Lars Frich

Radiofrequency ablation of liver tumors
An experimental and clinical study

Faculty of Medicine
University of Oslo
2007
RADIOFREQUENCY ABLATION OF LIVER TUMORS

An experimental and clinical study

LARS FRICH

The Department of Surgery
and
The Interventional Centre
Rikshospitalet University Hospital

Thesis submitted for the degree of Dr. med.

October, 2006

Faculty of Medicine
University of Oslo
Surgery is a conservative art. It takes to novel methods reluctantly as an old dog to new tricks. It was slow to adopt the ligature; slow to adopt the principles of antisepsis; slow to adopt the fastidious technique and painstaking haemostasis that have largely put a stop to operating by the clock. It has been equally slow to adopt the principles of electrosurgery which, from a technical standpoint, are likely to be no less revolutionizing.

— Harvey Cushing, 1928
ACKNOWLEDGEMENTS

The present work was carried out at the Department of Surgery and the Interventional Centre, Rikshospitalet University Hospital during the years 2001-2006. The work was financially supported by the Norwegian Cancer Society, the University of Oslo, Rikshospitalet University Hospital, Skaugens foundation (Bergliot og Sigurd Skaugens fond til bekjempelse av kreft) and Henrik Homans foundation (Legatet til Henrik Homans minde).

I would like to express my gratitude to my primary supervisor Ivar Gladhaug, whose unique combination of clinical proficiency, scientific judgment, openness to pursue new concepts and gentle personality is highly appreciated. Co-supervisor Professor Atle Bjørnerud provided invaluable guidance in the exciting world of magnetic resonance imaging.

My sincere thanks to Professor Erik Fosse at the Interventional Centre who provided excellent facilities for conducting experimental research in an inspiring multidisciplinary environment. I am in debt to former head of the Department of Surgery Anstein Bergan, present head of the Department of Surgery Karl-Erik Giercksky, and head of the Section for Gastrointestinal Surgery Øystein Mathisen for supporting the clinical study and for providing good working facilities.

As my predecessor research fellow, Tom Mala introduced me to the field of thermal ablation of liver tumors. His research and experience provided me with a foundation for which I am most grateful. I am also grateful to Bjørn Edwin whose early enthusiasm for thermal ablation and incorporation of these techniques into clinical practice was an important inspiration for conducting experimental research. I would like to thank the staff at the Interventional Centre whose professionalism and positive spirit have been a most valuable resource. Especially, I would like to thank Sumit Roy and Per Kristian Hol for inspiring scientific discussions and Terje Tillung for assistance in magnetic resonance imaging. It has been a pleasure to cooperate with Knut Brabrand, Gaute Hagen and Trond Mogens Aaløkken at the Department of Radiology in the treatment and follow-up of patients.
I have much appreciated discussions of electrosurgical principles with Professor Sverre Grimnes at the Department of Biomedical and Clinical Engineering. Sigrid Fossheim kindly provided liposomes. Laura Killingbergtrø and the staff at Section for Gastrointestinal Surgery provided unique care to our patients. Kristin Bjørnland and Solveig Pettersen provided expertise in zymography. I am grateful to Professor Ole Petter F. Clausen and the staff at the Department and Institute of Pathology for preparation and examination of histopathologic specimens. Dag Sørensen and the staff at the Center for Comparative Medicine provided excellent care of animals. My sincere thanks also goes to the patients who participated in the clinical study. Hopefully, you have benefited from our common research effort.

Finally, I would like to thank my family and my family-in-law. The ones not here are deeply missed. Special thanks to my children Ingeborg and Jens Andreas who have been a highly loved source of joy. I am immensely grateful for the support my wife Ellen provided during these years. This thesis would not have been possible without her encouragement, love and patience.
## CONTENTS

LIST OF PAPERS ............................................................... VII
ABBREVIATIONS .............................................................. VIII
INTRODUCTION ................................................................. 1
RADIOFREQUENCY ABLATION ................................................. 7
AIMS OF THE STUDY ........................................................... 21
SUMMARY OF PAPERS .......................................................... 23
DISCUSSION ................................................................. 29
FUTURE PERSPECTIVES ....................................................... 39
CONCLUSIONS ............................................................... 43
APPENDIX I - THE BIOHEAT EQUATION ................................. 45
APPENDIX II - A NOTE ON TERMINOLOGY ............................ 47
APPENDIX III - A NOTE ON ANIMAL MODELS ........................ 49
REFERENCES ................................................................. 51
ERRATA ................................................................. 67
LIST OF PAPERS

I  Frich L, Mala T, Gladhaug IP.
Hepatic radiofrequency ablation using perfusion electrodes in a pig model:
Effect of the Pringle manoeuvre.

II Frich L, Bjørnerud A, Fossheim S, Tillung T, Gladhaug I.
Experimental application of thermosensitive paramagnetic liposomes for monitoring
magnetic resonance imaging guided thermal ablation.

III Frich L, Hol PK, Roy S, Mala T, Edwin B, Clausen OPF, Gladhaug IP.
Experimental hepatic radiofrequency ablation using wet electrodes:
electrode-to-vessel distance is a significant predictor for delayed portal vein
thrombosis.

IV Frich L, Bjørnland K, Pettersen S, Clausen OPF, Gladhaug IP.
Increased activity of matrix metalloproteinase 2 and 9 after hepatic
radiofrequency ablation.

Radiofrequency ablation of colorectal liver metastases: evaluation of local tumor
recurrence by 3D volumetric analysis.
Submitted.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D</td>
<td>2-dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>3-dimensional</td>
</tr>
<tr>
<td>CEA</td>
<td>carcinoembryonal antigen</td>
</tr>
<tr>
<td>CRC</td>
<td>colorectal cancer</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>HCC</td>
<td>hepatocellular carcinoma</td>
</tr>
<tr>
<td>MDCT</td>
<td>multi-detector computed tomography</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
</tr>
<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>RF</td>
<td>radiofrequency</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>RPV</td>
<td>right portal vein</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SI</td>
<td>signal intensity</td>
</tr>
<tr>
<td>T₁</td>
<td>longitudinal relaxation</td>
</tr>
<tr>
<td>T₂</td>
<td>transversal relaxation</td>
</tr>
<tr>
<td>VOI</td>
<td>volume of interest</td>
</tr>
</tbody>
</table>
Colorectal cancer is the second most common malignancy in Norway, following cancer of the prostate in men and the breast in women. Average age-adjusted incidence rates in the period 2000-2004 for cancer of the colon were 27.0 per 100,000 person-years in men and 23.9 in women, with corresponding rates for cancer of the rectum 15.9 in men and 10.5 in women. Approximately 3,400 patients are diagnosed with colorectal cancer in Norway per year, and more than 1,600 deaths per year are attributed to this entity. The 5-year survival for localized colorectal cancer is 80-90%. The corresponding 5-year survival in patients with metastatic disease is below 10% [1]. Synchronous metastases, i.e. those recognized at the time of diagnosis of the primary tumor, are found in approximately 15-25% of patients with colorectal cancer. Metachronous metastases, those detected after diagnosis of the primary tumor, are found in additional 25-50% [2-5].

Metastasis (from Greek methistanai, change of place) is the result of a series of interrelated steps in which cancer cells leave the original tumor and migrate to distant organs via the blood or the lymphatic system [6, 7]. Different primary malignant tumors have a propensity to spread to specific organs [7]. The patophysiology of organ specificity in tumor metastasis can be considered in terms of two mutually not exclusive hypotheses: the “seed-and-soil” hypothesis and the “mechanical” hypothesis. The “seed-and-soil” hypothesis was proposed by Paget in 1889 and focuses on the interaction between the cancer cells and the specific organ microenvironment [8]. The “mechanical” hypothesis was proposed by Ewing in 1928, and suggests that the greater the number of tumor cells delivered to a specific site, the more likely it is that a metastasis will develop at that site. The “mechanical” hypothesis is therefore mainly concerned with the anatomy of possible dissemination routes from the organ containing the primary malignancy [7, 9, 10]. Primary tumors in organs drained by the portal circulation have a higher probability of metastasis in the liver without metastasis elsewhere than primary tumors in organs drained by the systemic venous circulation [11, 12]. In line with the “mechanical” hypothesis, this may be related to the fact that the liver is the first visceral organ that malignant cells encounter following release into the portal circulation [13].
data suggest that survival may be improved in selected patients if metastatic disease in the liver can be controlled.

The first documented liver resection was performed on a trauma patient in 1716 [14]. Successful elective liver resection for a mass lesion was performed in 1888 [15]. A total of 59 cases of partial hepatectomy had been described worldwide by 1899 [16]. Resection for metastatic tumors was first described in 1940. Cancer metastatic to the liver was for a long time considered incurable, and systematic attempts to treat liver metastasis by surgical resection were not made until the 1960s. In a retrospective study from 1963, an average survival of 35 months was found in 25 patients with liver metastasis from different primaries treated by surgical resection [17]. A 1978 literature review of more than 400 patients treated with liver resection for metastatic cancer from various primary tumors concluded that cure could be achieved, especially in patients with primary colorectal tumors [18]. Liver resection was associated with a mortality of nearly 30% in early series [14]. Several technical advances have gradually transformed liver resection into its present state as a safe routine surgical procedure [19]. Due to improvements in surgical technique and intra- and postoperative patient support, the current operative mortality rates are less than 5% with a 5-year survival of 30-50% [20-22]. Recent studies have shown improved survival compared with historical series, with 5-year survival of 58% [23-25]. With the reduced perioperative mortality, a more aggressive approach has emerged towards resection of colorectal liver metastases. Repeat liver resections in patients with isolated liver recurrence after the first hepatectomy have shown survival rates comparable to those achieved by first hepatectomies [26-28]. Curative hepatectomy has been performed in selected patients with initially unresectable colorectal liver metastases after downsizing by chemotherapy [29]. However, at present only 10-15% of patients with colorectal liver metastases are candidates for potential curative hepatic resection [5, 29].

Evaluating the possible benefit of a treatment requires knowledge of the natural course of the disease. Patients with colorectal liver metastases accepted for surgical treatment represent a subset with limited disease and favorable prognosis. Patient selection bias may therefore present problems in interpreting the results of interventions in patients with limited hepatic colorectal metastases. Most studies of the natural history of malignant liver metastases are retrospective studies conducted in the 1960s and 1970s, without the advantage of modern imaging modalities. Median survival for patients with untreated liver metastases from colorectal cancer rarely exceeds 9 months [30, 31]. In a retrospective study of 252 patients with biopsy proven hepatic metastases that were not resected, 5-year survival was less than 3% [32]. A prospective study of 484 patients conducted between 1980-90 found a median 3-year survival of 0.9% [33]. Improved short-term survival, but no 5-year survival was found in 62 patients.
with potentially resectable metastases that were not resected [34, 35]. Although the efficacy of liver resection versus no treatment has not been assessed in prospective randomized studies, it is generally accepted that resection of colorectal liver metastases improves long-term survival [21, 36, 37].

Surgical treatment is by its nature directed towards localized disease. Precise knowledge of the extent and anatomical location of malignant disease constitute a fundamental prerequisite for rational surgical management. Accurate preoperative staging is dependent on the sensitivity of the imaging modality used. Although the sensitivity of sonography, computed tomography (CT) and magnetic resonance imaging (MRI) is high for hepatic metastases above 1-2 cm diameter, hepatic metastases below 1 cm diameter can only occasionally be detected by these imaging modalities. Thus, colorectal cancer patients may have more widespread disease than anticipated from preoperative staging [38]. Several classification systems are currently used for staging of colorectal cancer. Dukes’ classification for rectal cancer, proposed in 1932 and modified in 1958 [39, 40], is widely used as a valuable prognostic indicator in patients with colorectal cancer. According to this classification, colorectal cancer is staged into A, B or C based on the tumor involvement of the wall of the colon and rectum. Dukes’ classification has later been expanded to include stage D for patients with distant metastasis. The TNM classification of malignant tumors was introduced by the International Union Against Cancer in 1958 [41]. The TNM system provides anatomic information on primary tumor, lymph node involvement and the presence or absence of distant metastasis. These staging systems do not separate between patients with resectable or non-resectable hepatic metastases. It has therefore been argued that new staging systems should be developed for patients with advanced disease [42].

Treatment of cancer can be provided as palliative or curative therapy. Palliative therapy is provided in the context that symptoms are present that can possibly be ameliorated by the treatment, but that eradication of the cancer is not expected. Curative therapy implies that a chance, albeit sometimes small, exists for complete eradication of the cancer. It is generally accepted that surgical resection for malignant liver disease should only be performed with a curative intent [31]. Criteria used for evaluating if a patient with malignant disease confined to the liver is eligible for hepatectomy can be categorized as technical criteria, such as tumor proximity to major intrahepatic vessels precluding safe resection, biological criteria assumed to be associated with inferior prognosis such as the number, size or distribution of tumors, or general criteria related to the physical condition of the patient. Disease in patients selected to hepatectomy was previously limited to one to three unilobar metastases, preferably presenting metachronously and resectable with a margin of 1 cm [42]. The definition of resectability has evolved, and the current definition of resectability with curative intent is
based on the ability of the surgeon to achieve clearance of all measurable disease from the liver, while leaving a healthy future remnant liver of at least 20% of the total liver volume [42, 43]. Extrahepatic malignant disease has been considered an absolute contraindication to hepatectomy for colorectal liver metastases [21, 44], but this dogma has recently been challenged [45, 46].

Methods for local ablation\(^\text{i}\) of malignant tumors were developed in order to achieve local tumor control and possibly increased survival in patients that could not be offered curative hepatic resection. Ablation techniques can be categorized into chemical or thermal methods according to the physical principle used for induction of tissue damage. Early attempts in using cold to treat liver tumors were crude and involved direct application of liquid nitrogen to tumors or to metallic instruments in contact with tumors [47]. Modern cryoablation devices use nitrogen or argon gas under high pressure, which is led into a cryoprobe whose tip is cooled down to subzero temperatures. However, the inability to visualize deep liver tumors limited the use of local ablation until intraoperative sonography became available in the late 1980s [48]. Several minimally-invasive methods producing localized heat such as microwave coagulation therapy [49], laser ablation [50] and radiofrequency ablation [51] are currently in clinical use for treatment for hepatic tumors. High-intensity focused ultrasound offers a non-invasive approach to tissue destruction, but has not yet come into clinical use for treatment of malignant liver tumors [52]. Several recent reviews present current methods for local ablation of hepatic tumors [53-57].

The therapeutic approach to colorectal hepatic metastases has changed dramatically over the last three decades, from an evaluation of whether surgical resection was indicated or not, to a complex multidisciplinary field involving surgeons, radiologists and oncologists offering an increasing number of treatment modalities [54, 58, 59]. Current treatment of colorectal hepatic metastases includes strategies for achieving hepatic resection in previously non-resectable patients such as neoadjuvant\(^\text{ii}\) chemotherapy, preoperative portal vein embolization to increase the future remnant liver [60], 2-stage resection approaches [61] and ex situ resection with liver autotransplantation [62]. Non-resection treatments currently used are tumor ablation [63, 64], locoregional or systemic adjuvant chemotherapy [65], local radiation by microspheres [66], external beam radiotherapy [67], immunotherapy [68] and gene therapy [69].

\(^{i}\) From Latin ablatio, removal or destruction. In the context of this thesis used to describe methods used for local tissue destruction by heat, cold or chemical substances.

\(^{ii}\) From Latin adiuvans, to help. Adjuvant chemotherapy: chemotherapy given after tumor surgery to prevent cancer recurrence. Neoadjuvant chemotherapy: chemotherapy given prior to surgical removal of a tumor to shrink the tumor, with the intent of making the patient operable or the surgical procedure less extensive.
Radiofrequency ablation is an electrosurgical method utilizing the flow of electrical current through tissue to dissipate heat. An electrode is placed inside the tumor, and tissue in the vicinity of the electrode is heated, producing localized tissue destruction [70]. This modality has recently been introduced in the treatment of patients with non-resectable hepatic metastases [51, 71].

This study was carried out to investigate various experimental aspects of hepatic radiofrequency ablation, as well as the clinical application of this technique in a well-defined patient population with colorectal liver metastases.

iii The term radiofrequency refers to the frequency of the alternating current used in these systems (300 - 500 kHz), which is within the radiofrequency spectrum.
ELECTROSURGICAL PRINCIPLES

Electrosurgery can be defined as the therapeutic use of heat dissipated by high-frequency electrical current passing through biological tissue. Electrosurgery can be performed with either monopolar or bipolar techniques; monopolar techniques are more commonly used for tumor ablation. For monopolar electrosurgery an asymmetrical electrode system is utilized where a small active electrode is placed in the tissue to be treated and a large dispersive electrode is placed on the skin of the patient. With the bipolar technique, two identical electrodes are placed in the tissue close to each other, and the current passes through tissue locally between the electrodes.

Fundamental quantities when describing an electrical circuit is the electrical potential (volt, V), the flow of electric charge per time unit (ampere, A), and the resistance through which the current passes (ohm, Ω). Electrical current may be unidirectional (direct current, DC) or may change direction (alternating current, AC). Alternating current can be characterized by the frequency of cycles per second (hertz, Hz). The opposition to alternating current is denominated impedance, and is determined not only by the resistance, but also by the reactance, which is the capacitive and inductive components of the electrical circuit. The reactance and hence the impedance is dependent on the current frequency. The impedance is represented by the symbol Z, and is measured in ohm (Ω).

The relationship between the electrical potential, current and resistance in a resistive direct current circuit is expressed by Ohm’s law, where V is the potential difference, I is the current, and R is the resistance:

\[ V = R \cdot I \quad (1) \]

Power in a purely resistive circuit is represented by the symbol P (watt, W), and is determined by the potential and current:

\[ P = I \cdot V \quad (2) \]

By combining Ohm’s law (1) with equation (2), the power can also be expressed as:

\[ P = I^2 \cdot R \quad (3) \]
The relationship between energy, current, resistance and time can be determined by Joule’s law where \( Q \) is energy (joule, J), \( I \) is current (A), \( R \) is electrical resistance (\( \Omega \)) and \( t \) is the length of time the current is permitted to flow (sec):

\[
Q = I^2 \cdot R \cdot t
\]

The central concept in electrosurgery is that current passing through tissue will dissipate electrical energy as heat. Equation (4) shows that energy generated in an electrical circuit is a function of the current to the second power. The current passing through the active electrode, the tissue and the dispersive electrode is the same per time unit. Because the resistance of the metal electrodes is negligible, virtually no heat will be dissipated in the electrodes. Both the active and the dispersive electrode are therefore cold during electrosurgery.

Biological tissue consists of cells containing ionic electrolytes, surrounded by cell membranes with capacitive properties. The cells are surrounded by extracellular electrolytes and organized into organs enclosed by membranes with different electrical properties than the intracellular and extracellular electrolytes. The impedance of tissues is much higher than the impedance of electrodes [72]. Hence, current passing through tissue will dissipate heat.

The current density (\( J \)), defined as the electric current per cross-sectional area (A/m²), is constant throughout a cable if the cross section of the cable is constant and the material of the cable is homogeneous. When current-carrying electrodes are brought into contact with tissue in a closed circuit, current spreads out in the tissue volume. An idealized model of a spherical electrode positioned in a homogeneous medium illustrate that the current spread out from the electrode in the shape of a sphere:

\[
J = \frac{I}{4 \cdot \pi \cdot r^2} \quad r \geq a
\]

where \( J \) is the current density, \( I \) is the current passing through the electrode, \( r \) is the distance from the electrode, \( a \) is the radius of the electrode.

In a monopolar system, the active electrode is small, leading to a high current density. In contrast, the dispersive electrode is large in order to permit the limited heat produced by the low current density to be dissipated by conduction [73]. Assuming adiabatic conditions, i.e. that no heat is transferred to or from the tissue (for instance by blood flow), and no phase difference between the current density and the electric field, the temperature rise in a tissue volume is a function of the current density to the second power:

\[
\Delta T = \frac{J^2 \cdot t}{\sigma \cdot c \rho}
\]

where \( \Delta T \) is the temperature rise, \( J \) is the current density, \( t \) is the time, \( \sigma \) is the tissue conductivity, \( c \) is specific heat capacity and \( \rho \) is the density.
The power density \( W_v \) (watt/cubic metres) is an expression of the electrical energy per time unit that tissue is exposed to under ideal conditions [74]. The power density falls off extremely rapidly with distance from the electrode, as it is inversely proportional to the distance from the electrode to the fourth power:

\[
W_v = \sigma V^2 \frac{a^2}{r^4}
\]

where \( \sigma \) is the tissue electrical conductivity, \( V \) the voltage, \( a \) the radius of the electrode, and \( r \) the distance from the electrode.

Although the relationship between the temperature rise in a tissue volume and the current density described by equation (6) is valid for an adiabatic model system, additional factors such as heat loss and metabolic tissue heating influence tissue temperature in vivo. The bioheat equation (Appendix I) is a mathematical model that incorporates these factors [76]. The general concept of the bioheat equation can be simplified to [55]:

\[
\text{extent of coagulation} = \text{energy deposited} \times \text{tissue interactions} - \text{heat loss}
\]

This model can be used to predict the extent of tissue coagulation produced by thermal energy under a variety of circumstances [77]. During thermal ablation in a given tissue volume in vivo, increase of coagulation volume can be achieved by increasing energy deposition or by reducing heat loss. During monopolar electrosurgery, the electrode-to-tissue interface at the active electrode is exposed to a high power density which may cause a rapid temperature rise. Consequently, tissue in proximity to the active electrode is susceptible to carbonization. Tissue carbonization leads to increased tissue impedance and an abrupt fall in the flow of electrical current, which limits tissue heating and thereby the extent of the coagulation [75]. The balance between obtaining a sufficient power density at a distance from the electrode while avoiding tissue carbonization at the electrode-to-tissue interface is a fundamental biophysical limitation of electrosurgery which was noted as early as 1928 [73].
TISSUE RESPONSE TO HEAT

The tissue response to heat is a function of temperature and exposure time. Tumors are believed to be more thermosensitive than normal tissue, possibly due to inability to dissipate heat effectively [78-80]. Cellular homeostasis is maintained with a mild elevation of temperature to approximately 40˚C. When temperatures are increased to 42-45˚C cells become more susceptible to damage by other agents such as chemotherapy and radiation [81]. Below a tissue temperature of 45˚C thermal changes are largely reversible [82]. With a temperature of 50-52˚C, cellular death occurs within 4-6 minutes [70, 80, 83]. At temperatures above 60˚C near instantaneous protein coagulation and irreversible cellular damage occurs [55]. The liquid in the tissue evaporates at temperatures above 90˚C, resulting in desiccation if the tissue is heated slowly, or vaporization if the heat is delivered rapidly [84, 85]. Carbonization of the tissue occurs at temperatures above 105˚C.

The aim of radiofrequency ablation is to achieve and maintain a temperature of 50–100˚C throughout the entire target volume. Thermally treated tissue is associated with different findings on postprocedural imaging (Appendix II). The term “coagulation” is used in this thesis to describe the localized tissue alterations caused by thermal ablation.

HEPATIC RADIOFREQUENCY ABLATION: A CONCEPTUAL HISTORY

Development of electrosurgery

The term electricity was introduced in 1600 by the natural philosopher and physician William Gilbert to describe the phenomenon that rubbing furs against amber (Greek elektron) would cause an attraction between the two materials. Gilbert found that a number of materials had similar abilities, but erroneously believed that the attraction was caused by a subtle effluvium that was released after rubbing had pushed away the air between the two materials [86]. The first device generating static electricity was invented in 1663 by Otto von Guericke by using a rotating sphere of sulphur on a shaft. By 1740 electrostatic machines were in widespread use in Europe. In 1745 the Leyden jar was invented, which made it possible to store and discharge static electricity [87].

In the early eighteenth century, dissected frogs were regularly used to explore the effects of static electricity. While investigating these effects the Italian physicist Luigi Galvani accidentally discovered in 1780 that muscle spasms were induced in frog legs after the sciatic nerve was brought into contact with two dissimilar metals. Galvani believed that the muscle contractions were caused by

iv The use of loco-regional or whole body hyperthermia with a temperature of 42-44˚C to sensitize tumor cells to the cytotoxic effects of ionizing radiation and chemotherapy should not be confused with thermal ablation, which require temperatures > 50˚C.
a disturbance of the equilibrium of electrical fluid carried to the muscles by the nerves. He coined the term *animal electricity* to describe what was believed to be a new form of electricity [85, 86]. This discovery and subsequent experiments led to the concept of electrical current and the birth of bioelectricity. In 1799 Alessandro Volta constructed the first device that produced continuous electrical current by a chemical reaction, thereby showing that this assumed new form of electricity could be generated independently of animals. The device produced by Volta, known as the *voltaic pile*, is the progenitor of modern batteries. The current produced by this device was known as Galvanic current, corresponding to the modern term *direct current*. Michael Faraday discovered electromagnetic induction in 1831 and thereby the principles necessary for construction of the dynamo, which was able to deliver *alternating current*.

Exposing living organisms to direct current or low-frequency alternating current is associated with neuromuscular stimulation and an involuntary tetanic response. In 1891 Jacques-Arsène d'Arsonval discovered that by using frequencies above 10 kHz, electrical current could be passed through tissue without producing other physiological effect than that of heat. Based on these findings, an apparatus was built that produced a spark across a gap, which could be used to generate superficial tissue destruction [88-90]. Carl Franz Nagelschmidt developed the theory that creation of heat observed during treatment was caused by molecular agitation induced by high frequency electrical currents, and introduced the concept *diathermy* in 1897 (from Greek *dia*, through; *therma*, heat) [86, 90]. The first surgical use of electrical current is believed to be the treatment of a carcinomatous ulcer of the hand by the French physician Joseph Rivière in 1900 [91]. During the next decade, the use of electricity in treating lesions of the skin, oral cavity as well as coagulation of vascular tumors and hemorrhoids became widespread [85]. Pozzi introduced the term *fulguration* (from Latin *fulgur*, lightning) referring to the superficial carbonization resulting when an electrical spark was used to treat skin [92].

In 1909 Eugéne-Louis Doyen introduced the term *electrocoagulation* (from Latin *coagulare*, to curdle) to describe a method where the tissue was touched directly with the treatment electrode and a neutral electrode was attached to the patient. This arrangement produced deep tissue coagulation rather than carbonization on the tissue surface [93, 94]. In 1911 William Lawrence Clark used a high voltage spark gap oscillator to remove skin tumors. By microscopy of the treated tissue he observed that it shrunk from dehydration, and used the term *desiccation* (from Latin *desiccare*, to dry out) to describe this process [95]. In 1923 George A. Wyeth used electrosurgery for cut-

---

v It has later been shown that nerve and muscle excitation can be elicited at frequencies well above 10 kHz, but that increasingly higher current density is needed to elicit such a response with increasing frequency (LaCourse, J.R., et al., Effect of high-frequency current on nerve and muscle tissue. IEEE Trans Biomed Eng, 1985. 32(1): p. 82-6.)
ting tissues, utilizing an apparatus he termed an endotherm knife (from Greek endo, within; thermé, heat) [96]. In 1925 Ward discovered that a continuous sine wave from a vacuum tube oscillator was effective for cutting tissue, whereas coagulation was achieved by a damped sinusoid from a spark gap oscillator [97].

These discoveries inspired the physicist William T. Bovie to develop a device that could be used both for cutting and for obtaining hemostasis by tissue coagulation (Fig 1). In 1926 the device, known as the Bovie unit, was used by the neurosurgeon Harvey Cushing for surgical treatment of patients with intracranial tumors that had previously been considered inoperable [73, 94]. The Bovie unit consisted of an alternating current generator, interchangeable small active electrodes mounted on an insulated pistol-grip of bakelite, and a large dispersive electrode placed on the skin of the patient. By varying the modulation of the current used, the unit was capable of superficial dehydration, cutting or coagulation of a limited tissue volume [73]. Modern electrosurgical units are based on the electrophysiological principles of the Bovie unit.

Development of hepatic radiofrequency ablation

Radiofrequency ablation was suggested for treatment of hepatic malignancies in 1990 [98, 99]. The same year an experimental study using a needle electrode with 10 mm non-insulated tip showed that it was possible to produce an ellipsoid coagulation in porcine liver with maximal diameter perpendicular to the electrode axis of 1.4 cm [99]. In 1992 percutaneous ultrasound guided radiofrequency ablation was performed in 10 pigs, with typical coagulation diameters of 1-2 cm [100]. The first publication presenting hepatic malignancies treated with radiofrequency ablation appeared in 1993, and presented 13 patients with hepatocellular carcinoma. The unit which was used achieved a coagulation volume of 1.8 mL [101], corresponding to a coagulation diameter of 1.5 cm. In a 1995 study using needle electrodes connected to a generator commonly used for cardiac ablations, various combinations of electrode tip exposure, gauge, duration of treatment and temperature were examined to assess the op-
timal settings for generating the largest possible tissue coagulation volume. The authors concluded that coagulations larger than 1.6 cm in diameter could not be produced by any combination of factors with a needle electrode [102]. Numerous patient series were published in the second half of the 1990s [103-110]. The populations treated were often heterogeneous with both primary hepatic tumors and metastases from various primary tumors. The majority of early reports are observational studies with short follow-up and missing data on long-term patient survival.

Established oncological criteria suggest that hepatic malignancies should be eradicated radically including a 1-cm margin of apparently healthy tissue to eliminate microscopic foci of malignant cells, and to compensate for the uncertainty in determining the exact tumor margin [111, 112]. In order to adhere to the principle of a 1-cm tumor-free margin, a tumor must be completely enveloped by a continuous coagulation that encompasses the tumor as well as a 1-cm margin on all sides. The diameter of a spherical coagulation would therefore need to be at least 2 cm larger than the largest diameter of the tumor (Fig 2) [113]. This implies that a coagulation volume several times the tumor volume must be created (Fig 3). The needle electrode was not capable of generating adequate coagulation volumes with one electrode insertion [55, 102]. Furthermore, the use of overlapping coagulations is not an effective method to increase coagulation volume (Fig 4) [113].

![Coagulation diagram](image)

**Figure 2.** The figure shows an electrode placed in a tumor. In order to obtain a 1-cm tumor-free margin, the tumor with a diameter of 2 cm requires a tissue coagulation with a diameter of at least 4 cm.


![Volume graph](image)

**Figure 3.** The figure shows the tumor volume (filled) and the volume of the corresponding 1-cm margin. The volume of a 2-cm spherical tumor is 4.2 mL, the volume of the surrounding 1-cm tumor free margin (excluding the tumor) is 29.3 mL. The required coagulation volume to treat a 2-cm spherical tumor is therefore 33.5 mL, approximately 8-fold the tumor volume. Present radiofrequency ablation systems produce coagulation volumes of approximately 40 mL.
Devices producing larger coagulations with one electrode insertion would therefore have to be developed to make radiofrequency ablation useful for treatment of most clinically relevant hepatic tumors (i.e. those measuring > 1-2 cm in diameter). Several strategies were proposed in the last half of the 1990s in order to increase the coagulation volume [114]. Although generators capable of adjusting power output according to tissue impedance and temperature were introduced, it was principally the rapid development in electrode design that enabled substantially larger tissue coagulations to be obtained.

**Loop electrode.**—The loop electrode consisted of a super-elastic metal with a distal loop with a radius of 1 cm, which was introduced into the tissue by a cannula. After the electrode had regained its loop shape, the electrode was rotated simultaneously with activation of the electrosurgical unit in cut mode. This produced a spherical coagulated rim of 1 mm which physically separated a non-coagulated spherical tissue volume with a diameter of 2 cm from the surrounding vital parenchyma [115, 116].

**Bipolar electrode.**—The use of bipolar electrodes, i.e. insertion of two identical electrodes in the target tissue, generates high current density at both electrodes and in tissue between the electrodes [117]. A bipolar system was found to produce larger coagulations than the needle electrode, but also more irregular coagulation geometry [118].

**Cluster electrode.**—The use of cluster electrodes, i.e. insertion of three or more electrodes that are activated simultaneously, was examined in 1995. Simultaneous application of energy to three electrodes placed 1.5 cm apart would lead to a coagulation diameter of 3 cm [119].

**Internally cooled electrode.**—The internally cooled electrode was described in 1996 [120, 121]. This design consisted of a hollow electrode with two internal lumens extending to the electrode tip. One lumen delivered cooled saline to the electrode tip, and the other lumen returned the saline to a

![Figure 4](image-url). The figure illustrates the result of overlapping coagulations for a device generating a spherical tissue coagulation of 2 cm (thick line). The coagulation diameter can be increased by positioning four coagulations in the x-y plane (thin lines) and two additional coagulations along the z-axis (not shown). The positioning of six coagulations will produce a spherical coagulation with a diameter of 2.8 cm (dotted line). A coagulation with a diameter of 2 cm correspond to a volume of 4.2 mL, whereas a coagulation with a diameter of 2.8 cm correspond to a volume of 11.5 mL. Hence, increasing the number of coagulations by a factor of 6 only increase the spherical coagulation volume by a factor of 2.7.

collection unit outside the body. The perfusate did not come into contact with patient tissues. A thermocouple was embedded at the tip of the electrode to allow for continuous temperature measurement. By cooling the electrode tip, the risk of excessive heating and carbonization was reduced, enabling a higher current density and higher deposition of electrical energy in the tissue than was tolerated with conventional electrodes. Using this principle coagulation with a diameter of 2.5 cm could be made in porcine liver in vivo [120].

**Perfusion electrode.**— It was noted in 1990 that adding saline to the electrode-to-tissue interface would increase coagulation volume [98]. The explanation proposed for this finding was that installation of saline produced a more electrically uniform tissue, thereby limiting tissue carbonization. It was further hypothesized that a high local ion concentration at the electrode-to-tissue interface would increase the effective surface of the electrode and thereby the coagulation volume. In 1997 the effect of continuous saline infusion during ablation was evaluated [105]. This study showed that radiofrequency ablation with a needle electrode produced coagulations with a diameter of less than 1 cm in vivo, whereas continuous intraparenchymal infusion of 0.9% saline at 1 mL/min during ablation increased maximum coagulation diameter to 4 cm.

**Multitined expandable electrode.**— A device utilizing four expandable electrodes was presented in 1998 [122, 123]. The device consisted of an insulated shaft containing four retractable electrodes which could be deployed to a maximum diameter of 3 cm. Each electrode tip contained a thermocouple for temperature monitoring. Coagulations with diameters of 3.1 cm were created in an experimental study [122]. Commercially available hepatic radiofrequency ablation systems have been developed based on the principle of the cluster electrode,

| Table 1. The four most commonly used systems for hepatic radiofrequency ablation. |
|---------------------------------|----------|----------|----------------|
| Generator name | Frequency (kHz) | Power output (W) | Electrode type |
| HiTT 106\(^1\) | 375 | 60 | Perfusion |
| Cool-tip RF\(^2\) | 480 | 200 | Internally cooled |
| RF 3000\(^3\) | 480 | 200 | Multitined expandable |
| 1500X\(^4\) | 460 | 250 | Multitined expandable |

\(^1\) Integra LifeSciences, Tuttlingen, Germany, formerly Berchtold Tuttlingen, Germany
\(^2\) Valleylab, Boulder, CO, formerly Radionics, Burlington, MA
\(^3\) Boston Scientific Corp., Boston, MA, formerly RadioTherapeutics Corp., Sunnyvale, CA
\(^4\) RITA Medical Systems, Mountain View, CA
the internally cooled electrode, the perfusion electrode, the multitined expandable electrode as well as combinations of these principles. Technical details of the four most commonly used systems for hepatic radiofrequency ablation are summarized in Table 1.

An impedance-controlled radiofrequency ablation system with perfusion electrodes (Elektrotom HiTT 106, Berchtold GmbH & Co, Germany) based on the principle of continuous intraparenchymal infusion of saline during ablation was used in this study (Fig 5).

**Figure 5.** The figure shows the perfusion electrodes used in this study.

a. The diameter of the electrodes are 1.7 mm with a insulated shaft with a length of 15 or 20 cm incorporating a lumen for saline perfusion.

b. The terminal non-insulated 1.5 cm segment of the electrode has three pairs of two side holes, placed at an angle of 120° from each other.

c. Saline is distributed to the tissue around the electrode through the side holes.
HEPATIC RADIOFREQUENCY ABLATION: CLINICAL CHALLENGES

Patient survival

Following the first report in 1996 of 6 patients with colorectal liver metastases treated with radiofrequency ablation [104], larger series were published in 1999 and 2000 [110, 124, 125]. Follow-up was short and survival at standardized time intervals (i.e. 1, 3 and 5 years) was not presented. More recent patient series indicate that radiofrequency ablation of non-resectable colorectal liver metastases is associated with a 5-year survival of approximately 30% [126-129].

Facilitated by downsizing as a result of neoadjuvant chemotherapy, and the ability of local ablation techniques to treat non-resectable tumors, patients with previously non-resectable tumors are offered therapy with curative intent. The natural course of the disease in this novel and highly selected patient category is not known. Treatment is offered within a complex multimodal framework consisting of repeat surgical interventions, local ablation procedures and different adjuvant and neoadjuvant chemotherapy regimens. The interventions may be implemented in varying order and at non-standardized time points. No randomized studies comparing radiofrequency ablation with hepatic resection or with chemotherapy in patients with colorectal hepatic metastases have been performed to date. Although preliminary data indicate that improved survival can be achieved in selected patients with non-resectable colorectal hepatic metastases treated with radiofrequency ablation, formal scientific proof is lacking.

Local tumor control

Complete eradication of malignant growth is considered mandatory to achieve increased survival in patients with colorectal liver metastases [35]. This assumption constitutes the oncological rationale for local ablation of non-resectable hepatic metastases. However, local tumor control, i.e. lack of tumor recurrence at the site of treated tumors, is not a sufficient condition for increased patient survival, as intrahepatic recurrence not related to the treatment site and extrahepatic recurrence is common in patients treated with R0-resection\(^vi\) [130] and radiofrequency ablation [24, 131] of colorectal liver metastases. Local tumor control should therefore be considered a surrogate endpoint, but is nevertheless a valuable indicator of the technical ability to eradicate a tumor.

Local tumor recurrence rates after radiofrequency ablation are higher than after hepatic resection, with reported rates varying from less than 5% to above 50% [110, 125, 132-134]. A recent meta-analysis of 5227 liver tumors treated with radiofrequency ablation found that tumor diameter > 3 cm, perivas-
cular tumor localization and multiple tumors were independent predictors for local tumor recurrence. Thus, the ability of radiofrequency ablation to eradicate liver tumors is limited by a number of tumor-related factors. Furthermore, higher local recurrence rates were found for procedures using a percutaneous approach [135].

In order to attain reliable tumor destruction by radiofrequency ablation, knowledge of the expected coagulation geometry and volume as well as the relationship of the coagulated volume to the position of the electrode is crucial. A systematic review of the literature from 1990 to 2003 found that adequate experimental data were missing for most of the commercially available electrodes [136]. Reproducibility of coagulation geometry and volume is low under standardized treatment protocols in experimental studies [137, 138] and in clinical series [139]. Reliable intra-procedural monitoring of the extent of tissue coagulation is therefore essential to ensure adequate treatment of a tumor and to avoid damage to abutting structures. Transabdominal sonography provide accurate guiding of the ablation electrode, but is unreliable in assessment of the extent of tissue coagulation [100, 140]. The use of CT [141], MR [142, 143] and contrast enhanced sonography [144] have been evaluated for intra-procedural monitoring of radiofrequency ablation. Real-time monitoring of the temperature distribution during the procedure would allow the operator to optimize the treatment and possibly avoid damaging normal structures adjacent to the treatment site. Temperature sensors are attached to or embedded in the ablation electrodes of most commercial radiofrequency ablation electrodes. However, thermal information is restricted to a predefined number of measuring points, and provide no information on tissue temperatures at a distance away from the electrode. Non-invasive temperature measurement is an attractive concept with the potential to visualize the three-dimensional temperature distribution during treatment [145].

Cooling caused by blood flow in large vessels passing through or near the treatment site is an important limitation of the extent of the coagulation in vivo [122, 146]. Several studies have examined the effect of temporary reduction of intrahepatic perfusion by pharmacological modulation [147, 148] or temporary mechanical occlusion of hepatic vessels [146, 149-152]. Interruption of hepatic inflow during ablation increases efficiency [153], and enables coagulations that are larger, with a more spherical geometry [149, 151], which would be beneficial in order to achieve complete treatment.

**Assessment of local tumor recurrence**

Malignant tissue treated by radiofrequency ablation is left in situ, necessitating repeated imaging follow-up to determine if the tumor has been eradicated. Early detection of local tumor recurrence may facilitate early re-intervention with potential benefits for patient survival. The use of fine needle aspiration cy-
tology is controversial due to the risk of needle tract seeding [154, 155]. Consequently, non-invasive methods should preferably be used for detection of persistent or residual malignant tissue at the coagulated site. However, it can be difficult to distinguish benign tissue alterations caused by radiofrequency ablation from residual tumor on post-ablation follow-up imaging [156-158]. Non-invasive methods for assessment of local tumor recurrence should therefore be refined.

Complications

Two large retrospective studies have assessed complications associated with hepatic radiofrequency ablation [159, 160]. The reported mortality in both studies was below 1%, with complications occurring in less than 10% of patients. Complications directly related to the application of thermal energy such as damage to bile ducts or intrahepatic vessels occurred in less than 1% of patients. In one of these studies of 3670 patients, 3 of 20 deaths was attributed to portal vein thrombosis and 1 death to bile duct stricture [159]. The risk of damage to intrahepatic vessels and bile ducts [150, 161-163], as well as the feasibility of protecting bile ducts by intraductal cooling have been examined in recent studies [164-166].

Biological alterations at the coagulated site

Although the gross and microscopic findings after hepatic radiofrequency ablation are well characterized [140], biological effects induced in the treated tumor and surrounding hepatic parenchyma have not been well characterized due to the fact that the treated tumors and surrounding parenchyma are left in situ. It has been hypothesized that radiofrequency ablation generates an antigen source that may induce antitumor immunity [167], which may be a beneficial effect. However, of special interest are recent experimental findings that radiofrequency ablation strongly promotes proliferation of residual neoplastic cells [168]. Examination of biological effects induced by radiofrequency ablation may be of importance for understanding mechanisms for post-ablation tumor recurrence, and should be further characterized.
This thesis examines the use of hepatic radiofrequency ablation in four experimental studies in non-tumor animal models and in one clinical study in patients with colorectal liver metastases. An impedance-controlled perfusion electrode radiofrequency ablation system was used in all studies. Specifically, the aims were:

1. Characterize coagulation geometry and coagulation volume generated in porcine liver during maintained and interrupted hepatic inflow.

2. Investigate the feasibility of a thermosensitive liposomal agent for thermal monitoring of MRI-guided hepatic radiofrequency ablation in rabbit liver.

3. Determine if electrode-to-vessel distance or the use of hepatic inflow occlusion are associated with acute or delayed portal vein thrombosis in porcine liver.

4. Examine if hepatic radiofrequency ablation increases the expression of matrix metalloproteinase-2 and matrix metalloproteinase-9 in the transition zone separating coagulated tissue from normal porcine hepatic tissue.

5. Determine if alterations in coagulation volume on post-ablation CT images acquired every third month facilitate early detection of local tumor recurrence in patients with colorectal hepatic metastases treated with radiofrequency ablation.
Coagulation geometry, coagulation volume and delivered energy per coagulated tissue volume was assessed in a non-tumor porcine model. Mechanical occlusion of the portal vein and the hepatic artery (Pringle maneuver) during ablation was performed in 6 of 12 animals. One coagulation was made in each animal close to the portal vein. All animals were sacrificed 4 days after ablation. Use of hepatic inflow occlusion increased the effective coagulation diameter by 1 cm, but was associated with elongated coagulations extending outside the expected treatment site. Displacement of the geometrical centre of the coagulation relative to the position of the electrode was observed. The energy delivered during ablation could not be used to predict coagulation volume.
Frich L, Bjørnerud A, Fosheim S, Tillung T, Gladhaug I.
Experimental application of thermosensitive paramagnetic liposomes for monitoring magnetic resonance imaging guided thermal ablation.

The utility of a thermosensitive liposomal agent to assess the extent of tissue coagulation during MRI-guided laser ablation and radiofrequency ablation in non-tumor rabbit liver was examined. One coagulation was made in each animal prior to administration of the agent, and two additional coagulations were made after administration of the agent. Tissue temperature during treatment was measured by a MRI-compatible temperature fiber. \(T_1\)-weighted MR images were acquired prior to heating, during heating and after heating for each coagulation. Signal intensity (SI) of the coagulations generated prior to injection of the agent decreased 7% below baseline during heating, but increased 5% above baseline at normalization of tissue temperature. Both coagulations generated after administration of the agent showed increased SI during heating (5% vs. 19%), followed by a further increase after tissue temperature had normalized (14% vs. 26%). Increased SI was found for coagulations made 50-55 min after injection of the agent compared to those made 12-14 min after injection of the agent. Persistent signal enhancement on \(T_1\)-weighted MR images was found in areas assumed to be exposed to a temperature above the phase transition temperature of 57˚C, indicating that thermosensitive liposomes may be used for monitoring of radiofrequency ablation.
Frich L, Hol PK, Roy S, Mala T, Edwin B, Clausen OPF, Gladhaug IP.
Experimental hepatic radiofrequency ablation using wet electrodes: electrode-to-vessel
distance is a significant predictor for delayed portal vein thrombosis.

Possible explanatory variables associated with acute and delayed portal vein thrombosis
after hepatic radiofrequency ablation close to portal vessels were assessed in an experi-
mental study. Coagulations were created within 1.5 cm of the right portal vein branch
in 12 pigs with (n=6) or without (n=6) hepatic vascular occlusion (Pringle maneuver).
Sham operations with Pringle maneuver were performed in four animals. Vessel patency
was assessed by rotational portal venography prior to ablation, 10 min after ablation and
4 days after ablation. Distance between the ablation electrode and the right portal vein
branch was measured from 3-dimensional reconstructions of the portal venograms. No
animals developed acute portal vein thrombosis. Delayed portal vein thrombosis oc-
curred in 2 of 6 animals in the Pringle group, and in 3 of 6 animals in the group where
coagulations were made during physiological hepatic circulation. All five occurrences of
delayed portal vein thrombosis occurred in animals with a distance between the ablation
electrode and the right portal vein branch of 5 mm or less. Delayed portal vein throm-
ysis may occur after hepatic radiofrequency ablation during physiologic hepatic circula-
tion. An electrode-to-vessel distance of < 5 mm was a significant predictor for delayed
portal vein thrombosis.
Activity of MMP-2 and MMP-9 in plasma and in tissue lysates from the transition zone surrounding coagulated hepatic tissue was examined in a non-tumor porcine model. Hepatic vascular occlusion was performed in 6 of 12 animals. MMP activity was quantified by gelatin zymography. Cellular localization of MMPs was determined by immunohistochemistry. MMP-2 and MMP-9 activity in tissue lysates from the transition zone was significantly increased compared to normal hepatic parenchyma with ratios of 3.0 and 2.6, respectively. MMP-2 and MMP-9 activity in plasma 1 h after radiofrequency ablation was significantly increased compared to baseline levels, with ratios of 1.2 and 1.5, respectively, but had normalized 4 days after radiofrequency ablation. Hepatic inflow occlusion during ablation did not influence MMP activity. Expression of MMP-2 and MMP-9 was localized to macrophages in the transition zone.
Radiofrequency ablation of colorectal liver metastases: evaluation of local tumor recurrence by 3D volumetric analysis.

Submitted.

In this clinical study, 11 patients with colorectal liver metastases were treated with radiofrequency ablation. Multi-detector computed tomography (MDCT) was performed at 1 and 3 months after radiofrequency ablation, and then at intervals of 3 months until 24 months, and each sixth month thereafter. A total of 81 post-ablation scans were analyzed, with a median follow-up of 27 months (17-43 months). The theoretical treatment margin 1 month after radiofrequency ablation was assessed retrospectively. Post-ablation coagulation volume was retrospectively determined using a semi-automatic three-dimensional (3D) method. Local tumor recurrence was found in 7 patients, and was detected median 9 months (6-21 months) after radiofrequency ablation. A positive margin was not achieved in 3 patients. In patients without local tumor recurrence, coagulation volume was less than 30% of baseline value at 24 months. In contrast, coagulation volume increased markedly in 6 of 7 patients with local tumor recurrence. Local tumor recurrence was identified simultaneously by conventional morphological criteria and semi-automatic 3D volumetric analysis. Semi-automatic 3D volumetric analysis provides added diagnostic information compared with conventional morphological evaluation, and may increase specificity of non-invasive diagnosis of local tumor recurrence after radiofrequency ablation of colorectal cancer liver metastases.
Experimental Studies

Study I

Hepatic radiofrequency ablation systems were not compared in experimental studies using standardized treatment protocols until 2003 [137, 138]. All evaluated systems showed substantial variability in coagulation volume and geometry. Furthermore, coagulation volumes were higher in ex vivo experiments than in vivo, leading to the conclusion that ex vivo findings did not predict the in vivo efficacy of these systems [137]. Although the systems were able to produce coagulations with transverse diameters in the range 2.5 to 4 cm, the coagulations were smaller than expected and deviated from the assumed spherical geometry. The perfusion electrode system used in the present study was the most efficient [153] (i.e. requiring the least amount of electrical energy per coagulated tissue volume), produced among the largest volumes of coagulated tissue [138], but with larger variations in coagulation geometry and coagulation volume than systems based on other principles [137, 138].

Active heating of tissue during radiofrequency ablation is counteracted by heat loss caused by circulating blood, as shown by the bioheat equation (Appendix I). Temporary interruption of hepatic blood flow during ablation would therefore be expected to influence coagulation geometry and coagulation volume. Interruption of hepatic inflow during ablation is associated with two different effects. The term heat-sink effect refers to cooling by adjacent blood vessels with diameter > 3 mm, which may lead to irregular coagulation geometry in proximity to the vessels [122, 169]. Additionally, parenchymal perfusion on the capillary level is reduced. In line with the bioheat equation, reduction of heat loss caused by parenchymal perfusion would be expected to lead to a general increase in the coagulation volume [146]. Interruption of hepatic inflow during ablation with multitined expandable electrodes and internally cooled electrodes produced coagulations that were larger, less elliptic and less distorted in experimental studies [149, 151, 170] and clinical series [152, 171]. In contrast, the impact of interruption of hepatic inflow on coagulation geometry
and coagulation volume made with the perfusion electrode system had not been investigated. In study I we examined coagulation geometry and coagulation volume produced by the perfusion electrode system in a porcine model with and without hepatic inflow occlusion (Pringle maneuver). The portal vein and the hepatic artery was mechanically occluded during ablation in 6 of 12 animals, based on the assumption that reduction of heat loss would lead to a coagulation geometry more in line with an ideal sphere as suggested by equation (7).

Our results confirmed that the use of a standardized ablation protocol during physiological hepatic circulation did not produce predictable coagulation geometry or predictable coagulation volumes. As expected, reduction of heat loss by Pringle maneuver was associated with increased coagulation volume, which may be considered a beneficial effect. However, the coagulation geometry was characterized by elongated coagulations extending outside the expected treatment site, and only a fraction of the increased coagulation volume contributed to a spherical shape. The explanation for the elongated coagulations observed in our study may be related to the effect of saline infusion during ablation. With the settings used in this experimental study, saline was infused at a rate of 105 mL/hour, corresponding to 16 mL during a 9 min coagulation, which was larger than the mean coagulation volume of 10 mL. A recent clinical study confirmed that saline was distributed outside the planned treatment volume with the perfusion electrodes used in this study [172]. Thermal damage was observed to abutting organs, which may be caused by direct thermal damage from heated saline. Additionally, uneven intraparenchymal distribution of saline may alter both the local current density and tissue conductivity, which would influence the tissue temperature, as seen from equation (6). After determination of the largest circle that could be inscribed in each slice from a coagulation, the term effective coagulation diameter was assigned to the diameter of the largest of these circles. This entity was used to calculate the effective coagulation volume, which is the volume of the largest sphere that could be inscribed into the coagulation volume. Knowledge of the largest spherical coagulation volume produced is of clinical interest as it would allow the operator to adequately coagulate a tumor without considering variation in coagulation geometry relative to the axis of the electrode. In this study, determination of geometrical properties were based on photographs of tissue slices from the coagulation which had been cut in parallel sections. A limitation of this method is that it is dependent on the plane of cutting, and that the accuracy decreases with increasing slice thickness. Nevertheless, the clinically important geometrical concepts introduced in this study are robust, easily comprehensible and may be applied for evaluation of geometrical properties of future ablation systems. The terminology to describe coagulation geometry is still under development. Of interest,
standardization of the methods and terminology for describing coagulation geometry has recently been proposed [173].

Our findings indicated displacement of the geometrical centre of the effective coagulation volume relative to the position of the electrode. Inability to predict the position of the coagulated area relative to the electrode constitutes a fundamental limitation in the use of local ablation techniques (Fig 6). The ablation electrode was positioned close to the right portal vein branch in all animals. It is reasonable to assume that displacement of the geometrical center of the coagulation relative to the electrode may have been caused by the heat-sink effect [146, 174]. If this is the case one would expect displacement to be less pronounced in the animals where hepatic inflow occlusion was performed during ablation. Displacement of the geometrical centre of the coagulation relative to the electrode could not be quantified reliably by the methods used in our study because the exact position of the ablation electrode was not known at reoperation 4 days after the ablation procedure. Lastly, due to the dependence on electrically conducting saline in generating a large coagulation volume, displacement of the coagulation volume may also be a limitation of the perfusion electrode design.

Study II

As shown in study I, coagulation geometry and volume is not predictable under standardized experimental ablation protocols. Furthermore, coagulations are smaller and more irregular than expected in clinical series of patients treated with hepatic radiofrequency ablation [139, 175]. Intraoperative visualization of the thermally induced necrosis may be beneficial to achieve complete treatment of the index tumor and to avoid damage to organs adjacent to the planned treatment site. Assessment of the extent of tissue coagulation by sonography, CT or MRI during and immediately after ablation is unreliable [55, 140, 141, 175]. Non-invasive thermometry is an attractive concept with the potential to visualize the three-dimensional temperature distribution created during ablation. Several methods have the potential to measure temperature non-invasively, including MRI-based thermometry, ultrasound thermometry, microwave-based thermometry and electrical impedance tomography [176, 177]. MRI-

Figure 6. The figure shows an electrode placed in a tumor and the position of the anticipated coagulation diameter (dotted line). The actual position of the coagulation is indicated by a circle. Due to displacement of the coagulation, a part of the tumor is incompletely treated.
based methods can provide non-invasive thermal mapping of relative temperature changes with acceptable accuracy, spatial resolution and temporal resolution [145]. However, radiofrequency ablation generates significant electromagnetic interference in the frequencies used for transmission and reception in MRI, precluding the use of several MRI-based methods [178]. Temperature-sensitive MRI contrast agents represent a novel approach for thermal imaging by indicating an absolute temperature threshold.

In study II we examined the feasibility of a thermosensitive liposomal agent with a membrane phase transition temperature of 57°C and a size of 110 nm to assess the extent of tissue coagulation during intraprocedural MRI-guided laser ablation and radiofrequency ablation. The liposomal membrane can be tailored to a predetermined transition temperature by modulating the composition of membrane phospholipids. The liposomes used in our study contained gadodiamide (GdDTPA-BMA), which is a MRI contrast agent that acts by reducing the longitudinal relaxation time ($T_1$) of tissue water protons. At physiologic temperatures the paramagnetic compound is retained within the liposome and does not influence the $T_1$-relaxation of the tissue water protons due to slow transmembrane water exchange conditions. At temperatures above the transition temperature the $T_1$-relaxation efficacy of the liposomal agent increases markedly either due to fast transmembrane water exchange conditions or leakage of gadodiamide into the tissue. $T_1$-shortening of the tissue water protons will be shown as high signal intensity (SI) on standard $T_1$-weighted MR images. A transition temperature of 57°C of the liposomal membrane was chosen because protein denaturation and irreversible cellular damage would be expected to have occurred above this temperature threshold [55]. Increased SI on $T_1$-weighted MR images would therefore indicate the extent of irreversible tissue damage. In study II coagulations made after injection of the agent showed slightly increased SI during heating, followed by a nonreversible increase in SI after the tissue had regained physiological temperature. We speculated that this may have been caused by leakage of GdDTPA-BMA from the liposome interior and subsequent entrapment due to coagulation of vessels. This mechanism has also been suggested by a recent study in which the feasibility of using these liposomes as indicators for local drug delivery during hyperthermia has been examined [179]. The diameter of the coagulations as measured from photographs of macroscopic specimens and as determined from MR images were inconsistent. This may possibly be explained by the fact that irreversible thermal damage may have occurred at a temperature below the phase transition temperature of the agent [80]. Nevertheless, a consistent pattern in the diameters was not observed. This finding might have been caused by the introduction of saline into the tissue during treatment, which would be expected to decrease SI on $T_1$-weighted images and lead
to local inhomogeneities in the concentration of the liposomal agent or of GdDTPA-BMA released in the tissue. Although the mechanisms responsible for our observed results are not completely understood, consistent results were found that confirm the ability of the liposomal agent to differentiate between tissue exposed to thermal treatment and normal liver tissue. The persistent signal enhancement in areas exposed to a temperature above the threshold temperature may be of clinical value during MRI-guided radiofrequency ablation.

**Study III**

The presence of large vessels in the proximity of tumors treated with radiofrequency ablation is associated with a high rate of incomplete tumor destruction and local tumor recurrence, assumed to be caused by cooling effects [135, 169, 180]. Temporary reduction of flow in the portal vein and hepatic artery during radiofrequency ablation may enhance coagulation necrosis close to hepatic vessels [169]. However, as maintained flow in large vessels serve to protect the vessels from thermal damage [181], vessels may be more vulnerable to thermal injury and subsequent thrombosis if radiofrequency ablation is performed during interruption of hepatic vascular inflow. Although segmental portal vein thrombosis may be asymptomatic, patients with a deficit in functional hepatic reserve may be at risk of postoperative hepatic failure after portal vein thrombosis. The safety of performing radiofrequency ablation in proximity to large intrahepatic vessels has not been established. It has been suggested that radiofrequency ablation should only be performed for tumors at least 2 cm from major intrahepatic vessels [105]. By adhering to this principle, potentially operable patients could be excluded from treatment. We therefore considered it important to examine factors associated with risk of damage to intrahepatic vessels during radiofrequency ablation in an experimental model.

In study III we examined risk factors associated with portal vein thrombosis after hepatic radiofrequency ablation, with special attention to the effect of temporary hepatic inflow occlusion and the electrode-to-vessel distance. The presence or absence of portal vein thrombosis was examined by performing rotational portal venography prior to radiofrequency ablation, 10 min after radiofrequency ablation and 4 days after radiofrequency ablation. Consequently, we could determine by an objective method in the living animal when portal vein thrombosis occurred and the anatomical location of a portal vein thrombosis. Acute portal vein thrombosis was defined as thrombosis on portal venograms acquired 10 min after completion of the ablation, whereas delayed portal vein thrombosis was defined as thrombosis on portal venograms acquired 4 days after ablation. Delayed portal vein thrombosis occurred in 2 of 6 animals in the group in which hepatic inflow occlusion was performed, and in 3 of 6 animals in the group where coagulations were made during physi-
ological hepatic circulation. Hence, the use of temporary hepatic inflow occlusion was not associated with portal vein thrombosis. We found that an electrode-to-vessel distance of less than 5 mm was a significant predictor for delayed portal vein thrombosis. Our findings conflict with those of a study which reported that portal vein thrombosis invariably occurred when hepatic radiofrequency ablation was performed during vascular inflow occlusion, and that no portal vein thrombosis was found during physiological hepatic circulation [163]. These apparently diverging results may be caused by differences in study design and the method used for detection of portal vein thrombosis. The electrode-to-vessel distance, which was quantified by 3D portal venography in our study, proved to be of significance for the occurrence of portal vein thrombosis. Due to variations in electrical power and electrode designs used in different ablation systems, we consider our findings to be valid only for the specific radiofrequency ablation system used in this study. Even though the electrode-to-vessel distance may be of importance in regard to portal vein thrombosis in humans, the pig is considered to be more susceptible to activation of the coagulation system following endothelial damage than humans [182]. Consequently, the electrode-to-vessel distance needed to induce post-ablation thrombosis in humans may be shorter than the 5 mm distance found to be of significance in our experimental study. Our results indicate that portal vein thrombosis may occur without temporary occlusion of hepatic vessels. Another finding of clinical importance is that normal portal venography 10 min after radiofrequency ablation does not exclude later occurrence of portal vein thrombosis. This finding may be of importance for postoperative evaluation of patients with assumed high risk of post-ablation portal vein thrombosis.

Study IV

Several established risk factors for local tumor recurrence after radiofrequency ablation of hepatic metastases exist, such as tumor diameter > 3 cm and tumor proximity to major vessels [135]. Local tumor recurrence after radiofrequency ablation can be attributed to incomplete treatment of the index tumor or growth of micrometastatic disease around the tumor [181]. Micrometastatic disease in the vicinity of the macroscopic tumor has been detected in 2% to 70% of patients who underwent liver resection for colorectal liver metastases [183, 184]. These contradictory results possibly illustrate methodological limitations. Therefore, the true rate of micrometastatic disease in this patient category remains uncertain. Patients treated with hepatic radiofrequency ablation have higher local, intrahepatic and distant recurrence rates, as well as lower overall survival than comparable resected patients [24]. A reasonable explanation for these findings is negative patient selection bias, with micrometastatic disease being present in a larger proportion of patients treated with radiofrequency ablation. An alternative
explanation is that thermal ablation may induce biological alteration in the tumor tissue or peritumoral hepatic parenchyma that facilitate spread and invasion of malignant cells. However, biological effects induced by radiofrequency ablation in the treated tumor and surrounding hepatic parenchyma are not well characterized due to the fact that the treated tumors and surrounding parenchyma are left in situ. In a recent experimental tumor model in mice, hepatic radiofrequency ablation promoted intrahepatic growth of residual neoplastic cells [168]. The mechanisms explaining these findings is not well understood, although increased expression of growth factors have been found after thermal ablation in experimental tumor models [185].

Matrix metalloproteinases (MMPs) are a family of matrix-degrading endopeptidases involved in a variety of physiological and pathological events [186-188]. The gelatinases MMP-2 and MMP-9 are involved in colorectal cancer tumor cell invasion, metastasis and angiogenesis [188]. In study IV we examined the activity of MMP-2 and MMP-9 in the transition zone separating the coagulated tissue and normal hepatic parenchyma after hepatic radiofrequency ablation in a non-tumor porcine model. MMP-2 and MMP-9 activity in tissue lysates from the transition zone 4 days after radiofrequency ablation was three-fold increased compared to normal hepatic parenchyma. Increased activity of MMPs probably represents an inflammatory response caused by radiofrequency ablation. However, up-regulation of MMP activity may provide a microenvironment that facilitates the risk of local tumor recurrence and distant metastasis by vascular invasion in the presence of viable malignant cells. This mechanism would be of particular importance if a tumor is incompletely treated by radiofrequency ablation, leaving viable malignant cells, or if micrometastases are present in or near the transition zone. Furthermore, hepatic radiofrequency ablation is increasingly combined with hepatic resection, which induces hematogenous dissemination of colorectal cancer cells [189, 190].

The data generated in this non-tumor preliminary animal study does not allow us to draw conclusions concerning the possible role of MMPs in local tumor recurrence, invasion or metastasis after radiofrequency ablation of colorectal liver metastases. However, based on the assumption that increased expression of MMPs in peritumoral tissue is associated with increased risk of growth and metastasis of malignant cells, our results indicate that hepatic radiofrequency ablation should only be performed when complete eradication of the target tumor including an adequate margin is possible.
CLINICAL STUDY

Study V

Hepatic radiofrequency ablation has been used for treatment of patients with non-resectable liver metastases from colorectal cancer at our institution since 2003. Prior to introduction of hepatic radiofrequency ablation in clinical practice, a non-randomized phase II study was designed and approved by the Regional committee for medical research ethics. Written informed consent was obtained from all patients prior to radiofrequency ablation. All patients in the study were systematically followed by multi-detector computed tomography (MDCT) of the liver at 1 and 3 months after radiofrequency ablation, and then at intervals of 3 months until 24 months, and each sixth month thereafter.

Non-invasive diagnosis of local tumor recurrence is desirable due to the risk of needle tract seeding and negative impact on patient survival if biopsy is performed in operable patients [191, 192]. However, it can be difficult to distinguish between local tumor recurrence and benign tissue alterations on post-ablation follow-up imaging [156-158]. In study V our hypotheses were that inadequate treatment margins as determined from scans 1 month after radiofrequency ablation may identify patients who would develop local tumor recurrence, and that increase in coagulation volume during follow-up may be used to detect local tumor recurrence earlier than conventional morphological criteria. The geometrical concepts of effective coagulation diameter and effective coagulation volume developed in study I were applied in the analysis of the treatment results.

Based on previous published results from other groups, occurrences of local tumor recurrence at the treated site were expected to be diagnosed during follow-up within the first 6 months. However, local recurrence had also been reported to appear up to 23 months after the procedure [125, 193, 194]. In order to compare groups with and without local tumor recurrence, we retrospectively identified 11 patients who had either developed local tumor recurrence or who had been followed for at least 24 months without local tumor recurrence, assuming that local tumor recurrence would not develop in this last category.

Local tumor recurrence was found in 7 of 11 patients. Intrahepatic recurrence not related to the radiofrequency ablation site was found in 6 patients, and extrahepatic tumor growth in 6 patients. Of the 7 patients with local tumor recurrence, 4 patients presented with inoperable intrahepatic or extrahepatic tumors at the time of diagnosis of local tumor recurrence. Fine needle aspiration cytology of the area treated with radiofrequency ablation was performed in 4 patients during follow-up. In 3 patients malignancy was verified, whereas no malignant cells were found in 1 patient who did not develop local tumor recurrence. Malignant growth is common in this patient category despite successful treatment of the index tumor, with inoperable intrahepatic...
or extrahepatic malignant growth occurring in 3 of 4 patients where the index tumor had been successfully treated with radiofrequency ablation. These findings are comparable to a previous study [24]. The largest coagulation diameter 1 month post-ablation was larger than the pre-ablation tumor diameter in all patients. However, when considering the effective coagulation diameter 1 month post ablation, a positive treatment margin had only been achieved in 8 patients. The 3 patients with an effective coagulation diameter smaller or equal to the largest tumor diameter developed local tumor recurrence. A negative treatment margin may be of clinical use to identify patients who later develop local tumor recurrence. However, the prognostic value of this parameter should be examined in prospective studies.

In the patients who did not develop local tumor recurrence, coagulation volume decreased during follow-up, with all values below 30% of baseline values at 24 months. In contrast, coagulation volume increased significantly in the patients who developed local tumor recurrence. Our findings suggest that the combination of semi-automatic 3D volumetric analysis and conventional morphological evaluation may increase the specificity of non-invasive diagnosis of local tumor recurrence after radiofrequency ablation of colorectal cancer liver metastases, thereby possibly avoiding unnecessary biopsies. Furthermore, systematic post-ablation follow-up is associated with detection of treatable malignant disease, with 3 of 11 patients re-treated with curative intent.

We found a high rate of local recurrence in this study, which can partly be explained by the fact that the selection criteria used in this study leads to an overrepresentation of patients with local tumor progression. Hepatic radiofrequency ablation using expandable and internally cooled electrodes increase parenchymal pressure [195], and it has been speculated that this may be a mechanism for spread of malignant cells to surrounding tissue. One would expect that intraparenchymal infusion of saline by the ablation system used in this study would lead to further increase in parenchymal pressure. Furthermore, study I confirmed that the perfusion electrodes produced irregular and non-predictable coagulations, which may be unfavorable in regard to achieving adequate treatment margins. This was in line with our clinical experience, and is one of the reasons we no longer use the perfusion electrode system in clinical treatment at our institution. Finally, a learning curve exists for new procedures. Therefore, as this patient series represents our initial experience with radiofrequency ablation, a lower rate of local tumor recurrence would be expected with increasing experience.

Study V is a small clinical study. Some authors regard small clinical studies as unscientific and unethical [196]. On the other hand, one could argue that in rapidly lethal diseases, one should look for large effects in small trials rather than small effects in large trials [197]. This non-randomized study with a median follow-up of 27 months does not allow us to determine long-term survival effects of
radiofrequency ablation of colorectal liver metastases. However, of the 11 patients included in the study, 9 patients are alive with a median follow-up of 28 months (20-43 months). This compares favorably to recent studies of patients with metastatic colorectal cancer treated with chemotherapy, in which a median survival of 20-22 months has been achieved [198, 199]. However, the patients in our study is a highly selected group, with liver-only involvement which may have better prognosis than patients included in chemotherapy studies. Patient selection bias can therefore not be excluded as an explanation for the apparent short-term benefit of radiofrequency ablation. In 7 of the 11 patients in study V, liver resection had been performed previously. Therefore, a time-effect is also present, with these 7 patients having a median survival of 12 months (2-77 months) after development of metastatic disease to the liver prior to entering our study.
New technology that offers the promise of improved patient care is attractive, and may be adopted despite lack of evidence of either efficacy or superiority over existing procedures [200]. When hepatic radiofrequency ablation was introduced in the early 1990s, it was viewed as a palliative minimally-invasive experimental method [135]. Within a decade after its introduction, the use of hepatic radiofrequency ablation is increasing despite lack of formal scientific evidence.

In order to investigate the therapeutic potential of the concept of local ablation of liver metastases, local tumor recurrence at the site treated with radiofrequency ablation should be minimized. This involves development of radiofrequency ablation systems capable of generating predictable tissue coagulations, and improved intra-procedural monitoring in order to secure adequate treatment margins. The ability of radiofrequency ablation to eradicate liver tumors is limited by a number of tumor-related factors [135] which should be taken into consideration when determining which patients are eligible for this treatment modality. Novel diagnostic modalities such as positron emission tomography (PET) may improve staging of malignant disease, which would facilitate selection of patients with localized non-resectable disease who may benefit from local tumor ablation [201].

It is important to assess whether high local control rates are of clinical significance in regard to long-term survival, since intrahepatic tumor recurrence not related to the area treated with radiofrequency ablation or extrahepatic tumor recurrence significantly limits the value of local tumor control. Furthermore, an unresolved apparent inconsistency exists in post-treatment recurrence rates in patients with colorectal liver metastases treated with hepatic resection or radiofrequency ablation, with significantly higher recurrence rates observed in patients treated with radiofrequency ablation [24]. If these differences cannot be attributed to biological differences in the treated tumors, differences in recurrence rates must be attributed to the treatment, and not the disease [202]. Further exploration of the biological effects induced by radiofrequency ablation may provide insights in the underlying mechanisms of these findings.
Long-term patient survival is the primary clinical endpoint in evaluation of potential curative interventions in cancer patients. Even though recent non-randomized series indicate that radiofrequency ablation of non-resectable colorectal liver metastases is associated with a 5-year survival of approximately 30% [126, 129], these patients belong to a highly selected group treated within a multimodal framework. Patient survival in non-randomized series cannot reliably be attributed to any single intervention, as the outcome may reflect patient selection bias and the cumulative effect of a multimodal treatment regime [51]. Randomized studies should therefore be performed to determine the possible impact of radiofrequency ablation on long-term survival. However, randomization of resectable patients to radiofrequency ablation is considered unethical, as superior long-term survival in well-defined patient populations is established for surgical resection [134, 202, 203]. Randomization of non-resectable patients to chemotherapy or radiofrequency ablation may be ethically acceptable. In 2002 a multicenter randomized phase III study examining the possible benefit of hepatic radiofrequency ablation was initiated by the European organization for research and treatment of cancer (EORTC). The objective of the study was to examine the effect of performing radiofrequency ablation in addition to chemotherapy by randomizing 390 patients with non-resectable colorectal liver metastases to chemotherapy vs. chemotherapy and radiofrequency ablation. However, in this multicenter study with nearly 50 participating centers, recruitment of patients was insufficient, and the study was redesigned to a randomized phase II study, with planned inclusion of 152 patients [204]. The lack of sufficient recruitment may reflect ethical or practical concerns of the individual physicians in charge of patient inclusion, or that patients refuse to volunteer for randomization. Nevertheless, this study will probably be able to determine if radiofrequency ablation provide an added survival benefit to chemotherapy.

Despite expanding indications for resection of colorectal liver metastases, several recent studies have shown improved survival compared with historical series, with 5-year survival of 58% [23-25]. These studies may establish a new gold standard for long-term survival after resection of colorectal hepatic metastases against which other potentially curative treatments must be compared. Long-term results after radiofrequency ablation are promising, but do not currently match those achieved by resection. Radiofrequency ablation has been promoted by some as an inexpensive minimal-invasive alternative to resection [205]. However, the short-term benefits of less invasiveness should not be considered a valid argument for performing a treatment which may be less effective than established treatment. Therefore, based on currently available studies, only non-resectable tumors should be treated with radiofrequency ablation [202, 206]. Considering the complexity of the multi-modal treatment
available to patients with colorectal liver metastases, radiofrequency ablation should only be performed in patients in which resection is not considered possible after evaluation by a multidisciplinary team including oncologists, radiologists and surgeons experienced in hepatic surgery [202].
CONCLUSIONS

1. The perfusion electrode system produces irregular coagulations during physiological hepatic perfusion. Mechanical occlusion of the portal vein and the hepatic artery during ablation increases the effective coagulation volume, but is associated with elongated coagulations extending outside the expected treatment site.

2. A liposomal thermosensitive agent showed persistent signal enhancement on T₁-weighted MR images of tissue exposed to a temperature above the phase transition temperature of 57°C, and may be of use during MRI-guided radiofrequency ablation.

3. Delayed portal vein thrombosis may occur after radiofrequency ablation during physiological hepatic flow. An electrode-to-vessel distance of < 5 mm was a significant predictor for delayed portal vein thrombosis.

4. Radiofrequency ablation of normal porcine liver is associated with significantly increased activity of matrix metalloproteinase-2 and -9 in the transition zone separating tissue coagulation from normal hepatic parenchyma. The expression of matrix metalloproteinases was localized to macrophages in the transition zone.

5. Semi-automatic 3D volumetric analysis of post-ablation CT images acquired every third month does not facilitate earlier detection of local tumor recurrence than conventional morphological evaluation, but may increase specificity of non-invasive diagnosis of local tumor recurrence after radiofrequency ablation of colorectal cancer liver metastases.

6. Local tumor progression was found in a high proportion of patients with colorectal liver metastases treated with radiofrequency ablation.
7. Intrahepatic and extrahepatic malignant growth is commonly found during follow-up in patients treated with radiofrequency ablation despite successful treatment of the index tumor.

8. Systematic post-ablation follow-up may identify patients with malignant disease that can be offered re-resection or re-ablation in curative intent.
In 1948 Pennes developed a mathematical model for heat transfer in perfused tissue based on temperature measurements in the human forearm [76]. Pennes suggested that the rate of heat transfer between blood and tissue is proportional to the product of the volumetric perfusion rate and the difference between the arterial blood temperature and the local tissue temperature. The equation proposed by Pennes is known as the bioheat equation [55]:

\[
\rho c \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) - c_p \rho_b m \rho T (T - T_b) + Q_p (r, t) + Q_m (r, t)
\]

where

- \( \rho \) density of tissue, blood (kg/m³)
- \( c \) specific heat of tissue, blood (W sec/kg °C)
- \( k \) thermal conductivity
- \( m \) perfusion (blood flow rate/unit mass tissue) (m³/kg sec)
- \( Q_p \) power absorbed per unit volume tissue
- \( Q_m \) metabolic heating per unit volume of tissue.

For the sake of this thesis, the most important contribution of the bioheat equation is to offer a biophysiological explanation of the effects of reduction of intrahepatic blood flow during radiofrequency ablation.
APPENDIX II - A NOTE ON TERMINOLOGY

The terminology used for description of radiofrequency ablation systems, electrodes, and postoperative image findings is inconsistent. Incompatible sets of terminology have been proposed by different groups [181, 207]. The terminology used in this thesis adheres to the proposal of the International working group on image-guided tumor ablation [181]. Due to the general evolution of the terminology and the fact that authors are encouraged to implement the terminology used by a specific scientific journal, the terminology used in the papers in this thesis is inconsistent.

1. Radiofrequency ablation system and electrodes

A single radiofrequency ablation system was used for all studies in this thesis. The current generator is denominated “impedance-controlled”, whereas the system including the electrodes is denominated as “saline-enhanced radiofrequency ablation system”. The preferred term for the electrodes used in this study is “perfusion electrodes”, which correspond to the term “saline-cooled” used in paper II and “wet electrode” used in paper III. In the literature, the terms “open perfused system” and “Berchtold system” are occasionally used to describe this system.

2. Thermally treated tissue

Postprocedural imaging may identify zones with altered signal intensity (at magnetic resonance imaging), echogenicity (at sonography), or attenuation (at computed tomography) that can be attributed to the thermal treatment. When describing findings on postprocedural images, the zone in which the induced treatment effect is present is called “coagulation” or “ablation zone”. This corresponds to the terms “lesion” or “ablation” used in paper II.
3. Failure of local therapy

A distinction is made between tumor growth within or at the site treated with radio-frequency ablation, intrahepatic foci of malignancy not related to the site treated with radiofrequency ablation, and extrahepatic tumor growth. The preferred terms for tumor growth within or at the site previously treated with radiofrequency ablation are “local tumor progression” and “local tumor recurrence.”
All animals were supplied by the Department of Comparative Medicine, Rikshospitalet University Hospital. Two different non-tumor animal models were used in this study.

In paper I, III and IV we used a pig model. The porcine liver is not morphologically similar to the human liver. Nevertheless, the pig is widely used as a model for hepatic thermal ablation. Furthermore, this model has proved to be valuable to our group in previous experimental research. A total of 26 pigs were operated. Eight pigs were included in a pilot study to establish the model. Of the consecutive 13 pigs who were randomized to hepatic vascular occlusion, one pig was euthanized within 2 hours of the primary operation due to respiratory distress attributed to complicated tracheal intubation. Consequently, 12 animals were successfully randomized, operated and reoperated 4 days later. Data from these 12 animals are included in paper I, III and IV. Additionally, 5 animals constituted a control group where only hepatic vascular occlusion but no radiofrequency ablation was performed. One of the animals in the control group was euthanized 2 days postoperatively due to symptoms of paralytic ileus. Data from the remaining 4 animals in the control group were used in paper III.

In paper II we studied a liposomal paramagnetic agent which was only available in limited quantities. In order to obtain adequate plasma levels, the experiments were performed in a small-animal model. The rabbit was chosen because it is a well-established liver model in magnetic resonance imaging research. A total of 14 rabbits were operated. Five rabbits were included in a pilot study to establish the model. Of the remaining 9 animals, one animal with preoperative weight loss was euthanized during the ablation procedure due to signs of distress. In the remaining 8 animals the procedure was successfully completed.
REFERENCES


55


157. Chopra, S., G.D. Dodd, 3rd, K.N. Chintapalli, J.R. Leyendecker, O.I. Karahan, and H. Rhim, Tumor recurrence after radiofrequency thermal ablation of hepatic tumors:


ERRATA

Paper III, page 1995

The correct heading of the second column of Table 2 is:

Control group (n=6)
Hepatic radiofrequency ablation using perfusion electrodes in a pig model: Effect of the Pringle manoeuvre

L. Fricha,b,*, T. Malab, I.P. Gladhaugb,c

a The Interventional Centre, Rikshospitalet University Hospital, N-0027 Oslo, Norway
b Department of Surgery, Rikshospitalet University Hospital, N-0027 Oslo, Norway
c Department of Surgery, Faculty Division Rikshospitalet, University of Oslo, N-0027 Oslo, Norway

Abstract

Aim: To assess the influence of the Pringle manoeuvre on volume and geometry of coagulations close to the portal vein using an impedance-controlled radiofrequency ablation system with perfusion electrodes.

Methods: Twelve pigs were randomly assigned to a control group (n=6) and a group where the Pringle manoeuvre was applied during ablation (n=6). One coagulation was made in each animal close to the portal vein. All animals were sacrificed 4 days after ablation, and the livers were removed for gross and histopathologic analysis.

Results: Effective coagulation volume in the Pringle group (10.8±5.0 cm³) was significantly increased (p=0.03) compared to the control group (4.1±4.1 cm³). The efficacy ratio, defined as the effective coagulation volume divided by the coagulation volume, was not significantly different in the Pringle group (0.47±0.27) compared to the control group (0.33±0.22). The geometrical centre of the effective coagulation volume did not correspond to the position of the ablation electrode. Thermal damage of the gallbladder was found in three animals, all belonging to the Pringle group.

Conclusions: The Pringle manoeuvre was associated with increased effective coagulation volume, but did not significantly influence the predictability of coagulation volume or geometry.

Keywords: Radiofrequency; Liver; Neoplasm; Animal experimentation

Introduction

Radiofrequency ablation (RF ablation) is used for in situ destruction of malignant liver tumours in patients who are not candidates for hepatic resection.1 RF ablation should be subject to established oncological criteria for surgical treatment of malignant liver tumours including complete tumour clearance and treatment margins of 0.5–1 cm.2,3 Coagulation geometry is as important as coagulation volume in order to achieve adequate treatment margins.3,5 Interruption of hepatic inflow during ablation enable RF coagulations that are larger, less elliptic and less distorted in experimental studies using multitined expandable electrodes.4,6 In contrast, effects of the Pringle manoeuvre on coagulation volume and geometry made with an impedance-controlled RF ablation system using perfusion electrodes have not been investigated.

Perfusion mediated tissue cooling is believed to be particularly prominent in the perivascular regions.7 It is therefore of special interest to characterize volume and geometry of coagulations made in proximity to large intrahepatic vessels. The aim of this study was to assess the impact of the Pringle manoeuvre on the volume and geometry of coagulations made with an impedance-controlled perfusion electrode system close to the right portal vein branch (RPV) in normal porcine liver.

Materials and methods

Study design

Twelve domestic pigs with a mean weight of 30.3 kg (SD=3.4 kg) were randomly assigned to a control group...
(n=6) and a group where the Pringle manoeuvre was applied during ablation (n=6). One coagulation was made in each animal. Coagulation volume and geometry was assessed from specimens retrieved 4 days after ablation.

**RF ablation protocol**

An impedance-controlled RF ablation system (Elektrotom 106 HiTT, Berchtold GmbH and Co., Germany) was used. The system incorporates a generator that delivers alternating current at 375 kHz. The cumulative energy output is displayed in real-time. The 1.7 mm diameter perfusion electrode (EZ 707-15-04, Berchtold, Germany) has a 15 cm long shaft incorporating a lumen for saline perfusion, with a terminal non-insulated segment of 15 mm. RF energy was applied for 1 min with an effect of 30 W, immediately followed by 8 min with 50 W.

**Operative procedure**

Surgery was performed during general anaesthesia induced by intravenous pentobarbital 150–400 mg and morphine 10–30 mg and maintained by inhaled isoflurane 1–1.2% in oxygen. The analgesic effect was supplemented with morphine infusion at 10 mg/h. A 134 cm² dispersive plate (EZ 344-02, Berchtold, Germany) was placed over the right hip. Laparatomy was performed and a silicone vessel loop (Sterion, MN, USA) was passed twice loosely around the hepatoduodenal ligament.

The ablation electrode was positioned 2 cm into the liver parenchyma in the anterior–posterior plane within 1.5 cm of RPV. In the Pringle group, the vessel loop was tightened around the hepatoduodenal ligament 1 min prior to application of RF energy and released immediately after ablation had been completed. The needle track was coagulated by applying 25 W during withdrawal of the electrode. The animals were euthanised 4 days after ablation and the livers were removed for gross and histopathological analysis.

**Assessment of coagulation volume and geometry**

All coagulations were excised with wide margins and cut at 4–6 mm intervals transverse to RPV. All fresh tissue slices were photographed with a L-shaped ABFO no. 2 photomacrographic scale (Lightning Powder Company, Jacksonville, FL, USA) (Fig. 1(a)). The images were imported into the scientific image analysis program ImageJ version 1.33 (National institute of mental health, Bethesda, MA, USA). The border of normal liver tissue vs tissue with macroscopically visible changes was traced on both sides of each slice, and the perimeter and area of the coagulation was measured. The geometrical centre of the inscribed circle does not correspond to the assumed position of the ablation electrode (black dot).

![Figure 1](image1.png)

**Histopathological examination**

Tissue slices were fixed in 4% formaldehyde for at least 10 days, then dehydrated and embedded in paraffin.
A 4–5 µm thick section was cut from each slice, stained with haematoxylin and eosin and examined by light microscopy by an experienced pathologist.

**Statistical analysis**

The animals were allocated to the two groups by block randomization, using a block size of two. SPSS for Mac OSX version 11.0.2 (SPSS, Chicago, IL, USA) was used for statistical analyses. Data are presented as mean ± SD unless otherwise indicated. Independent samples t-test was used for comparison of the two groups. A p-value < 0.05 was considered statistically significant.

**Results**

Coagulation characteristics and delivered energy per coagulation volume in the two groups are summarized in Table 1.

**Coagulation volume**

Coagulation volume was increased three-fold, although not significantly, in the Pringle group compared to the control group (Fig. 2(a)). Effective coagulation volume was significantly increased in the Pringle group (Fig. 2(b)). Delivered energy per coagulation volume was six-fold lower, although not significantly, in the Pringle group.

**Coagulation geometry**

Maximal coagulation diameter was increased, although not significantly, in the Pringle group. Effective coagulation diameter was significantly increased in the Pringle group. The efficacy ratio was not significantly different in the two groups. The geometrical centre of the effective coagulation volume did not correspond to the position of the ablation electrode (Fig. 1(b)).

**Complications**

Thermal damage to abutting organs was present on re-operation in three animals, all belonging to the Pringle group. In two animals, gallbladder perforation was caused by severely deformed coagulations with maximum lengths of 11.3 and 13.7 cm extending to the gallbladder (Fig. 3). Additionally, perforation of the gastric wall was found in one of these animals. Perforation of the gallbladder was also found in a third animal where the coagulation had not extended to the gallbladder.
Histopathology

A sharp demarcation between coagulated tissue and normal hepatic parenchyma was found at gross examination, corresponding to the 1–2 mm border between coagulated and non-coagulated tissue at histopathologic examination (Fig. 4). The hepatic microstructure was preserved in parts of the coagulated area. Cytoplasm of coagulated hepatocytes was more eosinophilic, and showed pycnotic nuclei compared to viable hepatocytes. In other coagulated areas, the hepatocytes had undergone karyolysis and were denucleated.

Discussion

Coagulation volumes in our control group were smaller than in previous experimental studies using perfusion electrodes, but in conformity with clinical results. These diverging findings can be attributed to the use of different ablation protocols and methods used for volume estimation. Furthermore, as the coagulations in our study were made close to a portal vein, perfusion mediated tissue cooling possibly limited the coagulation volume. These studies nevertheless support our findings of unpredictable ablation volumes when no hepatic vascular occlusion is used. Use of the Pringle manoeuvre was associated with three-fold increase in coagulation volume, which is comparable to previous studies with multitined expandable electrodes. The effective coagulation volume was nearly three-fold increased in the Pringle group, demonstrating a beneficial effect of the Pringle manoeuvre. The maximal coagulation diameter is of importance in order to avoid thermal damage to abutting organs, but is oncologically irrelevant. In contrast, knowledge of the effective coagulation diameter is important to avoid incomplete coagulation of the index tumour. Although the effective coagulation diameter was increased by 1 cm in the Pringle group, the Pringle manoeuvre was associated with elongated coagulations extending outside the expected treatment site.

The isoperimetric ratio has been used as an indicator of coagulation circularity, but is not appropriate for characterization of irregular non-spherical volumes as different ratios can be acquired depending on the orientation of the measurement planes. In this study, we, therefore, introduced the concept of the efficacy ratio which directly relates to the ability to produce a spherical coagulation volume (Fig. 5). A low efficacy ratio indicates that a large proportion of the coagulation occurs in locations not relevant for the oncological goal of the treatment. The efficacy ratio was below 0.5 in both groups, but tended to increase in the Pringle group. Our observation of a displacement of the geometrical centre of the effective coagulation volume relative to the position of the electrode may be caused by the proximity of the electrode to large portal vessels. This finding may be of clinical importance and should be further assessed.

Direct heat transfer from the treatment site can cause burns of adjacent viscera, which is a well known complication of hepatic RF ablation regardless of ablation system used. However, when using perfusion electrodes, diffusion of heated saline may cause thermal damage to structures not in direct contact with the treatment site.
the present study, thermal damage to abutting structures was observed in three animals. Gallbladder perforation was observed in one of these animals even though the coagulation did not involve the gallbladder, suggesting that this perforation may be caused by diffusion of heated saline.

The effect of saline infusion on coagulation volume and geometry has not been completely elucidated, but is believed to be three-fold: a liquid electrode effect where the effective surface of the ablation electrode is increased by the electrical conductive qualities of saline, reduction of charring in the proximity of the electrode due to cooling effects, and extended thermal damage by diffusion of heated saline into the tissue. A marked divergence from a spherical geometry for coagulations produced with perfusion electrodes during physiological liver perfusion has been reported previously, and may be caused by diffusion of saline along vessels, tissue planes or surfaces, generating low resistance electrical paths that could lead to heating outside the expected target area. In our study, use of the Pringle manoeuvre resulted in unpredictable coagulation volumes and geometry as well as thermal damage to the gallbladder and the gastric wall. A possible explanation for this finding is that reduced tissue capillary level microperfusion due to hepatic inflow occlusion may increase the risk of low resistance electrical paths or heated saline to cause thermal damage.

Our findings are valid only for the specific RF ablation system and electrodes used in the study. The lack of statistical significance for several of the examined variables may be explained by the limited statistical power of the study. In contrast to the human liver, the deep fissures and the thin lobes in the porcine liver may influence the volume and geometry of the coagulations. Extrapolation of our findings to clinical settings should, therefore, be done with caution.

In conclusion, the reproducibility of the coagulation volumes and geometry including the efficiency ratio was low in the control group. The Pringle manoeuvre was associated with increased effective coagulation volume, but did not significantly increase the predictability of coagulation volume, coagulation geometry or the efficacy ratio. Furthermore, the Pringle manoeuvre was associated with coagulations extending outside the expected treatment site causing thermal damage to adjacent organs.

Acknowledgements

We thank the staff at the Interventional Centre at Rikshospitalet for providing excellent assistance. We also thank the Department of Comparative Medicine at Rikshospitalet for support in animal care, and Ole Petter F. Clausen, Department of Pathology, Rikshospitalet University Hospital for examination of the histopathological specimens. This work was supported by the Norwegian Cancer Society, grant no. D02042/001.

References


Experimental Application of Thermosensitive Paramagnetic Liposomes for Monitoring Magnetic Resonance Imaging Guided Thermal Ablation

Lars Frich,1,2* Atle Bjørnerud,3 Sigrid Fossheim,4 Terje Tillung,1 and Ivar Gladhaug2

The use of a liposomal paramagnetic agent with a $T_1$-relaxivity that increases markedly at temperatures above the phase transition temperature ($T_m$) of the liposomal membrane was evaluated during magnetic resonance imaging (MRI) guided hyperthermia ablation. A neodymium-yttrium aluminum garnet (Nd-YAG) laser unit and a radiofrequency ablation system were used for tissue ablation in eight rabbit livers in vivo. One ablation was made in each animal prior to administration of the liposomal agent. Liposomes with a $T_m$ of 57°C containing gadodiamide (GdDTPA-BMA) were injected iv, and two additional ablations were performed. $T_1$-weighted scans were performed in heated tissue, after tissue temperature had normalized, and 15–20 min after normalization of tissue temperature. Increase in signal intensity ($\Delta SI$) for ablations prior to injection of the agent was 13.0% (SD = 5.7) for the laser group and 9.1% (SD = 7.9) for the radiofrequency group. Signal intensity after administration of the agent unrelated to heating was not statistically significant ($\Delta SI = 1.4\%, P = 0.39$). For ablations made after injection of the agent, a significant increase was found in the laser ($\Delta SI = 34.5\%, SD = 11.9$) and radiofrequency group ($\Delta SI = 21.6\%, SD = 22.7$). The persistent signal enhancement found in areas exposed to a temperature above the threshold temperature above $T_m$ allows thermal monitoring of MRI guided thermal ablation. Magn Reson Med 52:1302–1309, 2004. © 2004 Wiley-Liss, Inc.

Key words: thermometry; contrast agent; liver; radiofrequency ablation

Hyperthermia ablation is used for treatment of benign and malignant tumors (1–4). The tissue response to heating is related to the absolute temperature induced in the tissue and exposure time. Irreversible tissue destruction occurs when tissue is exposed to temperatures in excess of 50–55°C (5,6). Hyperthermia ablation has been widely used for treating malignant liver tumors not amenable to conventional surgical resection. Heating modalities in clinical use include laser, microwave, radiofrequency, and focused ultrasound (1,7–9). The exact size and shape of the resulting ablation is difficult to predict because of the heterogeneous distribution of the thermal energy due to local disparity in tissue composition, tissue anatomy, and vascularization (10,11). In clinical series, local tumor progression after ablation therapy has been reported to be as high as 30–60%, indicating the presence of viable malignant cells at the treated site (6,12,13). Improved intraprocedural monitoring may reduce the local tumor progression rates associated with thermal ablation and prove beneficial in regard to long-term patient survival.

Multiplanar imaging capabilities and excellent soft tissue contrast make magnetic resonance imaging (MRI) ideally suited for peroperative visualization, positioning of energy applicators, and monitoring of thermal ablation procedures. Additionally, noninvasive, three-dimensional mapping of relative temperature changes is feasible with MRI, based on the longitudinal relaxation time ($T_1$), the diffusion coefficient ($D$), or proton resonance frequency (PRF) of tissue water. These methods rely on image subtraction and are sensitive to tissue motion (14). Thermal imaging of the liver therefore presents certain challenges as the liver is displaced during respiration due to movement of the diaphragm. A substantial body of research exists on MRI-based methods for temperature mapping during laser ablation (15–17). These methods can be applied to thermal monitoring of other MRI-compatible local thermal ablation therapies such as focused ultrasound (18). Thermal monitoring of radiofrequency ablation poses certain challenges: MRI-based methods for thermal monitoring cannot be used during radiofrequency ablation, as the application of radiofrequency energy leads to electromagnetic noise that severely deteriorates image quality. Simple switching circuits (19) and filtering of the RF signal by modification of the hardware (20) have been developed that allow image acquisition in near real-time or during RF ablation. At present such solutions are not incorporated in commercially available systems used for RF ablation. Consequently, reliable MRI-based thermometry of radiofrequency ablation requires development of new methods for thermal imaging.

Thermosensitive MRI contrast agents represent a novel approach for thermal imaging that offers a robust, motion-insensitive method for indication of an absolute temperature threshold on standard imaging hardware using conventional MRI sequences. Mechanisms of action include temperature-induced transition from a diamagnetic to a paramagnetic state using bistable molecular complexes (21) and temperature-dependent relaxivity by incorporation of conventional MRI contrast agents within thermosensitive liposomes (22,23). In the present study we used liposomes with a phase transition temperature ($T_m$) of 57°C containing gadodiamide (GdDTPA-BMA) during thermal ablation in vivo. The agent is designed to be “MR dormant” at physiologic temperatures and “MR activated”
when reaching the predefined $T_m$ of the liposome membrane. At physiologic temperatures, the $T_1$-relaxation enhancement of the liposomal agent is low due to limited water exchange. As the temperature approaches $T_m$, the transmembrane water exchange kinetics is faster and the $T_1$-relaxivity increases rapidly and markedly before leveling off as $T_m$ is exceeded (Fig. 1). Tissue with a temperature above $T_m$ would therefore be expected to appear with increased signal intensity (SI) on T1-w images. An important secondary mechanism in vivo is leakage of GdDTPA-BMA from the liposomes into the surrounding tissue at temperatures above $T_m$. Following thermal coagulation of parenchyma and vessels, it is assumed that the agent cannot migrate and therefore will interact with the tissue where it was released. Consequently, a persistent signal intensity enhancement on T1-w images may verify that a temperature of $T_m$ or above was achieved.

The purpose of this study was to quantify relative signal intensity alterations associated with the use of paramagnetic thermosensitive liposomes during and after laser and radiofrequency ablation of rabbit liver in an interventional MRI system.

**MATERIALS AND METHODS**

**Liposomes**

Liposomal gadodiamide (GdDTPA-BMA), whose membrane consisted of 90% (w/w) distearoylphosphatidylcholine (DSPC) and 10% distearoylphosphatidylglycerol (DSPG), was prepared by the thin film hydration method (24). The liposomes were subsequently sized down by membrane extrusion and untrapped GdDTPA-BMA was removed by dialysis. Key physicochemical liposomal properties, as measured by standard methods described elsewhere (22,24), were effective Gd concentration 27 mM, extraliposomal pH 7.2, and osmolality 355 mosmol/kg. Intensity weighted liposome size was 110 nm with a polydispersity index of 0.16, indicating a highly monomodal size distribution. The transition temperature ($T_m$) of the liposomal membrane was 57°C with a SD of the $T_m$ measurements of 1%. The liposomal agent was injected iv at a dose of 1.1 mL/kg, corresponding to 30 µmol Gd/kg. The thermosensitive liposomal agent was supplied by Amersham Health AS. Figure 1 shows the in vitro temperature dependence of the $T_1$-relaxivity for liposomal gadodiamide (GdDTPA-BMA) used in this study. For the relaxometric measurements, the liposomal dispersion was diluted with pH-adjusted isosmotic glucose solution to an effective Gd concentration of 1 mM and dispensed in NMR tubes. Each tube was heated to a predetermined temperature within a temperature range of 37–57°C in a thermostated heating block at a heating rate of 10°C/min. When the desired temperature was reached, each sample was inserted into a 0.47-T relaxometer (Minispec PC-120b, Bruker GmbH, Germany) for immediate relaxometric measurements.

**Laser and Radiofrequency Equipment**

The laser apparatus consisted of a neodymium-yttrium aluminum garnet (Nd-YAG) laser unit with a wavelength of 1.064 µm (Dornier mediLas Fibertom 4100, Dornier MedTech GmbH, Germany). The laser light was distributed through a 1.9-mm-diameter flexible MRI-compatible light guide designed for interstitial tissue ablation (Dornier MedTech GmbH, Germany) with a 20-mm diffuser at the tip of the fiber.

A saline-enhanced, impedance-controlled radiofrequency system (Elektrotom HITT 106, Berchtold GmbH & Co., Germany) was used for radiofrequency ablation. The 375-kHz radiofrequency generator delivered a nonmodulated high-frequency alternating current to a maximum of 1.2 amp. Maximum power output of the generator could be set between 5 and 60 W. The actual power output was adjusted automatically in accordance with the impedance of the patient circuit. A syringe pump (Pilot C, Fresenius Vial, France) was connected by a RS-232 connection to the RF generator. Output of a saline solution of 154 mmol (0.9%) NaCl per liter water was controlled by an algorithm built into the RF generator. The saline-cooled MRI-compatible RF electrodes had a diameter of 1.7 mm and a 15-cm shaft. The saline solution was distributed to the tissue in the immediate vicinity of the tip of the electrode through three groups of two side holes each in the 15-mm exposed tip, placed at 120° angles from each other.

**MRI-Compatible Fiber-Optic Thermometry System**

Tissue temperature during the thermal treatment was measured by a MRI-compatible fiber-optic thermometry system consisting of a flexible fiber-optic probe and an optoelectronic signal-processing unit (Multitemp 1601, Optomed, Norway). The outer diameter of the temperature probe was 1.5 mm. The probe had 16 measuring points with a sensor interval of 3 mm. All 16 sensors could be read out simultaneously in real-time with a temporal resolution of 1 sec and an accuracy of ±0.1°C. The system was calibrated to a temperature range of 0–150°C. The temperature monitoring system was connected by a RS-232 interface to a laptop with specially designed software (Multitemp). Temperature readings for all 16 sensors were sampled each second.

**Animals and Operative Procedure**

The experimental protocol was approved by the Department of Comparative Medicine at Rikshospitalet Univers-
sity Hospital under the surveillance of the Norwegian Animal Research Authority. Eight chinchilla-bastard rabbits with a mean weight of 3.2 kg (2.7–4.0 kg) were treated in accordance with current legislation governing animal care. After premedication with a subcutaneous injection of 0.1 mL/kg fentanyl 0.315 mg/mL + fluanisone 10 mg/mL (Hypnorm, Janssen), an iv cannula (Nedelon, Becton–Dickinson) was inserted in the dorsal ear vein and 0.1 mL/kg fentanyl 0.315 mg/mL + fluanisone 10 mg/mL (Hypnorm, Janssen) was administered iv. All rabbits were shaved in the upper abdominal midline using a hair clipper (Oster Golden A5). The four rabbits to undergo radiofrequency ablation were shaved in an area of 10 × 20 cm on the lower back, and a 134-cm² dispersive plate (EZ 344–02, Berchtold, Germany) was applied. The rabbits were intubated with a 3- or 3.5-mm tracheal tube. All animals were anesthetized by Isoflurane 1–1.2%, 50% oxygen, using a minute volume of 1.2 liter and respiratory frequency of 30/min. A midline incision was made and the rabbits were placed inside the MRI head coil. To eliminate motion artifacts during scanning, the right medial, left medial, and left lateral liver lobes were exteriorized and placed on a horizontal 5-mm polycarbonate sheet fixed to the head coil. Care was taken not to compromise the circulation of the liver.

Four animals were treated with laser ablation and four animals with radiofrequency ablation. The laser fiber or the radiofrequency electrode was inserted into one of the liver lobes axially. The temperature fiber was inserted parallel in close proximity to the laser fiber or the RF electrode, with the tip of the temperature sensor extending 15 mm in front of the energy applicator. Each treatment session consisted of 9 min active heating. For the animals treated with laser ablation, a maximum power setting of 20 W and duration of 3 min was applied three times successively for each ablation. Automated stepwise reduction of the laser effect (20/15/10 W each for 30 sec and 7 W for 90 sec) was used. For the radiofrequency system a power setting of 20 W and a continuous treatment time of 9 min was used. One ablation was created in each of the three exterorized liver lobes of each animal. The first ablation in each animal, denoted lesion A, was made prior to injection of the agent. Tissue temperature during active heating and passive cooling was monitored by real-time readout from the temperature probe in the liver. When all sensors on the temperature probe were below 37°C, the laser fiber/RF electrode and the temperature probe were removed and inserted into one of the two remaining liver lobes. The liposomal agent was injected in a dorsal ear vein with an infusion speed of 1 mL/min, and the vein was flushed with 10 mL saline solution of 154 mmol NaCl. The second ablation, denoted lesion B, was made 12–14 min after administration of the agent, using the same energy modality and protocol as the first ablation. The tissue temperature was allowed to normalize, and the third ablation, denoted lesion C, was made accordingly in the remaining lobe 50–55 min after injection of the agent. The A, B, and C lesions were randomly assigned to each of the three exterorized liver lobes. After the MRI scans had been completed, the animals were killed by injecting 100 mg/kg pentobarbital iv.

MRI Protocol

All procedures were performed on a 0.5-T vertically open whole-body MRI system (Signa SP2, GE Medical Systems) using a transmit and receive head coil. An axial T$_1$-weighted fast spin echo (FSE) sequence was used for imaging. The imaging parameters were repetition time (TR) = 300 msec, echo time (TE) = 19 msec, flip angle (FA) = 90°, slice thickness 4 mm, spacing 4.5 mm, 256 × 256 acquisition matrix, field of view 180 × 180 mm, and four signal averages. Total duration of the sequence was 2 min, 16 sec. A scan was performed after each repositioning of the laser fiber or RF electrode, but before energy was applied. For the four animals operated with laser, one scan was performed every 3 min during heating for each of the ablations. The mean signal intensity of the three scans during laser heating was used for further analysis. Due to the electromagnetic noise generated by the radiofrequency circuit while operating, scanning in heated tissue during treatment was not possible for the four animals treated with radiofrequency ablation. A scan was therefore performed within 10 sec after termination of the radiofrequency treatment. The liver tissue was allowed to reach baseline temperature and a postablation scan at baseline temperature was performed for all animals. This scan protocol was repeated for each of the three ablations. Scans performed prior to heating, in heated tissue, and after cooling of the tissue were denoted f0, f1, and f2, respectively. The scan at f0 for B and C lesions was made 15–20 min after cooling of the tissue of the previous ablations. Signal intensity of A and B lesions 15–20 min after cooling of the tissue, denoted f3, could therefore be measured from the f0 scans of B and C lesions, respectively. No scan was done 15–20 min after normalization of the tissue temperature for C lesions. Hence, signal intensity at f3 is therefore not available for C lesions, and statistical analysis is performed only for the values measured at f1 and f2 for all lesion types. To evaluate signal intensity changes due to the agent unrelated to heating, signal intensity of the reference regions of interest (ROIs) was measured prior to administration of the agent and 3 min after injection of the agent, but prior to the generation of the B lesions.

Analysis of MR Images

The signal intensity of the ablated area versus the untreated tissue was measured from uncompressed DICOM images by image analysis software designed for quantitative image analysis (DIMview, Kikhsiptalet University Hospital, Oslo, Norway) running on a Windows XP computer system. Ablations as seen on T$_1$-w images consisted of a dark central area at the needle track with diameters of 1–5 mm surrounded by a brighter rim. Each ablation was segmented manually using a polygon tool, avoiding the central darker part. The mean value of the signal intensity of this ROI (S$_{ROI}$) was used for further analysis. To compensate for a potential general variation of signal intensity in the liver during the procedure, each signal intensity measurement was normalized using two independent regions of interest (S$_{ROI}$ and S$_{ref}$) from liver tissue not exposed to the local thermotherapy on the same image.
as the ablations. The subtraction of the mean signal intensity of these two reference ROIs from the signal intensity of the ablation was used as an expression of the relative alteration of signal intensity between the ablation and the surrounding liver tissue.

\[
\Delta SI_h = \left( SI_{h, ablation} - \frac{SI_{h, ref, 1} + SI_{h, ref, 2}}{2} \right) \times 100
\]

The largest diameter of each ablation was measured from the MR images by an investigator blinded to the results of the corresponding measurements on the macroscopic specimens.

Analysis of Liver Tissue

The livers were harvested immediately after the animals were killed. To retain the orientation of the ablated area, a marker was inserted into the tracks from the laser fiber or RF electrode, and each ablation was excised with wide margins. Using a custom-built slicer, the ablated tissue was cut in 4-mm slices axial to the inserted marker. All slices were photographed with a 2.1-megapixel digital camera (Canon, Tokyo, Japan) under standardized lighting conditions with a L-shape ABFO No. 2 photomacrographic scale (Lightning Powder Company, Jacksonville, FL). The images were imported into the scientific image analysis program ImageJ version 1.3 (National Institute of Mental Health, Bethesda, MD) running on an Apple Macintosh OSX computer system. Each image was calibrated by measuring the number of pixels per unit length of the photomacrographic scale. Image resolution was typically 10 pixel/mm. Slices from each ablation were manually segmented using the border of normal liver tissue versus tissue with macroscopically visible changes, and the largest diameter of the ablation was determined.

Statistical Analysis

SPSS for Mac OSX version 11.0.2 (SPSS, Chicago, IL) was used for statistical analysis. Analysis of the normalized signal intensity of the ablations was performed based on a variable component model, with animal as random component and lesion type and relative time as fixed components. This statistical model accounts for dependency in observations from the same animal. Post hoc tests were based on Scheffe’s method. Paired samples t test was used for analysis of significance of the observed signal intensity alterations of the agent unrelated to heating.

RESULTS

In one of the rabbits in the laser treatment group only 2 ablations were made due to the anatomy of the liver. In the remaining seven animals 1 ablation was made in each of the three exteriorized liver lobes. Hence, 11 laser ablations were made in four animals and 12 radiofrequency ablations were made in four animals. At the time of removal of the liver, macroscopic lesions were identifiable for all attempted ablations. Energy deposited during each radiofrequency ablation was typically 10,500 J. Examples of temperature distribution during laser and radiofrequency ablation are shown in Fig. 2a and b. During laser ablation, temperatures in close proximity to the laser applicator tip exceeded the 150°C calibration limit of the temperature sensor for several of the ablations. With the radiofrequency system, the highest registered temperature was 104°C. A temperature above 57°C was registered by at least one of the temperature sensors in the first 3 min of passive cooling following the treatment. During scanning in this thermal imaging window, increased signal intensity of the areas with a temperature above the transition temperature of the agent could be expected. Tissue temperature returned to baseline within 10 min following termination of the energy application.

Signal Intensity prior to Administration of the Agent

For the animals in the laser group, decreased SI was seen for the A lesions for scans at t1 during heating (ΔSI = −3.5%, SD = 6.4) followed by a signal increase (ΔSI = 12.2%, SD = 2.3) at t2 when the tissue had returned to baseline temperature. On scan t3 15–20 min after the tissue had reached baseline temperature, no further discernable increase in SI (ΔSI = 13.0%, SD = 5.7) was seen (Fig.
A similar pattern was seen for the A lesions in the animals in the radiofrequency group, where scans at t1 showed decreased SI (ΔSI = -6.9%, SD = 8), followed by a signal increase at t2 (ΔSI = 4.7%, SD = 10.5) and t3 (ΔSI = 9.1%, SD = 7.9) (Fig. 3b).

Signal Intensity after Administration of the Agent

The change in signal intensity prior to and 3 min after iv injection of the contrast agent unrelated to heating was not statistically significant (ΔSI = 1.4%, SD = 4.5, P = 0.35). For animals in the laser group a slight increase in signal intensity was measured for B lesions at t1 (ΔSI = 2.3%, SD = 6.1), followed by an increase at t2 (ΔSI = 18.8%, SD = 20.0) and a further increase at t3 (ΔSI = 34.5%, SD = 11.9). For C lesions an increase during ablation at t1 (ΔSI = 16.4%, SD = 7.1) followed by a further increase at t2 (ΔSI = 31.5%, SD = 8.9) was found (Fig. 3a). Test for comparisons of SI measurements found significance at the 0.05 level between A lesions and C lesions with a P value less than 0.05 and a confidence interval (CI) of 6.95–22.05. The difference between B lesions and C lesions was also significant (P = 0.049, CI = 0.03–15.13). Difference between A lesions and B lesions did not reach significance when performing an analysis including only SI measurements at t1 and t2 (P = 0.053, CI = -0.07–13.91). The results for the animals in the radiofrequency group (Fig. 3b) were comparable to those in the laser treatment group. The B lesions showed a minor signal increase at t1 (ΔSI = 5.4%, SD = 9.5) followed by a further increase at t2 (ΔSI = 13.7%, SD = 9.7) and at t3 (ΔSI = 21.6%, SD = 22.7). For C lesions an increase at t1 (ΔSI = 19.3%, SD = 12.7) was found, followed by a further increase at t2 (ΔSI = 26.3%, SD = 10.9). In the radiofrequency group, test for comparisons of means of SI found a significance between A lesions and C lesions with a P value less than 0.05 and a CI of 6.95–22.05. The difference between B lesions and C lesions was also significant (P = 0.048, CI = 0.09–20.08). The difference between A lesions and B lesions did not reach significance when performing an analysis including only SI measurements at t1 and t2 (P = 0.165, CI = -2.41–17.58). Typical MR images of an ablation made after administration of the agent are shown in Fig. 4. Figure 5 shows the corresponding macroscopic specimen.
Diameter of Ablations on MRI and Macroscopic Specimens

The largest diameter of the ablations perpendicular to the probe track was measured from the photographs of the macroscopic specimens by one of the investigators (LF). The mean diameter of the 11 laser ablations was 16.3 mm (SD = 1.2; 14–18 mm). The corresponding figure for the 12 radiofrequency ablations was 24.3 mm (SD = 3.8; 18–32 mm). Another investigator (AB) blinded for the results of the analysis of the macroscopic specimens measured the largest diameter for all ablations from the MR images at t2. For the 11 laser ablations the mean and SD of the difference between the diameter as measured from the photographs and the MR images were 1.7 ± 1.3 mm. For 3 of the 12 radiofrequency ablations, a reliable measurement of the largest diameter could not be established from the MR images. For the 9 radiofrequency ablations where a diameter could be determined, the corresponding figures were −0.3 ± 3.4 mm. Figure 6 shows a scatterplot of the diameter of the ablations as measured from the MR images and the photographs of the specimens.

DISCUSSION

Several active and passive methods for noninvasive thermometry within the human body exist, utilizing transmission or reflection of microwaves, ultrasound, or MRI (25). In the present study, we examined the feasibility of the liposome encapsulated paramagnetic agent gadodiamide (GdDTPA-BMA) as a thermosensitive probe during MRI-guided laser ablation and radiofrequency ablation of rabbit liver in vivo.

Local application of heat in tissues leads to alternations in metabolic, macromolecular, extracellular, and vascular parameters (26). Irreversible cellular damage is a function of temperature and exposure time and is dependent on the type of tissue. Denaturation of proteins occurs after exposure for temperature above 50–55°C and leads to macroscopically visible tissue changes in liver tissue. Tissue becomes desiccated when reaching 90–100°C and carbonized when temperatures exceed 150°C (5,27,28). Reversible and irreversible structural and biologic tissue changes related to ablation (26) as well as the influence of liposomal GdDTPA-BMA contributed to the alterations in signal intensity on T1-w images observed in the present study. Additionally, a direct thermal T1-effect was present for scans in heated tissue. The correlation between tissue T1-relaxation time and temperature is complex and nonlinear if one considers the entire temperature range investigated in the present study (37 to 150°C) (26,29). In the temperature range of 37–45°C, a linear increase in T1 with temperature is expected (14,30). At higher temperatures, coagulation and tissue denaturation occur, leading to nonlinear and irreversible T1 changes, and the T1-temperature correlation becomes complex and tissue dependent (29,31).

Alterations of T1 related to the ablation and a direct thermal T1-effect contributed to the observed signal intensity for A lesions made prior to injection of the liposomal agent. A lesions therefore provided a reference that facilitated quantification of T1-changes that could be attributed to the T1-effect of the liposomal agent for subsequent B and C lesions. Signal intensity of A lesions decreased to 4–7% below baseline in both the laser and the radiofrequency groups on scans at t1 performed in heated tissue. On scans at t2 after normalization of the tissue temperature, ∆SI increased 5–12% above baseline. These findings are in accordance with previous results from laser ablation of rabbit liver (32). No further increase in ∆SI was seen for A lesions at t3, 15–20 min after normalization of the tissue temperature. The slope between t1 and t2 can possibly be explained by the direct thermal T1-effect causing a net increase in T1 of T1 for scans acquired in heated tissue at t1. In scans at normalized temperature at t2, the direct thermal T1-effect is no longer present and the observed 5–12% increase in signal intensity at t2 can be attributed to structural and biologic tissue changes related to the ablation. Intravenous injection of the liposomal agent in tissue with normalized temperature unrelated to heating did not cause a general increase in signal intensity, confirming that the T1-relaxation enhancement of the agent was negligible at a temperature well below the phase transition temperature of the liposomal membrane (Fig. 1). For B lesions a mean increase in ∆SI of 2–5% compared to baseline was found at
The signal intensity for B lesions increased to 14–19% at T2 and further to 22–35% at T3. The nonreversible contrast enhancement after cooling could be explained by a leakage of GdDTPA-BMA from the liposome interior and subsequent entrapment due to coagulation of vessels. The increased signal intensity stabilized and was present on scans performed more than 60 min after termination of the thermal treatment. Signal intensity for C lesions was significantly increased to 16–19% at T1 with a further increase to 26–32% at T2. The magnitude of the normalized signal intensity enhancements for B and C lesions was comparable to that found in a recent study of focused ultrasound ablation of rabbit liver using the same liposomal agent (23). The slope between T1 and T2 observed for B and C lesions was comparable to the slope between T1 and T2 observed for A lesions, suggesting that the considerably lower SI seen during scanning in heated tissue at T1 compared to T2 for B and C lesions was primarily caused by two counteracting processes taking place during heating. The alterations of tissue parameters induced by the thermal treatment and the direct thermal T1-effect caused a net increase of tissue T1 as seen on T1 scans of A lesions, whereas the activated liposomal agent decreased T1 of the tissue.

Increased signal intensity by a constant offset was observed in C lesions compared to B lesions and in B lesions compared to A lesions in both the laser group and the radiofrequency group. Conventional liposomes are rapidly opsonized in blood after iv injection and taken up by the mononuclear phagocyte system (MPS) with the liver as a major organ. For a liposome size of 110 nm as used in this study, Kupffer cells are responsible for the liposome uptake in liver, but a minor uptake by the hepatocytes is also to be expected. This intracellular uptake increases with time postinjection and reaches a plateau, with the latter dependent on factors such as liposome size and coating (33). Bio-distribution studies of the current liposome formulation in rabbits using the same dosage as used in the present study show a rapid blood clearance with only 35% of the injected dose remaining in the blood 5 min postinjection. The liver uptake at 3 h postinjection was about 40%, a typical figure for the liposome size and membrane investigated (unpublished data). The offset between B lesions made 12–14 min after administration of the agent and C lesions made 50–55 min after administration of the agent may therefore be explained by a time-dependent hepatic intracellular uptake of the liposomal agent, where the intracellular concentration of GdDTPA-BMA was higher at the time of creation of C lesions compared to B lesions.

An increase in signal intensity was observed for B lesions at T3 compared to T2 in both the laser and the radiofrequency group (Fig. 3a and b). A corresponding slope between T2 and T3 was not present for A lesions. The reason for this delayed enhancement is not established. However, delayed focal signal increase can be seen on T2-w images in infarcted myocardial tissue following injection of extracellular gadolinium agents (34). This finding is believed to be caused by loss of integrity of the cell membranes in the infarcted tissue, with a subsequent local increase of the distribution volume of the agent. A hypothesis could be made that a similar local increase in the distribution volume occurs in liver tissue exposed to thermal ablation, giving rise to a time-dependent accumulation of GdDTPA-BMA released from the liposome interior into the surrounding tissue at tissue temperatures above Tm.

The correlation between the largest diameters of the ablations as measured from the MR images and the macroscopic specimens was not comparable to that found in a study examining the correlation between GdDTPA-enhanced MRI and histomorphologic findings of laser ablation in rabbit liver (35). Several factors might contribute to this inconsistency. The contrast agent used in this study enhances the ablation site, and not the surrounding normal liver tissue, as is the case for conventional MRI liver contrast agents. Irreversible tissue damage is dependent on both the temperature and the exposure time. The temperature needed for irreversible tissue damage with an exposure time of 9 minutes as used in this study might be lower than the 57°C temperature threshold of the liposomal agent (5). Hence, irreversible tissue damage could occur in areas exposed to a temperature below the transition temperature of the liposomal agent. Interestingly, the diameters measured from the laser specimens were larger than those measured from the MR images for all ablations except for one, suggesting that enhancement is only present in limited areas within the thermal lesion. For the radiofrequency ablations, which were larger and more irregular than the laser ablations, the correlation was poorer. During radiofrequency ablation, saline was introduced at the ablation site. Saline is associated with increasing T1-relaxation times and could cause a decrease in signal intensity on T1-w images. Additionally, the presence of saline might lead to local inhomogeneities in the concentration of the liposomal agent. The lack of correlation between images and specimens could also partly be explained by methodological limitations. For example, even if care was taken to retain the orientation of the ablated area, slicing of the liver specimens were not necessarily identical to the plane of the axial T1-w images.

This experimental study was performed in a small animal model subject to several limitations. Findings in rabbit liver tissue are not necessarily transferable to those in a human liver. Furthermore, all treatments were conducted in normal liver parenchyma. Vascular divergence between intratumoral and peritumoral blood flow and surviving normal liver (36) could lead to significant inhomogeneous distribution of the contrast agent of possible importance. In our study, the temperature-sensitive liposomal agent was not compared to other established MRI contrast-enhanced methods. Compared to our A lesions, conspicuousity can probably be improved by conventional MRI contrast agents (37) or by using other imaging modalities such as contrast-enhanced ultrasound (38). However, consistent results were found that confirm the ability of the liposomal agent to differentiate between tissue exposed to the thermal treatment and normal liver tissue.

In conclusion, a significant nonreversible increase in signal intensity was found for B and C lesions made after injection of the liposomal agent compared to control A lesions in both the laser ablation group and the radiofrequency group. The persistent signal enhancement in areas exposed to a temperature above the threshold temperature may be of clinical value during MRI-guided thermal ablation, especially for local heating modalities, such as radiofrequency ablation, where other MRI-based thermometry methods are not feasible.
ACKNOWLEDGMENTS

The authors thank Unni Nordby Wiggen and Astrid Rognes, Amersham Health AS, for the preparation and characterization of the liposomes. Thore Egeland, Rikshospitalet University Hospital, provided statistical assistance. We thank the staff at the Interventional Centre at Rikshospitalet for providing excellent assistance. We also thank the Department of Comparative Medicine at Rikshospitalet for support in animal care.

REFERENCES


Experimental hepatic radiofrequency ablation using wet electrodes: electrode-to-vessel distance is a significant predictor for delayed portal vein thrombosis

Abstract The aim of this study was to examine possible explanatory variables associated with acute and delayed portal vein thrombosis after hepatic radiofrequency (RF) ablation using wet electrodes. Coagulations were created within 1.5 cm of the right portal vein (RPV) branch in 12 pigs with \( n = 6 \) or without \( n = 6 \) Pringle manoeuvre. Sham operations with Pringle manoeuvre were performed in four animals. Rotational portal venography was performed prior to ablation, 10 min after ablation and 4 days after ablation. Vessel diameters and vessel patency was determined from the portal venograms. Distance between the ablation electrode and RPV was measured from 3-dimensional reconstructions of the portal venograms. The portal veins were examined by microscopy. Delayed portal vein thrombosis was found in two of six animals in the Pringle group and three of six animals in the control group 4 days after ablation \( (P = 1.0, \text{Fisher's exact test}) \). All five occurrences of delayed portal vein thrombosis were found in the six animals with a distance between the ablation electrode and RPV of 5 mm or less \( (P = 0.030) \), indicating that the electrode-to-vessel distance may be an independent explanatory factor for delayed portal vein thrombosis after RF ablation with wet electrodes.

Keywords Radiofrequency (RF) ablation - Portal vein thrombosis - Complication - Experimental animal study

Introduction Hepatic resection may be contraindicated in patients with tumours located close to central hepatic vessels, if resection of these vessels is associated with unacceptable risk of hepatic insufficiency due to extensive loss of functional liver parenchyma. For cirrhotic patients with a deficit in functional hepatic reserve this risk is greatly increased \([1]\). Radiofrequency (RF) ablation minimises loss of functional liver parenchyma and is a treatment option for patients with non-resectable malignant liver tumours \([2-5]\). Treatment of perivascular tumours not amenable to resection is a potentially important clinical application for hepatic RF ablation.

Large vessels in the proximity of tumours treated with RF ablation are a predictor of incomplete tumour destruction and, consequently, local tumour progression \([6-8]\). Ablation of perivascular tumours, i.e. tumours contiguous with a vessel with a diameter of 3 mm or more, is restricted by the heat-sink effect, where the region to be coagulated is cooled by blood flow in large vessels \([9, 10]\). Occlusion of hepatic vascular inflow, by clamping of the portal vein and the hepatic artery (Pringle manoeuvre), can be expected to decrease the heat-sink effect and reduce perfusion of the hepatic parenchyma. Use of the Pringle manoeuvre is associated with increased energy efficiency \([11]\) and increased ablation volume \([9, 12]\). Additionally, hepatic
vascular occlusion enhances coagulation necrosis close to hepatic vessels [6] and may be advantageous for ablation of perivascular tumours [8]. However, vascular inflow occlusion may make hepatic vessels more vulnerable to thermal injury and subsequent thrombosis [13]. Portal vein thrombosis related to RF ablation may lead to greater loss of functional liver parenchyma than anticipated, increasing the risk of postoperative hepatic insufficiency.

Risk factors associated with occurrence of portal vein thrombosis following RF ablation have not been fully established. The aim of this experimental study was to examine possible explanatory variables associated with acute and delayed portal vein thrombosis after RF ablation in proximity to the right portal vein (RPV) branch in porcine liver. Specifically, the impact of performing hepatic vascular occlusion, and the distance between the ablation electrode and RPV was investigated.

Materials and methods

Study design

The protocol was approved by the institutional Department of Comparative Medicine. Sixteen domestic pigs with a mean weight of 30.7 kg (SD=3.1) were included in the study. Twelve animals were randomly allocated to two experimental groups. In one group Pringle manoeuvre was applied during the ablation (n=6). In the control group, RF ablation was performed without Pringle manoeuvre (n=6). Four animals were assigned to a sham group where Pringle manoeuvre, but no RF ablation, was performed. Patency of portal vessels in all groups was determined by rotational portal venography prior to ablation, 10 min after ablation and 4 days after ablation. The portal veins were examined by microscopy after euthanasia.

Radiofrequency equipment

An impedance-controlled RF ablation system (Elektrotom HiTT 106, Berchtold GmbH & Co, Germany) was used for creating coagulations. The system incorporated a generator that delivered alternating current at 375 kHz to a maximum of 1.2 A. Power output of the generator could be set between 5 W and 60 W. The actual power output was adjusted by the generator in accordance with the impedance of the patient circuit. Tissue impedance and cumulative deployed energy were displayed in real-time. The active radio-opaque electrode was 1.7 mm in diameter, with a 15 cm long shaft incorporating a lumen for perfusion of saline solution. The terminal non-insulated segment of the electrode was 15 mm in length. A syringe pump (Pilot C, Fresenius Vial, France) was used for infusion of isotonic saline solution (0.9% NaCl) through the electrode. The saline solution was distributed to the tissue via three pairs of two side holes in the electrode tip, placed at a 120° angle from each other. The pump was connected by a RS-232 serial port to the RF generator. The infusion rate of saline solution was determined by the pre-selected power output of the generator and the circuit impedance. Under a power setting of 50 W, saline solution was infused at a rate of 105 ml/h, which corresponded to a volume of 16 ml during 9 min ablation. If an upper impedance threshold of 900 Ω was reached, five-times the value of the nominal flow was infused for 1.2 s. Radiofrequency energy was applied for 1 min with an effect of 30 W, immediately followed by 8 min with 50 W. Maximum cumulated electrical energy generated during one treatment cycle with this protocol was 25,800 J. A new electrode was used for each animal.

Angiography system

A floor mounted C-arm based angiography system (Angiostar O.R., Siemens, Erlangen, Germany) was used for acquisition of rotational venographic images of the portal veins. During each portal venography, 60 ml ioxixanol 320 mg I/ml (Visipaque, Amersham Health, Oslo) was injected into the splenic vein by an injector at 5 ml/s. Starting 3 s after the injection commenced, a series of 160 portal venograms was obtained while the C-arm rotated through 198° over 8 s. The set of image data was transferred to a post-processing workstation (Leonardo, Siemens), and a 3-dimensional (3D) portal venogram was created using a volume-rendering algorithm.

Operating procedure

After the animals had undergone premedication with intramuscular injection of ketamine 15–20 mg/kg (Ketalar, Pfizer), atropine sulphate 1 mg (Atropin, Nycomed Pharma) and azaperone 2–4 mg/kg (Stresnil, Janssen-Cilag), an intravenous cannula was placed in an ear vein, and the animal was transported to a combined angiography and operating theatre. Ampicillin 250 mg was administered intramuscularly prior to surgery and after the surgical procedure had been completed. General anaesthesia was induced with intravenous administration of pentobarbital 150–400 mg and morphine 10–30 mg. After endotracheal intubation, anaesthesia was maintained with isoflurane 1–1.2% in oxygen. The analgesic effect was supplemented with morphine infusion at 10 mg/h. A thermodilution Swan–Ganz catheter 7.5 F (Edwards Lifesciences, USA) was introduced via the right external jugular vein and advanced to the pulmonary artery for monitoring of vital signs. A 134 cm² dispersive plate (EZ 344-02, Berchtold, Germany) was placed over the right hip. A midline incision with a transverse right subcostal extension was made. The hepatoduodenal ligament, containing the hepatic artery, the portal vein and the bile duct, was identified, and a silicone
vessel loop (Sterion, MN) was passed twice around all structures. To minimise the risk of unintended damage to portal vessels, we did not use surgical diathermy when exposing the hepatoduodenal ligament. The spleen was mobilised, and a 5 F 100 mm long introducer (Radifocus, Terumo, Leuven, Belgium) was introduced into the distal 2–4 cm of the splenic vein and advanced 5 cm in an antegrade direction for administration of intraportal contrast medium. A dilute heparin solution 2.5 IU/ml heparin (Hepaflex, Baxter, Lessines, Belgium) was infused through the introducer at 60 ml/h to maintain catheter patency.

The porcine intrahepatic portal vein shows a consistent pattern of ramification into four segments with the RPV coming off at an almost right angle to the portal arch [14]. The extrahepatic portal vein with the bifurcation to the RPV was identified in all animals. The ablation electrode was positioned in the anterior–posterior plane under direct visual guidance based on the point of entry of the RPV into the hepatic parenchyma. The electrode was inserted 2 cm into the liver parenchyma between the portal arch and within 1.5 cm of RPV, perpendicular to the vessel (Fig. 1). The distance between the active tip of the ablation electrode and RPV was determined by portal venography. The electrode was repositioned if the active tip was not within 1.5 cm of the RPV. When the position of the electrode was found to be acceptable, rotational portal venography was performed. In animals randomly allocated to hepatic inflow occlusion, the silicone vessel loop was tightened around the hepatoduodenal ligament 1 min prior to application of RF energy and released immediately after ablation had been completed for a total duration of 10 min. In the sham group hepatic inflow was occluded for 10 min in all animals, without insertion of an ablation electrode into the liver. Portal flow was measured in the sham group by a transmit time flowmeter (VeriQ, Medi-Stim ASA, Norway) using a 12 mm flow probe (Cardiac Output, Medi-Stim ASA).

The acute effect of the ablation on the portal veins was assessed by portal venography 10 min after completion of the ablation, while the RF electrode was still positioned in the hepatic parenchyma. The electrode was removed after completion of the portal venography. To prevent bleeding from the needle track, we activated the electrode at 25 W during its withdrawal. The introducer in the splenic vein was removed, and the distal 2–4 cm of the splenic vein was ligated. The Swan–Ganz catheter was removed, and the right external jugular vein was ligated. Postoperatively, the animals were kept under observation until full recovery from anaesthesia and were allowed free access to food and water. Postoperative pain was managed with intramuscular injection of buprenorphine hydrochloride 0.01 mg/kg (Temgesic, Schering-Plough, N.J., USA) twice daily for 3 days. The animals underwent repeat laparotomy 4 days after ablation, and the delayed effect of the ablation was evaluated by rotational portal venography as described previously. The animals were subsequently euthanised with intravenous injection of pentobarbital 500 mg and potassium chloride 2–3 mmol/kg. The liver was excised immediately after asystole had occurred.

Interpretation of portal venograms

Diameter of the portal arch and RPV was measured at the perpendicular from the tip the ablation electrode to the vessel on native portal venograms by image acquisition software (Polytron T.O.P., Siemens). The 3D reconstructions of the portal venograms allowed multidirectional view of the volume data set consisting of the radio-opaque electrode and contrast medium-filled portal veins. The distance between the non-insulated 15 mm tip of the ablation electrode and the portal arch and RPV was determined by rotation of the 3D reconstruction to the projection where the largest perpendicular distance between the tip of the electrode and the corresponding portal vein segment could be measured. If areas relevant to the examination were hidden by other structures, the volume data could be trimmed and a volume of interest generated by post-editing of the volume data set (Fig. 2). To correct for possible displacement of the electrode relative to the vessels during ablation, we measured the electrode-to-vessel distance on portal venograms obtained both prior to and 10 min after ablation. The mean of these measurements was considered to be the electrode-to-vessel distance, and was used in the statistical analysis. Measured vessel diameters and electrode-to-vessel distances were rounded off to the nearest millimetre.
Occurrence of portal vein thrombosis was determined by independent examination of the native 2-dimensional (2D) portal venograms and the 3D reconstructions by a surgeon and two experienced radiologists unaware of the group to which the particular animal had been randomly allocated. Agreement between the examiners was reached by consensus. Owing to the pseudostenosis phenomenon observed at volume-rendered 3D angiography [15], native 2D images were used to confirm abnormal findings on the reconstructed 3D images. The splenic vein, the extrahepatic portal vein, the portal arch and the RPV were classified as normal or abnormal. Abnormal vessels were categorised as either having a filling defect or as being occluded. An occluded portal vessel with lack of peripheral filling on post-ablation portal venograms was considered to be a portal vein thrombosis. Acute portal vein thrombosis was defined as thrombosis on portal venograms acquired 10 min after completion of the ablation, whereas delayed portal vein thrombosis was defined as thrombosis on portal venograms acquired 4 days after ablation.

**Histopathological examination**

The portal arch was incised longitudinally, and the presence of intraluminal thrombi and macroscopic endothelial damage was noted. The opening of the RPV was identified. All coagulations were excised with wide margins and cut in 4–6 mm slices transverse to the RPV, fixed in 4% formaldehyde for at least 10 days, dehydrated, and embedded in paraffin. One 4–5 μm-thick section was cut from each paraffin block and stained with haematoxylin and eosin for microscopic examination by an experienced pathologist blind to the findings on the portal venograms. The presence of viable hepatocytes between the coagulated area and the portal vessel wall, the degree and extent of damage to the portal vessel wall itself, and the presence of thrombotic material adherent to the intimal surface of the vessel was noted.

**Statistical analysis**

SPSS for Mac OSX version 11.0.4 (SPSS, Chicago, Ill., USA) was used for statistical analyses. A $P$ value less than 0.05 was considered statistically significant. Data are
Presented as mean ± SD unless otherwise indicated. A sample size of six animals in each experimental group was needed to demonstrate a significant difference between the two groups with proportions of portal thrombosis of 0.05 and 0.95 using a two-group continuity corrected $\chi^2$ test with 80% power at the 0.05 two-sided significance level. The animals were allocated to the two experimental groups by block randomisation, using a block size of two. Occurrence of delayed portal vein thrombosis in the two experimental groups was analysed by Fisher’s exact test. Independent samples $t$-test assuming unequal variances was used for comparison of the two experimental groups with respect to energy delivered during the ablation, diameter of the portal arch, diameter of RPV, distance between the electrode and the portal arch and distance between the electrode and RPV. Subsequently, the animals were regrouped on the basis of presence of delayed portal vein thrombosis on post-ablation portal venograms, and the same variables were compared using independent samples $t$-tests. The distance between the RPV and the ablation electrode was recoded into a binary variable with a cut-off value of 5 mm. Fisher’s exact test with Bonferroni correction for multiple tests was used for examination of the relationship between Pringle manoeuvre and the recorded electrode-to-vessel distance as explanatory variables, and delayed portal vein thrombosis as outcome variable.

**Results**

One ablation was completed in each animal in the two experimental groups. Reversible systemic hypotension with a reduction of mean arterial pressure of 10 mmHg or more during the ablation was observed in five animals in the Pringle group and two in the control group. Increase in tissue impedance was frequently observed during ablation in the animals in the Pringle group.

Portal venograms acquired prior to RF ablation were considered normal in all animals. A filling defect in the vicinity of the electrode tip was seen 10 min after RF ablation in one animal in each experimental group. Neither of these two animals was considered to have an acute portal vein thrombosis. The remaining ten portal venograms acquired 10 min after ablation were normal. Delayed portal vein thrombosis was found 4 days after ablation in two of six animals in the Pringle group and three of six animals in the control group (Table 1). Delayed portal vein thrombosis was present in the two animals with abnormal portal venograms 10 min after ablation, and in three animals with normal portal venograms 10 min after ablation. Four of the occurrences of portal vein thrombosis were located in the RPV (Fig. 2). A fifth portal vein thrombosis was found in one animal in the Pringle group, where the portal arch with a diameter of 13 mm was found to be occluded. Gross examination of the liver of this animal revealed thermal damage to the luminal surface at the opening of the RPV, with a thrombus extending into the portal arch. The endothelium of the portal arch was macroscopically normal. Diameters of the occluded RPV branches ranged from 4–9 mm (5.6±1.9 mm). The splenic vein and the extrahepatic portal vein were normal in all animals in the two experimental groups.

No occurrences of thrombosis or filling defects were found in the splenic veins, extrahepatic portal veins or intrahepatic portal veins in the sham group 10 min after Pringle or at follow-up 4 days later. Portal flow measurements in the sham-group animals during Pringle manoeuvre confirmed cessation of portal flow. Ligation of the distal 2–4 cm of the splenic vein after removal of the introducer used for administration of intraportal contrast agent did not significantly decrease portal flow compared to baseline values (884±60 ml/min vs 934±146 ml/min, $P=0.455$).

**Histopathology**

A sharp demarcation between the coagulated area and normal hepatic parenchyma was found at gross examination, corresponding to the border between viable and non-viable tissue found at histopathological examination (Fig. 3a,b). The hepatic microstructure was preserved in parts of the coagulated area. The cytoplasm of coagulated hepatocytes was more eosinophilic and showed pyknotic nuclei compared to viable hepatocytes. In other coagulated areas, the hepatocytes had undergone karyolysis and were denucleated. Fibroblast proliferation with a variable degree of inflammation was prominent between the coagulation and the portal vein in all animals. The portal vein could not be identified in one animal in the Pringle group with delayed portal vein thrombosis due to extensive tissue necrosis. In the remaining four animals with delayed portal vein thrombosis, thrombosis was confirmed by microscop-y. In addition, subtotal thrombotic occlusion of the RPV was found in one animal in the Pringle group. The corresponding portal venogram was interpreted as normal, and this animal was therefore not considered to have a portal vein thrombosis in the statistical analysis.

**Table 1** Acute and delayed portal vein thrombosis in the two experimental groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pringle group</th>
<th>Control group</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$(n=6)$</td>
<td>$(n=6)$</td>
<td></td>
</tr>
<tr>
<td>Acute portal vein thrombosis$^a$</td>
<td>0/6</td>
<td>0/6</td>
<td>–</td>
</tr>
<tr>
<td>Delayed portal vein thrombosis$^b$</td>
<td>2/6</td>
<td>3/6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

$^a$Fisher’s exact test

$^b$Ten minutes after ablation

$^*$Four days after ablation
Viable hepatocytes were present between the vessel wall and the coagulated area in one of five animals in the Pringle group and in four of six in the control group. Damage to the endothelial lining was occasionally present without thermal damage to the adjacent hepatocytes. Necrosis of all layers of the portal vein wall covering 20–100% of the circumference was present in all five pigs in the Pringle group in whom the portal vein could be assessed. In the control group, necrosis of the vessel wall was seen in four of six animals, covering 20–80% of the circumference. In one animal in the control group with portal vein thrombosis, extensive inflammation of the portal vein was found. In the remaining three animals where the portal vein could be assessed, necrosis of all layers of the portal vein was present.

Factors associated with portal vein thrombosis

Mean energy delivered during ablation was lower in the Pringle group than in the control group (22,560±2,520 J vs 25,670±360 J, \( P=0.046 \)). No significant differences regarding preoperative vessel diameter or electrode-to-vessel distances were found in the two experimental groups (Table 2). Hepatic vascular occlusion was not associated with occurrence of portal vein thrombosis (\( P=1.0 \), Fisher’s exact test). The largest diameter of a thrombosed RPV with Pringle manoeuvre was 9 mm, whereas the corresponding vessel diameter in the control group was 5 mm. The longest distance between the ablation electrode and a thrombosed

Table 2: Deployed energy, preoperative vessel diameters and electrode-to-vessel distances in the two experimental groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pringle group ((n=6))</th>
<th>Control group ((n=6))</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>22,560±2,520 J</td>
<td>25,270±361 J</td>
<td>0.046</td>
</tr>
<tr>
<td>Diameter of portal arch</td>
<td>11.3±1.5 mm</td>
<td>11.2±1.6 mm</td>
<td>0.856</td>
</tr>
<tr>
<td>Diameter of right portal vein branch</td>
<td>5.8±1.6 mm</td>
<td>5.7±1.8 mm</td>
<td>0.867</td>
</tr>
<tr>
<td>Distance of electrode to portal arch</td>
<td>10.0±4.2 mm</td>
<td>17.2±7.4 mm</td>
<td>0.074</td>
</tr>
<tr>
<td>Distance of electrode to right portal vein branch</td>
<td>6.0±2.2 mm</td>
<td>6.8±5.6 mm</td>
<td>0.745</td>
</tr>
</tbody>
</table>

\( ^* \)Independent samples \( t \)-test with unequal variances assumed
Deployed energy, preoperative vessel diameters and electrode-to-vessel distances, using delayed portal vein thrombosis as the categorising variable

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Delayed portal vein thrombosis ((n=5))</th>
<th>No delayed portal vein thrombosis ((n=7))</th>
<th>(P^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>24.313±1.384 J</td>
<td>23.630±2751 J</td>
<td>0.586</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>23.630±2751 J</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Diameter of portal arch</td>
<td>11.6±1.8 mm</td>
<td>11.0±1.3 mm</td>
<td>0.547</td>
</tr>
<tr>
<td>Diameter of right portal vein branch</td>
<td>5.6±1.9 mm</td>
<td>5.9±1.5 mm</td>
<td>0.810</td>
</tr>
<tr>
<td>Distance of electrode to portal arch</td>
<td>10.2±6.7 mm</td>
<td>16.0±6.3 mm</td>
<td>0.167</td>
</tr>
<tr>
<td>Distance of electrode to right portal vein branch</td>
<td>3.6±1.5 mm</td>
<td>8.4±4.2 mm</td>
<td>0.023</td>
</tr>
</tbody>
</table>

\(^a\)Independent samples \(t\)-test with unequal variances assumed

Table 3

RPV with Pringle manoeuvre was 5 mm. The corresponding distance in the control group was 4 mm. The shortest distance between the electrode and the RPV without occurrence of portal vein thrombosis was 3 mm and was found in an animal with a RPV diameter of 5 mm in the Pringle group. This animal received lowest cumulated energy of all animals (Fig. 4).

The mean distance between the ablation electrode and the RPV was significantly shorter in the five animals that developed delayed portal thrombosis (3.6±1.5 mm) than in the seven animals with normal portal venograms (8.4±4.2 mm), with a \(P\) value of 0.023. No other significant differences were found in these two groups (Table 3). Delayed portal vein thrombosis occurred in five of six animals, with a distance between the electrode and the RPV of 5 mm or less, while no occurrences of portal vein thrombosis were found in the six animals where this distance was 6 mm or more (Fig. 4). A distance between the electrode and the RPV of 5 mm or less was a significant predictor for delayed portal vein thrombosis (\(P=0.030\), Fisher’s exact test).

Discussion

Although a non-cirrhotic liver may tolerate a resection of up to 80%, reduction of liver parenchyma by even 50% may be associated with postoperative hepatic insufficiency [1]. The hepatic artery normally supplies 25% of the total blood flow to the liver but may provide up to 30–50% of the normal oxygen requirement of the hepatic parenchyma, because of the higher oxygen content in the arterial blood than in the partly deoxygenated portal blood. Increased oxygen extraction from the hepatic arterial blood and increased arterial hepatic blood flow can minimise the effect of a significant reduction in portal flow [16]. Owing to these compensatory mechanisms the clinical implication of segmental portal vein thrombosis after RF ablation could be questioned. Although segmental portal vein thrombosis may be asymptomatic [13], transient liver failure has been reported after portal vein embolisation in patients with reduced hepatic reserve [17]. Liver failure caused by portal vein thrombosis was the most frequent cause of death in a recent study of 312 patients with hepatic tumours treated by radiofrequency ablation [18]. Patients with a deficit in functional hepatic reserve may be at risk of postoperative hepatic failure after portal vein thrombosis. Of special clinical concern are patients in whom an extensive hepatic resection has been performed and concomitant RF ablation in the remnant liver is planned [18]. Determination of risk factors associated with occurrence of segmental portal vein thrombosis related to RF ablation may, therefore, be of value for selecting patients suitable for RF ablation.

Use of the Pringle manoeuvre was not associated with increased risk of delayed portal vein thrombosis in this experimental randomised study. Histopathological examination confirmed necrosis of the portal vein wall in all pigs in the Pringle group where the integrity of the portal vein wall could be assessed. Viable hepatocytes between the vessel wall and the coagulated area were present in only one of five animals in the Pringle group, compared with four of six in the control group. These findings suggest that hepatic vascular occlusion during RF ablation enhances cellular devitalisation close to portal vessels and confirm the increased risk of thermal damage to vessels during hepatic inflow occlusion found in previous experimental studies [19, 20]. Nevertheless, this did not translate into a higher incidence of acute or delayed portal vein thrombosis in this study. Even though the distribution of all variables under consideration, except energy, was similar in the two experimental groups, as expected from randomisation (Table 2), these figures are derived from a small data set of five occurrences of delayed portal vein thrombosis in 12 animals and should be interpreted with caution. Hence, our finding that the Pringle manoeuvre was not associated with acute or delayed portal vein thrombosis may be explained by the limited sample size.

Less energy was delivered during ablation in the Pringle group than in the control group. This finding can be explained by the fact that the impedance-controlled radiofrequency system employed in this study reduced power output if tissue impedance increased during ablation. The reduced or absent hepatic blood flow caused by the Pringle manoeuvre can be expected to limit the perfusion-mediated cooling of the electrode-to-tissue interface, leading to rapid desiccation and subsequent impedance rise. As the ablation protocol used in this study consisted of a predefined time interval of 9 min and a fixed power setting of 50 W, reduced power output resulted in lower accumulated energy output. The importance of the
lower mean energy deposited in the Pringle group is unclear, as no statistical significant difference was found when we compared deployed cumulated energy in the animals with and without delayed portal vein thrombosis (Table 3).

A recent study by Ng et. al. reported that use of the Pringle manoeuvre during RF ablation invariably resulted in delayed portal vein thrombosis, whereas no such risk was present for ablation without hepatic vascular occlusion [20]. In our study, delayed portal vein thrombosis occurred only in two of six animals in the Pringle group. Perhaps of more importance was that three of six animals in the control group developed delayed portal vein thrombosis. These apparently diverging results may have several explanations. In the study by Ng et. al., the distance between the vessel and the electrode was stated to be 5 mm in all animals. Our results suggest that the distance between electrode and vessel may be of significant importance for development of delayed portal thrombosis. The RF ablation systems used in the two studies were different. In the study by Ng et al., an internally cooled electrode with a non-insulated tip of 30 mm was used, whereas we used a wet ablation electrode design with a non-insulated tip of 15 mm. The generator and generator setting used by Ng et. al. was not specified, but the electrodes used in the study are usually connected to a generator that can deliver up to 200 W, compared to the 50 W used in our study. The duration of ablation (12 min vs 9 min) also varied. In the study by Ng et. al. the presence of delayed portal vein thrombosis was investigated 1 week after ablation. Histopathological examination revealed complete necrosis of the portal vein wall in the majority of the animals 4 days after the ablation in our study. We cannot exclude that the proportion of portal vein thrombosis could have been higher if our animals had been examined at 7 days after ablation compared to 4 days after ablation. Other dissimilar aspects are the methods used for assessment of portal vein thrombosis. The study by Ng et al. measured portal flow by means of intraoperative Doppler ultrasonography, while repeat portal venography was used for determination of portal vein thrombosis in our study. The use of portal venography was based on the assumption that venography would facilitate objective detection of segmental portal vein thrombosis that might go unnoticed when alterations of flow velocity at the portal trunk were measured.

The approach used in our study involved exposing portal endothelium injured by ablation to a non-ionic contrast agent and ligation of the distal 2-4 cm of the splenic vein. Both the use of intraportal contrast medium and ligation of the distal splenic vein could be potential influential factors for portal vein thrombosis, especially in the group without Pringle manoeuvre. Even though the non-ionic contrast agent used in this study is associated with a low incidence of thrombus-related events [21], the safety profile of the agent has not been evaluated for porcine portal endothelium damaged by thermal ablation. We cannot, therefore, completely rule out the possibility that use of this contrast agent in the presence of portal endothelium damaged by thermal ablation could lead to formation of thrombi that may have influenced the results. Significantly reduced portal vein flow caused by decreased inflow from the splenic vein could be a contributing factor for intrahepatic portal thrombosis. However, flow measurements in the sham group showed that ligation of the distal splenic vein was associated with a statistically not significant reduction of portal flow of 5%. It is, therefore, not likely that reduced portal flow due to distal splenic vein ligation was a confounding factor for the development of portal thrombosis. The distal splenic vein is a possible source of emboli to intrahepatic portal veins. However, filling defects were not observed in the splenic veins or extrahepatic portal veins on any portal venogram. Furthermore, thrombosis developed in the RPV, which comes off at almost a right angle to the portal arch and is not a probable site of thrombus formation caused by distant emboli.

The absence of acute portal vein thrombosis following RF ablation in proximity to portal veins has been established by previous studies [20, 22]. Only two of the five animals with delayed portal vein thrombosis 4 days after ablation had abnormal findings on the portal venograms 10 min after ablation. Hence, a normal portal venography obtained 10 min after ablation did not exclude later occurrence of thrombosis, whereas an abnormal portal venography 10 min after ablation was associated with delayed portal vein thrombosis. Thrombus formation related to RF ablation is believed to be initiated by endothelial cell injury with interruption of endothelial continuity, leading to platelet adhesion and activation as well as thrombin production [23]. In the present study, histopathology confirmed that portal vein thrombosis was invariably accompanied by transmural necrosis of the portal wall or extensive inflammation of the portal endothelium. This suggests that acute endothelial damage caused by the ablation represents a risk for subsequent thrombosis of the vessel.

Our finding of an electrode-to-vessel distance of 5 mm or less as a significant predictor of portal thrombosis is biologically plausible. The relationship between factors that influence tissue temperature after deposition of energy is described by the bioheat equation [24]. According to this, an increase in tissue temperature is dependent on the energy deposited in the tissue, modified by local tissue interactions, minus the heat loss [25]. In accordance with the bioheat equation, our findings suggest that the critical distance from the electrode at which portal vessels are at risk of thrombosis may be increased if perfusion-mediated heat loss is minimised due to hepatic vascular occlusion. The tissue power density $W_v$ (watts/cubic metres) is an
expression of the electrical energy per time unit that tissue is exposed to under ideal conditions:

\[ W_v = \delta V^2 \frac{a^2}{r^4} \]

where \( \delta \) is the tissue conductivity, \( V \) the voltage, \( a \) the radius of the electrode, and \( r \) the distance from the electrode. Thus, the power density can be expected to fall off extremely rapidly with increasing distance from the electrode, as it is inversely proportional to the distance from the electrode to the fourth power [26]. The RF ablation system used in this study infuses saline solution to the tissue through the active electrode during ablation. Although the effect of saline infusion has not been completely elucidated, the conductive properties of saline solution are believed to increase the effective radius of the electrode [27] and thereby increase power density at a given distance from the electrode. The magnitude of this effect has not been quantified for the RF ablation system used in this study.

From an electrical point of view, the liver is inhomogeneous, with large variations in tissue conductivity. Consequently, the flow pattern of the electrical current around the electrode is determined by the tissue morphology and composition. The temperature distribution is also related to non-electrical tissue factors, such as the amount of heat loss by conduction. The complexity of the association between these factors and the resulting thermal tissue damage is demonstrated by the fact that size and shape of the coagulated volume cannot be predicted, even under standardised ablation protocols [28]. Although the relationship between these factors and the resulting tissue temperature is not a trivial one, it seems reasonable to assume that, when the electrode-to-vessel distance is below a critical limit, the vessel wall will be exposed to temperatures high enough to cause endothelial damage. Factors related to the specific RF ablation equipment used, such as the generator output, size and shape of the active electrode and the presence of internal or external cooling of the electrode, influence the energy efficacy [11] and the extent of the coagulation [29]. The RF ablation system used in this study has been shown to produce irregular coagulations when compared with other RF ablation systems [29, 30]. Therefore, our finding of an electrode-to-vessel distance of 5 mm or less as being associated with delayed portal vein thrombosis is only valid for the specific RF ablation system used in this study.

Several limitations of this study must be addressed. First, findings after experimental RF ablation in normal porcine liver are not necessarily applicable to clinical settings. The pig has a coagulation pathway similar to that of humans, but it is considered to be more susceptible to activation of the coagulation system following endothelial damage [31]. Hence, thermal ablation and endothelial damage causing thrombosis in pigs would not necessarily lead to portal thrombosis in humans. Secondly, for the prevention of clotting in the introducer in the splenic vein, heparin at 150 IU/h was administered through the introducer between the contrast examinations during the 1–2 h procedure. The accumulated heparin dose used in our study was less than 5% of the 100–150 IU/kg per hour considered a normal dose for surgical procedures in the pig [31]. We consider it unlikely that administration of heparin at 150 IU/h into the splenic vein would have influenced the occurrence of portal vein thrombosis or its natural course. Reversible systemic hypotension with a reduction of mean arterial pressure of 10 mmHg or more during ablation was observed in both experimental groups during ablation. Consequently, reduced hepatic blood flow may have occurred even in the control group. This study was designed to detect thrombosis in the portal veins. Concurrent thrombosis of segmental hepatic arteries would be expected to increase the loss of hepatic function but was not assessed in this study. Finally, the experiments were carried out in an animal model with an adequate hepatic reserve, which is unsuitable for demonstration of altered hepatic function after segmental portal vein thrombosis.

In conclusion, delayed portal vein thrombosis associated with hepatic RF ablation close to portal veins occurred in both the Pringle and the control group. Use of the Pringle manoeuvre during RF ablation was not associated with a higher risk of delayed portal thrombosis. On the other hand, our findings indicate that the Pringle manoeuvre rendered portal veins more susceptible to thermal damage. A distance between the ablation electrode and the RPV of 5 mm or less was significantly associated with development of delayed portal vein thrombosis after RF ablation with wet electrodes. Further research is warranted to examine the role of the electrode-to-vessel distance in the development of acute or delayed portal vein thrombosis for other hepatic RF ablation systems, and to assess alterations of hepatic function associated with segmental portal vein thrombosis.

**Acknowledgements** We thank the staff at the Interventional Centre for providing excellent assistance, and the Department of Comparative Medicine for support in animal care. Marijke Veenstra and Marte Olstad at the Institute of Biostatistics provided statistical assistance. This work was supported by the Norwegian Cancer Society, grant no. D02042/001.
References

Increased Activity of Matrix Metalloproteinase 2 and 9 After Hepatic Radiofrequency Ablation

Lars Frich, M.D.,*†1 Kristin Bjørnland, M.D., Ph.D.,†‡¶ Solveig Pettersen, B.S.,‡
Ole Petter F. Clausen, M.D., Ph.D.,§ and Ivar P. Gladhaug, M.D., Ph.D.†¶

*The Interventional Centre, †Department of Surgery, ‡Institute for Surgical Research, §Department and Institute of Pathology, Rikshospitalet University Hospital; and ¶Department of Surgery, Faculty Division Rikshospitalet, University of Oslo, Oslo, Norway

Submitted for publication December 22, 2005

Background. Radiofrequency (RF) ablation of hepatic metastases from colorectal cancer (CRC) is associated with a high rate of local and intrahepatic tumor recurrence. Matrix metalloproteinases (MMPs) play an important role in inflammation, tissue repair and tumor cell invasion and metastasis. MMP-2 and MMP-9 are associated with increased risk of recurrence and decreased survival in patients with colorectal cancer. The primary aim of the study was to determine if hepatic RF ablation increased MMP-2 and MMP-9 activity in the transition zone surrounding the coagulated hepatic tissue.

Materials and methods. Twelve pigs were randomized to hepatic RF ablation with (n = 6) or without (n = 6) hepatic vascular occlusion (Pringle maneuver). Four days after ablation tissue specimens were collected from the transition zone surrounding coagulated hepatic tissue, and from normal hepatic parenchyma. MMP activity was quantified by gelatin zymography. Cellular localization of MMPs was determined by immunohistochemistry using antibodies against MMP-2, MMP-9, and the macrophage marker CD68.

Results. MMP-2 and MMP-9 activity was increased in the transition zone compared to normal hepatic parenchyma, with ratios of 3.0 (P = 0.005) and 2.6 (P = 0.001), respectively. Pringle maneuver did not influence MMP activity. MMP-2 and MMP-9 expression was localized to macrophages in the transition zone.

Conclusions. Hepatic RF ablation is associated with increased expression of MMP-2 and MMP-9 in macrophages in the transition zone surrounding the coagulated hepatic parenchyma. These findings may contribute to the understanding of possible mechanisms for the high recurrence rates observed in patients after RF ablation of CRC hepatic metastases. © 2006 Elsevier Inc. All rights reserved.

Key Words: matrix metalloproteinases; radiofrequency ablation; liver; neoplasm; animal experimentation.

INTRODUCTION

Hepatic resection is the gold standard for treatment of liver metastases from colorectal cancer (CRC), with 5-year survival rates of approximately 30% in selected patients [1, 2]. The majority of patients with hepatic metastases from CRC are not candidates for potential curative hepatic resection because of bilobar tumor location, underlying hepatic disease or extrahepatic tumor growth. Radiofrequency (RF) ablation is increasingly used for in situ tumor destruction in patients with non-resectable metastases limited to the liver [3]. Focal tissue necrosis is achieved by applying alternating electrical current within a frequency range of 400 to 500 kHz to an active electrode positioned in the target tumor. At temperatures above 55°C, protein denaturation and cell death occur. Heating of tissue is associated with increased tissue impedance and char ring which limit the coagulation necrosis to 3 to 5 cm diameter [4]. Furthermore, in vivo the extent of tissue coagulation is restricted by the heat-sink effect, where the region to be coagulated is cooled by blood flow in large vessels [5, 6]. Increased coagulation volume can be achieved by reduction of hepatic perfusion during ablation by temporary mechanical occlusion of the portal vein and the hepatic artery (Pringle maneuver) [7–9].

Patients with CRC liver metastases treated with hepatic RF ablation have higher local, intrahepatic,
and distant recurrence rates, as well as lower overall survival than comparable resected patients [10]. A plausible explanation for these findings is negative patient selection bias, with micrometastatic disease being present in a larger proportion of patients treated with RF ablation [11]. An alternative explanation that warrants further investigation is that thermal ablation of malignant tumors may induce biological alterations in the tumor tissue or peritumoral hepatic parenchyma that facilitate spread and invasion of malignant cells [12, 13].

Matrix metalloproteinases (MMPs) are a family of zinc-dependent matrix-degrading endopeptidases involved in a variety of physiological and pathological events [14–16]. MMPs are associated with inflammation and tissue repair and play an important role in the normal physiological turnover of the extracellular matrix (ECM). Increased expression of MMPs is necessary for tumor cell invasion, metastasis and angiogenesis. The gelatinases MMP-2 and MMP-9 degrade type IV collagen, which is a major component of the basement membrane. Disruption of the basement membrane allows tumors to spread locally and distally. MMP-2 and MMP-9 contribute to CRC progression in experimental models [14], are overexpressed in patients with CRC hepatic metastasis [17–19], and are associated with increased risk of tumor recurrence and decreased survival in patients with CRC [16]. Increased MMP activity may provide a physiological mechanism of tissue remodeling that facilitates growth and spread of malignant cells.

Our study hypothesis was that hepatic RF ablation increases expression of MMPs and cytokines in the transition zone separating coagulated tissue from normal hepatic parenchyma. The primary aim of the study was to determine activity of MMP-2 and MMP-9, and expression of the cytokines tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10) and prostaglandin E₂ (PGE₂) in hepatic tissue lysates from the transition zone. Additionally, we wanted to examine if use of the Pringle maneuver during RF ablation was associated with alterations in MMP activity or cytokine levels. Tissue was harvested 4 days after ablation to allow examination of MMP activity and cytokine levels during an established inflammatory process with a well demarcated transition zone.

MATERIALS AND METHODS

Experimental Protocol

The protocol was approved by the institutional Department of Comparative Medicine. Twelve domestic pigs with a mean weight of 30.3 kg (SD ~ 3.4 kg) were randomized to two experimental groups. In the first group RF ablation was performed without occlusion of hepatic vessels (n = 6). In the second group Pringle maneuver was performed during RF ablation (n = 6). Premedication consisted of intramuscular injection of ketamine 15 to 20 mg/kg (Ketalar; Pfizer, Cambridge, MA), atropine sulfate 1 mg (Atropin; Nycomed Pharma, Rockside, Sweden) and azaperone 2 to 4 mg/kg (Stresnil, Janssen-Cilag). Amphicillin 250 mg was administered intramuscularly before and after the surgical procedure. Surgery was performed during general anesthesia induced by intravenous pentobarbital 150 to 400 mg and propofol 10 to 30 mg and maintained by inhaled isoflurane 1 to 1.2% in oxygen. The analgesic effect was supplemented with intravenous infusion at 18 mg/kg/h. A 7.5 F pulmonary artery catheter (CCOMbo 774HF75; Edwards Life sciences, Irvine, CA) was introduced via the right external jugular vein and advanced to the pulmonary artery for monitoring of vital signs. A 134 cm³ dispersive plate (EZ 344-02; Berchtold GmbH & Co., Tuttingen, Germany) was placed over the right hip. Laparotomy was performed and a silicone vessel loop (Sterion, Minneapolis, MN) was passed twice loosely around the hepatoportal duodenal ligament containing the hepatic artery, the portal vein and the bile duct.

An impedance-controlled RF ablation system (Elektrotom 106 HiPT, Berchtold GmbH & Co., Germany) was used for ablation. The system incorporates a generator that delivers alternating current at 375 kHz to a maximum of 1.2 amperes. The active electrode is 1.7 mm in diameter, with a 15 cm long shaft incorporating a lumen for saline perfusion, and a terminal non-insulated segment of 15 mm. A syringe pump (Pilot C; Fresenius Vial, Grenoble, France) was used for infusion of saline through the active electrode. The saline was distributed to tissue in the vicinity of the electrode tip through three pairs of two side holes. The electrode was positioned 2 cm into the liver parenchyma under visual guidance. RF energy was applied for 1 min with an effect of 30 Watts, immediately followed by 8 min with 50 Watts. In animals randomized to the Pringle group, the silicone vessel loop was tightened around the hepatoportal duodenal ligament 5 min before application of RF energy and released immediately after ablation had been completed. After ablation had been completed the pulmonary artery catheter was removed, and the right external jugular vein was ligated. Postoperatively, the animals were kept under surveillance until full recovery from anesthesia and were allowed free access to food and water. Pain was managed with intramuscular buprenorphine hydrochloride 0.01 mg/kg (Temgesic; Schering-Plough, Berkeley Heights, NJ) twice daily for 3 days. Four days after ablation, the animals were sacrificed by intravenous injection of pentobarbital 500 mg and potassium chloride 2 to 3 mmol/kg, and the livers were removed.

Blood Samples

Systemic MMP activity and cytokine expression was determined in blood samples collected at three time points as follows: baseline (i.e., 1 h after laparotomy, but before RF ablation), 1 h after RF ablation and 4 days after RF ablation. Blood samples were drawn into 5 mL citrate tubes, stored on ice during the operative procedure, and centrifuged at 1000 × g for 10 min. The plasma fraction was separated and stored at −70°C until analyzed. Protein content of each sample was measured with a Bio-Rad BCA assay.

Tissue Samples

Immediately after removal of the liver, fresh tissue cubes of 4 × 4 × 4 mm were excised from the transition zone, incorporating the edge of the coagulated area and the surrounding hepatic parenchyma. Corresponding samples were excised from normal hepatic parenchyma at least 5 cm from macroscopic signs of tissue coagulation. The samples were immediately frozen in liquid nitrogen. Proteins were extracted using 10 μL lysis buffer per 1 mg tissue and stored at −70°C until analyzed. Protein content was measured with a Bio-Rad BCA assay. As RF ablation denatures protein, measured protein content of the tissue lysates would not necessarily reflect the protein content before ablation. The amount of tissue lysates used in the gelatin zymography was therefore determined by dry weight of each tissue sample before homogenization.
Gelatin Zymography

MMP-2 and MMP-9 enzyme activity was analyzed using gelatin zymography. Equal amounts of plasma or liver tissue lysates were applied to a non-reducing 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel containing 0.1% gelatin (Bloom 300; Sigma-Aldrich, St. Louis, MO), and run as previously described with minor modifications [20]. The gelatin zymograms were calibrated with human MMP-2 and MMP-9 standard (CC073; Chemicon, Temecula, CA) and activated with 0.2% Brj 35 (Sigma-Aldrich). Gels were stained with Coomassie brilliant blue R-250 (Bio-Rad, Hercules, CA) and destained before being scanned. MMP enzyme activity was detected as clear bands against a staining background of undegraded substrate. The gels were photographed under standardized lighting conditions, and clear bands representing gelatinase activity was quantified by densitometry using TotalLab software (Nonlinear Dynamics, Newcastle upon Tyne, UK). Latent and active forms of MMP-2 and MMP-9 were pooled in the statistical analysis.

Enzyme-Linked Immunosorbent Assays

Cytokine expression in plasma and tissue lysates was determined by commercially available enzyme-linked immunosorbent assays (ELISAs) performed according to the instructions from the manufacturer (R&D Systems, Minneapolis, MN) for the following markers: TNF-α (PTA00), IL-10 (P1000) and PGE2 (DE0100). Values below the detection limit of an assay were treated as missing in the statistical analysis.

Immunohistochemical Analysis

Primary antibodies used in the study were a mouse anti-human-MMP-2 monoclonal antibody (MAB3308; Chemicon, Temecula, CA) and a rabbit anti-human-MMP-9 polyclonal antibody (AB13458, Chemicon), both diluted at 1/150. Western immunoblotting using a 7.5% polyacrylamide gel with a dual color protein standard (Precision Plus Dual Color, Bio-Rad) and antibodies against MMP-2 and MMP-9 was performed to verify that the MMP antibodies used in the study recognized porcine MMP-2 and MMP-9, with protein bands staining at approximately 72 kDa and 92 kDa, respectively. A monoclonal mouse anti-human CD68 antibody (M0876; DakoCytomation, Glostrup, Denmark) diluted at 1/100 was used as a macrophage marker.

Coagulations were excised from fresh liver tissue and cut into 24 6 mm thick slices. Each slice contained the central coagulated area and the surrounding transition zone between coagulated and normal hepatic parenchyma. The slices were fixed in 4% formaldehyde for at least 10 days, then dehydrated and embedded in paraffin. One 3 to 5 mm thick section was cut from each paraffin block and stained with hematoxylin and eosin. Additionally, 3 to 5 μm thick sections were mounted on SuperFrost Plus adhesion slides (Menzel-Gläser, Braunschweig, Germany). The sections were dried for 50 min at 50°C followed by 37°C overnight and deparaffinized, hydrated through alcohols and xylene and mounted in Eukitt Mounting Medium (Merck, Darmstadt, Germany). The sections were examined by light microscopy by an experienced pathologist.

Statistical Analysis

SPSS for Mac OSX version 11.0.4 (SPSS, Chicago, IL) was used for the statistical analyses. A P value of less than 0.05 was considered statistical significant. Data are presented as mean ± SD unless otherwise indicated. Plasma values of MMPs and cytokines 1 h after laparotomy, but before RF ablation were normalized to a value of 1 in each animal, and relative alterations in the corresponding plasma samples collected 1 h after RF ablation were analyzed by a one sample t test, using a test value of 1.0. MMP activity and cytokine expression in lysates from normal hepatic parenchyma were normalized to a value of 1 in each animal, and relative alterations in the corresponding values from the transition zone were examined by a one sample t test, using a test value of 1.0. Data from all animals were pooled in the statistical analysis. Differences in the two groups were analyzed by a two-group t test. Sample size of the study was estimated by nQuery Advisor 4 (Statistical Solutions, Saugus, MA). With a sample size of 6 in each experimental group, a single group t test with a two-sided significance level of 0.05 would have 80% power to detect a difference between a null hypothesis mean of 1 and an alternative mean of 1.75, assuming a standard deviation of 0.5. Concerning differences between the two experimental groups, a sample size of 6 in each group would have 80% power to detect a difference in means of 0.9 between the two groups assuming a common standard deviation of 0.5 when using a two group t test with a 0.05 two-sided significance level. The animals were allocated to the two experimental groups by block randomization, using a block size of two.

RESULTS

MMP Activity in Liver Tissue Lysates and Plasma

Five separate bands of gelatinolytic activity were detected on the zymograms. A band with an approximate molecular weight of 135 kDa represented the heterodimer of proMMP-9 and neutrophil gelatinase-associated lipocalin [21]. The next four bands represented proMMP-9 (92kDa), active MMP-9 (82 kDa), proMMP-2 (72 kDa), and active MMP-2 (62 kDa) (Fig. 1). The active form of MMP-2 was observed in the normal hepatic parenchyma and the transition zone in 10 animals, whereas the active form of MMP-9 was observed in 6 animals, and only in specimens from the transition zone.

MMP-2 and MMP-9 activity in tissue lysates from the transition zone was significantly increased compared to normal hepatic parenchyma with ratios of 3.0 and 2.6, respectively (Table 1). MMP-2 and MMP-9 activity in plasma 1 h after RF ablation was significantly increased compared with baseline levels with ratios of 1.2 and 1.5, respectively (Table 2). MMP-2 and MMP-9 activity in plasma was normalized 4 days after RF ablation.
Cytokine Expression in Liver Tissue Lysates and Plasma

PGE\(_2\) levels in tissue lysates from the transition zone was significantly increased compared to normal hepatic parenchyma with a ratio of 1.9. Corresponding tissue levels of TNF-\(\alpha\) and IL-10 were not increased, with ratios of 1.1 and 1.4, respectively (Table 1). Levels of TNF-\(\alpha\) and PGE\(_2\) were not increased in plasma 1 h after RF ablation compared with baseline levels, with ratios of 1.0 and 0.8, respectively (Table 2). PGE\(_2\) was only detected in three of 11 available plasma samples. IL-10 levels were below the detection limit in all plasma samples. Levels of TNF-\(\alpha\) and PGE\(_2\) in plasma were normalized 4 days after RF ablation.

Immunohistochemical Analysis

A sharp demarcation between coagulated tissue and the surrounding hepatic parenchyma was present at gross examination of the fresh tissue specimens in both experimental groups. This demarcation corresponded to a 1 to 3 mm transition zone between the coagulated tissue and surrounding hepatic parenchyma seen on hematoxylin and eosin stained sections (Fig. 2). The transition zone consists of an inner necrotic zone with dead hepatocytes, a rim of hemorrhagic material and thrombosed vessels, and an outer rim with hemorrhage and viable hepatocytes, as described previously [22]. Immunohistochemical analysis using antibodies directed against MMP-2 and MMP-9 in sections from the transition zone showed cells strongly positive of MMP-2 and MMP-9 surrounded by proliferating fibroblasts and inflammatory cells. Positive staining for MMP-2 or MMP-9 antibodies was not detected in normal hepatic parenchyma (Fig. 3).

TABLE 1

<p>| Lysates from Tissue Samples Obtained 4 Days After Radiofrequency Ablation. Activity of Matrix Metalloproteinase 2 and 9, and Levels of Tumor Necrosis Factor-(\alpha), Interleukin-10 and Prostaglandin E(_x) in Lysates from the Transition Zone Relative to Values in Normal Hepatic Parenchyma |
|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>n</th>
<th>Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2</td>
<td>12</td>
<td>3.0 ± 2.0 (0.96–8.57)</td>
</tr>
<tr>
<td>MMP-9</td>
<td>12</td>
<td>2.6 ± 1.1 (1.38–5.52)</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>8*</td>
<td>1.1 ± 0.3 (0.81–1.70)</td>
</tr>
<tr>
<td>PGE(_2)</td>
<td>12</td>
<td>1.9 ± 1.2 (0.32–3.81)</td>
</tr>
<tr>
<td>IL-10</td>
<td>12</td>
<td>1.4 ± 1.0 (0.27–3.91)</td>
</tr>
</tbody>
</table>

* TNF-\(\alpha\) was below the detection limit in 4 animals.

Note. MMP = matrix metalloproteinase; TNF-\(\alpha\) = tumor necrosis factor-\(\alpha\); IL-10 = interleukin-10; PGE\(_2\) = prostaglandin E\(_x\). Values are mean ± standard deviation (min, max).

TABLE 2

<p>| Plasma Samples Obtained 1 Hour After Radiofrequency Ablation. Activity of Matrix Metalloproteinase 2 and 9, and Levels of Tumor Necrosis Factor-(\alpha), Interleukin-10 and Prostaglandin E(_x) Relative to Baseline Values 1 hour After Laparotomy |
|---|---|---|</p>
<table>
<thead>
<tr>
<th>n</th>
<th>Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2</td>
<td>11</td>
<td>1.2 ± 0.2 (0.84–1.67)</td>
</tr>
<tr>
<td>MMP-9</td>
<td>11</td>
<td>1.5 ± 0.6 (0.84–2.45)</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>11</td>
<td>1.0 ± 0.2 (0.68–1.38)</td>
</tr>
<tr>
<td>PGE(_2)</td>
<td>3</td>
<td>0.8 ± 0.2 (0.66–0.93)</td>
</tr>
<tr>
<td>IL-10</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note. MMP = matrix metalloproteinase; TNF-\(\alpha\) = tumor necrosis factor-\(\alpha\); IL-10 = interleukin-10; PGE\(_2\) = prostaglandin E\(_x\). Values are mean ± standard deviation (min, max).

* Plasma was not available for analysis for one of the animals in the non-Pringle group.

* TNF-\(\alpha\) was below the detection limit in 4 animals.
Sections from the transition zone contained cells staining positive for the macrophage marker CD68, whereas cells in the normal hepatic parenchyma did not stain positive for the macrophage marker (Fig. 3).

**Effect of Pringle Maneuver**

Absolute MMP activity and cytokine values measured in lysates from normal hepatic tissue were not significantly different in the non-Pringle group and the Pringle group: MMP-2 \( (P = 0.542) \), MMP-9 \( (P = 0.592) \), TNF-\( \alpha \) \( (P = 0.951) \), IL-10 \( (P = 0.751) \), and PGE\( _2 \) \( (P = 0.282) \). No differences were found in tissue lysates from the transition zone compared to normal hepatic parenchyma: MMP-2 \( (P = 0.531) \), MMP-9 \( (P = 0.775) \), TNF-\( \alpha \) \( (P = 0.107) \), PGE\( _2 \) \( (P = 0.612) \), and IL-10 \( (P = 0.787) \). Thus, the Pringle maneuver did not affect the absolute values measured in normal hepatic tissue lysates or the ratio in tissue lysates from the transition zone compared to normal parenchyma. No qualitative differences between the two experimental groups were seen on immunohistochemical analysis of sections from the transition zone. No significant differences were found in plasma values for MMPs or cytokines in the non-Pringle group and the Pringle group (data not shown).

**DISCUSSION**

RF ablation of malignant liver tumors is associated with local recurrence rates ranging from 2% to 60% [23]. Tumor size larger than 3 to 4 cm, perivascular tumor localization and multiple tumors are established risk factors for local recurrence after hepatic RF ablation [23, 24]. Although local tumor recurrence rates comparable to those found after hepatic anatomical and wedge resection has been achieved [25], patients treated with RF ablation have higher intrahepatic recurrence rates and inferior survival rate compared with resected patients. This difference cannot fully be explained by selection bias or local tumor recurrence at the site of the

![Image](image_url)
coagulation [10]. The biological effects induced by RF ablation in the index tumor and surrounding hepatic parenchyma are not well characterized because of the fact that the treated tumors and surrounding parenchyma are left in situ. Although cellular necrosis occurs when tissue is exposed to temperatures above 50 to 55°C [26], cells in the periphery of a coagulated area are exposed to sub-lethal temperatures associated with a wide range of cellular responses including protein denaturation, inactivation of protein synthesis, cell cycle progression, and DNA repair processes [27]. Experimental animal data suggest that hepatic RF ablation promotes intrahepatic growth of residual neoplastic cells [12]. Although the biological mechanisms leading to induction of neoplastic growth after thermal RF ablation remain unresolved, experimental studies have shown that hepatic thermal ablation increase the expression of heat shock proteins [28] and growth factors [13] adjacent to the treated site.

In the present study MMP-2 and MMP-9 activity was significantly increased in tissue lysates from the transition zone 4 days after RF ablation. Increased MMP-2 and MMP-9 activity at the tumor-stroma interface in human tumor tissue sections are predominantly expressed by reactive stromal cells and represent a tumor-induced host response that can be adopted by malignant cells to promote tumor growth and invasion [29, 30]. The cellular origin of the increased MMP activity found in tissue lysates by gelatin zymography was examined in tissue sections from the transition zone by immunohistochemical staining with human MMP-2 and MMP-9 antibodies as well as the macrophage marker CD68. The results of the CD68 staining and the typical macrophage morphology clearly show that the cells staining for MMP antibodies in the transition zone were macrophages. Even though MMP-2 and MMP-9 activity was detected in normal hepatic tissue by zymography, no MMP-2 or MMP-9 staining was found in normal hepatic tissue by immunohistochemical analysis of tissue sections. Western blotting verified that the anti-human MMP-2 and MMP-9 antibodies used in the study recognized porcine MMP-2 and MMP-9. Therefore, we believe that the lack of staining for MMP-2 and MMP-9 in normal hepatic tissue was because of the very low MMP levels in normal tissue, which most probably were below the detection limit for immunohistochemistry.

Increased plasma MMP levels were found 1 h after RF ablation, possibly indicating that RF ablation induced acute MMP release from cells other than tissue macrophages at the treatment site. However, our approach of serial blood samples followed by analysis of hepatic tissue lysates after euthanasia 4 days after RF ablation does not allow us to draw conclusions regarding the temporal relationship between systemic and local tissue MMP activity.

Pringle maneuver during RF ablation is generally associated with increased coagulation volume [8], which is considered beneficial in treatment of large tumors to avoid viable residual tumor cells. To our knowledge, no randomized studies have evaluated the impact of hepatic vascular occlusion during RF ablation on local tumor recurrence. In a recent meta-analysis of local recurrence after hepatic RF ablation of liver tumors, univariate analysis found slightly lower local recurrence rates in patients in which hepatic vascular occlusion was used (9.3% versus 12.8%, $P = 0.038$). In a multivariate analysis of the same data, hepatic vascular occlusion was not significant [23]. Although RF ablation with Pringle maneuver has not been shown to have higher recurrence rates compared to RF ablation without Pringle, experimental data suggest that hepatic ischemia and reperfusion (I/R) in the presence of hepatic micrometastases is a strong stimulus of tumor growth [31]. This is of importance as hepatic RF ablation is increasingly combined with hepatic resection, which has been shown to induce hematicogenous and intrahepatic dissemination of CRC cells [32]. Increased activity of MMP-9 in serum 1 week after I/R has been reported [33]. We therefore considered it of interest to examine if Pringle influenced the tumor microenvironment by altering the expression of MMPs and cytokines. Our data suggest that Pringle maneuver for 10 min does not have an impact on absolute values of MMP-2 or MMP-9 activity or cytokine expression in normal hepatic parenchyma. These apparently diverging findings may be explained by the short duration of hepatic vascular occlusion used in our study.

The activity of MMPs is regulated at several levels including gene transcription of a number of growth factors, tumor promoters, oncoproteins, and hormones [34]. Additionally, MMPs are regulated by proenzyme activation and reversible inhibition of activated enzymes by tissue inhibitors of MMP [15, 35]. Increased MMP expression can be detected in repair or remodeling processes as well as in diseased or inflamed tissues and may act on pro-inflammatory cytokines, chemokines and other proteins to regulate inflammation [15]. TNF-α mediates many aspects of the acute inflammatory process, and is one of the earliest indicators of subsequent host responses, with peak plasma values 1 h after intravenous injection of endotoxin [36]. In our study, plasma levels of the pro-inflammatory mediator TNF-α or the anti-inflammatory mediators IL-10 and PGE$_2$ was not present in plasma 1 h after hepatic RF ablation, confirming findings in previous experimental [37] and clinical studies [38, 39]. Cytokine levels were only examined in blood samples drawn 1 h after ablation and 4 days postoperatively. We cannot exclude the existence of a cytokine response in between these time points. In fact, a recent experimental study examining
the systemic inflammatory response after hepatic RF ablation, cryotherapy or resection in porcine liver reported increased TNF-α levels 6 h after RF ablation [39]. These diverging findings may therefore be influenced by the time of blood sample collection relative to the procedure, but could also be related to different extent of the coagulation induced in these studies. In our study increased MMP activity in tissue lysates was found without a concurrent increase in tissue TNF-α levels. Increased levels of PGE2 were found in tissue lysates from the transition zone, in accordance with the roles of prostaglandins in inflammatory reactions after tissue damage [40].

MMP activity is up-regulated in many forms of cancer, including CRC [16, 30]. In a recent clinical study of 32 patients with CRC liver metastasis treated by hepatic resection, both active and latent forms of MMP-2 and MMP-9 were increased in tissues containing metastatic tumor compared to normal liver tissue, with median ratios of 17.6 for proMMP-2 and 2.9 for proMMP-9. Of importance, MMP expression was higher in the metastatic tumor itself than in the transitional tissue containing the invasive edge and immediate surrounding tumor and normal tissue [18]. Our results show that RF ablation of normal hepatic parenchyma is associated with a three-fold increased activity of MMP-2 and MMP-9. Therefore, if tumor tissue is incompletely coagulated, viable cancer cells are exposed to a microenvironment that may increase the risk of tumor progression and distant metastasis. On the other hand, one could hypothesize that in situ tumor destruction might provide a beneficial antigen source for induction of antitumor immunity [41]. However, based on the assumption that increased expression of MMPs in peritumoral tissue is associated with increased risk of tumor cell growth, our results indicate that RF ablation should only be attempted when complete eradication of the target tumor including a safety margin is possible.

This study was performed in an animal model subject to several limitations. All coagulations were performed in normal hepatic porcine parenchyma. MMP activity in tissue was assessed at one time point 4 days after ablation. Our study design does not allow us to draw conclusions regarding the prognostic role of the increased activity of MMP-2 and MMP-9 activity on clinical outcome data such as local tumor recurrence or patient survival. Furthermore, all coagulations were made with a RF ablation system using perfusion of saline into the tissue during ablation that may affect our results. Therefore, further research should be conducted to assess the expression of MMPs after hepatic RF ablation using other RF ablation systems.

In conclusion, hepatic RF ablation was associated with a three-fold increased activity of MMP-2 and MMP-9 in the transition zone separating coagulated tissue from normal hepatic parenchyma. The use of Pringle maneuver for 10 min during ablation did not influence MMP activity. The clinical significance of the combination of residual tumor cells and induction of a microenvironment that facilitates growth and of tumors is not fully explored and should be assessed in animal tumor models with extended observation time.

ACKNOWLEDGMENTS

We thank the staff at the Interventional Centre for providing excellent assistance, the Department of Comparative Medicine for support in animal care and Aasa R. Schjölberg at the Institute of Pathology for assistance with the immunohistochemical analysis. LP was supported by the Norwegian Cancer Society, grant no. D02042/001. KB was supported by a postdoctoral fellowship from University of Oslo, Norway.

REFERENCES


Radiofrequency ablation of colorectal liver metastases: evaluation of local tumor recurrence by 3D volumetric analysis

Lars Frich¹,²
Gaute Hagen ³
Knut Brabrand ³
Bjørn Edwin ¹,²
Øystein Mathisen ¹
Trond Mogens Aaløkken ³
Ivar P. Gladhaug ⁴,⁴

1. Department of Surgery, Rikshospitalet University Hospital, 0027 Oslo, Norway
2. The Interventional Centre, Rikshospitalet University Hospital, 0027 Oslo, Norway
3. Department of Radiology, Rikshospitalet University Hospital, 0027 Oslo, Norway
4. Department of Surgery, Faculty Division Rikshospitalet, University of Oslo, 0027 Oslo, Norway

Word count
Abstract 193 words
Manuscript including references 4548 words

Running title Volumetric evaluation of local tumor recurrence

Subject category Radiology, gastrointestinal, oncology

Corresponding author
Lars Frich
Department of Surgery
Rikshospitalet University Hospital
N-0027 Oslo
NORWAY
Tel. + 47 23071825
Fax. + 47 23072267
E-mail: lars.frich@labmed.uio.no

This work was supported by the Norwegian Cancer Society, grant no. D02042/001.
Abstract

**Background:** The purpose of this study was to characterize the relationship between local tumor recurrence after radiofrequency (RF) ablation of colorectal liver metastases and post-ablation alterations in coagulation volume.

**Materials and methods:** 11 patients with hepatic metastases from colorectal cancer were treated with RF ablation. All patients were followed for at least 24 months or until local tumor recurrence. Multi-detector computed tomography (MDCT) was performed at 1 and 3 months after RF ablation, and then at intervals of 3 months until 24 months and each sixth month thereafter. Median follow-up was 27 months (17-43 months). Local tumor recurrence was found in 7 patients. Coagulation volume was measured from 81 follow-up MDCT scans using a semi-automatic three-dimensional (3D) method.

**Results:** Patients with local tumor recurrence had a median increase in coagulation volume of 50% (range 0-378%) compared to images acquired 3 months previously. Local tumor recurrence was identified simultaneously by semi-automatic 3D volumetric analysis and conventional morphologic criteria.

**Conclusions:** Semi-automatic 3D volumetric analysis provides added diagnostic information compared with conventional morphological evaluation, and may increase specificity of non-invasive diagnosis of local tumor recurrence after RF ablation of colorectal liver metastases.

**Key words:** Radiofrequency ablation, liver, computed tomography
Introduction

Metastatic disease to the liver is a frequent event in patients with colorectal cancer [1]. Surgical resection is a potentially curative treatment of liver metastases from colorectal cancer, with 5-year survival rates of 30-50 per cent in selected patients [2-4]. The majority of patients with colorectal liver metastases are not candidates for hepatic resection. Radiofrequency (RF) ablation is increasingly used for in-situ tumor destruction in patients with non-resectable metastases limited to the liver [5]. In a recent meta-analysis of ninety-five series and 5224 liver tumors treated with RF ablation, the reported rate of local tumor recurrence varied between 2% and 60%. Follow-up was short in several studies, probably contributing to an underestimation of the true rate of local tumor recurrence [6]. Local tumor recurrence at the treated site usually occurs within the first 6 months, but have been reported to appear up to 23 months after the procedure [7-9].

Early detection of local tumor recurrence may facilitate early reintervention with potential benefits for patient survival. Malignant tissue treated by RF ablation is left in situ, necessitating repeated follow-up imaging or biopsy to determine if the tumor has been eradicated. Furthermore, biopsy of potentially operable colorectal liver metastases is controversial due to the risk of needle tract seeding and negative impact on patient survival [10, 11]. Consequently, non-invasive methods should preferably be used for detection of persistent or residual malignant tissue at the site of RF ablation. It can be difficult to distinguish between local tumor recurrence and benign tissue alterations on follow-up imaging [12-14]. Analysis of coagulation geometry and coagulation volume on early post-ablation scans may identify patients with inadequate treatment margins relative to the index tumor, which would be expected to have a high risk of local tumor recurrence. Various morphologic patterns are associated with local tumor recurrence on post-ablation computed tomography (CT) [13]. An enhancing peripheral tissue rim surrounding the non-enhancing coagulation may represent local tumor recurrence, but may also represent benign periablational enhancement, which is considered to be the result of a benign physiologic inflammatory reaction to thermally damaged cells [15, 16]. Additionally, local tumor recurrence may be diagnosed based on an increase in the overall coagulation size compared with previous scans [13]. It is difficult
to quantify small alterations in coagulation diameter reliably due to inter-scan displacement and
deformation of the liver. Increase in coagulation volume may be a more sensitive indicator of
growth than increase in coagulation diameter; for a spherical volume, an increase in diameter of
20% corresponds to a volume increase of 73%. However, the relationship between local tumor
recurrence after RF ablation of hepatic colorectal metastases and alterations in coagulation volume
is not well characterized.

In this clinical pilot study, patients with colorectal liver metastases treated with RF ablation were
followed for at least 24 months or until local tumor recurrence. To assess theoretical treatment
margins, we determined the largest tumor diameter on pre-ablation images, and the effective
coagulation diameter 1 month after RF ablation. Post-ablation coagulation volumes were measured
on repeat follow-up scans using a semi-automatic three-dimensional (3D) technique. Our study
hypotheses were: (1) inadequate treatment margins as determined from scans 1 month after
radiofrequency ablation may identify patients who would develop local tumor recurrence, and (2)
increase in coagulation volume during follow-up may be used to detect local tumor recurrence
earlier than conventional morphological criteria.
Materials and methods

Study population
This study was approved by the Regional committee for medical research ethics. From 2003 to 2006 a total of 23 patients underwent RF ablation for non-resectable malignant liver tumors at our institution. Written informed consent was obtained from all patients prior to the procedure. All patients were entered into a prospective database. We identified 11 patients who satisfied the following criteria: (a) non-resectable liver metastasis from colorectal cancer; (b) complete clearance of all liver tumors possible either by RF ablation alone or combined with surgical resection; (c) diameter of tumor treated with radiofrequency ablation less than 4 cm; (d) no extrahepatic disease; (e) follow-up > 24 months, or to local tumor recurrence. The reasons for exclusion were non-colorectal cancer liver metastasis (n=1), tumor diameter > 4 cm (n=2), extrahepatic tumor growth present at time of treatment (n=1), and follow-up less than 24 months without occurrence of local tumor recurrence (n=8).

All patients were treated with a curative intent. Patient age was 60.3 ± 8.0 yr (49-80 yr). The primary tumor location was the colon in 8 patients and the rectum in 3 patients. All patients had undergone curative resection of the primary colorectal tumor. Liver resection of colorectal liver metastases had previously been performed in 7 of 11 patients. The median interval between operation of the primary tumor (n=11) and RF ablation was 17 months (3-101 months). The median interval between liver resection (n=7) and RF ablation was 12 months (2-77 months). The patients included in this study had 1-4 liver tumors, with a median of 1 tumor. One tumor was treated by RF ablation in each of the 11 patients. Seven of these 11 tumors were classified as perivascular.

Operative procedure
Patients were treated with a RF ablation system with perfusion electrodes (Elektrotom HiTT 106, Berchtold GmbH & Co, Germany). This system incorporates a generator that delivers alternating current at 375 kHz to a maximum of 1.2 ampere, and a syringe pump (Pilot C, Fresenius Vial, France) for infusion of isotonic saline through the electrode tip during ablation. All procedures
were performed under general anesthesia. The approach used for RF ablation was percutaneous (n=4), laparoscopic (n=4) and by laparotomy (n=3). Intraprocedural image guidance was contrast-enhanced ultrasonography (n=6), intraoperative ultrasonography (n=4) and magnetic resonance imaging (MRI) (n=1). RF ablation and hepatic resection was performed during the same procedure in 4 patients, one of these resections was performed as a laparoscopic procedure. The median number of electrode activations in each procedure was 2 (2-4). Hepatic vascular occlusion was not used during RF ablation in any patients. CT or contrast-enhanced ultrasonography was performed in all patients prior to discharge to assess the completeness of the treatment.

Follow-up
All patients were followed with a standardized imaging protocol. Sixteen or 64-channel multidetector computed tomography (MDCT) (GE LightSpeed Pro 16 or GE LightSpeed VCT; GE Healthcare, Milwaukee, WI, USA), was performed using 150-200 mL iodixanol 320 mgI/mL (Visipaque, GE Healthcare, USA). Images were acquired in portal venous phase. Scans were performed at 1 and 3 months after RF ablation, and then every 3 months until 24 months, and every sixth month thereafter. A total of 81 post-ablation scans from 1 month after ablation and onwards were reviewed, with a follow-up of 27 months (17-43 months). CT of the chest was performed every sixth month. Carcinoembryonic antigen (CEA) with a cut-off level of 5 μg/L was used a marker of malignancy, and was measured at all time points. All patients were followed for at least 24 months or until local tumor recurrence.

Assessment of local tumor recurrence
Patients were considered to have local tumor recurrence based on at least one of three predefined morphological criteria on follow-up CT examinations; new lesion(s) in the periphery of the coagulated area; at least 20% growth of the largest diameter of the coagulated area; persistent attenuation in the periphery of the coagulated area different from the coagulated area and from normal hepatic parenchyma. During follow-up a strict distinction was kept between local tumor recurrence, i.e. tumor growth within or at the ablation margin, intrahepatic tumors not related to the areas treated with RF ablation, and extrahepatic tumor growth.
Treatment margin

The largest coagulation diameter and the effective coagulation diameter were retrospectively quantified from CT scans acquired 1 month after RF ablation using the scientific image analysis program ImageJ version 1.37 (National institute of mental health, Bethesda, MA, USA). A region of interest (ROI) was defined by tracing the border between normal liver tissue and alterations attributed to RF ablation on all slices where tissue coagulation was present. Measurement of geometric properties of each ROI was performed using automatic algorithms. The largest coagulation diameter was defined as the longest distance between any two points along the perimeter of the ROIs for a single coagulation. Effective coagulation diameter was defined as the diameter of the largest circle that could be fit into the ROIs defining a single coagulation (Fig 1). The largest pre-ablation tumor diameter was subtracted from the effective coagulation diameter, and this difference was divided by 2 to achieve the theoretical treatment margin surrounding the tumor on all sides.

Coagulation volume

All images were retrospectively reviewed by an experienced radiologist unaware of the course of the disease in each patient, who had not been involved in the RF ablation procedures or prospective interpretation of follow-up scans. Images of each patient were reviewed in chronological order. Tumors on preoperative images contiguous to a vessel with diameter of 3 mm or more were classified as perivascular. Volumetric analyses were performed on a workstation (GE Advantage Workstation 4.2; GE Healthcare, USA). The coagulation volumes were calculated by the software package Volume Viewer (GE Healthcare, USA). Using a seeding based semi-automatic technique, a volume of interest (VOI) was defined, using the border between the hypoattenuating volume representing tissue coagulation and normal hepatic parenchyma. The extent of the VOI was controlled on coronal and axial source images. The volume of the selected VOI was calculated by the software package.

Statistical analysis

SPSS for Mac OSX version 11.0.4 (SPSS, Chicago, Illinois, USA) was used for the statistical analyses. A p-value of less than 0.05 was considered statistical significant. Data are presented as
mean ± SD (range), or median (range). All post-ablation coagulation volumes were normalized to the 1-month baseline value for each patient. Independent samples t-test was used for comparison between groups. Wilcoxon signed rank test was used for repeated measurements within each group.
Results

Local, intrahepatic and extrahepatic tumor recurrence

Diameter of the tumors treated with RF ablation was $2.2 \pm 0.8$ cm (1.0-3.5 cm), with a tumor volume of $7.1 \pm 6.6$ mL (0.5-22.5 mL) (Table 1). Treatment of the index tumor was initially considered complete in all patients. Local tumor recurrence was found during follow-up in 7 patients, and was detected median 9 months (6-21 months) after RF ablation. Tumor diameter in the patients who developed local tumor recurrence was $2.2 \pm 0.3$ cm (1.0-3.5 cm) compared with $2.1 \pm 0.4$ cm (1.2-3.1 cm) in the patients without local tumor recurrence. This difference was not statistically significant. Fine needle aspiration cytology of the area treated with RF ablation was performed in 4 patients during follow-up. In 3 patients malignancy was verified, whereas no malignant cells were found in 1 patient who did not develop local tumor recurrence. Local tumor recurrence was found in 5 of 7 perivascular tumors. Of the 7 patients with local tumor recurrence, 4 patients presented with inoperable intrahepatic (n=1) or extrahepatic (n=3) malignant growth at the time of diagnosis of local tumor recurrence. The 3 patients with local tumor recurrence only were re-treated with resection (n=1) or repeat RF ablation (n=2). Considering all 11 patients, intrahepatic recurrence not related to the RF ablation site was found in 6 patients with a median of 14.5 months (4-24 months) after RF ablation, and extrahepatic tumor growth was found in 6 patients with a median of 11.5 months (4-22 months) after RF ablation, with the following distribution; lungs (n=4), lymph nodes (n=1), bone (n=1). Two patients who had developed extrahepatic disease died 17 and 20 months after RF ablation. The remaining 9 patients, including the 3 patients who were re-treated, were alive with a median follow-up of 28 months (20-43 months).

Treatment margin

Largest coagulation diameter 1 month after RF ablation was $4.8 \pm 1.4$ cm (2.9-7.5 cm), and was larger than the corresponding pre-ablation tumor diameter in all patients. Effective coagulation diameter was $2.9 \pm 0.8$ cm (1.6-4.2 cm). The effective coagulation diameter was larger than the corresponding tumor diameter in 8 patients (Table 1), with a positive margin of $0.6 \pm 0.3$ cm (0.3-1.0 cm). The 3 patients in which the effective coagulation diameter was smaller or equal to the tumor diameter developed local tumor recurrence. In the 7 patients with local tumor recurrence
the margin was 0.3 ± 0.6 cm (-0.4-1.0 cm), compared to 0.6 ± 0.3 cm (0.3-0.9 cm) in the 4 patients without local tumor recurrence. This difference was not statistically significant, (p=0.27).

**Coagulation volume**

Baseline coagulation volume 1 month after RF ablation was 29.7 ± 16.0 mL (13-70 mL), and was larger than the corresponding tumor volume in all patients. At 3 months, coagulation volume had decreased in all patients, and was 54.1 ± 16.9% (38-88%) of the baseline value. In the patients without local tumor recurrence (n=4), subsequent measurements showed generally decreasing coagulation volumes, with all values below 50% of baseline values at 12 months and below 30% of baseline values at 24 months. One patient had a volume increase of 3% between two successive measurements, but decreasing volume on the following measurement (Figure 2a). Patients who developed local tumor recurrence (n=7) showed a significant increase in coagulation volume of 85 ± 131% (0-378%) from the examination 3 months prior to diagnosis of local tumor recurrence until diagnosis of local tumor recurrence (p=0.028). The median volume increase in these patients was 50% (Figure 2b). One patient had local tumor recurrence without an associated alteration in coagulation volume (Figure 3). Coagulation volume measurements 3 months after diagnosis of local tumor recurrence were available in 5 patients, showing further increase in coagulation volume in 4 patients (Table 2). Of importance, 4 of the 7 patients with local tumor recurrence had a coagulation volume less than the baseline volume at time of diagnosis.
Discussion

Several methods have been proposed for early detection of local tumor recurrence after RF ablation on post-ablation CT scans, such as alterations in Hounsfield units [12] or analysis of coagulation geometry [17]. In the present study we retrospectively examined if inadequate treatment margins assessed 1 month post-ablation could predict local tumor recurrence, and if increase in coagulation volume on post-ablation MDCT images could be used to detect local tumor recurrence earlier than conventional morphological criteria.

Established oncological criteria suggest that hepatic malignancies should be eradicated radically including a 1-cm margin of apparently healthy tissue to eliminate microscopic foci of malignant cells, and to compensate for the uncertainty in determining the exact tumor margin [18, 19]. Considering the effective coagulation diameters produced in this study, we did not coagulate a spherical tissue volume sufficient to achieve a 1-cm margin on all sides of the tumor (i.e. a coagulation diameter at least 2 cm larger than the largest tumor diameter) in any patients. Theoretically, a margin of at least 0.5 cm could have been achieved in 5 patients, whereas 3 patients had a positive margin less than 0.5 cm. An effective coagulation diameter smaller or equal to the pre-ablation tumor diameter was found in 3 patients, and was invariably associated with local tumor recurrence (Table 1). However, prognostic significance of a negative margin remains to be examined in larger prospective studies. Even though one would expect a large margin to be beneficial to avoid local tumor recurrence, the patient with the largest margin in this study developed local tumor recurrence (Table 1). The calculated margins surrounding the tumors may have been overestimated if the tumor diameter was larger than anticipated from pre-ablation images. Furthermore, even if an adequate coagulation volume was achieved, suboptimal positioning of the ablation electrode or displacement of the geometrical center of the coagulated volume relative to the ablation electrode may result in inadequate treatment [20].

The coagulation volume, as defined in this study, might incorporate both viable and non-viable malignant and non-malignant tissue. Coagulation volume decreased from baseline at 1 month to
3 months in all patients. Consequently, decreasing coagulation volume from 1 to 3 months after RF ablation cannot be used as a predictor for the absence of later development of local tumor recurrence. A marked increase in coagulation volume when local tumor recurrence was diagnosed was seen in 6 of 7 patients. The lack of increase in coagulation volume in one patient was probably due to the occurrence of nodules at a small distance from the coagulated area which were not identified by the semi-automatic method used for volume estimation in this study (Figure 3). This indicates that semi-automatic volumetric analysis should only be used as a supplement to conventional morphological criteria. With a follow-up interval of 3 months, increase in coagulation volume was not present until local tumor recurrence was detected based on conventional criteria (Figure 2b).

The natural course of adequately treated colorectal metastases is a decrease in coagulation volume, as illustrated by the patients who did not develop local tumor recurrence. In the 6 patients where coagulation volume was increased at the time of local tumor recurrence, the increase in volume was at least 19% compared to the previous examination 3 months earlier, with a median increase of 50%. We attribute the observed volume increase to a growth of malignant tissue. It is difficult to determine with certainty whether local tumor recurrence was caused by incomplete coagulation of a tumor that continued to grow, or if a new tumor grew at or near the site of the adequately treated tumor [16]. Untreated colorectal liver metastases have a tumor volume doubling time of 3-5 months [21]. Viable malignant tissue after RF ablation would in most cases be expected to constitute a small fraction of the coagulated volume. It is therefore somewhat surprising that a median coagulation volume increase of 50% in 3 months was observed in our patients with local tumor recurrence. However, recent experimental research indicate that RF ablation induces biological alterations in the tissue of possible importance for tumor invasion and metastasis [22], and that thermal ablation promotes growth of residual neoplastic cells, possibly through stimulation by growth factors [23, 24]. Therefore, one could speculate that the growth rate of viable residual malignant cells exposed for thermal ablation may be higher than for untreated malignant tissue.

Three patients with local tumor recurrence were biopsied. In the remaining 4 patients with local tumor recurrence, biopsy was not obtained due to simultaneous intrahepatic or extrahepatic tumor
growth, which precluded potential curative surgical resection or local ablation. In these 4 patients
the result of a biopsy would not influence the planned treatment of the patient, and was waived. In
retrospect, operable patients with suspected local tumor recurrence by morphological criteria and
a concurrent marked increase in coagulation volume could be considered for repeated treatment
without performing biopsy, which delays potentially curative treatment and may lead to tumor
seeding [10, 11]. Our findings suggest that the combination of semi-automatic 3D volumetric
analysis and conventional morphological evaluation may increase the specificity of non-invasive
diagnosis of local tumor recurrence after RF ablation of colorectal cancer liver metastases, and
thereby possibly avoid unnecessary biopsies.

In this study we found local tumor recurrence in 7 of 11 patients, corresponding to a rate of 64%.
Reported local tumor recurrence rates varies from less than 20% [25], to recently reported rates
of 47% and 58% [26, 27]. Tumor proximity to major vessels is a well-established risk factor of
local tumor recurrence [6]. A high proportion of the tumors treated in this study were perivascular,
possibly influencing the rate of local tumor recurrence. The RF ablation system used in our study
produces irregular coagulations [20, 28, 29], which may contribute to the high rate of local tumor
recurrence. Furthermore, a learning curve exists for new procedures, including RF ablation [6, 30].
Therefore, as this patient series represents our initial experience with RF ablation, a lower rate of
local tumor recurrence would be expected with increasing experience.

This retrospective pilot study has some important limitations. It presents data from only 11 patients.
This sample size is too small to allow conclusions regarding the prognostic value of the treatment
margin. Quantification of largest pre-ablation tumor diameter, largest coagulation diameter and
effective coagulation diameter were made from 2D axial images. Local tumor recurrence was
verified by biopsy in 3 patients. Although we believe that the criteria used for identifying patients
with residual tumor have a high specificity, and the follow-up period of at least 24 months was
adequate to detect local tumor recurrence, the number of patients with malignant residual tumor
may be underestimated. The pattern of post-ablation volumetric alterations may be specific to
the perfusion electrode RF ablation system used in this study. Dual-modality positron emission
tomography (PET)/CT may have higher sensitivity to detect viable malignant tumor after RF
ablation compared to CT [31, 32], however this image modality was not evaluated in this study.

In conclusion, by acquiring follow-up MDCT images each third month after hepatic RF ablation, local tumor recurrence was identified simultaneously by the use of conventional morphological criteria and semi-automatic 3D volumetric analysis. In patients with local tumor recurrence an increase in median coagulation volume by 50% was seen, whereas in patients without local tumor occurrence decreasing coagulation volume during the follow-up period was observed, with all coagulation volumes less than 30% of baseline at 24 months. Semi-automatic 3D volumetric analysis provides added diagnostic information compared with conventional morphological evaluation, and may increase specificity of non-invasive diagnosis of local tumor recurrence after RF ablation of colorectal cancer liver metastases.
References


List of figures and figure captions

Figure 1  
a. 55-year old man with liver metastases from colorectal cancer. Portal venous phase multi-detector computed tomography acquired 1 month after resection of segment II/III and radiofrequency ablation of a tumor with a diameter of 3.1 cm in segment V shows a well-defined low-attenuation coagulation.

b. Geometric analysis of the coagulation in (a). The thick line denotes the border of normal liver tissue vs. alterations attributed to coagulation. $D_{\text{max}}$ denotes maximum axial coagulation diameter and $D_{\text{eff}}$ effective coagulation diameter. On the shown slice, $D_{\text{max}}$ is 4.6 cm, whereas $D_{\text{eff}}$ is 3.4 cm.

Figure 2  
Coagulation volumes 1-24 months after radiofrequency ablation of colorectal liver metastases. Coagulation volumes for each patient have been normalized to the corresponding coagulation volume 1 month after radiofrequency ablation.

a. Patients without local tumor progression (n=4). All values were below 50% of baseline values at 12 months and below 30% of baseline values at 24 months.

b. Patients with local tumor progression (n=7). The time of diagnosis of local tumor progression is marked by a circle. The patient marked by as asterisk had a volume increase to above 400% when local tumor progression was diagnosed at 6 months.

Figure 3  
55-year-old man with liver metastases from colorectal cancer in which local tumor progression was not detected by semi-automated 3D volumetric analysis. Portal venous phase multi-detector computed tomography. The preoperative CEA was 64 μg/L. Resection of segment II and III, local resection in segment VI and radiofrequency ablation of a tumor in segment VIII was performed in the same procedure. 1 month after radiofrequency ablation coagulation volume was 26 mL, and CEA was 3 μg/L. a. 9 months after radiofrequency ablation. The coagulation volume was
calculated to 7 mL. CEA was 5 μg/L. A well-defined low-attenuation coagulation without peripheral hyperemia is seen.
b. 12 months after radiofrequency ablation. The coagulation volume was calculated to 7 mL. CEA was 16 μg/L. A heterogeneous low-attenuation nodule representing local tumor progression is seen at the margin of the coagulated area located dorsal and medial to the well-defined coagulation (arrow).
Table 1
Patient characteristics.

Table 2
Post-ablation coagulation volumes in patients with local tumor progression (n=7).
Figure 1

a. 55-year old man with liver metastases from colorectal cancer. Portal venous phase multidetector computed tomography acquired 1 month after resection of segment II/III and radiofrequency ablation of a tumor with a diameter of 3.1 cm in segment V shows a well-defined low-attenuation coagulation.

b. Geometric analysis of the coagulation in (a). The thick line denotes the border of normal liver tissue vs. alterations attributed to coagulation. Dmax denotes maximum axial coagulation diameter and Deff effective coagulation diameter. On the shown slice, Dmax is 4.6 cm, whereas Deff is 3.4 cm.
a. Patients without local tumor progression (n=4). All values were below 50% of baseline values at 12 months and below 30% of baseline values at 24 months.

b. Patients with local tumor progression (n=7). The time of diagnosis of local tumor progression is marked by a circle. The patient marked by an asterisk had a volume increase to above 400% when local tumor progression was diagnosed at 6 months.
Figure 3

55-year-old man with liver metastases from colorectal cancer in which local tumor progression was not detected by semi-automated 3D volumetric analysis. Portal venous phase multi-detector computed tomography. The preoperative CEA was 64 μg/L. Resection of segment II and III, local resection in segment VI and radiofrequency ablation of a tumor in segment VIII was performed in the same procedure. 1 month after radiofrequency ablation coagulation volume was 26 mL, and CEA was 3 μg/L.

a. 9 months after radiofrequency ablation. The coagulation volume was calculated to 7 mL. CEA was 5 μg/L. A well-defined low-attenuation coagulation without peripheral hyperemia is seen.

b. 12 months after radiofrequency ablation. The coagulation volume was calculated to 7 mL. CEA was 16 μg/L. A heterogeneous low-attenuation nodule representing local tumor progression is seen at the margin of the coagulated area located dorsal and medial to the well-defined coagulation (arrow).
Table 1

Patient characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Tumor diameter (cm)</th>
<th>Largest coagulation diameter (cm)</th>
<th>Effective coagulation diameter (cm)</th>
<th>Theoretical treatment margin (cm)</th>
<th>Local tumor recurrence</th>
<th>Time to local tumor recurrence (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.3</td>
<td>6.7</td>
<td>4.2</td>
<td>1.0</td>
<td>Yes</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>4.8</td>
<td>3.0</td>
<td>0.3</td>
<td>Yes</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>2.9</td>
<td>2.1</td>
<td>0.5</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>3.9</td>
<td>2.7</td>
<td>0.6</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>4.2</td>
<td>2.5</td>
<td>0.9</td>
<td>Yes</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>1.8</td>
<td>3.9</td>
<td>2.5</td>
<td>0.4</td>
<td>Yes</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
<td>4.3</td>
<td>2.7</td>
<td>0.4</td>
<td>Yes</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
<td>4.3</td>
<td>2.7</td>
<td>0.4</td>
<td>Yes</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>3.1</td>
<td>4.6</td>
<td>3.6</td>
<td>0.3</td>
<td>Yes</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>2.3</td>
<td>4.9</td>
<td>2.6</td>
<td>0.3</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>2.1</td>
<td>4.2</td>
<td>2.1</td>
<td>0.0</td>
<td>Yes</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 2

Post-ablation coagulation volumes in patients with local tumor progression (n=7).

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Baseline volume at 1 month</th>
<th>3 months prior to diagnosis</th>
<th>At diagnosis</th>
<th>3 months after diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>19</td>
<td>27</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>30</td>
<td>47</td>
<td>54</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>10</td>
<td>93</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>11</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>2</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>11</td>
<td>22</td>
<td>9</td>
<td>14</td>
<td>17</td>
</tr>
</tbody>
</table>