applied energy and ablation time. To avoid type 1 error inflation caused by multiple testing, the Bonferroni Holm procedure was applied [163].

In addition the Pearson's correlation coefficient was calculated to investigate the relationship between coagulation volume, duration of RFA, energy applied and minimum resistance respectively.

Data analysis was done using Medcalc Version 8.2 (Medcalc software, Mariakerke, Belgium).

5.0 RESULTS

Volume [cm ³]								
Experiment	Reference standard	NaCl	Magnevist®	HAES	Glucose	Ethanol	Telebrix Gastro®	Aq. dest
1	1.05	2.12	2.27	6.02	2.64	2.46	1.96	3.47
2	1.43	1.73	2.55	5.24	3.03	3.56	2.55	1.98
3	1.42	1.74	2.55	4.97	2.83	4.24	2.36	2.35
4	1.28	1.50	3.03	3.40	3.92	2.83	2.93	2.11
5	1.95	0.79	2.05	4.52	3.35	4.71	2.83	3.52
Arithmetic mean	1.42	1.58	2.49	4.83	3.15	3.56	2.53	2.68
Standard deviation	0.33	0.49	0.37	0.97	0.50	0.94	0.39	0.75

Table 3: Volume per trial and average volume [cm³] of the lesions

Table 3 above shows the volume of the lesion created in each experiment. All fluids with the exception of ethanol produced regular lesions in the shape of a flattened sphere or ellipse. The lesions achieved using ethanol were irregularly shaped with tendril-like extensions.

The data can be roughly divided into three groups, according to average lesion size.

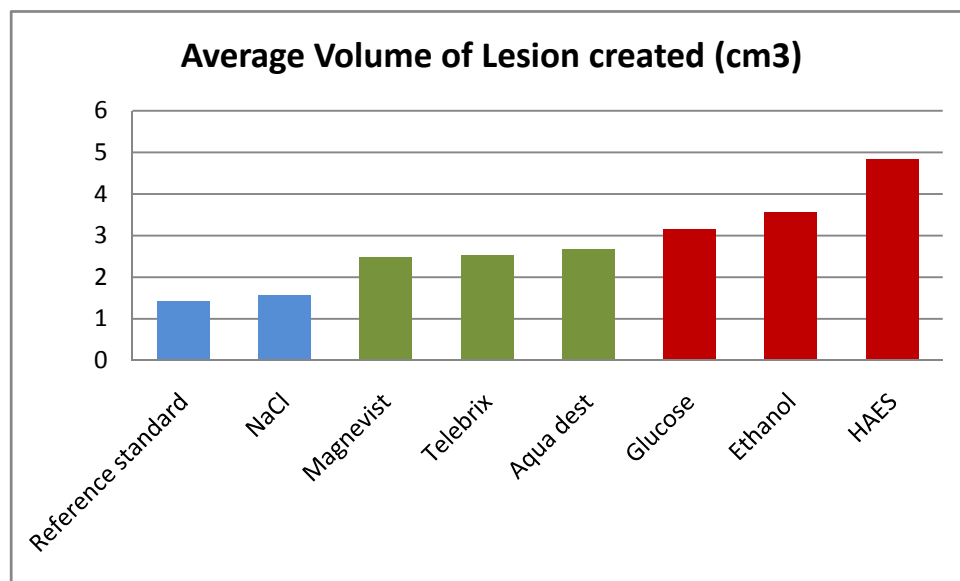


Figure 16: Average coagulation volume [cm³]. Lesion size was roughly divided into three groups and the bars in the chart above were colour coded accordingly. Blue indicates the fluids which produced the smallest lesions, green indicates fluids which produced lesions which were similar in size and red indicates fluids which produced the largest lesions.

The smallest lesions were created in the control experiment without the instillation of fluids, with an average volume of $1.42 \pm 0.33 \text{ cm}^3$. Instillation of isotonic NaCl solution produced lesions only marginally larger than those of the control experiments, with an average volume of $1.58 \pm 0.49 \text{ cm}^3$.

The second group of results is comprised of Magnevist®, TelebrixGastro® and distilled water. These three liquids created lesions within a similar range, Magnevist® with an average volume of $2.49 \pm 0.37 \text{ cm}^3$, TelebrixGastro® with an average of $2.53 \pm 0.39 \text{ cm}^3$, and distilled water with an average volume of $2.68 \pm 0.75 \text{ cm}^3$.

The largest lesions were achieved using HAES, with an average volume of $4.83 \pm 0.97 \text{ cm}^3$, which is almost 3 times larger than that of the control experiment. These results are closely followed by those of ethanol, which produced average lesions $3.56 \pm 0.94 \text{ cm}^3$ large, and glucose, which achieved an average of $3.15 \pm 0.50 \text{ cm}^3$ (see Figure 17).

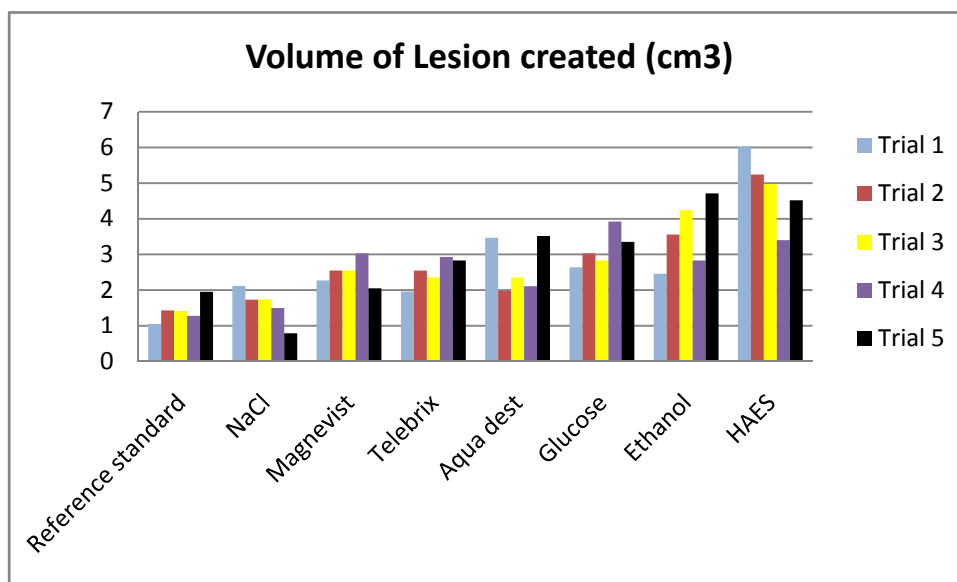


Figure 17: Coagulation volume [cm^3] per fluid and trial

The coagulation volumes varied within the trials for each liquid. The volume of lesions created under the instillation of NaCl were in the range of $1.5 - 2.1 \text{ cm}^3$ for the first four trials, but the last experiment produced a lesion of only 0.79 cm^3 which reduced the value of the arithmetic mean considerably.

Instillation of distilled water created lesions between 1.98 and 2.35 cm^3 in three experiments and considerably larger lesions of 3.5 cm^3 in experiment 1 and 5.

Volumes measured for Magnevist®, TelebrixGastro®, the reference standard, and to a lesser extent also glucose, were fairly homogeneous. Data for ethanol was the most inconsistent; however, lesions created under ethanol showed to be very irregular, with no spherical shape, and were difficult to measure (see Figure 19).

Data for HAES had the highest fluctuation with a range of 3.40 – 6.02 cm³, but the three largest volumes measured in this study were under instillation of HAES and the large range is mainly due to the smaller volume produced in experiment 4 (see Figure 20).

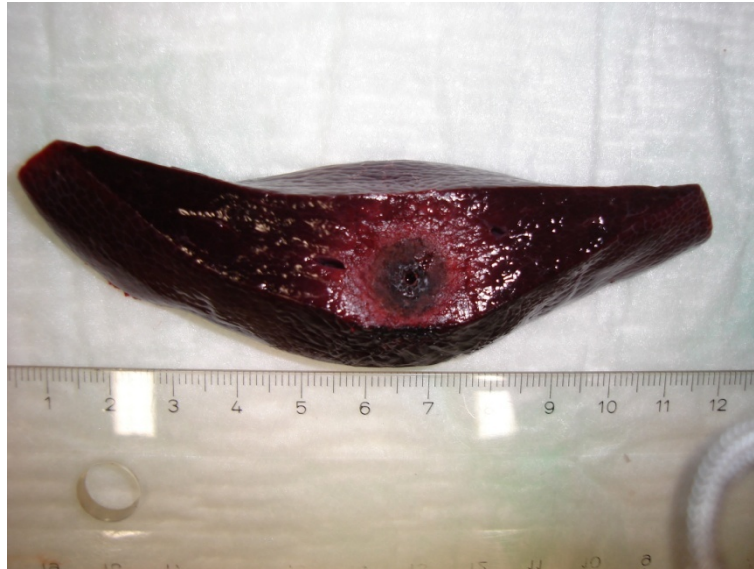


Figure 18: Coagulation necrosis achieved using RFA alone and dissected perpendicular to the applicator. The charcoal grey to tan area marks the area of coagulation



Figure 19: coagulation necrosis using RFA enhanced with ethanol. Note the irregularity of the coagulation area



Figure 20: Coagulation necrosis using RFA after injection of HAES. Note the central charring surrounding the puncture tract.

Energy [kJ]								
Experiment	Reference standard	NaCl	Magnevist®	HAES	Glucose	Ethanol	Telebrix Gastro®	Aq. dest.
1	2.4	2.61	2.56	5.88	6.11	7.08	3.38	2.78
2	2.5	2.42	4.28	5.52	5.19	9.32	3.65	2.72
3	3.3	3.58	4.56	5.53	4.86	5.93	4.90	2.82
4	2.5	2.04	4.77	7.39	8.08	4.31	4.26	4.65
5	3.4	2.97	2.53	5.17	6.96	5.26	5.17	3.76
Arithmetic mean	2.8	2.72	3.74	5.90	6.24	6.38	4.27	3.35
Standard deviation	0.49	0.58	1.10	0.87	1.32	1.93	0.77	0.85

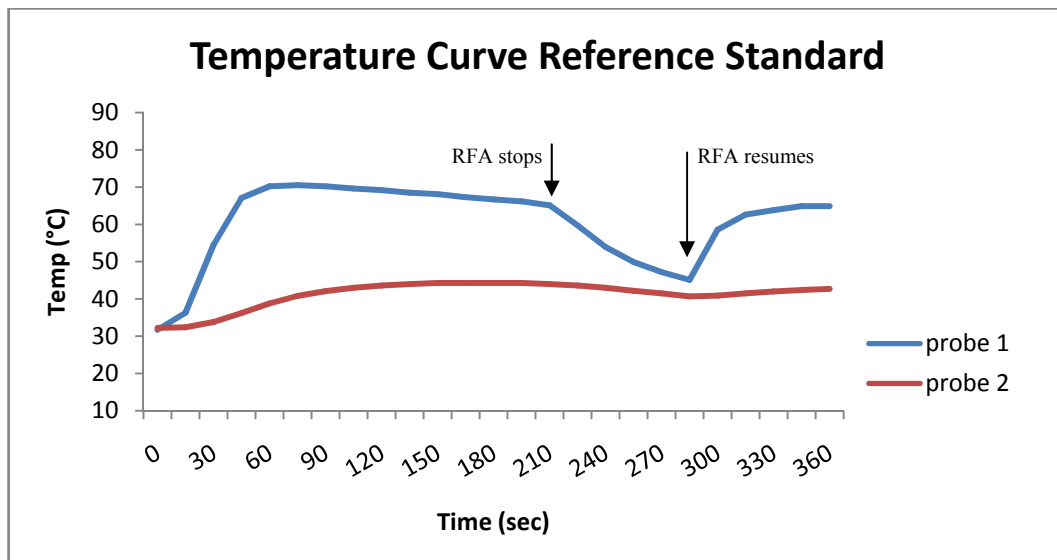
Table 4: Energy input [kJ] per trial and average energy input

Time [sec]								
Experi- ment	Reference standard	NaCl	Magnevist®	HAES	Glucose	Ethanol	Telebrix Gastro®	Aq. dest.
1	288	321	282	773	857	955	392	345
2	301	299	453	706	627	1218	458	355
3	361	405	592	702	646	604	636	391
4	306	237	645	785	1152	603	508	596
5	366	334	241	600	925	559	724	427
Arithmetic mean	324.40	319.20	442.60	713.20	841.40	787.80	543.60	422.80
Standard deviation	36.34	60.72	180.16	73.69	216.71	288.67	134.78	102.07

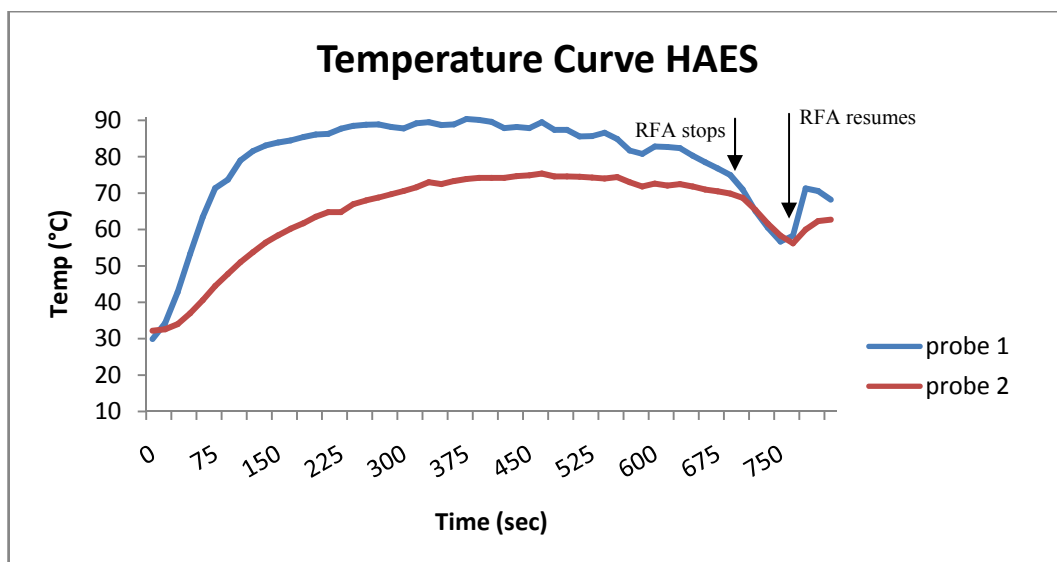
Table 5: Time of energy application [sec] and average time of energy application

The highest energy input was achieved with the use of ethanol, glucose and HAES. Instillation of glucose achieved an average energy input of 6.24 kJ, HAES of 5.90 kJ and ethanol of 6.38 kJ. These three fluids were also those with the longest average heating time; glucose achieved 841.4 sec, HAES 713.2 sec and ethanol 787.8 sec. However, of these three fluids glucose showed a lower energy input than ethanol, although it had the longest heating time (see table 4 and 5).

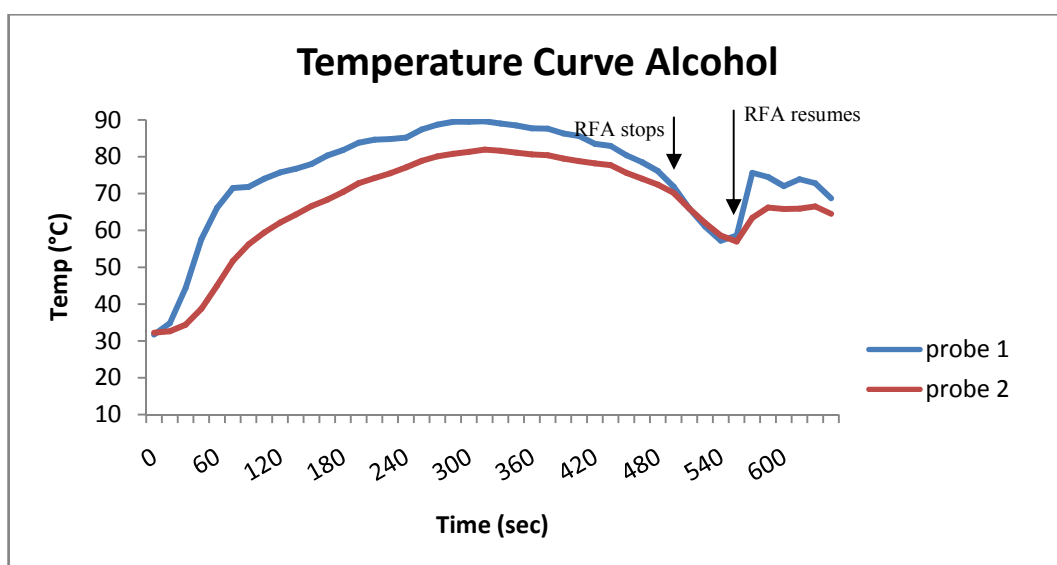
Temperature was recorded with two probes; probe 1 was at a distance of 5 mm from the electrode and probe 2 at 20 mm. RFA paused automatically when the impedance became too high and was manually restarted after 1 min. The resulting temperature curves were quite similar for all the liquids, with just slight differences in maximum temperatures. The graphs shown below were chosen as examples for temperature curves recorded using the different fluids.



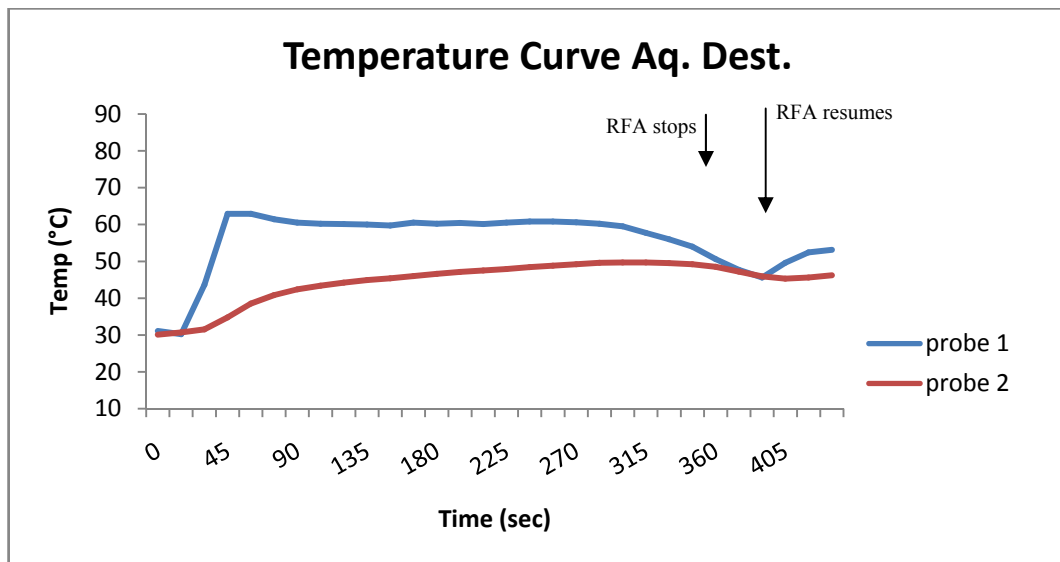
a)



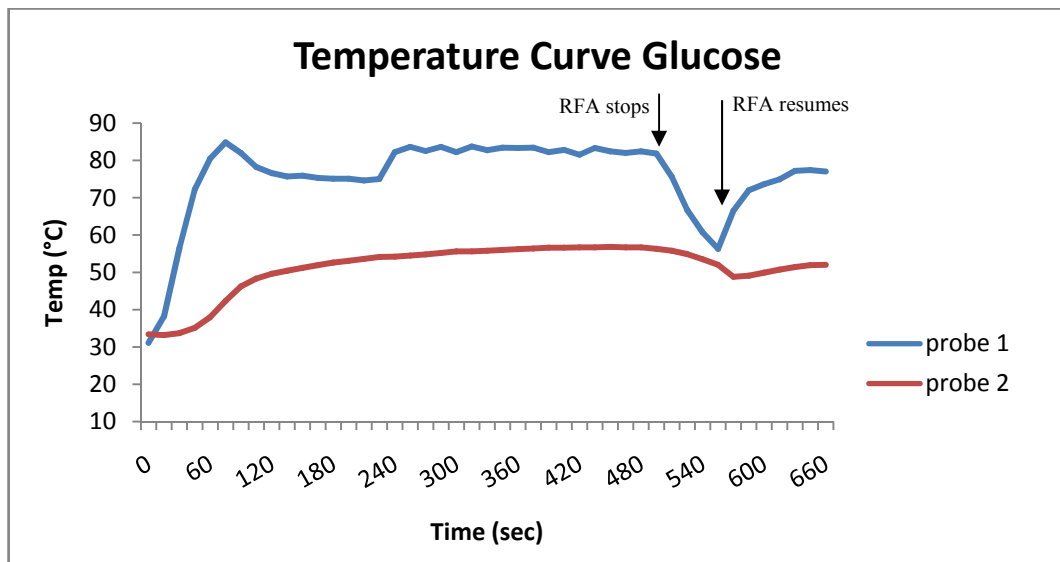
b)



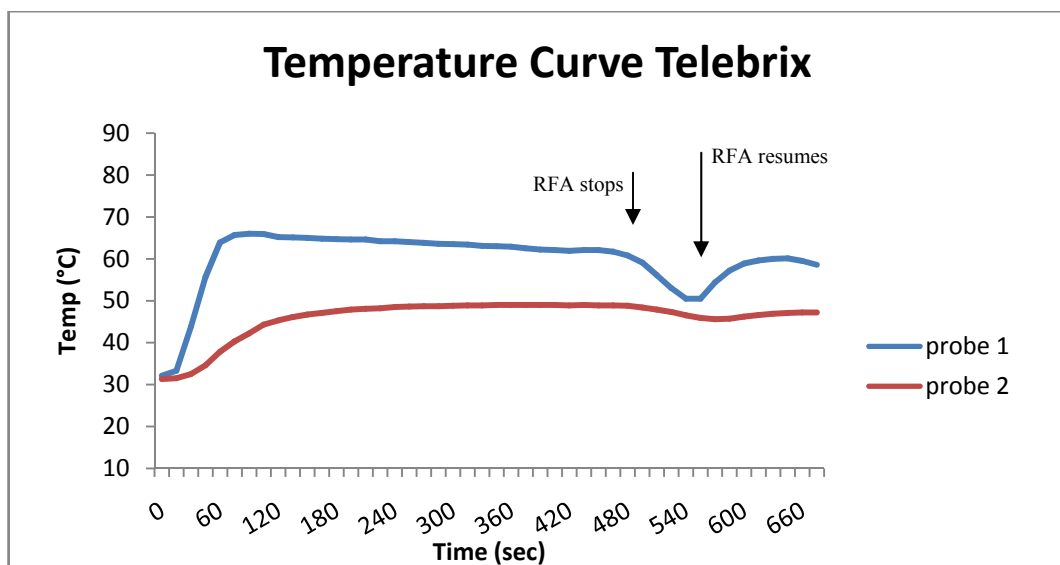
c)



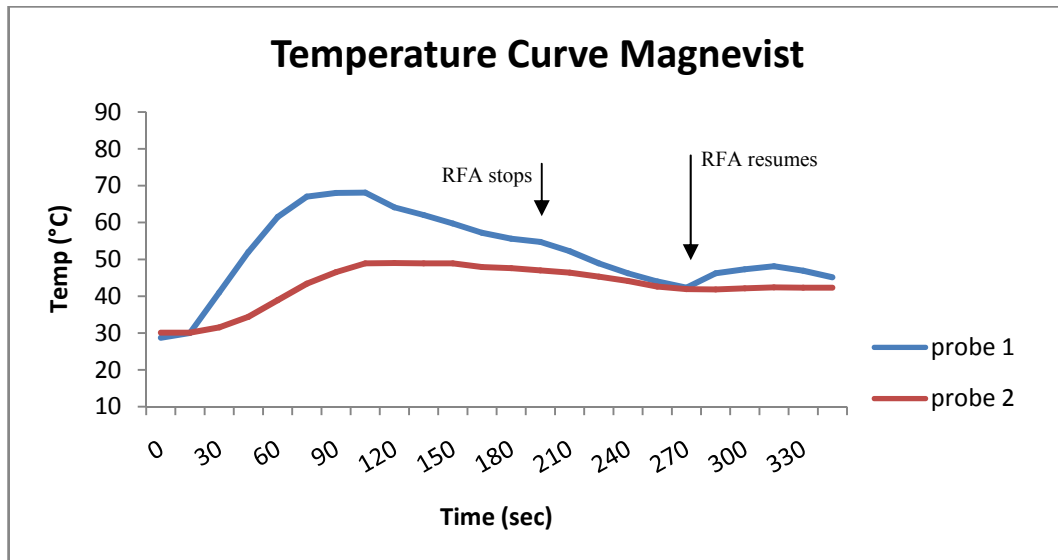
d)



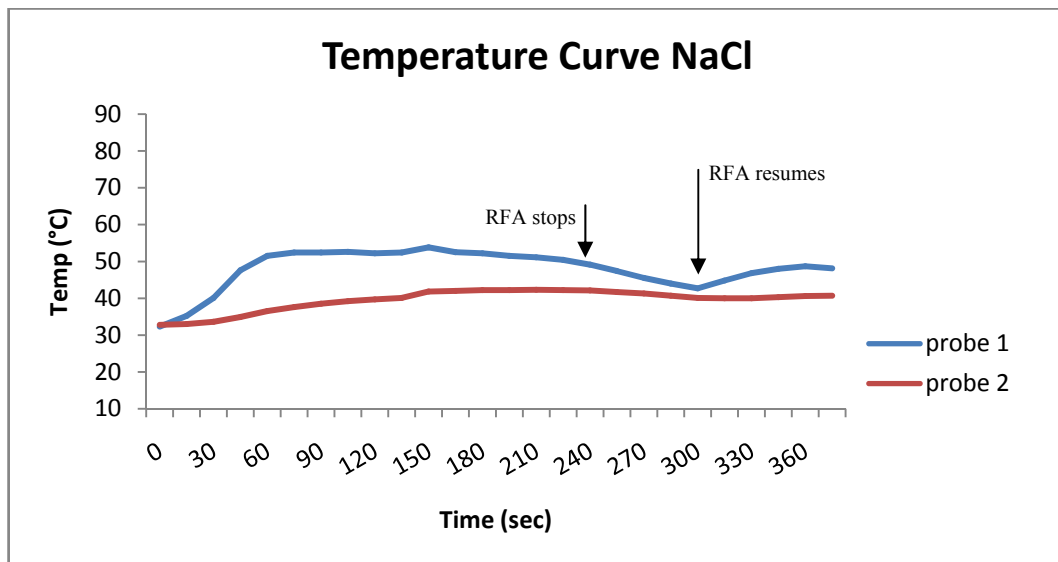
e)



f)



g)



h)

Figure 21 a-h: Examples of temperature curves recorded using the different fluids. Probe 1 was placed at a distance of 5 mm from the electrode, probe 2 was placed 10 mm away from the electrode. The first arrow marks the point where a rise in impedance caused an automatic power shut off. The second arrow marks the point where RFA was restarted manually after a 1 minute break.

The curve progression shows an initial steep rise in temperature until a plateau level is reached where it stays until the rapid rise in impedance causes RFA to stop. In some cases this pattern is very distinct while in others it is less visible. During the 1 minute break in the ablation cycle, the curve shows a drop in temperature before rising again after RFA is resumed, although not reaching the level of the previous plateau. The most distinctive drop in temperature can be seen in the curves described by HAES, ethanol, glucose and Magnevist (see Figures 21b, 21c, 21e, 21g). These are also the fluids which show the steepest initial rise in temperature at the beginning of the ablation cycle.

Efficiency Index [coagulation volume/ablation duration [cm ³ /sec]]								
Experiment	Reference standard	NaCl	Magnevist®	HAES	Glucose	Ethanol	Telebrix Gastro®	Aq. dest.
1	0.22	0.40	0.48	0.47	0.18	0.15	0.30	0.60
2	0.29	0.35	0.34	0.44	0.29	0.18	0.33	0.33
3	0.24	0.26	0.26	0.43	0.26	0.42	0.22	0.36
4	0.25	0.38	0.28	0.26	0.20	0.28	0.35	0.21
5	0.32	0.14	0.51	0.45	0.22	0.51	0.23	0.49
Arithmetic mean	0.26	0.30	0.37	0.41	0.23	0.31	0.29	0.40
Standard deviation	0.04	0.11	0.12	0.09	0.04	0.15	0.06	0.15

Table 6: Efficiency index [cm³/sec] per trial and average efficiency index

The Efficiency Index was calculated for each liquid and trial in order to evaluate how successfully it enhanced the radiofrequency ablation process.

As shown in table 6, Magnevist®, distilled water and HAES are the most efficient fluids, with efficiency indices ranging from 0.37 to 0.41 cm³/sec. Glucose was the least efficient fluid with an efficiency index of 0.23 cm³/sec., followed by the reference standard which has an efficiency index of 0.26 cm³/sec. TelebrixGastro®, saline solution and ethanol make up the midrange group with efficiency indices ranging from 0.29 to 0.31 cm³/sec.

Tissue resistance also affects the ablation process. The higher the resistance becomes, the less energy can be deposited into the tissue, as the current is not able to flow freely. Maximum resistance occurs around the point of carbonisation, because the tissue is then no longer conductive. Minimal resistance characterises the resistance found in the tissue before carbonisation has taken place and while energy can still be applied.

Minimal Resistance [Ω]								
Experiment	Reference standard	NaCl	Magnevist®	HAES	Glucose	Ethanol	Telebrix Gastro®	Aq. dest.
1	73	69	60	112	81	58	66	80
2	75	68	107	98	71	105	67	86
3	74	66	66	102	69	80	77	77
4	71	80	61	94	68	76	80	82
5	65	69	65	84	92	70	71	80
Arithmetic mean	71.6	70.4	71.8	98	76.2	77.8	72.2	81
Standard deviation	3.98	5.51	19.84	10.30	10.23	17.33	6.14	3.32

Table 7: Minimal resistance [Ω] per trial and average minimal resistance [Ω]

When looking at table 7 showing average minimal resistance for each fluid, three groups can be determined (see Figure 22).

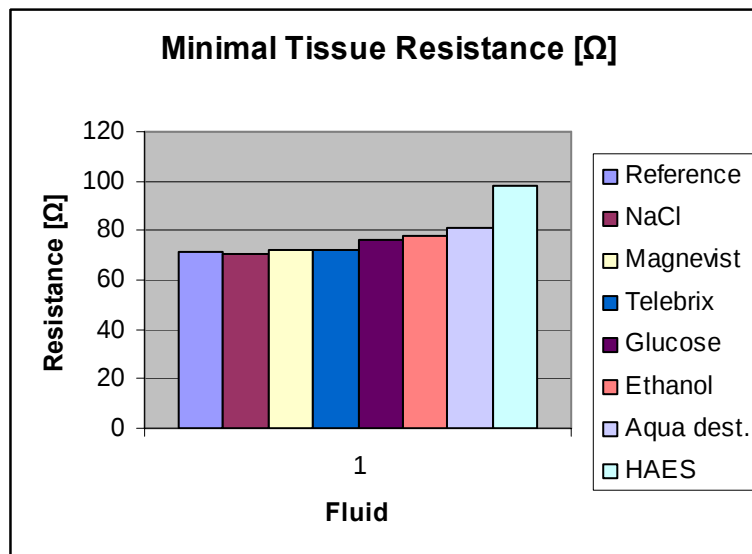


Figure 22: Average minimal tissue resistance [Ω] per fluid

The first group is formed by the reference standard, saline solution, Magnevist® and TelebrixGastro®, all achieving comparatively low minimal resistances ranging from 70.4 to 72.2 Ω.

The second group is comprised of glucose, ethanol and distilled water with resistances ranging from 76.2 to 81 Ω.

The highest minimal resistance in the tissue was recorded under the use of HAES with a resistance of 98 Ω.

The following differences in volume size were statistically significant:

Fluid I	Fluid II	P-Value	P-value according to Bonferroni Holms	Significance
reference standard	HAES	0.0001	0.0018	+
reference standard	Glucose	0.0002	0.0019	+
NaCl	HAES	0.0002	0.0019	+
Magnevist®	HAES	0.0010	0.0020	+
NaCl	Glucose	0.0010	0.0021	+
HAES	TelebrixGastro®	0.0011	0.0022	+
reference standard	Magnevist®	0.0013	0.0023	+
reference standard	TelebrixGastro®	0.0013	0.0024	+
reference standard	Ethanol	0.0014	0.0025	+
HAES	Aq.dest.	0.0019	0.0026	+

Table 8: Results of two sample t-test analysis of relationship between coagulation volume achieved and fluid used

Table 8 above shows the differences in coagulation size between the reference standard and HAES, Glucose, Magnevist®, TelebrixGastro® and Ethanol respectively to be statistically significant. Although the coagulation volume using distilled water was considerably larger than in the reference standard, it was not statistically significant. Coagulation necrosis created under HAES are significantly larger than those created under the use of saline solution, Magnevist®, the reference standard, TelebrixGastro® and distilled water. However, there is no significant difference between the lesions created under HAES and Glucose.

The following differences in the amount of energy applied can be considered statistically significant:

Fluid I	Fluid II	P-Value	P-value according to Bonferroni Holms	Significance
reference standard	HAES	0.0001	0.0018	+
NaCl	HAES	0.0001	0.0019	+
NaCl	Glucose	0.0006	0.0019	+
reference standard	Glucose	0.0006	0.0020	+
HAES	Aq.dest.	0.0015	0.0021	+

Table 9: Results of two sample t-test analysis of relationship between energy input and fluid used

Table 9 shows that a statistically significantly higher amount of energy was deposited into the tissue under the use of HAES, than under the use of saline solution, distilled water or the reference standard. The use of glucose solution allowed significantly higher amounts of energy to be deposited than the use of saline solution or the reference standard.

There was no significant difference between the reference standard and any of the other fluids and, other than expected, there was no statistically significant difference between the reference standard and saline solution.

The following differences of heating time can be considered statistically significant:

Fluid I	Fluid II	P-Value	P-value according to Bonferroni Holms	Significance
NaCl	HAES	0.0001	0.0018	+
Reference standard	HAES	0.0001	0.0019	+
NaCl	Glucose	0.0008	0.0019	+
Reference standard	Glucose	0.0008	0.0020	+
HAES	Aq. dest.	0.0009	0.0021	+

Table 10: Results of two sample t-test analysis of relationship between duration of energy applied and fluid used

The instillation of HAES enabled a statistically significantly longer time of energy application, than the instillation of saline solution, the reference standard or distilled water. Glucose instillation also allowed a significantly longer heating time than the use of saline solution or the reference standard.

The following differences in minimal resistances can be considered statistically significant:

Fluid I	Fluid II	P-value	P-value according to Bonferroni Holms	Significance
NaCl	HAES	0.0007	0.0018	+
Reference standard	HAES	0.0007	0.0019	+
HAES	TelebrixGastro®	0.0013	0.0019	+

Table 11: Results of two sample t-test analysis of relationship between minimal resistance achieved and fluid used

The average minimum resistance achieved under HAES was significantly higher than under saline solution, the reference standard or TelebrixGastro®. Differences in minimum resistance between HAES, glucose, Magnevist® and distilled water were all not statistically significant.

In order to evaluate the relationship between the coagulation size achieved and the duration of RFA, the energy applied and the minimal tissue resistance was calculated.

		Correlation coefficient r	Significance level P	95% confidence interval for r
Volume	Duration	0.5751	<0.0001	0.3211 to 0.7519
	Energy	0.6215	<0.0001	0.3844 to 0.7817
	Minimum resistance	0.5555	<0.0002	0.2950 to 0.7391

Table 12: Results of the calculation of Pearson's correlation coefficient for volume and duration, volume and energy, volume and minimal resistance

The calculations show a moderate positive correlation between the coagulation volume, the duration of the procedure, the energy applied and the minimum resistance registered within the tissue.

6.0 DISCUSSION

Hepatocellular carcinoma and hepatic metastases make up the majority of liver malignancies today. The late discovery of most of these diseases as well as the patient's compromised state of health often make radical or curative surgery difficult. This creates the need for alternative therapy methods. Image-guided RFA has proven to be an effective alternative method to treat HCC's and liver metastases. However, the application of RFA is still limited by the size of the necrosis achieved, which is seldom larger than 3.5 to 4.5 cm in diameter, minus the necessary safety zone of 10 mm [164, 147, 165].

Several methods have been devised to improve the outcome of RFA. Some techniques focus on the design of the electrode itself, and have led to the development of internally cooled electrodes, bipolar electrodes, cluster arrays, multiple expandable electrodes and open perfused or wet electrodes [132, 109, 106, 136, 142]. All these techniques aim at enlarging the tissue area affected by the electrode to reduce the risk of tissue boiling and charring around the electrode tip, which decreases thermal and electrical tissue conductivity.

Other techniques focus on improving the electrical and thermal conductivity of the targeted tissue by introducing conductive fluids, either through continuous infusion or by preinjection [106].

The aim of the presented study was to identify fluids which improve the effect of bipolar RFA substantially. Studies done up to now on fluid modulated RFA focussed on the use of hypertonic saline solution, acetic acid and ethanol in combination with RFA. In 2003, Lee et al. performed a study using hypertonic saline solution to enhance radiofrequency ablation and achieved significantly larger lesions with a saline solution enhanced bipolar electrode than with a saline enhanced monopolar electrode [141]. In 2004 Lee et al. conducted a further study, comparing wet RFA to dry RFA and RFA with preinjected boli of saline solution. They reached the conclusion that wet RFA using an open perfused electrode with 5% or 36% NaCl solution creates significantly larger ablation zones than dry RFA or RFA with preinjections of NaCl solutions [166]. Saline solution is used to enhance RFA because hypertonic saline solution increases the electrical and thermal conductivity of the tissue. Furthermore, the higher boiling point of saline solution helps to avoid rapid boiling of tissue adjacent to the electrode tip, thus preventing a rapid build-up of insulative gas [167, 168].

Other researchers enhanced RFA with an acetic acid-hypertonic saline solution, combining a cytotoxic agent with a conductive fluid, and achieved larger ablation volumes in a single session than with bipolar RFA alone [159].

Studies using ethanol injections in combination with RFA have also achieved larger lesions than just percutaneous ethanol injection or RFA alone [169, 170].

The assumption of these studies was that the introduction of fluids into the tissue lowers the rate of tissue desiccation and charring and, in the case of hypertonic saline solution, also improves tissue conductivity and therefore allows a larger amount of energy to be deposited. In the case of ethanol and acetic acid the tissue damage was even more increased by their cytotoxic properties. None of the studies in the past have systematically evaluated the influence of the fluids' conductivity on the ablation size.

The investigation of this study was based on the hypothesis that injecting highly conductive fluids increases the conductivity of the tissue and therefore leads to larger areas of ablation. A variety of fluids, which had been tested for their conductivity earlier, were injected into porcine liver specimens prior to RFA and the effects of these fluids on the ablation size were compared. These fluids were Telebrix Gastro, Magnevist, 5% glucose solution, 0.9% saline solution, distilled water, 95% Ethanol and HAES.

Bruners et al. found out in their study on fluid modulated RFA that HAES, Magnevist and Telebrix appeared to be the most suitable fluids for enhancing the ablation process. In their in-vitro experiments, these fluids showed to have the fastest heating time and appeared the most promising to enhance RFA. In the same study, glucose showed to have the longest heating time and was discussed to have a possible protective effect during RFA. Distilled water, as a non-ionic and therefore non-conductive fluid was also considered to have a possible protective effect during RFA. The effects of ethanol and 0.9% NaCl solution on RFA have already been investigated in several studies and were included in this particular study as comparison to the other fluids.

This study showed promising results on the effects of fluid modulated RFA. The results showed that RFA achieved considerably larger coagulation volumes with any of the fluids under investigation compared to RFA alone. The largest ablation volumes were

achieved under the use of HAES with an average volume of $4.83 \pm 0.97 \text{ cm}^3$. The coagulation volumes achieved using HAES were not only significantly larger than those achieved under the reference standard, with a p-value of 0.0001, but were also significantly larger than the ablation volumes achieved by any of the other fluids except glucose. This means that the injection of HAES prior to RFA achieved coagulation volumes approximately 3.4 times larger than the ablation volumes achieved under the reference standard. This observation goes in line with the observations made by the study of Bruners et al, where HAES showed to have the fastest heating time, second to TelebrixGastro®, as it was more conductive than the majority of the other fluids used in their study.

The ionic contrast agents Magnevist® and TelebrixGastro® also produced statistically significant larger coagulation volumes than non-enhanced RFA, with an average coagulation volume of $2.49 \pm 0.37 \text{ cm}^3$ and $2.53 \pm 0.39 \text{ cm}^3$ respectively. With the introduction of these fluids into the tissue, the ablation volumes were increased almost twofold, the average volume achieved using RFA alone being $1.42 \pm 0.33 \text{ cm}^3$. A further advantage of these fluids is that their distribution within the tissue can be displayed during image-guided RFA.

Isotonic 0.9% saline solution produced an average coagulation volume of $1.58 \pm 0.49 \text{ cm}^3$, which was only marginally larger than that achieved under the reference standard. Compared to other studies where saline solution enhanced RFA produced statistically significant larger ablation volumes than dry RFA, the results achieved in this study using 0.9% NaCl were not statistically significant.

The observation that highly conductive and ionic fluids such as HAES, TelebrixGastro® and Magnevist® produced statistically larger coagulation volumes than dry RFA or RFA enhanced by isotonic saline solution, confirm the considerations set up in this study's hypothesis. It appears that, due to their high conductivity, these fluids lead to an improved tissue conductivity and thus to larger coagulation volumes. This consideration is further supported by the observation that these fluids also led to a statistically significant increased energy input compared to the reference standard. HAES achieved an average energy input of $5.90 \pm 0.87 \text{ kJ}$, which presents an increase by a factor of 2.1 compared to the reference standard, which achieved an average energy input of $2.8 \pm 0.49 \text{ kJ}$. With an average energy input of $4.27 \pm .077 \text{ kJ}$, TelebrixGastro®

lead to an increase of energy input by a factor of 1.5 and Magnevist increased the energy input by a factor of 1.34, achieving an average of 3.74 ± 1.10 kJ.

Even though the fluids named above appear to have led to a statistically significant enhancement of RFA, they did not shorten the ablation time. Compared to the reference standard, HAES, Magnevist® and TelebrixGastro® led to longer times of energy application, although here only the difference in time between HAES and the reference standard proved to be statistically significant. Of all the fluids used in this study, only isotonic NaCl solution showed a significantly shorter heating time than the reference standard, with an average time of 319.2 ± 60.72 sec, compared to an average time of 324.40 ± 36.34 sec in the reference standard. The longer heating time of HAES, Magnevist® and TelebrixGastro® is most likely explained by the fact that due to the increased ion concentration in the tissue, the current was conducted further away from the electrode's tip, reducing fast temperature increases directly adjacent to the active tip, allowing a longer time of energy application and thus also a larger amount of energy deposition.

Unexpected results

Surprisingly the instillation of glucose also produced significantly larger lesions than non-enhanced RFA, even though it is not an ionic solution and should therefore act more as an insulant. In theory, infusion or injection of ionic solutions should improve electrical and thermal tissue conductivity and should lower tissue resistance. The fluid should also extend the surface of the electrode, creating a so-called virtual electrode, and preventing rapid tissue desiccation and a rise in impedance. Therefore, injection of insulating or non conductive fluids should lower tissue conductivity and therefore the current flow in the tissue. This should lead to comparatively small lesions.

But in the case of this study, quite large ablations were achieved using glucose, with an average coagulation volume of 3.15 cm^3 . Similarly large ablation volumes were also achieved using distilled water, which is originally also non-conductive and presented with an average coagulation volume of 2.68 cm^3 .

One explanation for this could be that non-ionic fluids were diluted by ionic tissue fluid, achieving an overall ion concentration which was still high enough to enable effective tissue conductivity. Another explanation could be that even though 5% glucose solution

did not improve tissue conductivity, it still helped to create a “virtual electrode” and as the fluid reached its boiling point, enlarged the tissue area affected by the heat.

This could be an important limitation in the practice of using 5% dextrose solution (D5W) during RFA as an insulator to protect adjacent structures of the ablation site. The risk of gastrointestinal wall perforation during RFA procedures occurs in 0.7% of all cases [171]. 5% Dextrose solution is supposed to mechanically form an insulative envelope and therefore protect untargeted tissue from thermal injury [172]. The solution is already used for the purpose of protecting perihepatic structures from thermal damage [173]. D5W redirects the current away from the site of fluid instillation and so creates a better thermal protection than saline solution. Increasing the thickness of the D5W layer around the protected structures provides additional protection, even though a fluid layer of 1 cm in thickness is considered adequate for protection, even if the RF electrode is placed only 0.5 cm away from the insulating layer [174].

The fact that during these experiments 5% glucose solution was able to achieve quite a large ablation volume, and therefore a longer heating time, might limit the use of D5W as a protective insulant.

It appears that non conductive fluids are able to act as a reservoir for heat, increasing the tissue’s thermal conductivity and could therefore cause a convection of heat to distant structures from the ablation site.

Even though the difference in coagulation size achieved using distilled water was not significantly larger than that of the reference standard, the result of RFA enhanced with distilled water was still surprisingly good. One reason for the increased coagulation size could be that distilled water, as a hypotonic solution, could have led to a shift of water into the cells, causing a cell hydrops, which would then lead to cell membrane rupture. This would set free intracellular ions into the tissue, which might be the cause for prevailing tissue conductivity and tissue heating.

It could also be that heating the liver samples to physiological temperatures in the water bath containing 0.9% saline solution increased the ion concentration in the tissue and that this affected the conductivity of the distilled water injected into the samples. However, the water bath in physiological saline solution was supposed to mimic the

physiological environment in in-vivo tissue, so this effect could also be observed in-vivo due to the blood flow.

Another explanation might be that, in accordance with the consideration set up regarding the results achieved using 5% glucose solution, the distilled water helped create a “virtual electrode” and so could heat a larger tissue area.

The conclusion drawn from these surprising outcomes using non-conductive fluids could be that the instillation of any fluid increases the boiling point of the tissue and leads to increased thermal stability of the tissue.

The instillation of HAES achieved significantly larger coagulation volumes than all the other fluids used in this study. This is in accordance with the observations set up in the in-vitro study carried out by Bruners et al, which showed HAES to have one of the fastest heating times and also the most rapid increase in temperature. As HAES is a hyperosmolar solution, it leads to a shift of intracellular water into the extracellular compartment. The use of HAES to treat hypotension during shock is based on this effect, as it increases blood volume in the blood vessels, when given intravenously, thus raising the blood pressure. This effect would also improve thermal conductivity in the injection area and would also prevent fast tissue dessication as it leads to a shift of tissue fluid into the injected area.

Another interesting result of the presented study is that, in contrast to the results published in other studies, the coagulation volumes achieved under the instillation of physiological saline solution are not significantly larger than those obtained through dry RFA alone. It is possible that the use of 0.9% saline solution is not sufficient to raise tissue conductivity, as it merely increases the volume of tissue fluid present, but not the conductivity. The majority of the other studies, for example the study published by Lee et al. [141], used a hypertonic saline solution to enhance the RFA process. It is therefore possible that isotonic saline solution does not increase electrical tissue conductivity enough to achieve greater coagulation volumes. This observation, however, differs from the conclusion set up by Aubé et al. in their study on the effect of different NaCl concentrations on RFA [175]. The results of their study showed no clear benefit of any concentrations higher than isotonic. Another possibility is that the water bath in isotonic

saline solution already saturated the liver samples and that any further addition of saline solution did not make a difference to tissue conductivity.

It could also be that the injection of 1 ml of fluid was not sufficient to raise thermal and electrical conductivity of the tissue and that larger volumes are required.

The manual injection of 1 ml of fluid could have affected the distribution of fluid in the tissue as it was difficult to always maintain the same, steady injection rate. This made it difficult to ensure a homogenous distribution of fluid in the tissue, which could also explain the differences in coagulation sizes. The use of openly perfused electrodes would lead to a permanent, steady infusion of conductive fluid into the targeted tissue, thus making a homogenous distribution more probable and would probably improve electrical and thermal conductivity even further. It may also lead to a constant cooling of the tissue adjacent to the electrode tip preventing a rapid build-up of insulating gas and inhibiting the carbonisation process. The heated fluid would be carried further into the tissue, allowing a larger area to be affected by the heat.

RFA has already been presented as a minimal invasive technique, which is especially suited for patients with a high co-morbidity, who are not candidates for curative, radical surgery. However, due to the limits of achievable coagulation volumes, several long intervention sessions are needed to ensure complete tumour ablation. This in turn increases the stress imposed on the patient, through general anaesthesia and mechanical ventilation during the intervention and also increases the therapy costs. It is therefore desirable to shorten the intervention time for achieving effective coagulation volumes.

A possibility to achieve larger coagulation volumes is to combine different percutaneous intervention strategies. Here again the aim is to increase the efficiency of the treatment during one single session by combining different methods rather than repeating the same treatment over several sessions.

One combination is that of acetic acid and RFA. Acetic acid is ionic and water soluble and has a similar cytotoxic effect as ethanol, but can be used in lower concentrations [159, 176]. Lee et al. showed in their study that low concentrations of acetic acid-hypertonic saline solution are just as effective as high concentrations, but involve fewer complications such as chemical peritonitis [159]. The effect is that of a synergistic mechanism, acetic acid improves the conductivity of the tissue and leads to thrombosis

of small tumour vessels, prior to RFA. This helps to reduce the heat sink effect, caused by blood perfusion in the tumour tissue [177].

Arrivé et al. evaluated the distribution of acetic acid in hepatic tissue and pointed out the need for homogenous distribution [176]. In 35 of 57 sessions, acetic acid leaked into tissue outside the tumour, leading to possible complications such as peritonitis, hepatic infarction or liver perforation [178].

The combination of percutaneous ethanol injection (PEI) and RFA has also shown larger coagulation volumes, but only if PEI is performed before RFA. Ethanol seems to induce immediate changes in the tissue, resulting in increased heating and coagulation [169]. It induces coagulation necrosis and thrombosis of blood vessels, thus reducing the blood flow within the tumour [179]. PEI holds major advantages over other methods to reduce the blood flow, such as the Pringle Manoeuvre, as it is a minimal invasive technique [169]. In a 5 year follow up study, Vallone et al. determined the combination of PEI and RFA to be an effective treatment for large hepatocellular carcinomas. This combination treatment showed an overall survival rate of 92% after one year, 87% after two years and 83% after three years [170].

However, in this study, ethanol produced irregularly shaped ablation zones, different from the regular ellipsoid shape formed by the other fluids used in this study. This is in accordance with the observation by Arrivé et al. as described above. The tendency of ethanol to produce irregular ablation zones makes it difficult to ensure complete ablation of tumours, most of which have a regular spherical shape. It also makes it difficult to control which tissue area is to be affected by the ablation process and damage to adjacent structures would be difficult to predict.

Limitations of this study

There were several limitations to this study. It was based on ex-vivo experiments, lacking the conditions present in a living organism. For example, blood flow in in-vivo tissue leads to a cooling effect and could transport heat away from the targeted site. This has already been described as the so called heat sink effect. Furthermore, it is possible that the ex vivo tissue had already changed its ionic composition. Even though steps were taken to mimic the physiological conditions in a living organism by placing the liver specimen into a water bath containing isotonic saline solution, it is still possible that the ionic composition of the tissue differs so greatly that it has an effect on the ablation process. These factors make it difficult to transfer the results of this study to a clinical situation. Therefore, further in-vivo experiments might be needed to verify the results of this study.

Also, only the preinjection technique was used, where a small volume of the fluid (1ml) was injected into the tissue prior to RFA. One of the disadvantages of this technique is that the injected volume disperses widely into the tissue and is not confined to the ablation site. During the experiments, the injected fluid sometimes oozed out of the liver specimen even though it was injected slowly. It was therefore difficult to control exactly how much of the fluid stayed within the tissue. During in-vivo conditions the pre-injected enhancing agent might diffuse out of the tumour or be washed out by blood perfusion. There is also no continuous flow of liquid to fill the gap between the electrode and the tissue, which would prevent the build up of insulative gas around the electrode tip as it is the case in continuous wet electrode perfusion. The preinjection technique also lacks the cooling effect provided by a continuous infusion of liquid. Previous studies show an advantage of continuous fluid infusion over preinjection [166]. Pre-injection of enhancing agents is an advantage when using cytotoxic substances such as ethanol or acetic acid, as a continuous infusion of these substances increases the risk of critical amounts passing into the blood circulation and harming the organism.

Furthermore, the number of experiments (n=5) carried out for each fluid, are possibly not sufficient to demonstrate a trend in coagulation size. For example Lee et al. performed 10 trials per variable [166], which might provide a more accurate trend in coagulation size. It also appears that 5 trials per variable were not sufficient to compensate for outliers which distorted the trend. This was the case for example in the

experiments carried out with isotonic saline solution, where the last trial produced an ablation volume considerably smaller than the previous trials (0.79 cm^2 as opposed to volumes $>1 \text{ cm}^2$ in trials 1 to 4).

Another limitation may be that only healthy liver tissue was used, and pathologically altered tissue might react differently to the RFA process. Mertyna et al. [180] conducted a study investigating the heat sensitivity of different ex-vivo tissue and in-vivo tumour models and results showed that, for example, healthy kidney tissue appears to have higher heat sensitivity than tumour tissue. This suggests that the type of tissue does indeed affect the efficacy of RFA. Tumour tissue often has more blood vessels than healthy tissue, which might affect the distribution of heat within the ablation zone due to increased tissue perfusion. HCC lesions are also known to have a pseudo-capsule which leads to the so-called “oven effect”, as the pseudo-capsule confines the heat, leading to much larger ablation volumes than expected by the electrode design.

The use of mere tap water to perfuse the internally cooled electrode might also have a limiting effect on the ablation process. Even though the effect is expected to be only minimal, it is possible that the perfusate was not cold enough to prolong the carbonisation process around the active tip, which led to an earlier rise in impedance, ending the ablation process. Perhaps longer ablation times and thus larger ablation zones would have been achieved if chilled perfusate had been used, which would cool the electrode tip more effectively.

Further investigations needed

Although this study shows promising results on the effects of fluid modulated RFA, there are still some aspects that need to be investigated in more depth. It seems that the effects of injecting fluids to enhance the RFA process are more complex than hitherto anticipated. Other factors than the ionic composition and conductivity of the fluid seem to play an important role and will need further investigation.

The surprising results that were received when using 5% glucose solution and distilled water as non ionising fluids, lead to the assumption that the instillation of any fluid increases the boiling point of tissue and thus enables a better thermal stability. Further studies on the effects of non ionising fluids on RFA need to be conducted in order to better understand these phenomena.

The results of this study are encouraging, but they will need to be made more reliable. The effects of the fluid should be reanalysed using in-vivo tissue and possibly even pathologically altered tissue, such as liver tumours. It would also be interesting to find out in how far the continuous infusion of the fluids improves the results of the RFA.

Fluid modulated RFA has shown to be a promising method, by which the coagulation volumes achieved during the ablation process can be increased. However, more research is needed in order to fully understand the effects these fluids might have on the affected tissue and the organism as a whole. Further research is also needed in order to explore the possibilities of using other fluids, which might have an even greater impact on RFA.

7.0 SUMMARY

Percutaneous ablative therapies continue to be of growing importance in tumour therapy and are already part of clinical practice. The benefits derived from these minimally invasive techniques, such as reduced intra-and post-interventional morbidity and mortality and cost effectiveness, make them particularly attractive, as they reduce the length of stay in hospital and can be applied on an outpatient basis.

Minimally invasive tumour intervention techniques include percutaneous ethanol or acetic acid injections, transcatheter arterial chemoembolisation and thermal tumour ablation such as cryoablation, laser induced thermotherapy, microwave coagulation therapy and radiofrequency ablation (RFA).

RFA has proved to be of special importance in the treatment of hepatic malignancies. The most common hepatic malignancies are hepatocellular carcinoma and hepatic metastases, which mostly originate from colorectal cancer or breast cancer.

The principle of RFA is the use of a high frequency alternating current, at a frequency of 350 to 460 kHz, to create heat induced coagulation necrosis. The radiofrequency current is applied into the tissue using a RF applicator, and flows towards a grounding pad or dispersive electrode. This is also known as monopolar RFA. In bipolar RFA, the dispersive electrode is incorporated into the tip of the needle electrode, eliminating the need for a ground pad.

RFA using monopolar electrodes produces limited coagulation sizes of only 0.6 to 1.7 cm in diameter [108]. In order to overcome this limitation different modes for applying RFA were tested. Multiple electrodes in free standing arrays, cluster arrays or in form of expandable umbrella electrodes aim at enlarging the area onto which energy is applied. Wet electrodes increase energy deposit into the tissue by lowering tissue resistance and increasing tissue conductivity. Internally cooled electrodes reduce the risk of charring close to the electrode, which causes a massive increase in tissue impedance and limits energy deposit.

Despite of these modifications to RFA techniques, the diameter of the coagulation seldom exceeds 5 cm [7]. This limits indications for RFA to small, single HCCs and small hepatic metastases.

The aim of this study was to investigate how the injection of conductive fluids into tissue before RFA, would affect to ablation process. The hypothesis being, that fluids with a high conductivity should enhance tissue conductivity and should therefore increase energy input into the tissue, achieving larger coagulation volumes.

The fluids used in this study were: 0.9% NaCl solution, Magnevist®, Telebrix Gastro®, HAES, 5% glucose solution, 95% ethanol and distilled water.

Bipolar RFA was carried out on freshly excised porcine liver specimen using a CelonLabPower RF-system and the internally cooled CelonProSurge T20 applicator. Before each ablation process, 1 ml of the fluid to be tested was injected into the specimen using a G21 hypothermic needle. Temperature within the specimen was recorded by inserting two fiberoptic thermocouples parallel to the applicator. Data concerning impedance, resistance and energy input was recorded using the CelonPower Monitor software. After RFA was completed, the specimens were dissected and the lesion size was assessed macroscopically using a ruler.

Compared to RFA alone (mean coagulation size 1.42 cm³), significantly larger coagulations ($p < 0.001$) were achieved using HAES, glucose, Magnevist, TelebrixGastro® and Ethanol. The largest coagulation volumes were achieved with HAES, with a mean volume of 4.83 cm³, the smallest were achieved using RFA alone with a mean volume of 1.42 cm³. The highest energy input was achieved using ethanol with a mean energy input of 6.38 kJ and the longest time of energy application was achieved using 5% glucose solution with a mean heating time of 841.40 sec.

Unexpected results were achieved using non-conductive fluids such as glucose solution or distilled water, indicating that the use of any fluid, irrelevant of its conductivity, seems to have an enhancing effect on RFA.

This study on fluid-modulated RFA indicates that the injection of conductive fluids can increase the size of coagulation necrosis and energy input, by improving not only electrical conductivity, but possibly also thermal conductivity.

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9.0 LIST OF ABBREVIATIONS

Aq. dest.: aqua destillata, distilled water

BCLC: Barcelona Clinic Liver Cancer classification system

BRCA: Breast Cancer Susceptibility Gene

CT: Computed Tomography

D5W: 5% Dextrose Solution

DNA: Deoxyribonucleic Acid

FAP: Familial Adenomatous Polyposis

HAES: Hydroxyethyl Starch

HBS-antigen: Hepatitis B Surface-Antigen

HBV: Hepatitis B Virus

HCC: Hepatocellular Carcinoma

HNPCC: Hereditary Nonpolyposis Colorectal Cancer

LITT: Laser Induced Thermotherapy

MCT: Microwave Coagulation Therapy

MRI: Magnetic Resonance Imaging

NaCl: Sodium chloride

NASH: Non-Alcoholic Steatohepatitis

PEI: Percutaneous Ethanol Injection

RF: Radiofrequency

RFA: Radiofrequency Ablation

TACE: Transcatheter Arterial Chemoembolisation

TAE: Transcatheter Arterial Embolisation

TNM classification: Tumour, lymph Nodes, Metastasis

UICC: Union International Contre le Cancer

VEGF: Vascular Endothelial Growth Factor

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11.0 AUFLISTUNG DER EIGENEN PUBLIKATIONEN

Bruners P, Müller H, Günther RW, Schmitz-Rode T, Mahnken AH. Fluid-modulated bipolar radiofrequency ablation: an ex-vivo evaluation study. Acta Radiol. 2008 Apr;49(3):258-66.

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13.0 ERKLÄRUNG § 5 Abs. 1 ZUR DATENAUFBEWAHRUNG

Hiermit erkläre ich, dass die dieser Dissertation zu Grunde liegenden Originaldaten bei mir, Halina Müller, Dresdener Ring 51, 47441 Moers, hinterlegt sind.

14.0 LEBENS LAUF

Name: Müller
Vorname: Halina
Geburtsort und -datum: Berlin, 12.07.1982
Familienstand: ledig
Nationalität: deutsch

Ausbildung:

Juni 2002: Abschluss des Abiturs und des International Baccalaureate
Oktober 2002 bis Mai 2009: Medizinstudium an der RWTH Aachen
März 2005: Ablegen der ärztlichen Vorprüfung (Physikum)
Februar 2008-Januar 2009: Praktisches Jahr
1. Terial: Klinik für Anästhesiologie und Operative Intensivmedizin am St. Antonius Krankenhaus Eschweiler, bei Chefarzt Dr. med. Hans Georg Lühr
2. Terial: Klinik für Chirurgie am Selian Lutheran Hospital in Arusha, Tansania. Chefarzt Dr. Paul Kisanga.
3. Terial Klinik für Innere Medizin am St. Antnius Krankenhaus Eschweiler, bei Chefarzt Prof. Dr. med. Uwe Janssens
Mai 2009: Ablegen der ärztlichen Prüfung

Berufspraxis

Seit August 2009: Assistenzärztin in der Abteilung für Innere Medizin des Krankenhaus Maria Stern in Remagen, bei Chefarzt PD Dr. Michael Neubrand

Seit November 2011: Assistenzärztin in der Klinik für Anästhesie und Intensivmedizin des Evangelischen Krankenhauses BETHESDA Duisburg, bei Chefarzt Prof. Dr. med. Jörg Meyer