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IRREVERSIBLE ELECTROPORATION IN THE LIVER

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POPULAR SCIENCE SUMMARY OF THE THESIS

Malignant liver tumours can be divided into primary liver cancers and liver metastases, where the primary tumour has spread to the liver. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. It is one of the most common types of cancers globally and the fourth most common cause of cancer related death. Liver cirrhosis is often the underlying cause and common risk factors for cirrhosis are alcohol abuse, infection with hepatitis B and C, and obesity. Liver metastases are common and up to 50% of patients with cancer in the colon or rectum, will develop liver metastases during the course of their illness. Other cancers can also metastasise to the liver, mostly with a very poor prognosis.

The treatment for liver tumours has developed during the last decades. Traditionally, surgery has been the primary treatment, where the part of the liver containing the tumours is removed. Limiting factors for liver surgery are the volume and function of the liver that is left in the patient after surgery. For patients where surgery is not possible, different methods for local treatments has been developed. Thermal ablation is a term meaning heating or cooling of the tumour and a surrounding healthy tissue, killing the cells in the target area. In the liver, radiofrequency- or microwave energy are commonly used to kill tumours with heat. These methods often cause less collateral damage to the liver and is often used after recurrence of tumours after previous surgery, and in patients with underlying liver disease where surgery is deemed unsuitable. A limitation of these thermal methods is that the heat also affects surrounding tissues, causing potential problems when the tumours are close to larger blood vessels, the central bile ducts, and organs adjacent to the liver.

Irreversible electroporation (IRE) is an ablation method that does not use heat to destroy the tumour cells. Multiple needle electrodes are placed around the tumour and short pulses of direct current is administered between them. The electrical field affects the cells and small holes are created in the cell membranes, disturbing the salt- and fluid-balance within the cells leading to cell death (apoptosis). Since only little heat is created around the electrodes, IRE can be used close to heat sensitive structures with preserved blood- and bile flow.

The placement of the electrodes in IRE-treatment is demanding. The electrodes must be placed as parallel as possible and at equal depth. As tumours treated with IRE are often located in the central parts of the liver, close to larger blood vessels and bile ducts, the accuracy of the placement is imperative, as not to damage these structures. To guide the electrode placement, different radiological modalities are used to visualise the tumours, the electrodes, and the surrounding tissues. The most commonly used method is ultrasound. Alternative methods have been developed to increase the accuracy in placing the electrodes, among them systems using three-dimensional image reconstructions of the patient's liver.

The energy delivery during IRE treatment is set in the hardware which records how much current the patients has received. Studies has shown that a decrease in the electrical resistance in the tissue is related to treatment success. The level of reduction necessary for minimising the risk of local recurrence is not yet clear.

This thesis addresses different aspects of IRE for liver tumours. *Paper I* is a study of the first 50 IRE treatments of liver tumours performed at Danderyd Hospital and shows that it is safe, with few major complications, and efficient on liver tumours that are not possible to treat with other methods. *Paper IV* is a national multi-centre study consisting of all patients treated with IRE for liver tumours in Sweden from the first patient treated in May 2011 until the end of 2018. This study presents data on the largest number of patients treated with IRE so far published. The results further establish IRE as a safe and efficient method of treating liver tumours located in the liver where other treatment-options are unsuitable.

Paper II and *III* focus on guiding the electrodes accurately and safely to the correct locations around the tumours with the use of a stereotactic 3D navigation system. *Paper II* describes the accuracy of electrode placement in a liver phantom, comparing the navigation system with ultrasound guidance. The study shows that the electrode placement is more accurate with the navigation system at the cost of longer procedure times. *Paper III* studies the patients treated with IRE using the navigation system and shows that the electrodes are placed in the liver according to plan with small margins of error and shows no placement-related complications.

In *paper V* all IRE treatments in Sweden between 2011 and 2018 were examined regarding how the electrical resistance in the tissue changes during the IRE treatment and compared this to the risk of local recurrence of the treated tumours. For metastases from cancers in the colon and the rectum, a correlation between larger decrease in tissue resistance and longer time to local recurrences of the tumours was shown.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Maligna levertumörer kan delas in i primär levercancer och levermetastaser. Vid primär levercancer utgår cancern från cellerna i levern och vid levermetastaser har en cancer i ett annat organ spridit sig och givit dottertumörer i levern. Hepatocellulärt carcinom (HCC) är den vanligaste formen av primär levercancer. Det är en av de vanligaste cancerformerna i världen och är den fjärde vanligaste orsaken till cancerrelaterad död, globalt sett. Skrumplever eller levercirrhos, är en vanlig bakomliggande orsak till HCC och levercirrhos i sin tur orsakas bland annat av alkohol, virussjukdomarna hepatit B och C samt övervikt. Cancer i tjocktarm och ändtarm är en vanlig orsak till att utveckla levermetastaser, och hos dessa patienter kommer upp till 50% drabbas av levermetastaser någon gång under sjukdomsförloppet. Även många andra typer av cancer kan orsaka levermetastaser, tyvärr ofta med dålig prognos.

Behandlingen av levertumörer har utvecklats de senaste decennierna. Traditionellt har förstahandsbehandlingen varit leverkirurgi. Man opererar då bort den del av levern där det finns tumörer. För att kunna behandla tumörer som inte går att operera bort har olika metoder av lokalbehandling utvecklats. Ablation innebär att man lokalt förstör vävnad i kroppen på

olika sätt. I levern är det vanligast att man använder radiovågor eller mikrovågor för att lokalt hetta upp tumören så att cellerna dör. Detta påverkar oftast en mindre del av den friska levern jämfört med att operera bort en tumör med tillräcklig marginal.

En begränsning med dessa metoder är att värmen även kan skada omkringliggande vävnad. När tumörerna växer intill större blodkärl, centrala gallgångar eller i närheten av andra organ i buken kan dessa skadas av värmen.

Irreversibel elektroporation (IRE) är en ablationsmetod som inte utnyttjar värme för att döda tumörcellerna. Nålformade elektroder sticks in i levern runt tumören och korta likströmspulsar skickas mellan dem. Det elektriska fältet påverkar cellerna så att de får hål i cellmembranen och dör. Då metoden nästan inte orsakar någon värmeutveckling alls, annat än precis kring elektroderna, kan IRE användas där det finns värmekänsliga strukturer med bibehållet blod- och gallflöde.

Placeringen av IRE-elektroderna är utmanande. Elektroderna ska placeras så parallellt som möjligt och på samma djup runt tumören i levern för att uppnå optimal behandling. För att placera elektroderna används olika radiologiska metoder för att se tumören, elektroderna och omkringliggande vävnader, vanligast är ultraljud. För att förbättra träffsäkerheten kan olika typer av navigationssystem användas.

Vid IRE-behandling ställs energinivåerna in och kan justeras under behandlingens gång. I tidigare studier har man visat att det elektriska motståndet i vävnaden bör sjunka under behandlingen för att den ska anses som lyckad. Hur mycket den ska sjunka för att minska risken för återfall av tumören är inte klarlagt.

Denna avhandling handlar om olika aspekter av IRE behandling av levertumörer.

Studie I är en genomgång av de första 50 patienterna som behandlades med IRE på Danderyds Sjukhus. Den visar att IRE är en säker behandlingsmetod med goda resultat vid behandling av tumörer som inte var aktuella för operation eller andra ablationsmetoder. *Studie IV* är en nationell multicenterstudie där alla patienter som behandlats med IRE för levertumörer i Sverige, från första behandlingen i maj 2011 till och med 2018, har följts upp. Det är den hittills största studien i ämnet som publicerats. Resultaten är ytterligare bevis för att IRE är en säker behandlingsmetod med goda resultat för de patienter med levertumörer som är svåra att behandla med andra metoder.

Studie II och III har studerat en metod för avancerad guidning av elektrodplacering vid IRE-behandling med hjälp av stereotaktisk datortomografibaserad 3D-navigering. *Studie II* studerade noggrannheten vid elektrodplacering i en modell och jämförde navigationssystemet med ultraljudsguidning och visade bättre träffsäkerhet med navigationssystemet på bekostnad av längre behandlingstid. *Studie III* studerade de patienter som hade genomgått IRE-behandling med hjälp av navigationssystemet och visade att elektroderna placerades i levern där det var planerat, med liten felmarginal och utan komplikationer relaterade till elektrodplaceringen.

I *studie V* har samtliga IRE-behandlingar i Sverige mellan 2011 och 2018 studerats avseende hur det elektriska motståndet i vävnaden förändrats under behandlingarna. Detta har sedan jämförts med risken för och tiden till återfall av tumörerna. För levermetastaser från tjock- och ändtarmscancer kunde ett samband visas mellan större minskning av motståndet i vävnaden och längre tid till lokala återfall av tumörerna.

ABSTRACT

Introduction: Malignant tumours in the liver are divided into primary liver cancers and liver metastases. Among primary liver cancers, hepatocellular carcinoma (HCC) accounts for 75-85% and is globally the fourth most common cause of cancer related death. Colorectal cancer (CRC) is the second most common cause of cancer related death. As many as 25-50% of all CRC-patients will be diagnosed with liver metastases, either at the time of diagnosis of their primary cancer, or later during progression of their disease.

Surgery is the primary treatment option for colorectal liver metastases (CRCLM). When surgery is not a possible, either due to an insufficient future liver remnant (FLR), or that the patient is not fit for extensive surgery, ablation therapies can be an alternative. Thermal ablation strategies can widen treatment options for extensive liver disease, for oligometastatic disease and for small, potentially resectable tumours, to spare liver parenchyma.

The most common thermal ablation techniques for liver tumours are radio-frequency ablation (RFA) and microwave ablation (MWA). The goal is to induce heat within the tumour including a surrounding margin of normal liver to create coagulative necrosis.

Irreversible electroporation (IRE) is a non-thermal ablation technique that uses multiple electrodes to administer short pulses of direct current at high voltage to induce permanent pores in the lipid bilayer of the cell walls, disrupting the homeostasis and making the cells go into apoptosis. Since there is only a small amount of heat created just around the electrodes, IRE can be used to treat tumours close to heat-sensitive structures such as major bile ducts and larger hepatic vessels.

The placement of the applicators in ablative treatment is one of the main parameters for success, even more so in IRE where several electrodes are placed around the tumour. For optimal treatment effect, they need to be as parallel as possible and at equal depth around the tumour. Different radiological guiding methods are used, ultrasound being the most common. To improve accuracy of applicator placement, stereotactic CT-based navigation systems have been developed.

Aims: The aim of *Study I* was to report the outcome and complications of the first 50 IRE treatments of liver tumours at a national referral centre. *Study II* compared the accuracy of multiple IRE-electrode placements with ultrasound guidance versus a stereotactic CT-based navigation system. In *Study III* the accuracy of multiple IRE-electrode placements with stereotactic CT-based navigation was evaluated in clinical practice. *Study IV* is a national multicentre study assessing complications and survival after IRE treatment of liver tumours. In *Study V* the aim was to investigate the relationship between decrease in tissue resistance and local tumour progression after IRE treatment of liver tumours.

Materials and methods: In *Study I* the 50 first patients treated with IRE of liver tumours at a national referral centre were included. Retrospective data on patient characteristics, guidance methods and treatment data were collected as well as all relevant radiological follow ups. For *Study II*, a liver phantom was used, containing an artificial liver with tumours as well as structures mimicking a rib cage, lungs, and large vessels. Around each of five tumours the interventionists placed four IRE-electrodes in a two-by-two-centimetre pattern using either US guidance or stereotactic CT-based navigation and the accuracy was compared between the two groups. In *Study III* 60 patients that had been treated with IRE for liver tumours using stereotactic CT-based navigation were included. All procedural CT-scans were retrospectively analysed and comparisons between the planned electrode placements and the actual placements were done regarding lateral and angular error, both for every single electrode and for all electrode pairs. *Study IV* and *Study V* are nationwide multi centre studies including all patients treated with IRE of liver tumours in Sweden from the first case in 2011 until the end of 2018. In *Study IV* patient-, tumour-, and treatment characteristics were collected through a retrospective search of patient records. Short- and long-term data were collected. Sub-group analyses were made for HCC- and CRCLM-patients. In *Study V* the recurrence patterns were compared to data collected from the IRE hardware regarding delivered currents and changes in tissue resistance.

Results: *Study I* analysed 60 tumours in 50 treatments and showed that IRE treatment of liver tumours is safe and with acceptable recurrence rates, 37% local tumour progression at 12 months in the whole group and 20% complications within 30 days, with two of them being Clavien-Dindo grade 3b-4b, and no 30-day mortality. *Study II* showed greater accuracy both in the pairwise electrode distances and angles in the stereotactic CT-based navigation group compared to US navigation, at the cost of longer procedural times. *Study III* found a good accuracy regarding stereotactic CT-based navigation with a median lateral error of 3.6 mm (range 0.2-13.6 mm) and a median angular error of 3.1 degrees (range 0.2-18.9 degrees) comparing planned and validated electrode placement. No electrode placement-related complications were recorded. *Study IV* analysed all 183 patients treated with IRE for 257 liver tumours in Sweden between 2011 and 2018, the majority with CRCLM or HCC. Median follow-up time was 59 months, and 30-day complications were reported in 18% of the patients with 1.2% being Clavien-Dindo grade 3b-5. Overall survival for the whole cohort was 32.6 months (95% CI 28.3-42.6), longer for HCC than CRCLM, 42.3 months (95% CI 27.8-65.7 months) compared to 27.9 months (95% CI 20.4-35.6 months). For CRCLM the time to local tumour progression was significantly longer for tumours smaller than 20 mm compared to larger tumours and it was also longer for patients with IRE as their primary treatment in the liver compared to patients previously treated with other modalities. *Study V* used the same cohort as *study IV* and data from 132 patients with 203 tumours that was complete regarding delivered energy during the IRE treatments. The analysis showed that impaired change in resistance over at least one electrode pair was correlated with the time to local tumour progression for liver metastases, but not for HCC.

Discussion:

Study I, presenting the initial experiences of IRE of liver tumours, and *study IV*, a nationwide multicentre study, further establish IRE as a safe and efficient ablation method for primary and secondary hepatic tumours. The complication rates in these studies are lower compared to previously published papers, especially for major complications. The overall survival and time to local tumour progression are comparable to previous studies.

Study II and *III* demonstrate that a stereotactic CT-based navigation system is safe to use and gives good accuracy in electrode placement with no electrode placement related complications. The navigation system is superior to US in placing IRE electrodes in a liver phantom regarding parallelism and lateral deviation of electrodes.

Study V demonstrates a relationship between change in tissue resistance and time to local tumour progression in CRCLM and NCRCLM, but not for HCC. There are multiple factors affecting tissue resistance during IRE, the formation of nanopores and altered electrical properties around the cells is one, the change in temperature in the ablation zone is another.

Further studies are needed to find the optimal electrode placement and energy settings in IRE treatment of liver tumours. Real-time measurement of tissue resistance could be one factor to investigate in the future.

LIST OF SCIENTIFIC PAPERS

I. **Initial experience with irreversible electroporation of liver tumours**
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Eur J Radiol Open. 2019 Jan 22;6:62-67. doi: 10.1016/j.ejro.2019.01.004. eCollection 2019.

II. **Stereotactic navigation versus ultrasound guidance in placing IRE applicators in a liver phantom.**
Stillström D, Eigl B. Freedman J
Scientific Reports. (2021) 11:21031. https://doi.org/10.1038/s41598-021-00505-1. Epubl 2021 Oct 26.

III. **Accuracy of Electrode Placement in IRE Treatment with Navigated Guidance**
Stillström D, Sandu RM, Freedman J.
Cardiovasc Intervent Radiol. 2021 Jun;44(6):968-975. doi: 10.1007/s00270-020-02762-5. Epub 2021 Jan 20.

IV. **Irreversible electroporation of liver tumours: A nation-wide multicenter study with long term follow-up**
Stillström D, Frühling P, Holmquist F, Nilsson A, Freedman J.
Manuscript

V. **Change in tissue resistance during IRE of liver tumours as an indicator of treatment success, a multicentre analyse with long term follow-up**.
Stillström D, Frühling P, Holmquist F, Nilsson A, Freedman J
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ALPPS	Associating Liver Partition and Portal vein Ligation for Staged hepatectomy
BCLC	Barcelona Clinic Liver Cancer
BMI	Body mass index
CCC	Cholangiocellular carcinoma
CE-CT	Contrast enhanced computed tomography
CEUS	Contrast enhanced ultrasound
CI	Confidence interval
CRC	Colorectal cancer
CRCLM	Colorectal cancer liver metastases
CT	Computed tomography
ECG	Electrocardiogram
FLC	Fibrolamellar hepatocellular carcinoma
FLR	Future liver remnant
GIST	Gastrointestinal stromal tumour
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
HFJV	High frequency jet ventilation
HKLC	Hong Kong Liver Cancer
HIFU	High Intensity Focused Ultrasound
IBD	Inflammatory bowel disease
iCCA	Intrahepatic cholangiocarcinoma
INR	International Normalised Ratio
IR	Infrared
IRE	Irreversible electroporation
LITT	Laser-induced thermotherapy
LTP	Local tumour progression
MDT	Multi-disciplinary team

MRI	Magnetic resonance imaging
MWA	Microwave ablation
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NCNNLM	Non-colorectal non-neuroendocrine liver metastases
NCRCLM	Non-colorectal cancer liver metastases
NELM	Neuroendocrine liver metastases
NET	Neuroendocrine tumour
OS	Overall survival
PEI	Percutaneous ethanol injection
PET	Positron emission tomography
PFS	Progression free survival
PVE	Portal vein embolisation
RFA	Radiofrequency ablation
TACE	Trans-arterial chemoembolisation
TAE	Trans-arterial embolisation
UCSF	University of California, San Francisco
US	Ultrasound
3D	Three dimensional

1 INTRODUCTION

The treatment of liver tumours has changed during the last decades. For colorectal cancers, liver metastases can be treated with curative intent and the treatment options are expanding. Other cancers with metastases to the liver are can also be treated with a curative intent in selected cases. For Hepatocellular cancer (HCC) the treatment options are also expanding.

For many years, surgery was the only curatively intended treatment, but other options are increasingly used e.g., ablation and radiotherapy with good results.

Ablation therapies were first used in the mid 1980-ies with ethanol injections. Today thermal ablations are in clinical practice and for the last 20 years irreversible electroporation (IRE) has been used in the treatment of liver tumours as a non-thermal ablation technique. IRE is still under development and the optimal treatment settings are still to be decided.

Navigating the applicator or applicators accurately in relation to a target and/or a predefined place is one of the key points in successful ablative treatment. Different systems for aiding the interventionist in placing the applicator have been developed and shown to be accurate and helpful, and ultimately giving the patient a safer and more efficient treatment.

This thesis focuses on IRE in the clinical setting, optimising the placement of IRE-electrodes, and the optimal settings for energy deliverance during treatment.

2 LITERATURE REVIEW

2.1 LIVER TUMOURS

2.1.1 Primary liver cancer

Primary liver cancer includes various types of malignant tumours with different histological features. Most common is hepatocellular carcinoma (HCC) that accounts for about 75-85% of all malignant liver tumours. Other types are intrahepatic cholangiocarcinoma (iCCA) accounting for 10-15% of cases, mixed hepatocellular carcinoma (HCC-CCA), fibrolamellar HCC (FLC) and hepatoblastoma, the most common type in children under 5 years of age [1,2].

Liver cancer is the sixth most common cancer worldwide, with approximately 841,000 new cases annually, and the fourth leading cause of cancer related death, resulting in approximately 782,000 deaths annually. The incidence among men is 2 to 3 times higher than women in most parts of the world, making it the fifth most common cancer and second most common cause for cancer related death among men. In some countries in Northern and Western Africa and Eastern and South-Eastern Asia, it is actually the most common type of cancer [2].

2.1.1.1 Hepatocellular carcinoma

HCC is the most common type of liver cancer with an incidence over 700.000 per year, and the fourth most common cause of cancer-related death globally [2]. In Sweden, the annual incidence is 5/100.000.

The incidence and underlying risk factors vary geographically. The incidence is highest in Sub-Saharan Africa and eastern Asia, with 80% of cases, where the major underlying cause is hepatitis B virus (HBV) infection and aflatoxin B1 exposure [2,3].

The main risk factors for HCC are viral (Hepatitis B and C), toxic (alcohol and aflatoxins) and metabolic (diabetes, steatohepatitis, hemochromatosis, and porphyria). Chronic hepatitis B and C are the underlying causes in > 80% of all HCC [4]. In countries with high incidence of viral hepatitis, 80-90% of HCC patients have cirrhosis. In Sweden about 20% of all HCC is seen in non-cirrhotic livers and non-viral liver disease is the most common cause of liver cirrhosis [5,6]. Fatty liver is seen in 10-20% of a western population. It is caused by alcohol, type 2-diabetes and metabolic syndrome [7]. The increasing incidence of HCC in low-risk HCC countries is considered to be related to the increasing prevalence of obesity, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD) leading to non-alcoholic steatohepatitis (NASH) [8].

Mortality by HCC can be prevented in different ways. In Taiwan, a nationwide vaccination of infants against HBV reduced the incidence of HCC in a cohort of people aged 6-26 years old from 0.92/100,000 among unvaccinated to 0.23/100,000 among vaccinated [9]. Prevention of

HCV infection includes avoiding transmission of contaminated blood-, and sexual transmission. There is an effective antiviral treatment available which prevents development of cirrhosis and in the end HCC. Reducing alcohol consumption and leading a healthier lifestyle to avoid obesity and metabolic syndrome also reduces the risk of cirrhosis and thus HCC [3]. Surveillance for HCC with ultrasound every 6 months in patients with cirrhosis, regardless of underlying cause, and patients with chronic HBV infection has been found to reduce mortality [10,11].

The prognosis for HCC is mainly dependent on whether curative surgery or transplantation is possible. In turn this is dependent on tumour burden, liver function and the patient's performance status. The median survival for untreated HCC in advanced stage is 8 months compared to a 5-year survival of 40-75 % after curative treatment at an early stage [5].

There are several systems for classification and staging of HCC, but the most widely used is the Barcelona Clinic Liver Cancer classification (BCLC classification), both in clinical practice and for trial design [12,13].
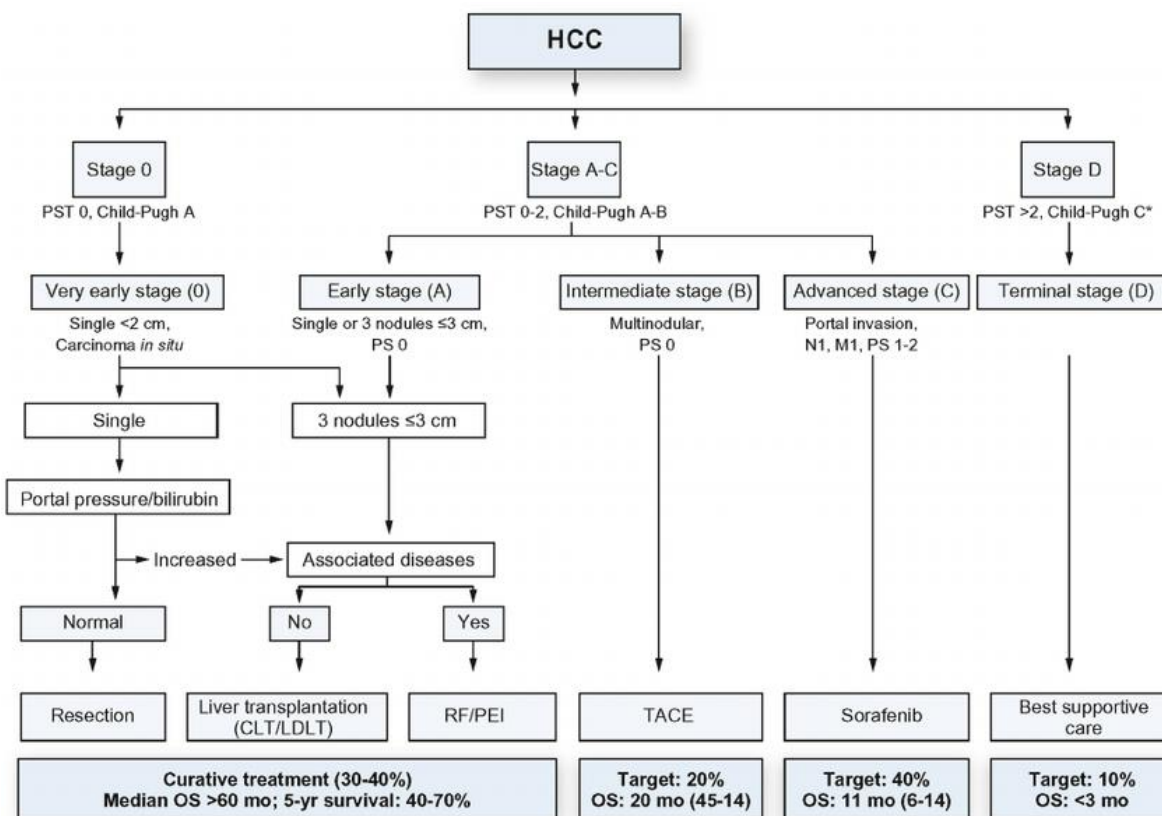
Figure 1: Barcelona Clinic Liver Cancer classification. HCC = hepatocellular carcinoma, PST = performance status, CLT = cadaveric liver transplantation, LDLT = living donor liver transplantation, RF = radio frequency, PEI = percutaneous ethanol injection, TACE = trans arterial chemoembolisation.

The Hong Kong Liver Cancer (HKLC) staging system has shown to be superior to the BCLC in predicting prognosis but is not entirely validated in a European population [14]. In HKLC, ablation is recommended for tumour sizes up to 5 cm.

4

For evaluating the liver function in the BCLC flow-chart, the Child-Pugh classification is used [15,16]:

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin, mg/dL	≤ 2	2-3	>3
Albumin, g/dL	>3.5	2.8-3.5	<2.8
Prothrombin time * Seconds over control * INR	 1-3 <1.8	 4-6 1.8-2.3	 >6 >2.3
Encephalopathy	None	Grade 1-2	Grade 3-4

Table 1: Child-Pugh classification

A total score of 5-6 constitutes grade A (well-compensated disease); 7-9 is grade B (significant functional compromise); and 10-15 is grade C (decompensated disease). These grades correlate with one- and two-year survival.

Grade	Points	One-year patient survival (%)	Two-year patient survival (%)
A: well-compensated disease	5-6	100	85
B: significant functional compromise	7-9	80	60
C: decompensated disease	10-15	45	35

Table 2: Prognosis for Child-Pugh classes

According to the BCLC classification, the curative treatment options include liver resection, liver transplantation and ablation. Trans-arterial chemoembolisation (TACE) can be used to downstage before transplantation and as a palliative treatment. Systemic drugs are given in palliation [5].

Ablation is the first line treatment of smaller HCC with up to three lesions not larger than 3 cm in diameter.

Liver resection is considered for patients with normal underlying liver function or compensated cirrhosis, with no evidence of portal hypertension and without metastases.

Liver transplantation is the only definitive cure for both the HCC and the underlying liver disease. In many countries the Milan criteria for liver transplantation are used (1 tumour ≤ 5 cm or up to 3 tumours none > 3 cm) with 5-year overall survival (OS) of 65-78% [17,18]. In Sweden, the University of California, San Francisco (UCSF) criteria (one tumour ≤ 6.5 cm, or ≤ 3 tumours the largest ≤ 4.5 cm, and total added diameter ≤ 8 cm) are used to decide which patients can be eligible for liver transplantation [19].

2.1.2 Colorectal liver metastases

Colorectal cancer (CRC) is the third most common type of cancer, following lung and breast cancer, and is also the second most common cancer-related cause of death [2]. CRC accounts for about 10% of all new cancers and cancer-related deaths. The incidence is highest in Western Europe, Australia, and New Zeeland, and lowest in Africa. About 0.9 million patients per year will die from CRC [2]. The incidence worldwide is predicted to increase due to economic development in many parts of the world to numbers as high as 2.5 million new cases in 2035 [20].

The incidence of colon cancer in Sweden is 43/100 000, similar between men and women. For rectal cancer it is 25/100 000 among men and 17/100 000 among women. It is the third most common cancer after prostate cancer and breast cancer. In 2012-2016 CRC contributed to 11% of all cancer-related deaths in Sweden, making it the third most common cause of cancer-related death [21].

There are several risk factors for CRC. Male sex and increasing age have shown strong associations with incidence in epidemiological studies [20]. Hereditary factors contribute to 10-20% of all cases and 5-7% of patients are affected by a well-defined hereditary colorectal cancer syndrome. Inflammatory bowel disease (IBD) is also a well-known risk factor. IBD patients have a 2- to 6-fold increase in risk of CRC compared to the general population [22]. IBD-related CRC accounts for approximately 2% of CRC-mortality. High intake of red and processed meats increases the risk of developing CRC [21]. Alcohol consumption also increases the risk of CRC, as do smoking. High body mass index (BMI) increases the risk for

CRC and physical activity lowers it [21]. Diabetes is an independent risk factor with a 30% increased risk after adjustment for smoking, obesity, and physical activity [21,23].

About 18-25% of all CRC-patients will be diagnosed with liver metastases at the time of diagnosis of the primary tumour (synchronous) and up to 25-50% will at some point develop liver metastases [24-26]. Colorectal cancer liver metastases (CRCLM) are more common in men and in patients with left sided CRC [24,25]. Metachronous metastases are often diagnosed within the first years after surgery of primary tumour, 76-85% within 1 year, and 83-98% within 3 years. Only about 2% of patients will present with CRCLM 5-10 years after resection of the primary tumour [26].

The prognosis of CRC is mainly dependent on tumour staging and tumour biology. In Sweden the 5-year survival rate in colon cancer in 2018 was 64% for men and 68% for women. For rectal cancer, the 5-year survival was 66% for both genders [21]. Among patients with CRCLM the 5-year survival rate is over 50% after liver resection, compared to approximately 5% after palliative treatment [24]. The prognosis is worse in right-sided colon cancers compared to left-sided and rectal cancers. Despite radical surgery the recurrence rate after liver surgery is over 50% [26].

The primary treatment for CRCLM has traditionally been surgery with resection of affected liver segments. In recent years ablative techniques have developed and shown oncological results comparable to liver resection in selected cases [27-31].

The current Swedish guidelines for curative intended surgery can be summed up as [21]:

- The patient is likely to tolerate surgery.
- All metastases, liver and elsewhere, can be resected with radicality.
- At least two adjoining liver segments with blood supply, venous outflow and bile drainage are left after surgery.
- Adequate liver volume is left after resection, 20-30 % of liver volume if the liver parenchyma is normal. If the patient has received pre-operative chemotherapy the liver remnant has to be larger.
- Taking tumour biology into account, it is still reasonable to resect the patient considering prognostic factors.

Over time, the indications for liver resection have widened, and today also bi-lobar metastases and multiple metastases can be treated. The most important factor to determine if surgery is possible is the size of the future liver remnant (FLR) [32]. To achieve a radical resection, several methods have been developed to increase the FLR e.g., portal vein embolisation with or without liver partition (ALPPS) as well as strategies with combinations of ablation and surgery.

2.1.3 Other liver metastases

Apart from CRC many other types of cancers can spread to the liver. Up to 50% of all cancer patients will present with, or develop, liver metastases during the course their disease and it is

generally associated with a poor prognosis. Untreated the 5-year survival is near zero [33]. The most common primary tumours spreading to the liver are CRC, pancreas, breast, melanoma, and lung, see table 3.

Primary tumour	Percentage
Small cell lung cancer (SCLC)	17%
Non-small cell lung cancer (NSCLC)	4%
Cutaneous melanoma	10-20%
Breast cancer	6-38%
Colorectal cancer	30-50%
Pancreatic cancer	30-40%
Gastric cancer	5-40%
Neuroendocrine tumour	20-46%

Table 3. Primary cancers and percentage that metastasise to the liver. Adapted from Tsilimgras *et al.,* with permission [33].

In a study on 2.4 million cancer patients, synchronous liver metastases were found in 5.14% [34]. The 1-year survival was 15.1% among patients with liver metastases compared to 24.0% with metastases other than liver. The median survival time was half in the liver metastasised group, 4 vs 8 months.

Treatment options for liver metastases varies depending on the primary tumour. Apart from CRC, neuroendocrine liver metastases (NELM) is an accepted indication for curative and palliative intended treatment with a 5 -year survival of 60-80% [34].

For non-colorectal non-neuroendocrine liver metastases (NCNNLM) surgery can be considered in well selected cases, but most often the prognosis is poor [34]. For breast cancer liver metastases resection, ablation or other local treatments can be considered in an oligometastatic setting with prolonged survival or possibility of pausing chemotherapy [35].

2.2 LIVER SURGERY

The main issue in liver surgery is the functional capacity of the remaining part of the liver. A sufficient size of the future liver remnant (FLR) must be ascertained before decision of surgery, or the patient might go into post-operative fatal liver failure. The FLR volume can be measured on cross-sectional imaging, commonly computed tomography (CT) or magnetic resonance imaging (MRI). Other methods focusing on the assessment of liver function are used or under development e.g., clearance of indocyanine green (ICG), technetium-99m-mebrofenin hepatobiliary scintigraphy (HBS) and technetium-99m-diethylenetriaminepentaacetic acid-galactosyl human serum albumin single photon emission computed tomography (GSA SPECT), and MRI with Gadolinium ethoxy benzyl diethylenetriamine pent acetic acid (Gd-EOB-DTPA) [36].

After liver injury the hepatocytes enlarge and proliferate. In a previously healthy liver, there is a regeneration of un-injured hepatocytes. If the hepatocytes are damaged before the resection the regeneration process is impaired. Because of this, the underlying liver disease

and function alters the required FLR volume for a safe resection. In patients without liver disease, as little as 20% FLR can be considered safe. At least 30% FLR is required after liver injury, e.g., after chemotherapy, in the presence of steatosis, infection, or other iatrogenic injuries. In patients with advanced liver disease, including cirrhosis, 40% FLR is considered a minimum [36].

If the FLR is deemed sufficient liver resection can be performed up front, but if it is not further steps are needed to increase the FLR before surgery. Since more than 30 years, portal vein embolisation (PVE) has been performed to increase the FLR. The portal veins to the part of the liver to be resected are embolized via a transhepatic percutaneous puncture. Over time this leads to hypertrophy of the FLR, hopefully making it large enough to proceed to liver resection. One of the limitations of the method is the risk of tumour growth in the time between embolisation and resection.

To overcome this problem and further increase the growth of the FLR other methods have been developed. A straightforward two stage procedure is sometimes possible and has been further developed with associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), which is performed through portal vein ligation and in-situ separation of the tumour-bearing segments of the liver from the FLR in a first stage followed by a second stage with liver resection. ALPPS gives a faster and greater growth of the FLR but has higher complication rates. Combining PVE with trans arterial embolisation (TAE) increases the growth of the FLR since the degree of hypertrophy is correlated to the extent of ischemia [37]. Hepatic vein embolisation has also been used in conjunction with portal vein embolisation to increase the FLR in selected cases with good outcome [38].
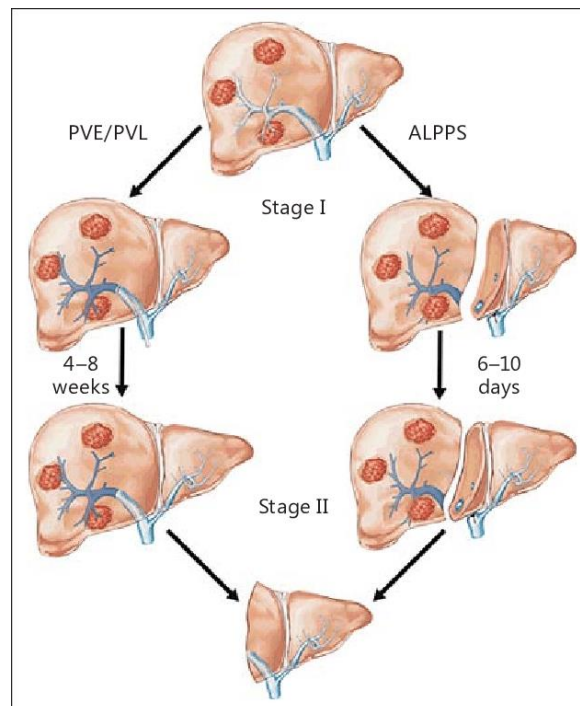
Figure 2. Conventional PVE techniques vs. associating liver partition and portal vein ligation for staged hepatectomy (ALPPS). PVE, portal vein embolisation; PVL, portal vein ligation, from Budai *et al.*, with permission [39].

Liver resection can be done according to anatomical segments in the liver or as parenchyma sparing local resections. There is no clear evidence to which method is best from an oncological point of view, although some studies indicate a prolonged tumour free survival after segmental resection compared to local resection of HCC measuring 2-5 cm in size [5]. Apart from adequate FLR, there has to be two adjoining liver segments with sufficient blood flow and bile drainage left [21].

2.3 LIVER ABLATION

Thermal ablation techniques include RFA, MWA, laser-induced thermotherapy (LITT), high intensity focused ultrasound (HIFU) and cryoablation. The goal with these techniques is to induce permanent cell damage within the tumour and a surrounding safety margin by increasing or decreasing the temperature. To achieve permanent cell damage the temperature must be increased above 50°C [40] or below -5 to -50 °C [41].

2.3.1 Radio frequency ablation

RFA is the most well-studied thermal ablation method. For small HCC it is one of the primary treatment options [12,13]. RFA can be regarded as an electric circuit with a generator, cabling, electrodes, and the tissue as a resistor [40]. The patient has a grounding pad attached to the body and an electrode connected to a generator is inserted into the tumour. Electromagnetic energy at 300-500 kHz is applied. The flow of current leads to increased temperature due to frictional agitation in the tissue at ionic level (the Joule effect), the heat then spreads to the surrounding tissue through conduction, causing cellular death through thermocoagulation necrosis [40,42-44]. One of the limitations of the technique is the cooling of tissues surrounding larger vessels, the so-called heat-sink effect [42]. In a study using histological examination of the ablation zones in explanted livers there was a significantly higher rate of unsuccessful ablations of tumours close to vessels larger than 3 mm in diameter, 47% versus 88% (p=.009) [45]. The maximum temperature that can be achieved by RFA is 100°C, above 100-110°C tissue vaporisation and charring occur which increases tissue impedance and limits thermal conductivity [40,43,46].

2.3.2 Microwave ablation

Microwave refers to electromagnetic energy in the 300 MHz to 300 GHz range [40]. Microwave ablation refers to the use of electromagnetic energy with frequencies above 900 MHz to induce tumour destruction [47]. For regulatory and practical reasons, commercially available devises operate at 915 MHz or 2.45 GHz.

Water molecules (H_2O) are polarised, the hydrogen side has a positive charge, and the oxygen side has a negative charge. The electromagnetic field has an oscillating electrical charge that changes from positive to negative. In this electromagnetic field, the positions of

the water molecules flips every time the electrical field changes. The microwave energy oscillates at 915 MHz or 2.45 GHz making the water molecule flip almost 2-5 billion times per second causing friction and thus heat, leading to coagulative necrosis of the surrounding tissue [47]. MWA can create temperatures within the tumour of >150°C and create larger ablation zones compared to RFA. The method is also less sensitive to heat sink and requires shorter ablation times. Tumours close to vessels that are not suitable to treat with RFA can sometimes instead be treated with MWA [48]. As the electromagnetic energy does not require direct current flow, MWA does not have the limitations of charring of tissue as do RFA [49].

2.3.3 Other ablative methods

Laser-induced thermotherapy (LITT)

Laser induces electromagnetic heating by using 600-1000 nm wavelength light energy and it is delivered into the tumour through optical fibres. Because light is scattered and rapidly absorbed by most body tissues the energy penetration is limited. Ablation zones after LITT are small, about 1-2 cm in diameter [40]. One advantage of LITT is that the optical fibres do not contain metal and can be used in an MRI and creates no artefacts on CT and MRI images. To achieve larger ablation zones, multiple fibres can be used and zones up to 5 cm in diameter are possible [50].

High Intensity Focused Ultrasound (HIFU)

HIFU uses ultrasound of high power in multiple beams that converge at a focal point. One can compare HIFU to a magnifying glass where the solar light is focused at one point where the energy is high enough to start a fire. The HIFU focal point is placed inside the soft tissue and the temperature there can rise to above 56°C and thus create thermal necrosis [51]. The use of HIFU in liver tumours can be difficult due to limited access between the ribs where there is a risk of soft tissue burns from reflection of high energy ultrasound waves by the ribs [52].

Cryoablation

Permanent cell death is achieved by freezing the cells below -20°C. Cryoablation uses the Joule-Thomson effect. When compressed gas rapidly expand there is a change in temperature. When argon gas expands it cools down, whereas when hydrogen or helium expands, they get warmer.

To achieve cooling within a liver tumour a cryo-probe is inserted, and compressed argon gas is led into the tip of the probe where there is a small chamber where the gas can expand and the temperature drops. This can create temperatures down to -140°C inside the centre of the tumour. The rapid cooling of the cells leads to intracellular ice crystal formation [40,53]. To further increase the effect, the tumour is thawed through the same probe when high-pressure helium is sent through the system heating up the cells. The ice within the cells melts, leading

to intracellular hypotonicity, osmotic fluid shift, and cell swelling or bursting. Then a second cycle of cooling is performed. The tissue cells are killed directly by cryoablation-induced injury or later by apoptosis [53].

Percutaneous Ethanol Injection (PEI)

The era of local tumour ablation of liver tumours started with percutaneous ethanol injection in the mid 1980-ies [54,55]. Alcohol induces cell death through dehydration of the cytoplasm, protein denaturation and coagulative necrosis. Alcohol also enters the local circulation causing necrosis of the vascular endothelium leading to platelet aggregation, vascular thrombosis, and ischemic necrosis of the tissue [40,54]. PEI is more efficient in treating HCC compared to liver metastases. This is due to the softer tissue within HCC compared to the more fibrotic tissue in metastases, and to the capsule or pseudo capsule surrounding the HCC which contains the alcohol within the tumour [40]. The initial results with PEI for HCC were promising, but the thermal ablation methods have shown to be more efficient [40,43] . PEI is still used on small HCC and on HCC where thermal ablation is not possible. It is considered a safe and cheap method with few side effects.

2.3.4 Irreversible electroporation

Electroporation takes place when short electrical pulses create pores in the plasma membrane of a cell, increasing its permeabilisation [56,57]. The process cannot be visualised in real time under microscopy and the mechanism is not fully understood [56,58]. Electroporation can be either reversible i.e., the pores closes when the electrical field is shut off, or irreversible when the pores in the plasma membrane stay open. This happens when the strength of the electrical field is high enough for a sufficient period of time, at least 10kV/cm and over 300 nano seconds [57,59,60]. The permanent opening of pores in the cell membrane leads to a disruption of the cell's homeostasis causing it to go into programmed cell death, apoptosis.

When electroporation is used for transfection or for introduction or removal of macromolecules from individual cells, the irreversibility is an unwanted side effect whereas in IRE treatment it is the goal.

History

The first description of a phenomenon resembling IRE was published in 1754 by J. A. Nollet, and in 1898 Fuller showed that multiple high voltage discharges had bactericidal effects on a water sample. The first modern studies on effects similar to IRE were published in the 1950's [61]. Since the 1960's the food industry has used IRE for sterilisation of water and foods [62].

The first studies on IRE as an ablation technique was published 2005 by Davalos, Mir and Rubinsky [57]. The development process went from *in vitro* experiments to small animal models to large animal models in the liver and the heart [61]. The effect of IRE on blood vessels was investigated and showed that all cells, including vascular smooth muscle cells,

were ablated, but the IRE did not affect the vessel matrix and the patency of the vessels were intact. This led to the conclusion that IRE treatment is safe in proximity of larger blood vessels and bile ducts [63].

The first publication of IRE performed on human patients was published 2010 by Ball *et al*. [64]. Several studies thereafter have investigated the safety and efficacy of IRE, a recent systematic review of IRE treatment of malignant liver tumours listed 25 studies [65]. The conclusion from these studies is that IRE shows encouraging data on overall survival (OS) and progression free survival (PFS) with an acceptable complication rate. There are still few published studies on long-term effects on OS and PFS.

IRE has been used in the treatment of primary and secondary liver cancers, locally advanced pancreatic cancer, prostate cancer, renal cancer, lung cancer, and sarcomas.

Technique

The most commonly used commercial IRE generator is the NanoKnife® (AngioDynamics, Latham, NY, USA). The IRE system consists of the generator, an ECG monitor and 2 to 6 electrodes.

The electrodes are placed around the tumour. In the liver they are placed approximately 20 mm apart. To optimise the effect of the treatment they should be placed as parallel as possible and at equal depth around the tumour [66,67]. This is performed under radiological guidance, either percutaneously or during open or laparoscopic surgery (see section 2.5). The desired voltage for every electrode pair is set, and normally 10-20 test pulses of 1,500 V/cm of direct current are delivered in pulses with a duration of 70-90 µs. The delivered current is reviewed, and the goal is 20-40 ampere for each pulse. If it is too low there will not be an irreversible electroporation, too high and the system will shut down as a safety measurement, to avoid induction of heat in the tissue. The voltage is adjusted if needed and then 70-90 treatment pulses are delivered. To avoid cardiac arrhythmias the pulses are administered in the refractory phase of the cardiac cycle, 50 ms after the R-wave [68,69]. The procedures are performed under general anaesthesia and under deep muscular relaxation to avoid muscular contractions due to the high direct current.

IRE in the liver

IRE is mainly used in the liver on tumours close to the hilum or major vessels or bile ducts. The safety and efficacy of IRE has been studied in several papers both prospectively and retrospectively [70-92]. The largest of them included 71 patients with a follow-up period of just under 3 years [81]. A meta-analysis of 25 studies included 776 patients with HCC, CCC and liver metastases [65]. The authors present pooled data on OS and PFS that are hard to draw conclusions from since the disparity in diagnosis is so wide. The complications reported are mostly minor, and it is concluded that IRE can be efficient in selected tumours, especially when the diameter is less than 3 cm.

2.4 ABLATION VERSUS RESECTION

Or rather ablation AND resection. The treatment for every patient should be tailored for maximal treatment effect. Though there are guidelines and recommendations for different types of tumours.

In HCC ablation is an accepted part of the algorithm and the primary choice for treatment of smaller tumours [13,93]. Also in more advanced cirrhosis, resection can be extremely risky and ablative treatment has its role.

For CRCLM surgery is still considered the gold standard [28,30]. The COLLISION Trial Group has suggested a treatment algorithm similar to the BCLC for liver only CRCLM [30] where resection is the first-hand choice and ablation is considered for unresectable metastases, mainly due to insufficient FLR, or in deep sited metastases. IRE is considered for tumours unsuitable for thermal ablation (See figure 3)
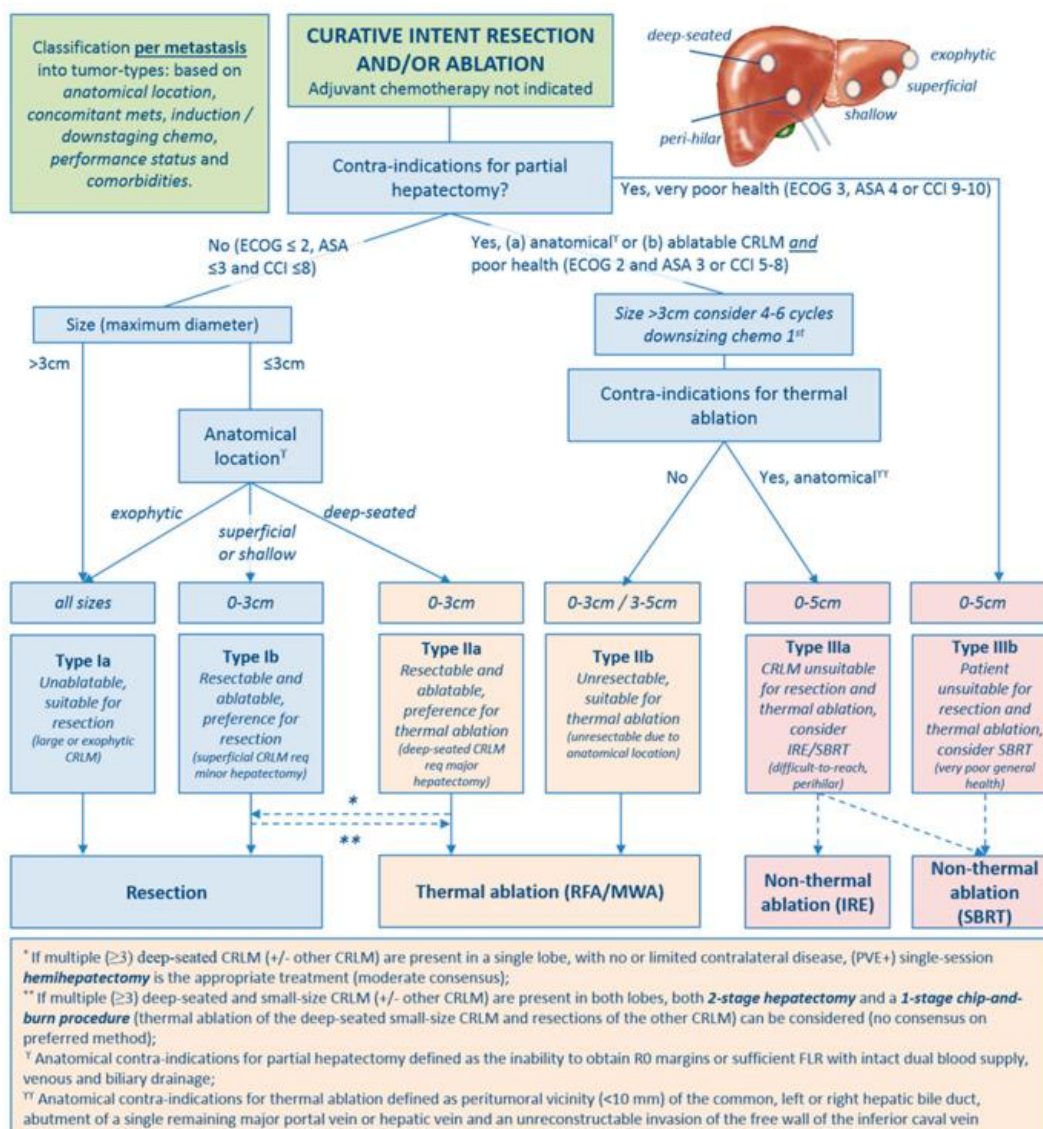
Figure 3. Per-tumour flowchart, from Nieuwenhuizen *et al.* [30] with permission.

A confounding factor in most publications comparing thermal ablation with resection is patient selection. Patients are most often selected for ablation when they are deemed unresectable, making comparison of groups of patients severely biased due to confounding by indication [27,28,31,94]

In a propensity score analysis of MWA versus resection for primarily resectable CRCLM smaller than 3cm, Tinguely *et al.* showed no significant difference in OS suggesting that MWA can be a valid first-line treatment for patients with small CRCLM [27]. Severe complications within 30 days (Clavien-Dindo grade IIIa-V) were significantly lower in the MWA group (7% vs 16%, $p<0.046$) and the median length of hospital stay was 1 day in the MWA group versus 7 days in the resection group. RFA has also been compared to resection for CRCLM in a propensity score analysis and for tumours smaller than 2 cm the OS was comparable between the groups [95].

No studies comparing IRE versus resection has been published. Since IRE is primarily used for unresectable tumours, such a study will probably not be conducted in the near future.

2.5 GUIDING THE APPLICATORS IN ABLATIVE TREATMENTS

The goal with all guidance methods is to place the applicator in the desired position without damaging any vital structures. Different techniques are used to reach this goal, and the choice regarding method is up to the treating interventionist. The technical progress is rapid and increasingly sophisticated methods are developed.

2.5.1 Tactile and visual

In open surgery, tumours can be palpated and the applicator placed directly, and in both open and laparoscopic surgery applicators can sometimes be placed under direct optical view, making the need for radiological guidance unnecessary.

2.5.2 Ultrasound

Ultrasound (US) as guidance for percutaneous ablation is cheap, fast, repeatable, and does not expose patient and interventionist to ionising radiation. For these reasons US has remained the most commonly used imaging technique used for local ablative treatments [96,97]. US also presents the interventionist with real-time imaging when placing the applicators.

In a study on US detection rate on small HCC, ≤3 cm, in cirrhotic livers, US had inferior detection rate compared to computed tomography (CT) and magnetic resonance imaging (MRI) [98]. The detection rate was lowest for tumours ≤1 cm and tumours located close to the diaphragm.

To further enhance the visibility of tumours during US guided ablations contrast-enhancement can be used. Contrast-enhanced ultrasound (CEUS) has shown increased

visibility of MRI-detected tumours from 27% of hepatic malignancies with US to 95% with CEUS [99].

2.5.3 Computed tomography

Contrast-enhanced computed tomography (CECT) provides a good view of most tumours, surrounding tissues, applicators, and ablation zones. It requires multiple scans for applicator positioning and exposes the patient and the interventionist to ionising radiation [97]. CT has a higher detection rate compared to US, especially on smaller tumours and those located in the hepatic dome and is not impaired by air and bone from the lungs and the thoracic wall [100-102].

2.5.4 Magnetic Resonance Imaging

MRI guided ablations have a high technical success rate, no ionising radiation, and has a high sensitivity for smaller lesions [100,103]. It is only performed in a few centres, mainly due to lack of access to MR scanners. It demands special MR-compatible applicators and requires multiple positionings. Compared to CECT, where the contrast agent is visible only for a short time, MRI gives the possibility of repeated scans during the session offering a better view of the tumour [103]. In a comparison between MR-guided and CECT-guided RFA, MR-guidance showed higher technical success rate and reduced number of sessions for complete ablation, but since it is sophisticated, expensive, and specialised technical equipment is needed, this modality is currently limited to only a few referral centres [104].

2.5.5 Fusion techniques

Some tumours are visible on CT, positron emission tomography (PET), and MRI but not on US or CEUS. To ablate these tumours with US guidance, techniques have been developed to fuse real time US with reconstructed images from CT, PET, and MRI [105-107].

The fusion system consists of a magnetic field transmitter fixed to the operation table and electromagnetic sensors applied to the US probe and to the ablation applicator. This gives the system a 3D position of the applicator in relation to the transmitter and the operation table where the patient is placed. By registration of anatomical landmarks, e.g., focal lesions and vascular branches, both on US images and cross-sectional images from CT or MRI, the modalities are fused and the interventionist will get simultaneous displays of US images and reconstructed CT/MRI scans that moves with the movement of the ultrasound probe [105,108].

2.5.6 Navigation systems

To further optimise applicator positioning in ablative treatments, navigation systems for applicator placement has been developed. These systems allow treatment planning in a 3D view and an exact planning for the placement of the applicators. There are several systems available, e.g., CAS-One (CAScination AG, Bern, Switzerland), IQQA-Guide (EDDA

Technology, Inc, NJ, USA), IMATICS CT-navigation™ system (BVM Medical Ltd, Leicestershire, UK), MAXIO robotic system (Perfint Healthcare, Indiana, USA).

The use of a navigation system for single applicator placement in MWA and RFA has been studied in several publications. Engstrand *et al.* [109] and Lachenmayer *et al.* [110] both showed that the CAS-One system provides accurate and safe applicator placement in MWA. Beyer *et al.* compared the CAS-One system with conventional CECT-guidance and showed no difference in accuracy, but significantly lower radiation dose in the CAS-One group [111]. Another advantage of the CAS-One system was shown by Perrodini *et al.* in a study on MWA with stereotactic navigation of NCRCLM. The system provides good opportunity for precise diagnostic biopsies of small lesions prior to ablation [112].

Bhatacharji *et al.* demonstrated the safety and accuracy with the IQQA-Guide for ablation of lung and liver tumours in a study both on a phantom and in patients [113]. A system with an electromagnetic navigation system, IMATIS CT-navigation™, was compared to conventional CT-guided procedures by Duran *et al.* and the navigation system improved the accuracy of applicator positioning [114]. Mbalisike *et al.* demonstrated increased accuracy with the MAXIO robotic system compared to standard CT-guided navigation [115].

In IRE treatment the demands for accurate electrode placement are even higher. There are multiple electrodes, up to six or even seven, and they need to be placed at the same depth around the tumour and parallel to each other [66,116]. With US the aiming is "off target" since the electrodes preferably are placed around the tumour, and the assessment of parallelism is difficult.

There are only a few studies on navigation systems for IRE treatment. The CAS-One system was compared to standard CT guidance by Beyer *et al.* and showed greater accuracy and reduced procedure length [117]. The same group used the MAXIO robotic system and compared this with conventional CT-guidance and the robotic system showed higher accuracy, faster procedures with lower radiation [118]. Published data on CAS-One guided electrode placement in IRE treatment of 84 tumours showed that the accuracy was good with no electrode placement related complications [119].

CAS-One

CAS-One (CAScination AG, Bern, Switzerland) is a stereotactic CT-guided navigation system that uses a CT scan of the target area (e.g., liver, lung or kidney), two infra-red (IR) cameras in combination with retro-reflective skin-markers that gives the interventionist a 3D view of the organ intended to treat.

The patient is placed on the CT table and put under general anaesthesia. To minimise the movement of the liver, high frequency jet ventilation (HFJV) or tracheal tube disconnection with apnoea can be used. The HFJV-technique uses a small catheter introduced through the normal endo-tracheal tube and air is inflated under high pressure in high frequency bursts. In this way the liver is almost totally stationary during the procedure compared to the 2-3 cm

movement seen with a normal ventilator [120-123]. Small retro-reflective spheres are attached to the skin and a diagnostic contrast enhanced CT scan is performed. The CT scan is uploaded to the CAS-One system. The interventionist uses the images to identify the target and plans the placement of the electrode/electrodes, both the entry point through the skin and the target. An adjustable arm with an electrode guide is placed in position in the 3D view and then the electrode is placed. For multiple electrode placement there is a software application that can plan for up to 7 parallel electrodes in different configurations (triangle, square, pentagram, etc). When the electrodes are placed, their actual position is verified by a new CT-scan without contrast enhancement that is overlayed the initial CT scan and compared to the planed electrode placement. When all electrodes are in an accepted position the actual treatment is performed.

Figure 4a. CAS-One, reprint with permission from CAScination AB, Bern

Figure 4b. Planning the needle trajectory. Coloured lines show the planned electrode paths.

Figure 4c. 3D view of the patient with retro-reflective skin markers (green) and planned electrode positioning

The CAS-One system can be used to place single or multiple ablation electrodes/antennas or for placement of biopsy needles. The system can be used for percutaneous ablation or biopsies as well as laparoscopic or open surgery.

3 RESEARCH AIMS

3.1 STUDY I

To report feasibility, short-term outcomes, and complications in 50 treatments of liver tumours with irreversible electroporation, with focus on hepatocellular carcinoma and colorectal cancer liver metastases, where resection or thermal ablation was not possible.

3.2 STUDY II

To compare the accuracy of multiple IRE-electrode placements in a liver phantom guided by ultrasound or a stereotactic computed tomography-based navigation system.

3.3 STUDY III

To evaluate the accuracy of multiple IRE-electrode placement in the liver guided by a stereotactic computed tomography-based navigation system in clinical practice.

3.4 STUDY IV

In a national multicentre study assess the short- and long-term outcome, complications, and survival after irreversible electroporation of liver tumours.

3.5 STUDY V

To investigate the relationship between change in tissue resistance during irreversible electroporation of liver tumours and local tumour progression.

4 MATERIALS AND METHODS

4.1 STUDY I

4.1.1 Patient selection

All patients were referred for IRE treatment after evaluation at a dedicated hepatobiliary multi-disciplinary team (MDT) conference. If the patients were not candidates for liver resection or transplantation, they were assessed for thermal ablation and only those where this was unsuitable were candidates for IRE. The main reasons for choosing IRE were tumour-location in the vicinity of central vessels or bile ducts, or to hollow viscera. The 50 first patients treated with IRE, beginning in February 2014 were included in this study.

4.1.2 Procedures and data collection

All patients were treated under general anaesthesia and the electrodes were placed with guidance of US, CEUS, US-CT/MR fusion or the CAS-One stereotactic CT-based navigation system. The percutaneous procedures were performed in the radiology department and the procedures that required open surgery were performed in an operating room. All patients had a post-intervention CT-scan performed, immediately after the ablation if the renal function allowed for a second contrast dose, otherwise on the first post-operative day.

All patients were followed up every 3 months for the first year, or until recurrence, with CT for HCC, and MRI for liver metastases. Further follow up was decided by the referring physician.

Data collection

All data from patient records were retrieved retrospectively. The electronic patient records were reviewed regarding pre-operative investigations, any prior interventions in the liver, per-operative as well as 30-day complications graded using the Clavien-Dindo classification [124], length of hospital stay, additional treatments and mortality.

All relevant radiological pre- and per-operative images were reviewed to validate tumour size, segmental localisation and per operative complications. All available imaging of the liver was reviewed for ablation site recurrences or new tumours in un-treated parts of the liver. Ablation site recurrence was defined as recurrence within 1 cm of the previously treated tumour. The minimum follow-up time was 12 months.

Statistics

Data for patient characteristics were presented using descriptive statistics. The Mann-Whitney U test or Fisher's exact test were used to present differences between groups. The threshold for statistical significance was set to $\alpha < 0.05$. Kaplan-Mayer estimates were used to present survival time, time to local recurrence, and loss of control.

For this study ethical approval was obtained from the Regional Ethic Review Board in the Stockholm-Gotland region (EPN Dnr 2016/2212-31/1).

The need for informed consent and permission to publish was waived by the review board related to the retrospective nature of this study and anonymisation of all data.

4.2   STUDY II

4.2.1   The phantom

In this study, no interventions were made on human individuals. All procedures were performed on a liver phantom: Triple modality 3D abdominal phantom, model 057A (CIRS Tissue Simulation & Phantom Technology, Norfok, VA, USA). The phantom's internal structures included the liver, the portal vein, two partial kidneys, a partial lung, the abdominal aorta, the vena cava, a simulated spine and six ribs. In the liver there were 6 lesions, and all lesions and surrounding tissues were visible with both CT and US.

To be able to perform interventions with the CAS-One a simulated thorax was made and added to the phantom, and the retro-reflective markers were attached to this, see figure 5.

Figure 5: The simulated thorax to the left with the skin markers attached and the phantom to the right.

4.2.2   Procedures and data collection

Five of the six tumours in the phantom's liver were used. Around each tumour, regardless of tumour size, four IRE electrodes were placed with the goal to place them in a perfect 20x20 mm square and at equal depth with the centre of the tumour 10 mm from the tip.

The electrodes were placed in two sessions, around three tumours in the first, and around two tumours in the second. After each session, a CT-scan of the phantom was performed with a slice thickness of 1 mm.

The interventionists used either US or CAS-One to place the electrodes. The interventionists that used US were all radiologists and the CAS-One interventionists were either radiologists or surgeons. All interventionists had prior experience in their respective modality, see table 4.

Interventionist	US ablation	US biopsy	CAS ablation
1. Radiologist	>100	>1000	>100
2. Radiologist	>100	>1000	>100
3. Radiologist	>100	>1000	>50
4. Radiologist	>50	>1000	
5. Radiologist	0	>100	
6. Surgeon			>100
7. Surgeon			>100
8. Surgeon			>100

Table 4. Interventionist's specialty and prior experience.

The ultrasound guided electrodes were placed with the help of a needle guide using the GE Logic™ E10 (General Electric Health Care, Boston, Massachusetts, USA) with the curved C1-6 probe or the C2-7 probe, as preferred by the interventionist.

The stereotactic navigation was performed with the CAS-One system in the way previously described in this thesis.

Data collection

All CT-scans were uploaded to the CAS-One IR software. The tip of each electrode and entry point into the phantom was manually defined. The geometry of each set of four electrodes was calculated giving inter-electrode distances and angles between each pair of electrodes.

Statistical analyses

The interventionists experience was presented using descriptive statistics. Lateral deviation and parallelism between electrode pairs were presented in boxplots. Student's t-test was used for comparing means between groups and the threshold for statistical significance was set to $\alpha < 0.05$. For analyses of interindividual differences within the groups a one-way ANOVA was used with a Tukey post-hoc test.

Ethical considerations

This study was only performed on a liver phantom. No human patients were treated and therefore no ethical approval was applied for.

All procedures with electrode placement were performed outside clinical work and the use of equipment and space in the radiology department did not affect any patients' treatments. The CT-scans to evaluate the positioning of the electrodes were performed between the scheduled patients in the CT-lab when there was a slot available, and no booked exams were delayed due to this study.

4.3 STUDY III

4.3.1 Patient selection

All patients were referred for ablative treatment after a dedicated hepatobiliary MDT conference. The patients referred for ablative treatment were not eligible for liver resection or transplantation for a variety of reasons. The decision for IRE was made when the tumour was not suitable for thermal ablation due to proximity to heat sensitive structures.

4.3.2 Procedures and data collection

The procedures were performed under general anaesthesia and HFJV was used to minimize movement of the liver. Initially a contrast enhanced CT-scan was performed, and the images were uploaded to the CAS-One system. All electrode trajectories were planned in the systems 3D viewer and the electrodes were placed guided by the system. When all electrodes were in place, a CT-scan without contrast enhancement was performed and these images were overlayed the initial scans with a user-defined transparency gradient, aided by the system software application. The interventionist could then decide if the electrodes were adequately placed and continue with the ablation. If needed, one or more electrodes were repositioned, and a new validation-CT was performed before treatment.

All treatments were performed with the NanoKnife® (Angiodynamics, Queensbury, NY, USA) according to the manufacturer's instructions. After 10-20 test pulses, and subsequent adjustment of energy levels, the treatment was performed with 70-90 pulses per electrode pair. The delivered current was analysed regarding amount and level of change in delivered current according to the manufacturer's recommendations and additional pulses were delivered if deemed necessary.

Before ending the procedure, with the patient still under general anaesthesia, another CT-scan was acquired to detect early complications and evaluate immediate ablation results. These scans were acquired using contrast enhancement if the renal function allowed for it.

Data collection

Using the software in the CAS-One system, all procedural CT-scans, with a slice thickness of 0.5-1 mm, were retrospectively analysed. The planned electrode trajectories for all tumours were compared to the actual placement. For each electrode, the angular error in degrees and lateral error in mm were calculated, see figure 6.
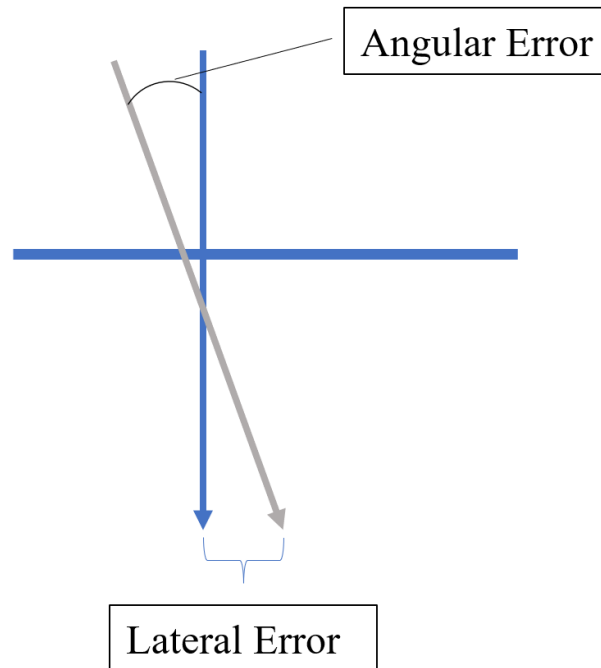

Figure 6: Angular error in degrees and lateral error in mm between planned and actual electrode placement.

For each electrode pair, the angular error between the validated placement of the electrodes was calculated as a measurement of parallelism, see figure 7.
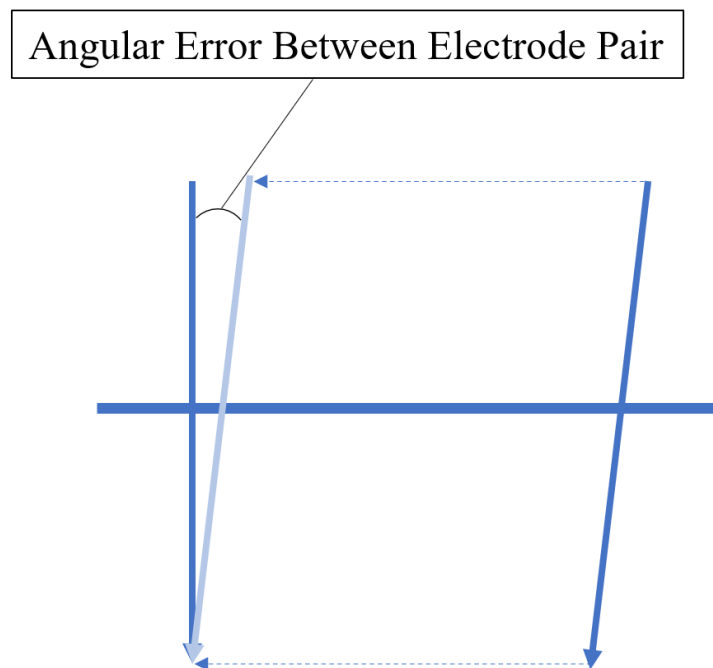
Figure 7: Measurement of angular error between the validated electrodes in a pair.

For each tumour where more than two electrodes were used, a virtual reference electrode was placed in the centre of the tumour around which all the other electrodes were planned. In the few cases where there was a need for an electrode placed in the centre of the tumour this actual electrode was used as a reference. The angular error between each placed electrode and the reference electrode was measured, see figure 8.
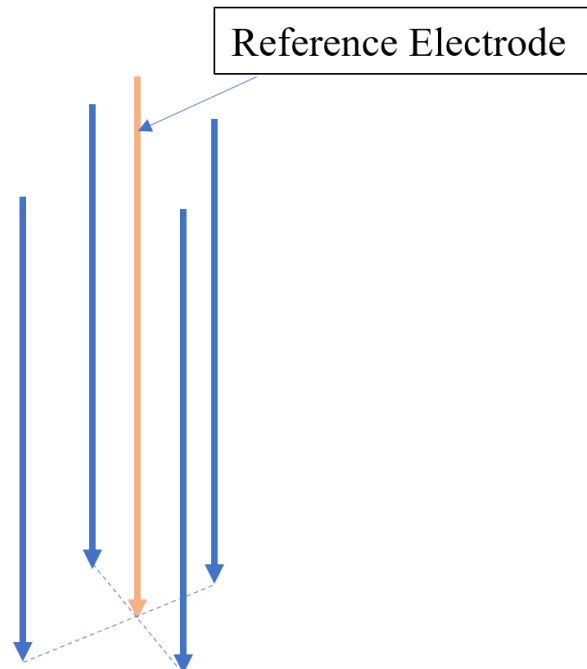
Figure 8: Relationship between reference electrode and validated electrode placement.

Statistical analyses

Descriptive statistics were used for presentation of patient characteristics. Lateral errors in mm and angular errors in degrees were presented as median and range and graphically presented in boxplots.

Ethical considerations

Ethical approval for this study was obtained from the Regional Ethical Review Board in the Stockholm-Gotland region (EPN Dnr 2016/2212-31/2). Due to the retrospective nature of the study and the use of anonymised data, the need for informed consent and permission to publish was waived by the review board.

4.4 STUDY IV

4.4.1 Patient selection

IRE has been performed at three hospitals in Sweden (Uppsala University hospital, Danderyd hospital, and Skåne University Hospital) since 2011. All patients treated for liver tumours with IRE until the end of 2018 were included in this study. Prior to treatment, all patients had

been discussed at multidisciplinary team conferences, and IRE was performed on patients that were not eligible for liver resection, liver transplantation, or thermal ablation.

4.4.2 Procedures and data collection

The patients were treated using the NanoKnife® IRE system (AngioDynamics, Queensbury, NY, USA) and all settings were in accordance with the instructions from the manufacturer and pulse-delivery synchronised with the patients' echocardiogram. All procedures were performed under general anaesthesia with deep neuromuscular block. The guiding of electrode placements was performed percutaneously, with US, CEUS, US-CT/MRI fusion, or stereotactic CT-based navigation with the CAS-One system, or intraoperatively with US or under direct visualisation.

Within the first three months a radiological follow up was performed with CT, CEUS, or MRI, and approximately every 3 months during the first year. After the first year, follow-up was decided by the referring physician.

Data collection

The medical records and all relevant radiological imaging for the patients were reviewed regarding patient demographics, tumour related data, local tumour progression (LTP), and survival as well as any adverse events. Time to LTP was analysed on tumour level and defined as the time from treatment until signs of recurrence. Overall survival was analysed on patient level and defined as time from first IRE treatment until death. Median follow-up time was calculated according to Schemper *et al*. using the Kaplan-Meier estimate of potential follow up [125].

Statistics

Descriptive statistics was used for patient characteristics. Categorical values were presented with proportions and percentages with median and range. OS and time to LTP was presented using Kaplan-Meier estimates and a Cox-regression model was used to assess relationship between clinical data and OS or LTP. The level for statistical significance was set to $\alpha < 0.05$. Time was censored at the last follow up for survival for those patients that were still alive.

Ethical considerations

This study was performed after ethical approval from the Regional Ethical Review Board in the Stockholm-Gotland region (EPN Dnr 2019-00871). The review board waived the need for informed consent and permission to publish based on the retrospective nature of the data collection and anonymity for all included patients.

4.5 STUDY V

4.5.1 Patient selection

The same cohort of patient as in *study IV* was used in this study. All patients treated with IRE of liver tumours, from the first case in May 2011 up until end of 2018, were included in this nationwide multicentre study. All patients had been assessed at a hepatobiliary multidisciplinary tumour board prior to treatment.

4.5.2 Procedures and data collection

The patients were treated at three hospitals in Sweden. The NanoKnife®-system was used for all procedures. Under general anaesthesia with deep neuromuscular block, the electrodes were placed guided by US, CEUS, US-CT/MRI fusion or the CAS-one stereotactic CT-based navigation system when using the percutaneous approach. For procedures performed under open surgery, US or direct visualisation was used.

The follow-up was performed with CT, CE-US, or MRI approximately every three months for the first year, and after that in intervals decided by the referring physician.

Data collection

A retrospective review of the medical records for all patients was performed, collecting data regarding gender, age, primary tumour, previous interventions in the liver, choice of modality for electrode guidance, size of tumour, procedure time, number of electrodes, length of hospital stay, complications, records of liver transplantation, and mortality.

All relevant radiological imaging was reviewed for per- and post-operative complications, signs of LTP, and other intra- and extrahepatic recurrences.

All data regarding the delivered pulses was stored in each NanoKnife®-equipment. Each delivered pulse had 90-115 registrations of voltage and current from which the resistance was calculated using Ohm's law ($U=R*I$). The first and last 20 registrations for each pulse was not used because of the uncertainty of these registrations. During the treatment, ten pulses are delivered with decreasing voltage before the system recharges. The comparison between pulses was performed between the first pulse and the first pulse of the last set of ten pulses, see figure 9.

Procedure Voltage/Current Graph
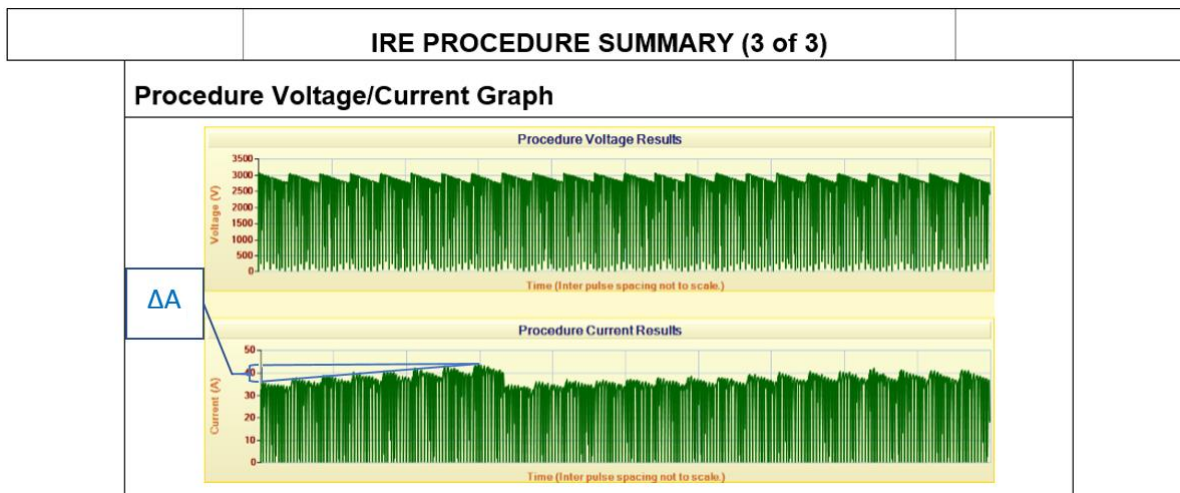
Figure 9: Procedure summary from NanoKnife® showing voltage and amperage for each pulse in a three-electrode treatment with 80 pulses. ΔA is the rise in amperage between pulse 1 and pulse 71.

For each tumour the electrode pair with the least decrease in resistance was identified. This measurement was then used in the analyses and compared to time to LTP.

Statistics

Descriptive statistics were used for patient and tumour characteristics. Student's t-test was used for comparison between groups. A Cox regression model assessed the relationship between change in resistance and time to LTP. The threshold for statistical significance was set at $\alpha < 0.05$.

Ethical considerations

Ethical approval was obtained from the Regional Ethical Review Board in the Stockholm-Gotland region (EPN Dnr 2019-00871). There was no need for informed consent or permission to publish.

5 RESULTS

5.1 STUDY I

Patients

From February 2014 until May 2017, 50 treatments were performed on 41 patients, 12 women (29%) and 29 men (71%), with a total of 60 tumours, the majority of the treated tumours were CRCLM (51%) and HCC (34%). The treatment was performed with a curative intent in 85% of the patients. The patient characteristics are summarised in table 5.

Table 5	
Sex, no. of treatments (%)	
Male	34 (68%)
Female	16 (32%)
Age (y), median (min-max)	63 (38–86)
Tumour type, no (%) of tumours	
Colorectal cancer liver metastases	30 (50.8%)
Hepatocellular carcinoma	20 (33.9%)
Cholangiocarcinoma	2 (3.4%)
Liver metastases from CCC	2 (3.4%)
Leiomyosarcoma	1 (1.7%)
Sarcoma	1 (1.7%)
Adrenocortical carcinoma	4 (6.8%)
Tumour diameter (mm), median (min-max)	20 (5–60)
Previous interventions, no. (%) of patients	
Resection	24 (46.2%)
MW ablation	23 (46.0%)
RF ablation	3 (6.0%)
IRE	8 (16.0%)
TACE (if HCC)	4 (21.1%)
Purpose no. (%), tumours	
Curative	50 (84.7%)
Stage 1	8 (13.6%)
Debulking	1 (1.7%)

Table 5: Patient and tumour characteristics in 50 interventions. Cholangiocarcinoma (CCC), Microwave (MW), Radio frequency (RF), Hepatocellular carcinoma (HCC), Irreversible electroporation (IRE), Trans-arterial chemo embolisation (TACE).

Electrode guidance

The first patient was treated during open surgery with intraoperative ultrasound, thereafter all but one were treated percutaneously, initially using ultrasound or ultrasound fusion with CT or MRI for electrode placement. In September 2014, the first patient was treated using stereotactic guidance with the CAS-One system. Subsequently, more than 80% of the patients were treated using stereotactic guidance for electrode placement.

After a system update in December 2015, including a new applicator guide and a software for planning multiple electrodes, the procedure time for the CAS-One guided procedures was significantly reduced from 198 min to 95 min ($p<0.05$). Part of this improvement is thought to be due to the learning curve of the procedure. There was no difference in recurrence rate before and after the update.

Due to proximity of tumour to bowel, two patients were treated with open surgical approach and intraoperative ultrasound was used to guide the electrode placement.

Complications

Two patients had intra-operative complications. One had a rise in blood-pressure to over 200 mmHg when a tumour close to the right adrenal gland was treated. The hypertension was normalised as soon as the pulse delivery was aborted. The second patient had a sub-capsular bleeding noticed on the CT-scan done to verify the electrode positioning. The procedure was aborted before treatment. This patient had a MWA a couple of weeks later and eventually a liver transplantation.

Table 6

Image guidance	
CAS -One	35 (70.0%)
Ultrasound-fusion	11 (22.0%)
Ultrasound	2 (4.0%)
Open surgery	2 (4.0%)
Procedure time, All 50 treatments (min), median (min-max)	167.5 (44–324)
Procedure time, CAS 35 treatments (min), median (min-max)	135 (44–304)
Procedure time, non-CAS 15 treatments (min), median (min-max)	210 (102–324)
Number of needles, median (min-max)	4 (2–7)
DPL, CAS n=35 (mGy x cm), mean (±SD)	1399.4 ± 515.6
DPL, radiated non-CAS n=10 (mGy x cm), mean (±SD)	906.6 ± 404.2
Length of hospital stay (days), median (min-max)	1 (0–10)
Complications, 30 days	
Pneumothorax	3
bleeding	1
liver failure	1
portal vein thrombosis	2
infection	1
brachial plexus injury	3
Clavien-Dindo	
1-3a	7
3b-5	2

Table 6: Procedure characteristics and list of complications. Patients' radiation dose for patients that underwent CT-scan during the procedure. Computer Assisted Surgery (CAS), Dose-length product (DLP)

Complications within 30-days were seen in 10 patients (20%). Only two of them were regarded as major, Clavien-Dindo grade 3b-4b. One patient with pneumothorax requiring drainage and one patient developed liver failure. No 30-day mortality was seen. One patient died from liver failure due to his underlying cirrhosis 46 days after his procedure. Procedural characteristics and complications are summarized in table 6.

Treatment results

On the first radiological follow-up, 11 patients (19%) had residual tumour. Of these 9 patients received additional ablative treatment, three with IRE and six with MWA. One patient with a residual CRCLM had chemotherapy after the IRE treatment and three months later had a hemi-hepatectomy. One patient with a residual HCC was treated first with TACE and after another recurrence with MWA and one year after the IRE treatment had a successful liver transplantation.

Local tumour progression, defined as recurrence within 1 cm of the ablated tumour, for the remaining patients was 3% at 3 months, 26% at six months and 37% at 12 months. Figure 10 shows the graph of recurrence following the first IRE for each tumour, additional IRE of recurrent tumours is not analysed. No patients with NCRCLM are analysed in this graph since the numbers are too few.

There were no signs of recurrence along the electrode tracts (seeding) in this material.
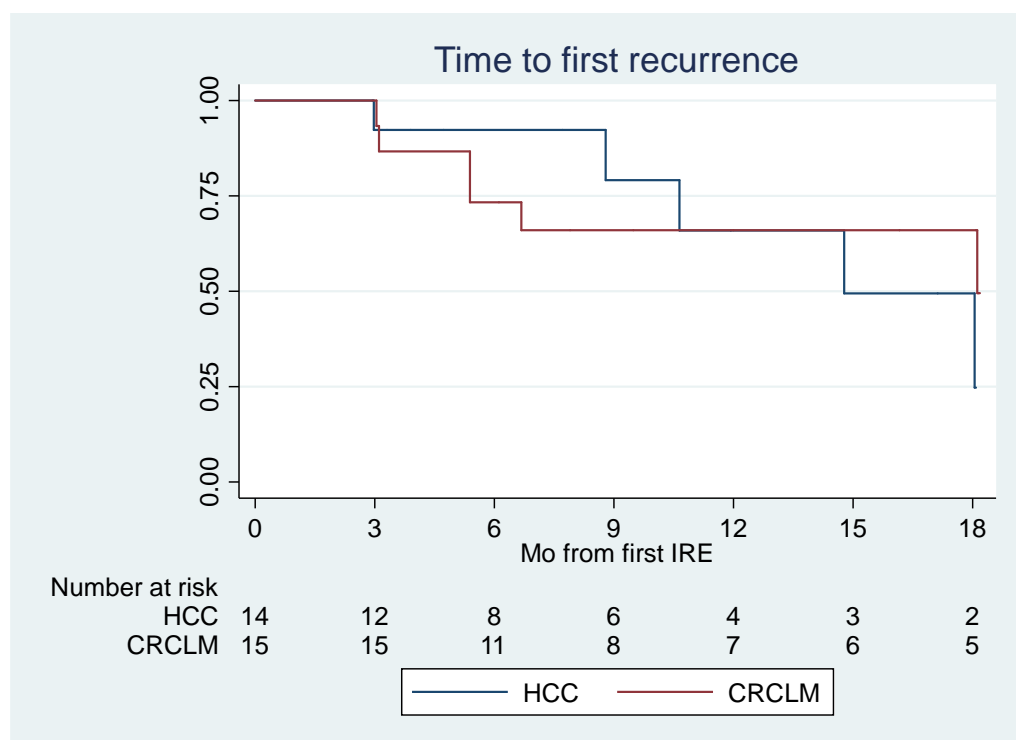
Figure 10: Kaplan-Mayer estimates showing time from first IRE treatment to first local recurrence for patients with HCC (Hepatocellular carcinoma) and CRCLM (Colorectal cancer liver metastases). IRE (Irreversible electroporation).

First stage procedures

Six patients with CRCLM were treated with IRE as a first step before planned additional treatments. Three patients went on to successful hemi-hepatectomy. One patient had a portal vein embolisation, but when surgery was performed after six weeks there was widespread metastatic disease. One patient was planned for resection, but had widespread metastases on the first radiological exam after the IRE. One patient had IRE as a first step and continued with multiple MWA.

5.2   STUDY II

Treatments

In the phantom, five of the six tumours were used, and the interventionists placed 4 electrodes around each tumour. Five interventionists used US, contributing a total of 25 tumours, 100 electrodes and 150 electrode pairs to analyse. After this extensive needle penetration of the phantom there was too much air inside it for a sixth interventionist to get good visualisation with ultrasound. Six interventionists used CAS-One for guidance, with a total of 30 tumours, 120 electrodes and 180 electrode pairs for analysis.

Accuracy of electrode placement

The absolute values for median deviation from the optimal (20 mm between electrodes in a square and 28 mm for the diagonal in the square) was 1.3 mm (range 0.0 to 11.3 mm) in the CAS-One group and 7.1 mm (range 0.3 to 18.1 mm) in the ultrasound group.
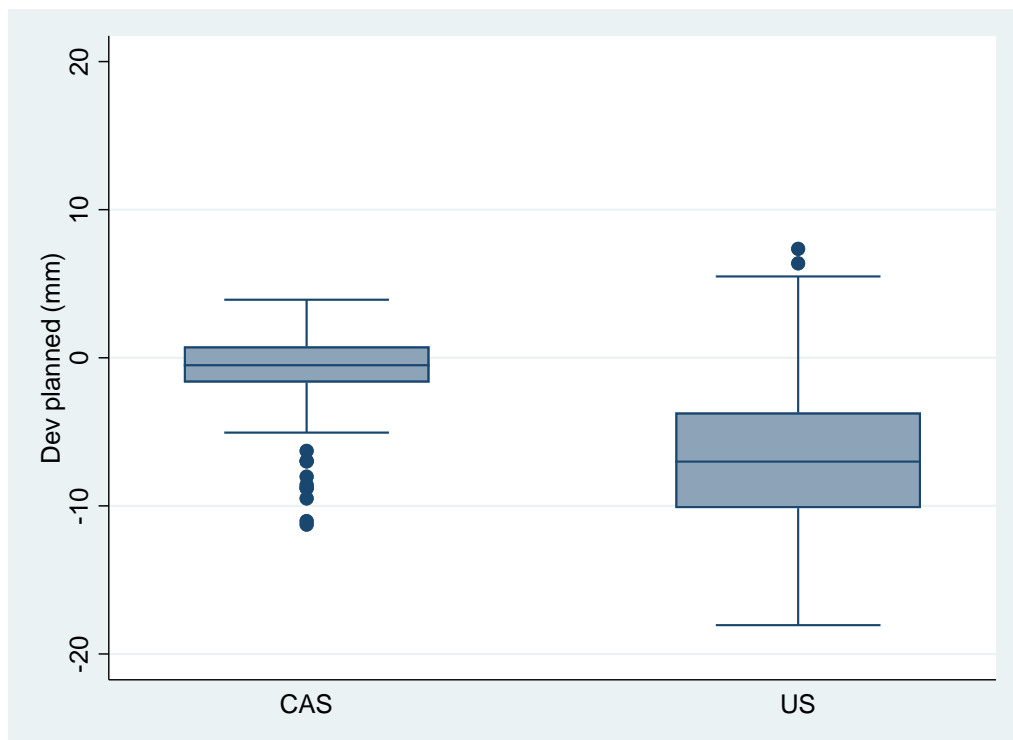
Figure 11: Median deviation in millimetre from the optimal distance between the electrode pairs, CAS-One group and US group.

The interindividual differences between interventionists in each group was tested in a one-sided ANOVA test, and this showed unequal variance. A Tukey's Post-Hoc analysis showed that one interventionist in the US group stood out. Even after excluding this interventionist the median of absolute values 1.3 mm (range 0.0 to 11.3 mm) in the CAS group versus 6.7 mm (range 0.3 to 18.1 mm) in the Ultrasound group (p<0.001).

Also, the difference in angular error differed between the groups with superior accuracy in the CAS-One group, where the mean angle between electrode pairs was found to be 2.7 degrees (95% CI 2.4 to 3.1 degrees) versus 5.5 degrees (95% CI 5.0 to 6.1 degrees) in the US group (p<0.001).

The time for placement of the four electrodes around each tumour was faster in the US group, 6:40 min (95% CI 5:28 to 7:52 min) versus 15:11 min (95% CI 13:05 to 17:18 min) in the CAS-One group (p<0.001). No correlation between time and accuracy was found in either group when analysed separately.

5.3 STUDY III

A total of 60 patients with 84 tumours were treated, 43% were HCC and 36% were CRCLM, in all segments of the liver. The mean tumour diameter was 21 mm and the median number of electrodes used were 4. A total number of 300 electrodes were analysed, see table 7 and figure 12.

Table 7	
Sex, no. of patients (%)	
Male	58 (81%)
Female	14 (19%)
Age (y), mean (±SD)	65,0 ± 11,0
Tumour type, no (%) of tumours	
Colorectal cancer liver metastases	30 (35.7%)
Hepatocellular carcinoma	36 (42.9%)
Cholangiocarcinoma	4 (4.8%)
Liver metastases from CCC	2 (2.4%)
Leiomyosarcoma	1 (1.2%)
Sarcoma	1 (1.2%)
Adrenocortical carcinoma	7 (8.3%)
Pancreatic NET	2 (2.4%)
Tumour diameter (mm), median (min-max)	19 (2-60)
Number of electrodes/tumour, median (min-max)	4 (2-6)
Number of electrodes, total	300
DLP, Patient radiation dose (mGy x cm), mean (±SD)	1654.9 ± 686,0

Table 7: Patient characteristics. Cholangiocarcinoma (CCC), Neuro endocrine tumour (NET), Dose-length product (DLP).
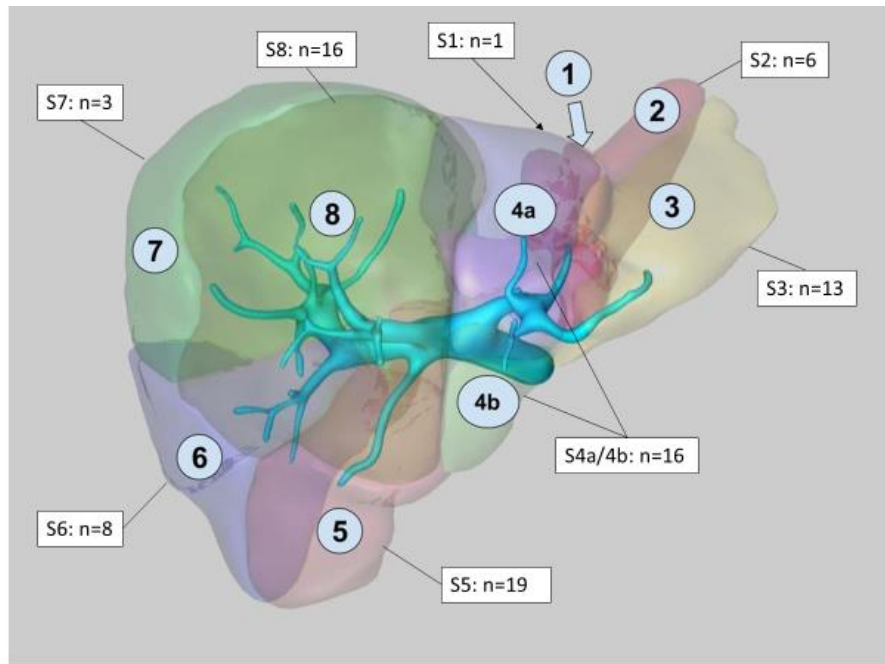
Figure 12: Distribution of tumours in the liver according to liver segments, as defined by Coinaud [126]. S stands for segment, and the digit for liver segment.

Electrode accuracy

Due to missing data and inaccurate registrations several treatments had to be excluded, resulting in 51 treatments with 206 electrodes and 336 electrode pairs for analysis.

Comparing the planned and validated electrode placements, the median lateral error was 3.6 mm (range 0.2-13.6 mm), and the median angular error was 3.1 degrees (range 0.2-18.9 degrees), see figure 13.
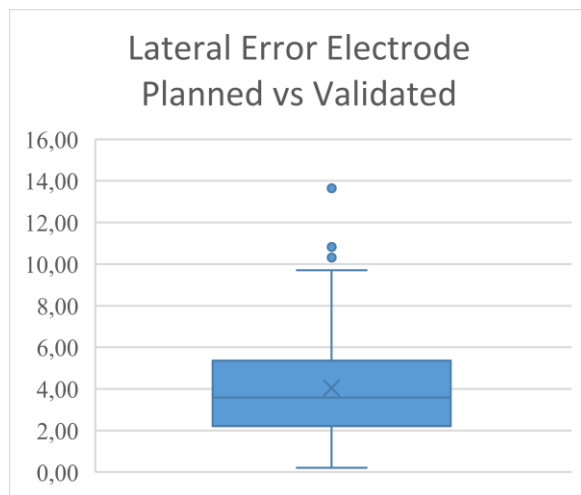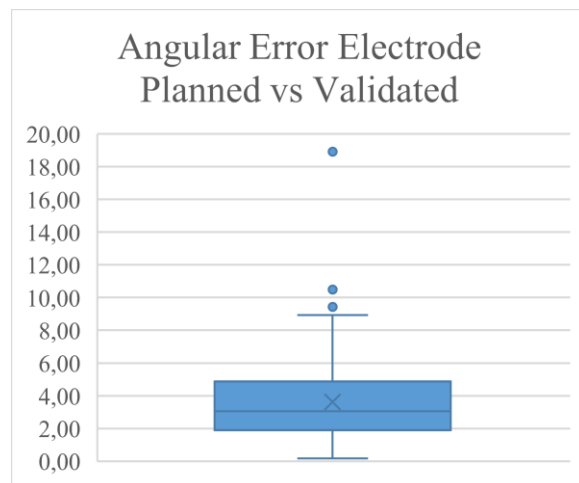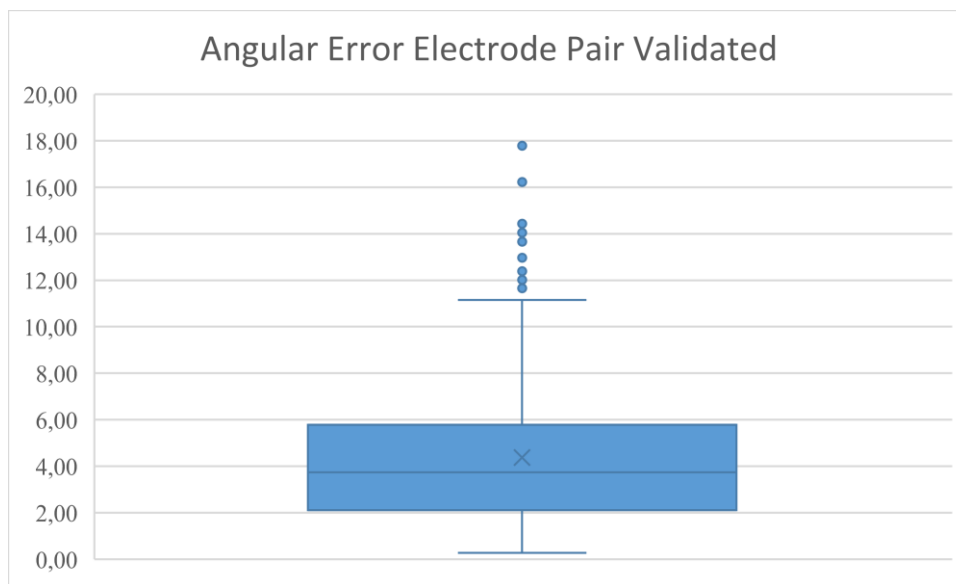

Figure 13a   **Figure 13b**

Figure 13a: Lateral error in mm between planned and validated electrode placement.

Figure 13b: Angular error in degrees between planned and validated electrode placement.

The median angular error between electrodes in each pair, a measurement of parallelism, was 3.8 degrees (range 0.3-17.2 degrees), see figure 14.

Figure 14: Angular error in degrees between two validated electrodes in a pair.

All electrode placements were validated with a CT-scan before treatment. All electrodes with a lateral error >10 mm were considered sub-optimally placed, and either repositioned or excluded from the treatment. None of these patients suffered from any placement related complications.

5.4 STUDY IV

Patients and tumours

In all 183 patients with 257 tumours were treated from May 2011 until December 2018. CRCLM and HCC were the most common diagnoses, 47.5% and 33,9% of the patients respectively. One fourth of the patients had IRE as their primary treatment for their liver tumour, while three quarters were previously treated with an intervention in the liver (resection, ablation or TACE). For patient and tumour characteristics see table 8.

Complications

29 patients (11.3%) had minor complications (Clavien-Dindo grade 1-3a) and four patients had a major complication (Clavien-Dindo grade 3b-5). Among them one death occurred due to a thromboembolic event, one patient with liver failure, one patient with pneumothorax requiring a thoracic drainage, and one patient with a bile duct stricture.

Table 8

Sex, no. of patients (%)	
Male	**140 (76.5%)**
Female	**43 (23.5%)**
Age (y), median (range)	**65 (15 - 87)**
Tumour type, <u>no (%) of</u> patients	
Colorectal cancer liver metastases	**87 (47.5%)**
Hepatocellular carcinoma	**62 (33.9%)**
Cholangiocarcinoma	**8 (4.4%)**
Non-Colorectal cancer liver metastases	**26 (14.2%)**
Tumour type, <u>no (%) of</u> tumours	
Colorectal cancer liver metastases	**133 (51.8%)**
Hepatocellular carcinoma	**73 (28.4%)**
Cholangiocarcinoma	**12 (4.7%)**
Non-Colorectal cancer liver metastases	**39 (15.2%)**
- Adrenal cortex cancer metastases	*8 (3.1%)*
- Small intestine NET metastases	*6 (2.3%)*
- Tubarian cancer metastases	*3 (1.2%)*
- Cholangiocarcinoma metastases	*2 (0.8%)*
- Sarcoma metastases	*2 (0.8%)*
- Haemangioendothelioma metastases	*2 (0.8%)*
- Pheochromocytoma metastases	*2 (0.8%)*
- Tonsillar cancer metastases	*2 (0.8%)*
- Leiomyosarcoma metastases	*1 (0.4%)*
- Pancreas NET metastases	*2 (0.8%)*
- GIST	*1 (0.4%)*
- Pseudomyxoma metastases	*1 (0.4%)*
- Breast cancer metastases	*1 (0.4%)*
- Metastases from cancer of unknown origin	*1 (0.4%)*
- Renal cell cancer metastases	*1 (0.4%)*
- Rectal carcinoid metastases	*2 (0.8%)*
- Papillary cancer metastases	*1 (0.4%)*
- Uveal melanoma metastases	*1 (0.4%)*
Tumour diameter (mm), median (min-max)	**18 (2 - 60)**
Number of electrodes, median (min-max)	**4 (2-7)**
Previous interventions, no. (%) of patients	**138 (75.4%)**
Resection	*78 (42.6%)*
MW ablation	*43 (23.5%)*
RF ablation	*16 (8.7%)*
IRE	*5 (2.7%)*
TACE (if HCC)	*13 (21.0%)*

Table 8: Patient and tumour characteristics. NET=neuroendocrine tumour, GIST=gastrointestinal stromal tumour, MWA=microwave ablation, RFA=radiofrequency ablation, TACE=trans arterial chemoembolisation, HCC=hepatocellular carcinoma.

Overall survival

Median OS from the first IRE was 32.6 months (95% CI: 28.3-42.6 months) for the whole cohort, see figure 15 for survival curves by tumour type.
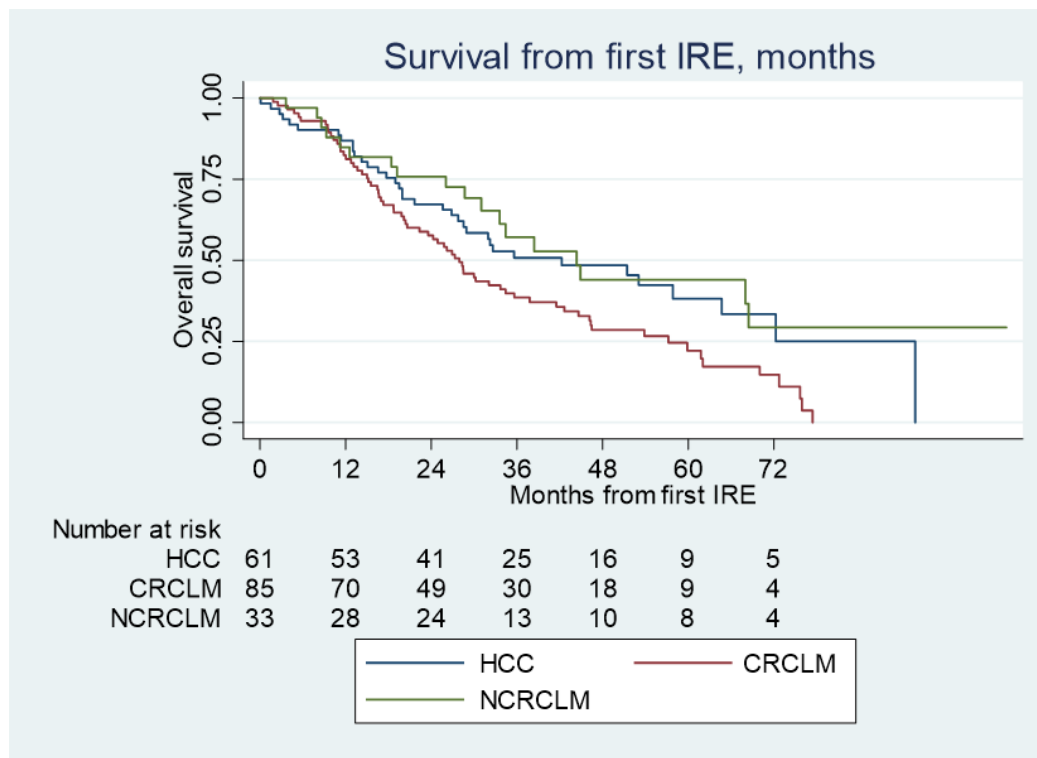
Figure 15: Survival time from first IRE in months by tumour type. HCC=hepatocellular carcinoma, CRCLM=colorectal cancer liver metastases, NCRCLM=non-colorectal cancer liver metastases, IRE=irreversible electroporation.
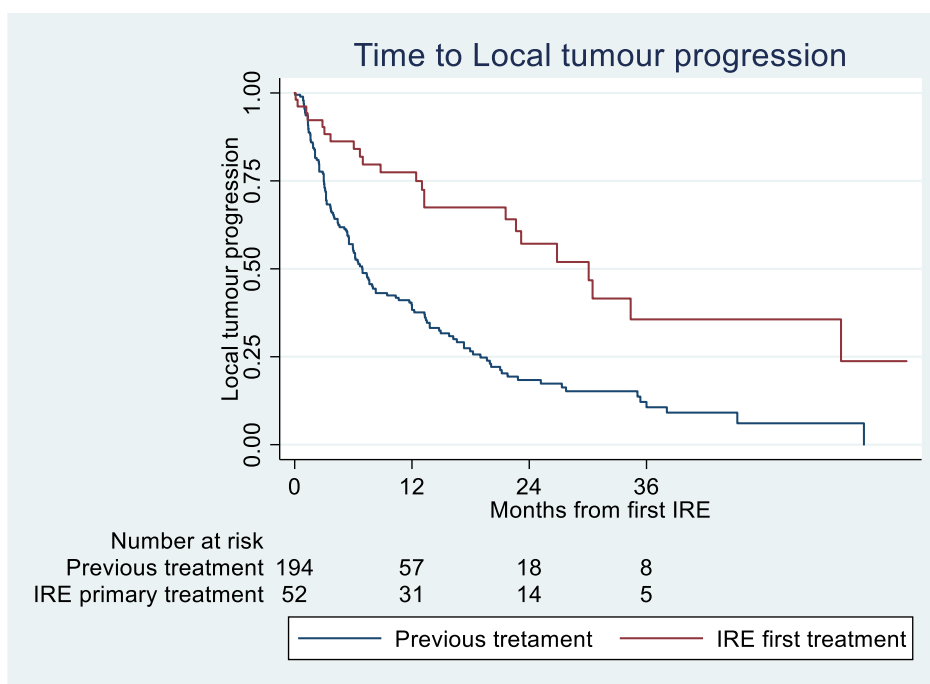
Local tumour progression

The median time to LTP was 21.6 months (95% CI: 13.3-36 months) for HCC and 6.9 months (95% CI: 5.5-10.3 months) for CRCLM, see figure 16.
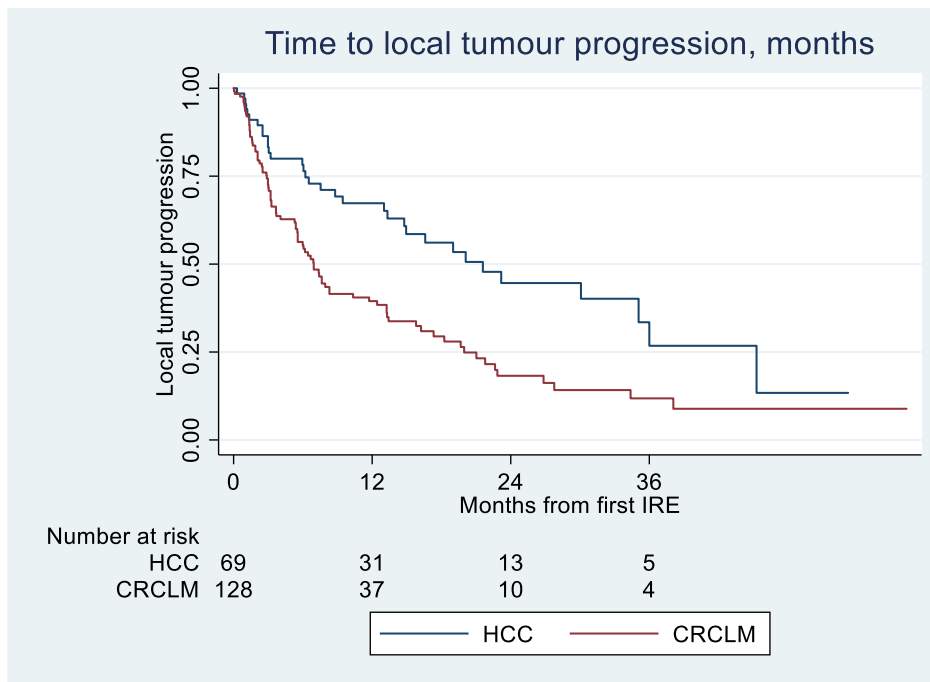
Figure 16: Time to local tumour progression by tumour type. HCC=Hepatocellular carcinoma, CRCLM=Colorectal cancer liver metastases.

A comparison between patients with IRE as first intervention in the liver and those with prior treatments shows a significant longer time to LTP for those without prior treatment (p<0.001), see figure 17.

Figure 17: Time to local tumour progression comparing patients previously treated in the liver with IRE as primary intervention. IRE=irreversible electroporation.

In a Cox-regression model tumour size <20 mm compared to ≥20mm and IRE as primary treatment had longer time to LTP in the CRCLM group, this was not shown with statistical significance for HCC.

## 5.5 STUDY V

Patients and tumours

The same cohort as in *study IV* was used. 183 patients were treated for 257 liver tumours. Patient characteristics are shown in table 9.

Table 9

Sex, no. of patients (%)	
Male	**140 (7.,5%)**
Female	**43 (23.5%)**
Age (y), median (range)	**65 (15 - 87)**
Tumour type, no (%) of patients	
Colorectal cancer liver metastases	**87 (47,5%)**
Hepatocellular carcinoma	**62 (33,9%)**
Cholangiocarcinoma	**8 (4,4%)**
Non-Colorectal cancer liver metastases	**26 (14,2%)**
Tumour diameter (mm), median (min-max)	**18 (2 - 60)**
Number of electrodes, median (min-max)	**4 (2-7)**
Previous interventions, no. (%) of patients	**138 (75,4%)**
Resection	78 (42,6%)
MW ablation	43 (23,5%)
RF ablation	16 (8,7%)
IRE	5 (2,7%)
TACE (if HCC)	13 (21,0%)
Purpose no. (%), tumours	
Curative	232 (90,3%)
Stage 1	20 (7,8%)
Debulking	4 (1,6%)

Table 9: Patient characteristics. MW ablation=microwave ablation, RF ablation=Radio frequency ablation, IRE=Irreversible electroporation, TACE=trans-arterial chemoembolisation, HCC=hepatocellular carcinoma.

Forty-one patients were excluded from the analysis due to missing or incomplete data from the NanoKnife®-system, and another ten patients were lost to follow up with no information more than three days after the procedure, most often due to the patient living abroad. This resulted in 132 patients with 203 tumours eligible for analysis, see figure 18.
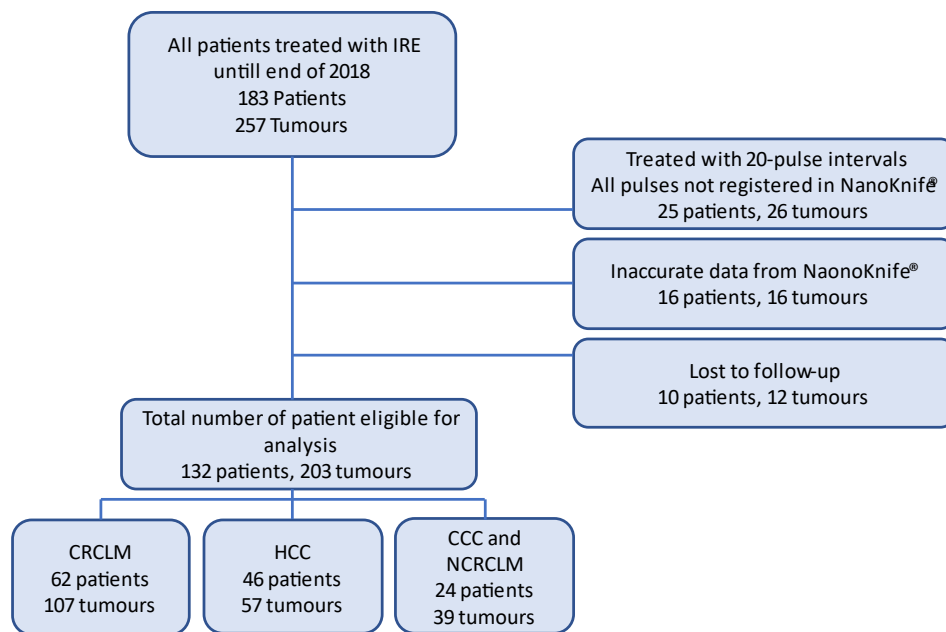
Figure 18: Consort flow diagram showing patients eligible for analyses. IRE=Irreversible electroporation, CRCLM=Colorectal cancer liver metastases, HCC=Hepatocellular carcinoma, CCC=Cholangiocellular carcinoma, NCRCLM=non-Colorectal cancer liver metastases.

Change in resistance and local tumour progression

When analysing the electrode pair with the least decrease in resistance for each tumour, there was a statistically significant correlation between decrease in tissue resistance and time to LTP for CRCLM and NCRCLM, but not for HCC. For CRCLM, there was also a correlation between larger size of the tumour and shorter time to LTP, see table 10.

Cox-Regression analysis, time to Local tumour progression							
	Covariates	Univariable			Multivariable		
		HR	95% C.I.	p-value	HR	95% C.I.	p-value
HCC	Age	1.00	0.96 - 1.05	0.96	1.01	0.97 - 1.06	0.60
	Sex (male vs female)	0.36	0.048 - 2.62	0.31	0.35	0.047 - 2.59	0.30
	Size, mm	1.02	0.97 - 1.07	0.51	1.00	0.95 - 1.06	0.42
	Δ Resistance (Ω)	0.98	0.97 - 1.00	0.11	0.99	0.98 - 1.01	0.13
CRCLM	Age	1.00	0.97 - 1.02	0.74	0.99	0.97 - 1.02	0.60
	Sex (male vs female)	1.40	0.85 - 2.32	0.19	1.32	0.80 - 2.18	0.29
	Size, mm	1.04	1.02 - 1.07	**<0.001**	1.04	1.01 - 1.06	**0.002**
	Δ Resistance (Ω)	0.98	0.96 - 0.99	**0.001**	0.98	0.97 - 0.99	**0.006**

Table 2: Uni- and multivariable Cox-regression analysis on time to local tumour progression.
HCC=hepatocellular carcinoma, CRCLM= colorectal cancer liver metastases.

6 DISCUSSION

6.1 SAFETY AND EFFICACY OF IRE

Study I was a follow-up of the 50 first patients treated at a national referral centre. It showed that IRE treatment is safe and comes with acceptable recurrence rates at short-term follow up. These findings were in line with previously published papers. Few patients had a follow-up time over 36 months. Gupta *et al.* included this study and 24 others in a systematic review that included 776 patients [65]. The largest of these studies, the one by Niessen *et al.* included 71 patients [81]. *Study IV* continues the studies on IRE of liver tumours in clinical practice and is a nationwide multi-centre study that includes 183 patients with 257 tumours and is the largest series published so far, with the longest median follow up, 59.0 months (52.1-65.9 months).

The complication rates found in *study IV* are lower than the reported pooled data from the 25 studies included in the meta-analyses by Gupta *et al*., especially for major complications, 1.2% compared to 7.0% [65].

Only a few studies have presented long-term follow up after IRE of liver tumours. Niessen *et al*. showed a median survival time of 26.3 months for primary malignancies and 19.9 months for liver metastases compared to 38.4 months and 31.0 months, respectively as found in *study IV* [81]. Mafeld *et al*. presented a median survival time of 38 months for patients with both primary and secondary liver tumours compared to 32.6 months (95% CI 28.3-42.6 months) in *study IV* [84]. Schicho *et al.* presented data on 24 patients with CRCLM. Mean OS was 26.5 months (range 2.5-69.2), in *study IV* the mean OS for the whole cohort was 35.1 months (95% CI 29.9-40.3 months) [86]. The presented 5-year survival of 8.3% shown by Schico *et al.*, compared to 22.1% in *study IV*, must be interpreted with caution since the number of patients is low in both studies.

Study I presented a relatively high number of incomplete ablations, seen on the first radiological examination. This could be explained by the location of the tumours close to vital structures in central parts of the liver. This was not addressed in *study IV*, but looking at three months, 25.6% of the cohort had LTP. The majority of these recurrences were due to residual tumour. These numbers will probably tend to be higher compared to thermal ablations because of the location of the tumours treated with IRE and the more demanding electrode placement with multiple electrodes compared to single applicators in thermal ablation.

Re-ablations are common in ablative treatment. In *study I*, 46% of the patients were previously treated with MWA and in *study IV*, 23.5% had a previous MWA, 8.7% had previous RFA, and 43.6% had previously undergone liver resection at least once. For liver resections, despite radical surgery, the recurrence rate for liver metastases is over 50 % [26]. The possibility to re-resect recurrences after previous liver resection is more limited compared to ablative therapies, especially after major resections. Multiple ablations can be

done as a parenchyma sparing procedure with preserved vascular structure in the liver, and IRE has its clear role in treatment of tumours close to heat-sensitive structures in and around the liver.

One study published by Freeman *et al.,* compares local recurrence-free survival (LRFS) between IRE and RFA on previously untreated HCC in a propensity-matched analysis [127]. They found no difference in LRFS between IRE and RFA at 1-, 2- and 5-years, and this study supports IRE as an effective tool in the multi-modal toolbox for treatment of liver tumours.

6.2 ELECTRODE PLACEMENT

To reach the optimal effect of IRE treatment, the correct placement of the electrodes is vital. The placement is more demanding compared to MWA and RFA, where most often one applicator is used, but for IRE the multiple electrodes should be placed as parallel as possible with the active tips at equal depths around the tumour. The power settings in the IRE-system are matched to a given distance between the electrodes. If the electrodes are converging or deviating there is a risk of over- or under-treatment, the former risking higher current with short circuit or heat development, and the latter the risk of to low current leading to reversible instead of irreversible electroporation and thus risk of recurrence. The relationship between parallelism and oncological outcome is not well described in the literature and needs further investigations

For guidance of IRE electrodes, US, CE-US, US fusion with CT/MRI, and CT-guidance is used in clinical practice. These methods are highly dependent on the interventionists experience and skills. Known challenges with all US guided IRE treatments is that the electrode is placed "off target" i.e., outside the tumour, and the limited space when placing multiple electrodes. One disadvantage of CT-guidance is the ionising radiation for both patient and interventionist.

Stereotactic CT-based navigation systems have been used for MWA and RFA with good accuracy [109-111,113-115,128]. The use of navigation systems for IRE treatment has been addressed by Beyer *et al.* in two publications with two different systems, CAS-One system, and a robotic system (Maxio, Perfint Healthcare, Florence, Oregon, USA [112,118]. Both studies showed greater accuracy with the navigation systems compared to CT-guided electrode guidance, with a lower radiation dose. *Study III* is so far the largest study of CT-based stereotactic navigation. The accuracy is measured on thin CT-slices regarding angular error and lateral deviation for each electrode and angular error and the distance between the two electrodes in each pair. This gives an accurate description of the electrode placement, and it is well within the error margin for safe and accurate IRE treatment of tumours, often located in central parts of the liver. On clinical follow up, no electrode-placement related complications were registered.

To compare CT-based stereotactic navigation with US guided navigation, a phantom was used in *study II* where IRE electrodes were placed either with the CT-based navigation

system (CAS-One) or with US. This study shows greater accuracy regarding parallelism and lateral deviation in the CT-based navigation group.

The CT-based navigation system has shown to be safe and accurate in patients and with higher accuracy in the phantom. Another advantage with the system is the ability to plan the electrode placement on the screen where the patient's tumour and surrounding tissues are clearly visible and facilitates a discussion within the team on how to optimise the electrode placement before placing them in the patient. The use of contrast enhanced CT for the scan used for planning also gives a good view of the rest of the liver where new tumours may have developed that will impact the treatment decisions.

Not all patients can be treated with one navigation tool. Some tumours are poorly visible on US or CT, some patients cannot undergo MRI because of metal implants, and some cannot have contrast enhanced CT due to impaired renal function or contrast allergies. There is not one way to perform IRE electrode placement, but if an ablation team have skills and equipment to use several modalities, their chance for successful treatments will increase.

6.3 ENERGY SETTINGS

The placement accuracy of the electrodes is vital for successful treatment, but the settings of the delivered energy must also be optimised. A decrease in tissue resistance has been described as an indicator of treatment success. Dunki-Jacobs *et.al.* have presented a study on local disease-free survival after IRE of pancreatic cancer and showed a correlation between mean change in tissue resistance and risk of local failure or recurrence [129]. They suggest that an increase in current of 12-15 ampere during the treatment can be used as a surrogate marker for change in tissue resistance. Raurs *et al.* studied temperature changes in the ablation zone and change in tissue conductivity, both *in vivo* and in patients with CRCLM [130]. The change in tissue resistance is affected both by the permeabilisation of the cell membrane and the increased heat. They found a positive relationship between change in resistance and time to local tumour progression. Both these studies use the mean change in resistance for the whole tumour. If this is the correct way to measure can be discussed. The IRE treatment can be seen as the sum of the treatment between each pair of electrodes that need to cover the whole tumour. For larger tumours, more pairs are used. The treatment effect on the tumour will never be better than the effect of the worst electrode pair. If a mean effect is calculated for the whole treatment, one single electrode pair with poor effect can be disguised if several electrode pairs have large changes in resistance.

In *study V* the change of resistance for the electrode pair with the lowest change is used in the analyses. A correlation between a decrease in resistance during the treatment and time to LTP could be shown for CRCLM and NCRCLM, but not for HCC.

There seems to be a correlation between decrease in tissue resistance and time to LTP in IRE treatment for liver tumours. Prior to the treatment the voltage is set, and sometimes changed during the procedure. The interventionist then interprets the delivered current to assess if the treatment can be regarded as adequate. Perhaps this should be changed, and the

interventionist should look at the change in resistance instead, since this is affected both by voltage and amperage that changes during the treatment and also during each pulse.

7 CONCLUSIONS

Study I

Irreversible electroporation is a valuable tool in the treatment of liver tumours close to heat-sensitive structures where thermal ablative methods are unsuitable.

Study II

Stereotactic computed tomography-based navigation is more accurate than ultrasound as guidance for placing electrodes for irreversible electroporation in a liver phantom.

Study III

The stereotactic computed tomography-based navigation system is accurate, safe, and user-friendly in placing multiple electrodes for irreversible electroporation in the liver.

Study IV

Irreversible electroporation is safe with acceptable complication rates in this nationwide multi-centre study.

Irreversible electroporation has its clear role in the multi-modal treatment approach for liver tumours when surgery and thermal ablation are unsuitable.

Study V

For colorectal and non-colorectal cancer liver metastases there is a correlation between decrease in tissue resistance and time to local tumour progression, this could not be shown for hepatocellular carcinoma.

The change in tissue resistance, instead of change in delivered current, could be the parameter that the interventionist should assess during the treatment to determine if the treatment is successful.

To find the optimal settings for irreversible electroporation of liver tumours further studies are needed.

8 POINTS OF PERSPECTIVE

Clinical implications

The treatment of liver tumours is a complex task. There are different approaches to surgery, different ablation techniques, TACE, and systemic chemotherapies. All patients should be discussed at a dedicated hepatobiliary multidisciplinary team conference where knowledge in all different treatment options should be represented. Patients that are candidates for ablative therapies should be referred to a dedicated ablation team with radiologists and interventionists that have a broad experience in both different guiding techniques and different ablation techniques.

The introduction of a stereotactic CT-based navigation system needs good cooperation between interventionists and anaesthesiologists since there is a need for a way to avoid movement of the liver during respiration. HFJV is efficient in doing so and has been shown to be safe for the patients [120-123]. Building an ablation team around the patients makes the introductions of new methods easier. Taking help from centres that already use the systems is also valuable.

The optimal settings for the energy deliverance are yet to be found.

Future research

Continuing the work of finding the optimal energy settings is necessary. A study on real-time changes in resistance during pulse delivery could help finding out if this is the most efficient way to assess successful ablation.

The optimal time to follow-up the treatment results is not clear and needs further studies.

IRE is used on tumours where thermal ablation techniques are considered unsuitable and there are no randomised trials comparing the short- and long-term results after IRE and MWA or RFA. The IRE treatment is more time consuming, the electrode placement is more difficult, and the treatment is more expensive. This could be some of the explanations why a randomised study has not been performed. It would however be interesting to compare IRE with thermal ablation for tumours that are easily accessible with a single applicator in a randomised, prospective study where patients with tumours suitable for thermal ablation are randomised to either IRE or MWA/RFA, since there might be a difference in LTP and with easily accessible tumours the procedure time might not be a major issue.

The immunological response after IRE, MWA, and RFA is probably different. The tumour cells are killed through thermal necrosis with thermal ablation, and with IRE through induced apoptosis. There could be a difference in immunological responses depending on what the immune cells are exposed to. A study of this is about to start, with an ethical approval already obtained.

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