From the DEPARTMENT OF CLINICAL SCIENCE,
INTERVENTION AND TECHNOLOGY
DIVISION OF RADIOLOGY
Karolinska Institutet, Stockholm, Sweden

TRANSARTERIAL CHEMOEMBOLIZATION IN PRIMARY LIVER AND KIDNEY MALIGNANCIES

AMAR KARALLI

Stockholm 2019

Published by Karolinska Institutet.
Printed by Eprint, Stockholm, Sweden

ISBN 978-91-7831-329-1

TRANSARTERIAL CHEMOEMBOLIZATION IN PRIMARY LIVER AND KIDNEY MALIGNANCIES

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Amar Karalli

Principal Supervisor:
Professor Rimma Axelsson
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology
Division of Radiology

Co-supervisors:
Associate professor Torkel Brismar
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology
Division of Radiology

Associate professor Per Stål
Karolinska Institutet
Department of Medicine Huddinge,
Unit of Gastroenterology and Rheumatology

Opponent:
Professor Lars Lönn
Rigshospitalet, National University Hospital of Copenhagen
Department of Interventional Radiology
Division of Radiology

Examination Board:
Professor Lennart Blomqvist
Karolinska Institutet
Department of Molecular Medicine and Surgery
Division of Diagnostic Radiology

Associate professor Inger Keussen
Sahlgrenska Academy
University of Gothenburg
Sahlgrenska University Hospital

Associate professor Lars Henningsohn
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology
Division of Urology

ABSTRACT

Hepatocellular carcinoma (HCC) is the most common type of liver cancer, and the most current treatment for unresectable hepatocellular carcinoma is transarterial chemoembolization using drug-eluting beads (DEB-TACE). The concept of TACE might also technically be applicable to other types of hypervascular cancers such as renal cell carcinoma (RCC). The overall aims of this project were to improve the understanding of DEB-TACE as a treatment of unresectable HCC and to assess its feasibility in treating RCC.

By using computerized tomography (CT) imaging before and early after DEB-TACE, in addition to the routine follow-up imaging, it was shown in study I that high arterial and low portal perfusion early after TACE indicated incomplete response with good diagnostic accuracy. Interestingly, it was also shown that portal perfusion of HCC was significantly higher in treated HCC compared to non-treated HCC ($p = 0.01$).

In study II the drug delivery performance, safety, and the grade of necrosis after DEB-TACE were compared to those of an alternative treatment, transarterial infusion (TAI) of doxorubicin-in-lipiodol emulsion. TAI is applied by injecting the emulsion in the vessel supplying a liver lobe without performing an embolization. Free doxorubicin and its metabolites were collected locally by placement of a pigtail catheter adjacent to the orifice of the liver veins and peripherally through standard venous blood samples and in urine samples. It was shown that the release of doxorubicin from the drug-eluting embolic agent was more controlled and prolonged. It was also shown that DEB-TACE caused milder adverse effects than TAI. The overall response (complete and partial response combined) for DEB-TACE was 91% compared to 67% for TAI, but this difference was not statistically significant.

TAI of the doxorubicin-in-lipiodol emulsion followed by embolization of the HCCs' feeding artery is known as conventional TACE (cTACE). In our center a switch from cTACE to DEB-TACE was made in 2009. A retrospective comparison between cTACE and DEB-TACE in this tertiary center (study III) showed no significant difference in overall survival between the two treatments. The adverse effects were significantly less common ($p < 0.05$) after DEB-TACE compared to after cTACE.

In study IV, randomizing 12 patients with RCC eligible for surgery to either DEB-TACE or transarterial embolization (TAE) using the same embolic agent as in DEB-TACE, but unsaturated with doxorubicin, made it possible to evaluate the feasibility of these two techniques in treating RCC and to assess their effect using CT preoperatively and microscopy postoperatively. Both treatments were feasible. DEB-DEB-TACE caused significantly more tumor necrosis ($p < 0.018$ on CT and $p < 0.016$ on microscopy) than TAE. The results of the evaluation by CT correlated significantly with the results of the evaluation by the microscopy ($p < 0.005$), suggesting that CT can be used to evaluate the effect of embolization on RCC. The fact that viable cancer cells were seen on microscopy even when CT showed total necrosis of the treated RCC limits DEB-TACE to palliation.

In conclusion, DEB-TACE changes HCC perfusion, causes fewer adverse effects compared to TAI and cTACE, and can be used to treat RCC.

LIST OF SCIENTIFIC PAPERS

I. Marquez, H. P., **Karalli, A.**, Haubenreisser, H., Mathew, R. P., Alkadhi, H., Brismar, T. B., Henzler, T. & Fischer, M. A. (2017). Computed tomography perfusion imaging for monitoring transarterial chemoembolization of hepatocellular carcinoma. *European Journal of Radiology*, *91*, 160-167.

II. Lilienberg, E., Dubbelboer, I. R., **Karalli, A.**, Axelsson, R., Brismar, T. B., Ebeling Barbier, C., Norén, A., Duraj, F., Hedeland, M., Bondesson, U., Sjögren, E., Stål, P., Nyman, R. & Lennernäs, H. (2017). In Vivo Drug Delivery Performance of lipiodol-Based Emulsion or Drug-Eluting Beads in Patients with Hepatocellular Carcinoma. *Molecular Pharmaceutics*, *14*(2), 448-458.

III. **Amar Karalli**, Johan Teiler, Mojgan Haji, Elin Seth, Torkel Brismar, Staffan Wahlin, Rimma Axelsson & Per Stål.
Comparison of lipiodol Infusion and Drug-Eluting Beads Transarterial Chemoembolization of Hepatocellular Carcinoma in a Real-Life Setting.
Manuscript under submission.

IV. **Karalli, A.**, Ghaffarpour, R., Axelsson, R., Lundell, L., Bozoki, B., Brismar, T., & Gustafsson, O. (2017). Transarterial chemoembolization of renal cell carcinoma: a prospective controlled trial. *Journal of Vascular and Interventional Radiology*, *28*(12), 1664-1672.

CONTENTS

LIST OF ABBREVIATIONS

ALP	Arterial liver perfusion
ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase
ASD	Age standardized incidence rate
AUC	Area under the curve
BCLC	Barcelona Clinic Liver Disease
BMI	Body mass index
CEUS	Contrast enhanced ultrasound
Cmax	Maximum concentration
CR	Complete response
CRP	C-reactive protein
CT	Computed tomography scan
cTACE	Conventional transarterial chemoembolization
DEB-TACE	Drug-eluting beads transarterial chemo embolization
EASL	European Association for the Study of the Liver
ECOG	Eastern Cooperative Oncology Group
HCC	Hepatocellular carcinoma
HI	Hepatic index
ICC	Intraclass correlation coefficient
INR	International normalized ratio
MIP	Maximum intensity projection
mRECIST	Modified Response Evaluation Criteria in Solid Tumors
MRI	Magnetic resonance imaging
PD	Progressive disease
PLP	Portal liver perfusion
PR	Partial response
RCC	Renal cell carcinoma

RECIST	Response Evaluation Criteria in Solid Tumors
ROC	Receiver operation characteristics
± SD	Standard deviation
SD	Stable disease
SIR	Society of Interventional Radiology
T2	Transverse relaxation time
TACE	Transarterial chemoembolization
TAE	Transarterial embolization
TAI	Transarterial infusion
TNM	Tumor node metastasis
VAS	Visual analog scale
WHO	World Health Organization

1 INTRODUCTION

1.1 INCIDENCE OF HEPATOCELLULAR CARCINOMA (HCC)

Liver cancer is a common malignancy (figure 1). HCC represents up to 85% of all primary liver cancers [1]. The incidence of HCC is increasing in Europe and globally [2]. In most cases, HCC occurs in the presence of cirrhosis [2]. Viral hepatitis (B or C), alcoholism, and non-alcoholic steatohepatitis are the most common underlying etiologies for cirrhosis[2, 3]. Vaccination against hepatitis B is recommended to all newborns, and antiviral therapies are recommended in patients with chronic hepatitis before cirrhosis can be established[2].

Diagnosis of HCC can usually be made non-invasively based on history, physical examination, blood tests, and radiology. There is a high diagnostic accuracy in patients with α-fetoprotein >500 ng/dl having a liver mass with a contrast enhancement pattern that suggests HCC as seen on computed tomography (CT) or magnetic resonance imaging (MRI) [3].

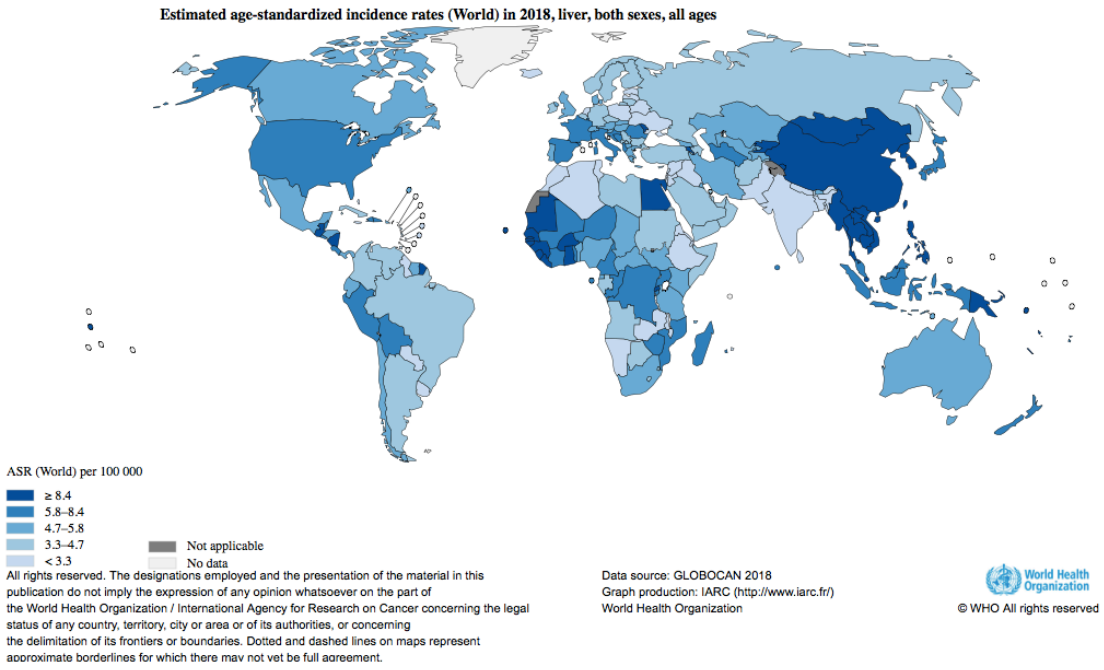

Figure 1. The age-standardized incidence rate (ASR) of liver cancer 2018 (source: GLOBOCAN 2018, Graph production: IARC, http://gco.iarc.fr/today, world Health Organization).

1.2 PROGNOSIS OF HCC

The majority of patients suffering from HCC have an underlying liver disease that can impair liver function, cause symptoms, and affects patient´s survival rate. That is why the prognostic evaluation of HCC must take into consideration the degree of liver function impairment and the cancer-related symptoms in addition to the basic tumor stage. Patient survival is related to the HCC stage, which is also important when choosing the treatment (figure 2). This can explain why survival ranges from 3 months in patients with late-stage HCC to more than 60 months in patients with very early stage HCC.

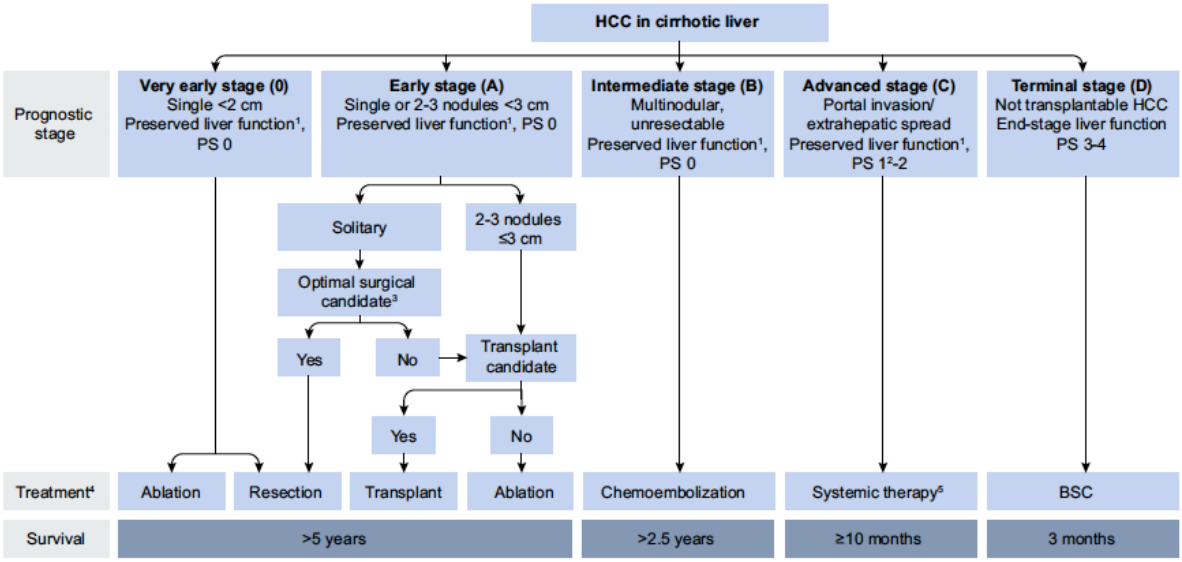

Figure 2. The European Association for the Study of the Liver (EASL) Clinical Practice Guidelines[2]: Management of hepatocellular carcinoma (Hepatology, 2018). PS = Performance status. BSC = Best supportive care.

1.3 STAGING OF HCC

The Tumor, Node, Metastases (TNM) staging system does not provide information about liver function or the symptoms the patient is suffering from. These are two crucial factors when determining which treatment is suitable for a certain patient with HCC. The Child–Pugh system is widely used to grade the impairment of liver function. It divides patients into three groups, A, B and C (Table 1). This system scores blood bilirubin, blood albumin, prothrombin time or international normalized ratio (INR), ascites, and encephalopathy as demonstrated in Table 2.

Table 1. The three classes of Child–Pugh scores according to the Barcelona Clinic Liver Cancer staging system.

Class A	Normal liver function (scoring 5–6 points)
Class B	Mild to moderate deterioration of liver function (scoring 7–9 points)
Class C	Severe deterioration of liver function (scoring 10–15 points)

Table 2. The Child–Pugh scores for five clinical measurements according to the Barcelona Clinic Liver Cancer staging system. Each is scored 1–3.

	1 point	2 points	3 points
Blood bilirubin mmol/L (mg/dL)	<34 (<2)	34-50 (2-3)	>50 (>3)
Blood albumin g/dL	>3.5	2.8–3.5	<2.8
Prothrombin time or INR	<4.0 <1.7	4.0-6.0 1.7-2.3	>6.0 >2.3
Ascites	None	Mild	Moderate to severe
Encephalopathy	None	Grade I-II	Grade III-IV

1.4 PERFORMANCE STATUS

The patient's performance status (PS) is used to grade the symptoms the patient is suffering from. PS is graded from 0 to 5 according to the Eastern Cooperative Oncology Group (ECOG) where grade 0 represents the status of a fully active individual and grade 5 represents the status of a dead individual (Table 3).

Table 3. Performance status system developed by the Eastern Cooperative Oncology Group (ECOG) published in 1982.

GRADE	ECOG PERFORMANCE STATUS
0	Fully active.
1	Restricted in activity but ambulatory and able to carry out work of a light or sedentary nature.
2	Ambulatory and capable of self-care but unable to do any work. Ambulatory more than 50% of waking hours.
3	Capable of only limited self-care. Confined to bed or chair for more than 50% of waking hours.
4	Completely disabled.
5	Dead.

1.5 BARCELONA CLINIC LIVER CANCER (BCLC) STAGING SYSTEM

The BCLC is a staging system consisting of five stages that are determined depending on the size of the HCC, number of lesions, PS, and liver function [4].

- **Stage 0**. Tumor size is less than 2 cm, the individual feels well (PS 0), and the liver function is normal (Child–Pugh A)
- **Stage A**. A single tumor less than 5 cm, or up to three tumors all less than 3 cm. The person feels well and is active (PS 0), and the liver is working well (Child–Pugh A or B)
- **Stage B**. Multiple tumors in the liver, but the person feels well (PS 0) and the liver is working well (Child–Pugh A or B)
- **Stage C**. The cancer has spread into the blood vessels, lymph nodes, or other body organs. Or the individual does not feel well (PS 1 or 2). The liver is still functioning (Child–Pugh A or B)
- **Stage D**. Severe liver damage (Child–Pugh C) or the person is not well and needs help in being looked after (PS 3 or 4).

1.6 DIAGNOSIS OF HCC

The classical presentation of a patient with HCC is upper abdominal pain, weight loss, and blood tests suggesting impaired liver function. Some patients present with acute abdominal catastrophe due to HCC rupture with bleeding. Nowadays many HCCs are detected on ultrasound screening of patients with liver cirrhosis [5], i.e. patients at risk.

1.7 IMAGING OF HCC

Imaging such as CT or MRI is required in order to diagnose HCC, to evaluate the effect of the given line of therapy, and for the follow-up [6]. The CT examination should include a non-contrast phase, a late arterial phase, a venous (parenchymal) phase, and a late venous phase (excretory). As for MRI, a dynamic contrast-enhanced MRI is needed [6]. In the setting of liver cirrhosis, international guidelines have set contrast enhancement in the arterial phase (wash in) and hypoattenuation (CT) or hypointense signal (MRI) in the portal or delayed phase (wash out) as the criteria for HCC diagnosis [5]. Both for CT and MRI the hyper- and hypo- appearances are determined in relation to adjacent parenchyma. In HCC with atypical contrast enhancement (about 30% of all HCCs), biopsy can settle the diagnosis [2,7] .

1.7.1 Contrast enhanced ultrasound (CEUS)

CEUS is not recommended as a first-line modality to diagnose HCC because it is difficult to scan the whole liver during the short arterial phase[2]. Some studies [2,7,8] showed that cholangiocarcinoma sometimes has a similar enhancement pattern (wash in and wash out) and might simulate HCC when using CEUS. Ultrasound is commonly used for surveillance for at-risk patients every 6 months[2, 7], and CEUS can be useful when CT and MRI are contraindicated or inconclusive[2].

1.8 LIVER PERFUSION CT

In 2001, Cuenod et al. published their article “Early changes in liver perfusion caused by occult metastases in rats: detection with quantitative CT” [9]. Since then, liver perfusion CT has been a subject of several studies, some of which were conducted at

Karolinska Institutet. This new technique is basically a repetitive imaging (e.g. 28 scans over 50 s) of the liver to obtain CT images during the contrast enhancement of this organ (and eventual tumors in it) [10-12, 14]. This technique can be used to quantify tumor vascularization (i.e., to measure the enhancement and obtain curves) (figure 3). Placing the marker on the region of interest on the CT image is done manually. The computer will then place the marker on the same region in all other maps (figure 4). The computer calculates arterial liver perfusion (ALP), portal liver perfusion (PLP), and the hepatic perfusion index (HPI), which is equal to ALP / (ALP + PLP) (figure 5).

The evolution toward HCC is characterized by arterialization of the blood supply and sinusoidal capillarization [13]. Quantitative assessment of HCC using liver perfusion CT was shown to have the potential to reduce false-positive findings, thus improving the specificity of HCC diagnosis[14].

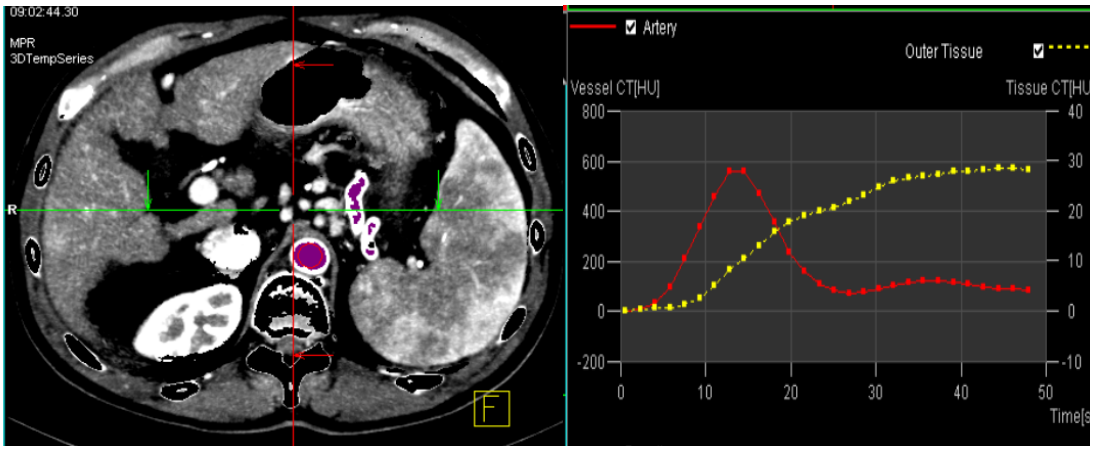

Figure 3. A liver perfusion CT image (left) and the graph (right) obtained. The red line represents arterial enhancement (obtained from placing the marker of the region of interest on the aorta, as shown on the CT image on the left), while yellow line represents the portal enhancement obtained from placing the marker on the portal vein (not shown on this CT image).

The fact that increased attenuation is related to the amount of the contrast uptake by the tissue makes it possible to use conventional contrast-enhanced CT to detect metastasis and HCC in the liver (and other tumors in other organs) where the enhancement at a certain time is captured on an image, e.g. at the arterial phase. Dynamic acquisition of the liver (as in perfusion CT) before, during, and after intravenous contrast administration allows for the recording of changes in attenuation during the contrast perfusion across the capillary basement membrane from the intravascular to the extracellular space. Changes in attenuation when the contrast is in the intravascular space reflect the blood flow, while the attenuation in the same tissue when the contrast is in the extracellular space reflects the blood volume[15]. The liver has a dual blood supply (arterial and from the portal vein). Placing the marker of the region of interest (the software calculates the values within this marker) on an artery (e.g. the aorta) would produce a graphic function showing the changes in attenuation. The same applies to the portal vein (figure 3). Maximum splenic or renal contrast enhancement is used to mark the end of the arterial perfusion and the start of the portal perfusion when calculating ALP and PLP[12].

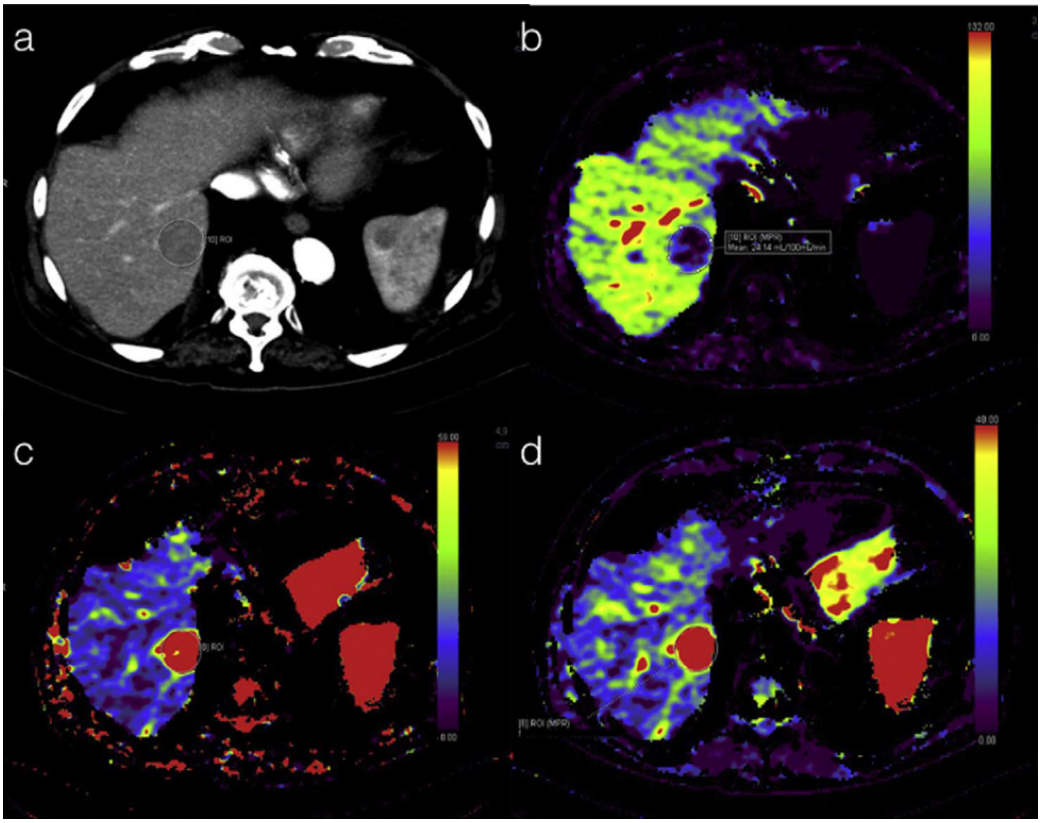

Figure 4. Axial CT images from liver perfusion CT (a). The region of interest (HCC treated by DEB-TACE) is marked by a circle on the PLP map (b). The software marks the same region on the ALP (c) and the HPI (d) color maps.

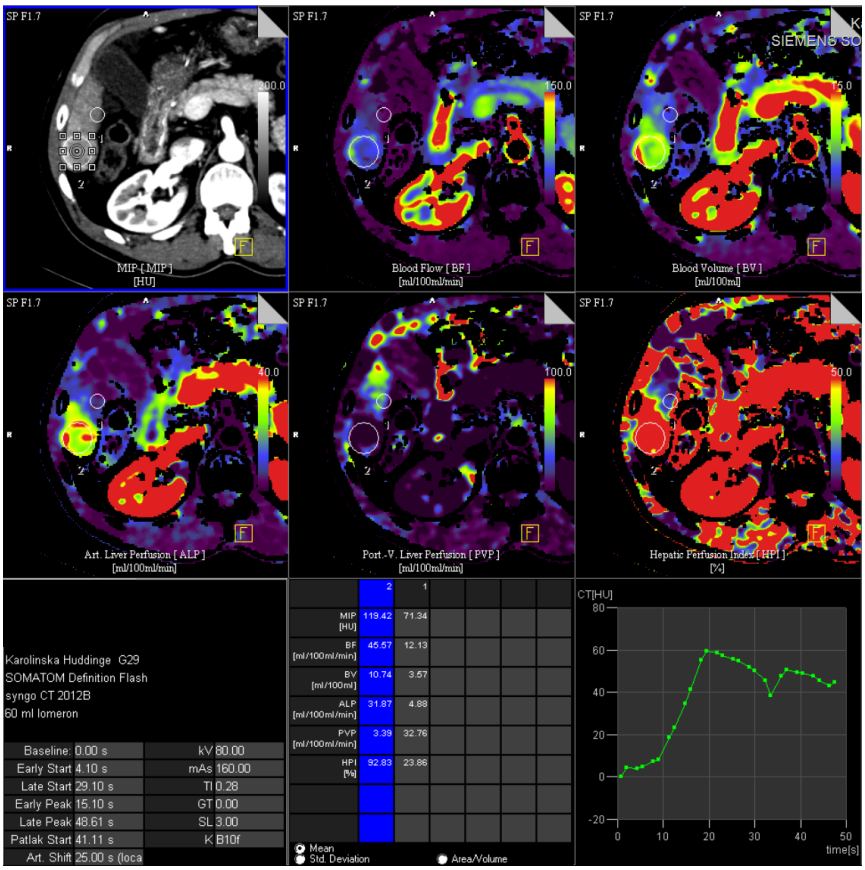

Figure 5. From left to right; upper row: maximal intensity projection (MIP) image, Blood flow color map, Blood volume color map; middle row: ALP color map, PLP color map, HPI color map; lower row: parameters table, values, graph.

When performing liver perfusion CT to diagnose a liver tumor (primary or metastatic) and/or to assess the effect of the given treatment, the contrast concentration should not be less than 300 mg/ml, and the optimal contrast dose is 40–60 ml with an injection rate of 4 ml/second[15].

1.8.1 MRI

MRI has gained increasing importance in HCC diagnostics and for assessing the effect of a given therapy. HCC is known to have a slightly higher signal than adjacent liver tissue on non-enhanced T2-weighted images and to have a hyperintense signal during the arterial phase due to HCC's known hypervascularity [2]. Although the sensitivity and specificity of MRI are similar to or slightly higher than CT, MRI's sensitivity is lowest when the tumor is less than 2 cm in size [3,6].

Diffusion weighted imaging is an MRI sequence that can assess the random motion of water molecules in a tissue. Tumors tend to have high signal intensity on such imaging due to restriction of the motion of water molecules [7].

1.9 TREATMENT OF HCC (IN LINE WITH EASL RECOMMENDATIONS)

Up to 27% of patients with HCC are considered operable. The rest might be candidates for thermal ablation, trans-arterial chemoembolization (TACE), radio embolization, percutaneous ethanol injection, or systemic sorafenib treatment. Of these therapies, TACE is the most commonly used therapy to treat HCC [2], and TACE is recommended for patients with HCC at BCLC stage B (figure 2).

1.9.1 Surgery and transplantation for HCC

Surgical resection is the treatment of choice in patients without liver cirrhosis, without extrahepatic spread, and who present with a solitary HCC. This includes patients with Child–Pugh class A [16]. Liver transplantation is reserved for patients who do not consume alcohol or other addictive substances and in whom the HCC meets the Milan criterion (single HCC with a size of 5 cm or less or 2–3 HCCs each with a size of 3 cm or less). Liver transplantation might be the optimal treatment for patients with Child–Pugh class C because it provides the patient with a non-cirrhotic liver[17]. Surgery and transplantation result in a 5-year survival rate of 60–80% [18,19].

1.9.2 Ablation of HCC

In patients unfit for surgery, ablation is the first line of treatment [2]. There are different techniques of local ablative therapies that are used today, including radiofrequency, microwaves, percutaneous ethanol injection, cryoablation, and interstitial laser coagulation irreversible electroporation. The choice of therapy has been the subject of much research [20, 21]. Of these ablative therapies, radiofrequency ablation is the most-assessed alternative [2]. Surgery and ablation in conjunction are chosen to treat 30–40% of patients with HCC [2].

1.9.3 TACE

TACE is a novel minimally invasive palliative treatment of unresectable HCC. TACE is also used to reduce the HCC burden enabling the patient to wait for liver transplantation[22-24]. TACE has been used to treat colorectal liver metastasis, uveal malignant melanoma liver metastasis, and neuroendocrine liver metastasis[6-8]. By inducing ischemia in combination with having a high intratumoral concentration of a cytotoxic agent, total or partial tumor necrosis can be obtained. This high concentration of the cytotoxic agent cannot be reached by administrating the agent intravenously without severe side effects[25].

The endovascular procedure is carried out under fluoroscopy guidance by an interventional radiologist. A sheath (usually 5 Charrière in caliber) is inserted in the common femoral artery to gain vascular access. Through this sheath, a guiding catheter is passed on a guiding wire and placed with its tip preferably in the common hepatic artery. A micro catheter is then placed through the guiding catheter on a micro guiding wire with its tip in the tumor-feeding vessel(s) (figure 6).

TACE is performed by injecting lipiodol (a poppy seed oil) mixed with a cytotoxic agent, followed by injection of an embolic agent made of an absorbable gelatin sponge (Gelita-spon®, Gelita Medical GmbH, Eberbach, Germany). This procedure is currently named conventional TACE (cTACE) [26]. Drug-eluting beads are an embolic agent consisting of micro particles made of polyvinyl alcohol saturated with a cytotoxic

agent. When the drug-eluting beads are injected via the micro catheter, the procedure is called drug-eluting beads TACE (DEB-TACE) [25]. Both procedures are carried out until total stasis in the feeding artery is reached. The micro catheter can be re-placed in another tumor-feeding artery in order to inject the same mixture/suspension into another vessel supplying another portion of the same HCC or supplying a different HCC.

Micro spheres made of polyvinyl alcohol cross-linked with acrylic polymer can be saturated with doxorubicin. When these micro spheres are injected into the arterial mesh supplying the HCC, they cause ischemia (due to embolization) and a simultaneous intratumoral release of doxorubicin.

The embolic agent (particles) is available in different sizes, including 75–150, 100–300 and 300–500 μm. Recently several manufacturers have even produced 40 μm particles. The particles (2 ml) are saturated with 75 mg doxorubicin at the pharmacy and delivered to the intervention suite in a 5 ml syringe containing 2 ml particles and 3 ml supernatant. The suspension is mixed with contrast agent before it is injected via the micro catheter. This makes the suspension detectable by fluoroscopy and makes the injection more controllable because it is not desired to inject the suspension vigorously and force it to reflux and fill arteries supplying non-tumorous tissue.

Lipiodol is a mixture of di-iodinated long-chain ethyl esterases of fatty acids [27-28]. The fact that lipiodol contains 480 mg iodine/ml makes lipiodol detectable by fluoroscopy. Lipiodol generates a transient embolization of the sinusoidal vessels in animal studies [29,30], and lipiodol has been reported to accumulate in HCCs for up to several months[28].

The capability of lipiodol as a liquid substance to penetrate deeply into the sinusoidal vessels and perhaps also to portal vessels as suggested in animal studies[30- 32] led to the assumption of its superiority to DEB-TACE, where the particles, saturated with the cytotoxic agent, remain in the arterioles. An earlier comparison of doxorubicin pharmacokinetics released from drug-eluting beads with that released from doxorubicin-in-lipiodol emulsion (in transarterial infusion (TAI)) in healthy pigs indicated that the release of doxorubicin when applying TAI is faster and more extensive in vivo than the doxorubicin release from the beads, resulting in higher systemic plasma exposure [32, 33].

The evaluation of the results of the endovascular treatment (TACE/TAI) is carried out using CT or MRI. Because lipiodol delivered to the HCC is radio-opaque and might mask contrast enhancement on CT, MRI is considered to be a better choice to evaluate the effect after cTACE and TAI. Both CT (figure 7) and MRI are suitable to evaluate the effect after DEB-TACE because of the lack of radio-opaque mass in the treated tumor at the time of evaluation, which is preferably four weeks after the endovascular treatment.

There are several systems to evaluate therapy response, including the World Health Organization (WHO) criteria, Response Evaluation Criteria in Solid Tumors (RECIST), modified RECIST (mRECIST), and the European Association for the Study of the Liver (EASL) criteria (Table 4).

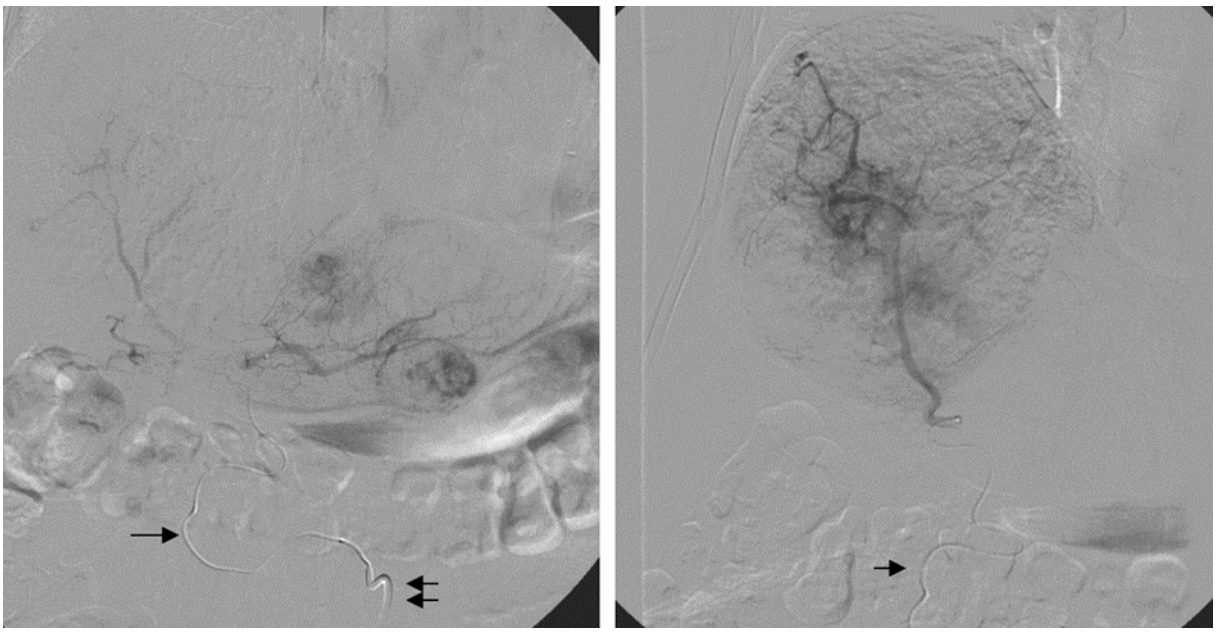

Figure 6. Subtraction angiography images showing the contrast enhancement in three HCCs, the micro catheter (black arrow), and the guiding catheter (double black arrows).

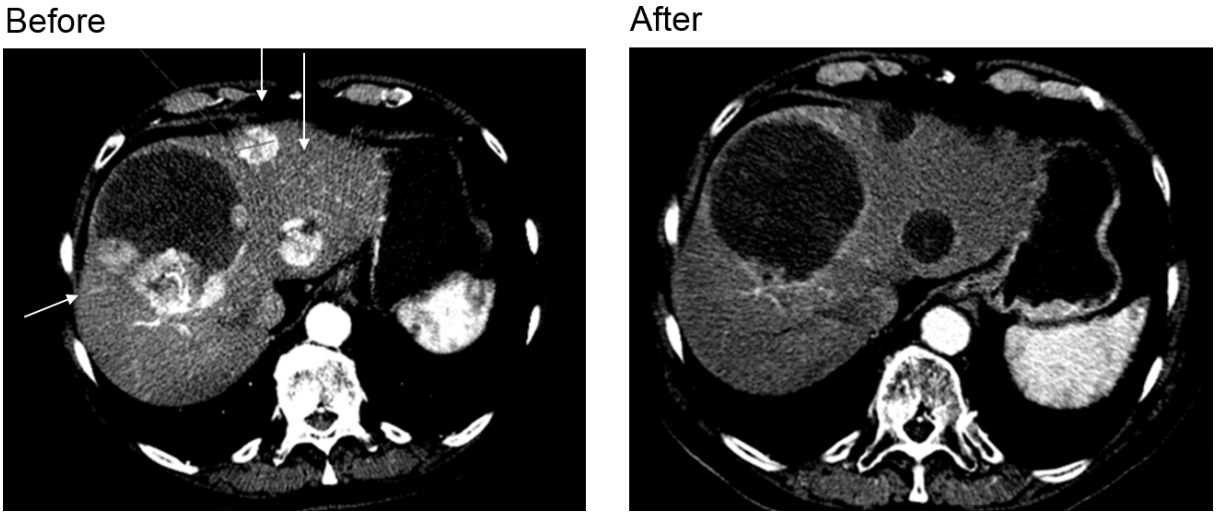

Figure 7. CT images before and after TACE (the same case as in figure 6). The HCCs are indicated by arrows.

Table 4. WHO, RECIST, mRECIST, and EASL criteria

Response	WHO	RECIST	mRECIST	EASL
Complete	Disappearance of the lesions	Disappearance of the lesions	No contrast enhancement in the lesion on CT or MRI	No contrast enhancement in the lesion on CT or MRI
Partial	50% or more decrease in the sum of the cross-products of the target lesion	30% or more decrease in the sum of the diameters of the target lesion	50% or more decrease in the sum of the diameters of the area showing contrast enhancement	30% or more decrease in the sum of the diameters of the area showing contrast enhancement
Stable disease	Neither partial response nor progressive disease	Neither partial response nor progressive disease	Neither partial response nor progressive disease	Neither partial response nor progressive disease
Progressive disease	25% or more increase in the cross-product of target lesions or the appearance of new lesions	20% or more increase in the sum of the diameters	25% or more increase in the sum of the diameters of the area showing contrast enhancement	20% ore more increase in the sum of the diameters of the area showing contrast enhancement

In our center, we shifted from cTACE to DEB-TACE in February–April 2009. The comparison between cTACE and DEB-TACE has been the subject of several studies regarding aspects such as overall survival, toxicity, and adverse effects. The results have differed from showing the superiority of DEB-TACE regarding survival and complications to showing no difference between the two regimes (Table 5A and 5B). None of these studies were conducted in Scandinavian patients.

Table 5A. Examples of studies regarding patient survival after cTACE vs. DEB-TACE

Type of study	Survival (cTACE vs. DEB-TACE)
Prospective studies	No significant difference in overall survival (Reyes et al. 2009, Lammer et al. 2010, Sacco et al. 2011, Van Malenstein et al. 2011, Golfieri et al. 2014).
Retrospective studies	Three studies showed significantly longer survival in patients treated with DEB-TACE (Dhanasekaran et al. 2010, Wiggermann et al. 2011, Nicolini et al. 2013). One study showed significantly longer survival in the cTACE group (Scartozzi et al. 2010). Two studies showed no difference in survival between the two treatments (Megías Vericat et al. 2015, Kloeckner et al. 2015).

Table 5B. Examples of studies regarding adverse events after cTACE vs. DEB-TACE

Type of study	Adverse events (cTACE vs. DEB-TACE)
Prospective studies	Three studies showed fewer adverse events with DEB-TACE (Van Malenstein et al. 2011, Ferrer et al. 2011, Lammer et al. 2010). Three studies showed no difference in adverse events (Golfieri et al., 2014; Sacco et al. 2011; Wiggermann et al. 2011).
Retrospective studies	Four studies showed higher incidences of post-embolization syndrome in the cTACE group (Varela et al. 2007, Megías Vericat et al. 2015, Arabi et al. 2015, Liu et al., 2015). One study showed no difference in adverse events between cTACE and DEB-TACE (Scartozzi et al. 2010).

It is also imperative to mention that TACE generally lacks a standard protocol [34]. Variables like patient selection, preparation of the emulsion, embolization endpoint, use of the supernatant in the syringe, and size of the embolic agent differ from one center to another.

Doxorubicin is a multitarget, broad-spectrum cytotoxic drug that can be delivered intra-arterially, either as a doxorubicin-in-lipiodol emulsion or by injecting polyvinyl alcohol particles saturated with doxorubicin. These particles act as a drug-eluting embolic agent. The polyvinyl alcohol particles incorporate chains of acylamido-2-methylpropane sulfonate sodium salt [35], and the positively charged doxorubicin binds to

the negatively charged sulfonate groups in the particles. The saturation and the release of doxorubicin from this embolic agent (particles) are driven by ion-exchange and diffusion [36, 37, 38].

Early pharmacokinetic studies showed slow release of doxorubicin from the particles with subsequent low systemic plasma exposure [36-38] causing milder doxorubicin-related side effects compared with doxorubicin-in-lipiodol emulsion.

1.9.4 Systemic therapy for HCC

This course of action is considered when other treatments are not suitable, e.g. the presence of distant metastases or tumor thrombus. Sorafenib (which is an oral multi-tyrosine kinase inhibitor) is still considered to be the first-line therapy that can increase the survival by 2.8 months in patients with advanced HCC. This was shown by a double-blinded, placebo-controlled phase III study [40].

1.10 OTHER TRANSARTERIAL THERAPIES FOR HCC

1.10.1 TAI

TAI is also known as transarterial oily infusion and is distinguished from TACE in that no embolization is performed [41]. This is a transcatheter treatment carried out by injecting an emulsion of doxorubicin-in-lipiodol either segmentally (in an artery supplying a liver segment) or lobar (in the right or left hepatic artery) without performing further embolization (apart from the transient embolization caused by the emulsion). This method of infusion makes it possible to treat several tumors in one liver lobe (or in a liver segment), but it also means placing the lipiodol with the cytotoxic agent in the entire right or left liver lobe and exposing a large portion of the cirrhotic liver to the cytotoxic agent. This might lead to further deterioration of liver function.

1.10.2 Trans arterial embolization (TAE)

TAE using particles that are not saturated with cytotoxic drugs is also used to treat HCC and is referred to as bland embolization in the literature. In fact, the advantage of TACE over TAE has been controversial [42]. Some studies have suggested that DEB-TACE and TAE have similar necrotic effects and some suggest that DEB-TACE contributes to longer time to disease progression [43].

1.10.3 Radioembolization

Transarterial radioembolization is performed by injecting a radioactive substance (Iodine-131-labelled lipiodol) [44] or by injecting microspheres containing Yttrium-90 [45] into the hepatic artery. The portion of the radioactive substance reaching the HCC emits low-penetration radiation causing tumor necrosis. This treatment is preceded by a prophylactic trans-catheter coiling of the extrahepatic branches and/or gastroduodenal artery if necessary. After coiling these arteries, 99Tc macroaggregated albumin is injected into the hepatic artery. The patient is then examined using single-photon emission CT imaging to calculate the dose around the HCC and the hepato-pulmonary shunt fraction. The result of these calculations might be the basis on which the treatment would be contraindicated (if dangerously high). One liver lobe is treated at a time.

Although radiation segmentectomy seems very promising, this technique has only very recently emerged and there are no randomized controlled trials testing embolization with particles containing Yttrium-90 [46].

1.11 RENAL CELL CARCINOMA (RCC)

Incidence

The age-standardized incidence rate of kidney cancer globally is shown in figure 8. Approximately 90% of all kidney cancers are RCCs [47], and 20–30% of all patients are diagnosed with metastatic disease[48]. All solid renal masses are suspected to be RCC until proven otherwise. Researchers have established that the incidental detection of renal cell carcinoma is rising because of increased use of imaging procedures [49].

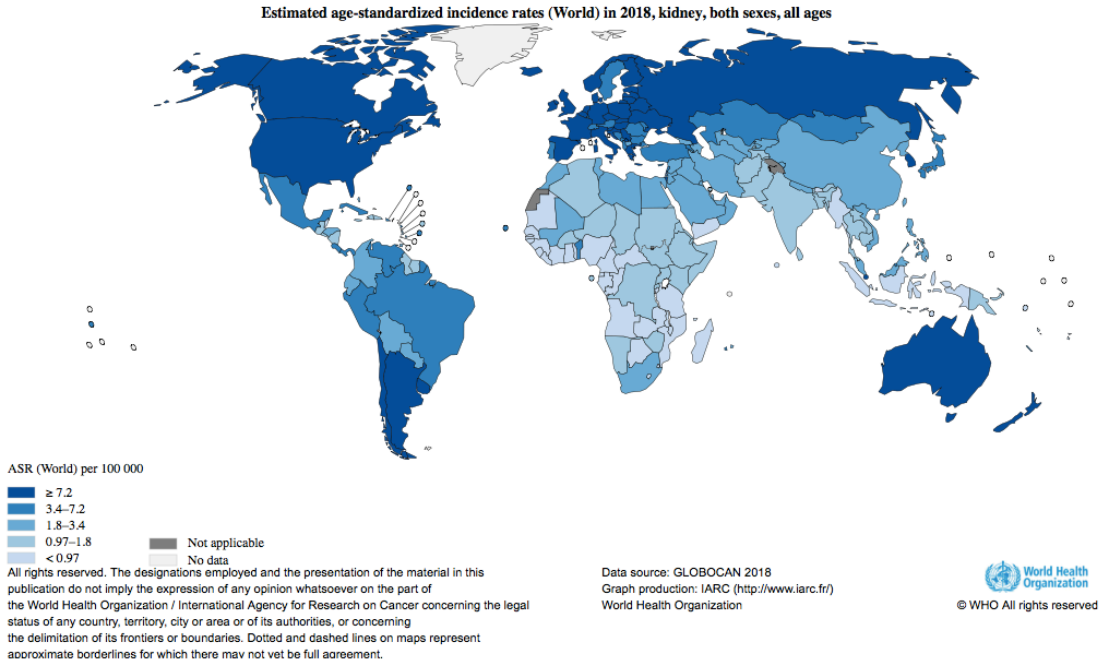

Figure 8. The age-standardized incidence rate (ASR) of kidney cancer 2018 (source: GLOBOCAN 2018, Graph production: IARC, http://gco.iarc.fr/today, world Health Organization).

1.12 DIAGNOSIS OF RCC

The typical presenting symptoms of renal malignancy are flank pain, macro hematuria, and a palpable abdominal mass. These symptoms are correlated with poor prognosis [50]. RCC does not have to be symptomatic in itself, and sometimes the reason the patient seeks care is symptoms related to metastases from RCC such as skeletal pain or respiratory tract symptoms [51].

1.13 IMAGING OF RCC

The modalities of ultrasound, CT, and MRI are used for the detection of renal masses. Contrast-enhanced ultrasound is the first choice in patients with impaired renal function

or when iodinated contrast media or gadolinium are contraindicated. For detecting lung metastasis (and affected mediastinal lymph nodes), CT is the most commonly used modality [52]. Diagnoses of small renal masses using both CT and MRI is based on the contrast enhancement pattern of the renal mass in order to differentiate these masses from non contrast-enhancing renal cysts. In our center we recommend a CT scan protocol that includes non-contrast phase, late arterial phase, parenchymal phase, and excretory phase imaging. Scan start for the phases with contrast media was determined using a bolus-tracking technique, but diagnosing angiomyolipoma with minimal fat component and oncocytoma is still a challenge for radiologists regardless of modality, i.e. CT or MRI [53,54]. All three modalities mentioned above can nowadays be used when applying image-guided therapies.

1.14 BIOPSY OF RCC

Percutaneous needle biopsy is usually efficient to settle the diagnosis of renal masses and helps to avoid unnecessary surgery for benign renal masses. Biopsy helps us to make a treatment decision especially in cases where the patients are not fit for surgery. Core biopsies have high specificity (98–100%) and high sensitivity (86–100%) for the diagnosis of malignancy [54].

1.15 PROGNOSIS AND STAGING OF RCC

Unlike patients with localized RCC, the prognosis for patients with metastasized RCC is extremely poor [48]. Due to the increasing use of radiological imaging, 15–48% of RCCs are nowadays detected incidentally in earlier stages [51]. As mentioned earlier, all renal masses are suspected to be malignant, but cysts and lesions with cystic components are challenging for the evaluating radiologist. The Bosniak classification (table 6) of cystic renal masses is a widely used instrument that categorizes cystic renal masses into five groups based on CT findings. This classification is used to estimate the risk of malignancy and to indicate the management line [55].

Table 6. The Bosniak classification of cystic renal masses [55].

Bosniak class	Risk for malignancy (%)
1	0
2	0
2F	25
3	54
4	100

F = follow-up.

The TNM 2010 classification is still of very high value [56] as a possible tool to assist in choosing between nephrectomy and nephron-sparing surgery. The prognosis of RCC is affected by histological, clinical, and molecular factors [57], and researchers have

suggested that in addition to PS, paraneoplastic signs such as hypoalbuminemia, weight loss, and malaise are significant predictors of worse survival in patients with RCC [56]. R.E.N.A.L score (Table 7) reflects the anatomical factors and their impact on post-treatment complications. R.E.N.A.L scores of 4–6, 7–9, and 10–12 are referred to as low, medium, and high respectively. Higher R.E.N.A.L scores suggest more difficult and more complicated operations. This system can be helpful in choosing between nephrectomy, nephron-sparing surgery, and thermal ablation [57].

Table 7. R.E.N.A.L scoring system [57]

		1 point	2 points	3 points
R	Maximum radius (cm)	≤4	>4 but <7	≥7
E	Exophytic	≥50%	<50%	
N	Nearness/Distance to collecting system (mm)	≥7	>4 but <7	≤4
A	Anterior (A) Posterior (P) Nether A nor P (X)	A, P, or X instead of pints	A, P, or X instead of points	A, P, or X instead of points
L	Location in relation to polar lines	Above upper or below lower polar line	Lesion crosses polar line	>50% of the mass crosses the axial renal midline or is lying between the polar lines

1.16 TREATMENT OF RCC

Localized RCCs are usually managed by nephron-sparing surgery or by radical nephrectomy [58-62]. Active surveillance and thermal ablation have become the preferred choices of management for patients who are unfit for surgery and having small renal masses [63-65]. The size of an RCC, its localization, and the distance between the RCC and other structures, such as the ureter, renal blood vessels, or intestine, are factors that can rule out ablation as a therapy choice [66-68].

The efficacy of embolization to treat bleeding angiomyolipoma and RCC-related hematuria has been studied [69], but earlier studies were not randomized [24] and did not discuss the cytoreductive effect of embolization [70-72].

1.16.1 Systemic and adjuvant therapy

Significant uncertainties remain, although retrospective cohort studies suggest a potential benefit from combining systemic therapies in different orders [73]. Despite the fact that RCC is known to be resistant to chemotherapy [74], the combination of systemic doxorubicin and gemcitabine in treating sarcomatoid RCC has been reported to result in a partial response albeit with a high toxicity profile [75]. Thus, it seems reasonable to study whether doxorubicin delivered at high local concentrations to the tumor combined with embolization of the tumor-feeding vessel, i.e. using the DEB-TACE technique, can overcome the RCC's resistance to chemotherapy [75]. No evidence supporting the use of adjuvant therapy after surgery is available. Radiotherapy is limited to treating pulmonary and skeletal metastasis from RCC [53] mainly to decrease the pain these skeletal metastases cause or to decrease the risk of a pathological fracture.

2 AIMS

2.1 GENERAL AIMS

The aims of this thesis were to increase the understanding of doxorubicin-eluting beads transarterial chemoembolization (DEB-TACE) as a treatment for intermediate stage hepatocellular carcinoma (HCC) and to investigate the possibility of its use in the treatment of renal cell carcinoma (RCC).

2.2 SPECIFIC AIMS

Paper I

To prospectively monitor changes in HCC perfusion early after DEB-TACE and to evaluate possible parameters that might predict the response to the teatment.

Paper II

To study the in vivo delivery of doxorubicin, short-term treatment safety and tumor response after transarterial infusion and DEB-TACE in HCC.

Paper III

To compare DEB-TACE with conventional transarterial embolization regarding overall survival and adverse events in HCC patients.

Paper IV

To assess the feasibility, safety, and cytoreductive effect after DEB-TACE and transarterial embolization to treat localized RCC.

3 MATERIALS AND METHODS

All studies were approved by the regional ethical review board and complied with the Declaration of Helsinki. Informed consent was obtained from all patients except for those included in paper IV (retrospective study) where data were retrieved from patient records.

3.1 PAPER I

This was a prospective dual-center observational study conducted at Karolinska University Hospital, Stockholm, and University Medical Center Mannheim, Mannheim. During the period June 2013–February 2015, 24 patients (15 men and 9 women with a mean age of 69 years; range 54–79 years) eligible for DEB-TACE were recruited.

3.1.1 Inclusion criterion

- Liver cirrhosis with unresectable or multi-nodular HCC eligible for DEB-TACE according to the BCLC criteria [4].

3.1.2 Exclusion criteria

- Atypical lesions, including hypovascular HCC

- Previous radiofrequency ablation

- Previous systemic treatment (e.g. anti-angiogenic therapy)

- Known hypersensitivity to iodide contrast agent

- Glomerular filtration rate below 45 mL/ min

- Contraindications for CT such as pregnancy

3.1.3 The endovascular procedure

DEB-TACE was carried out, after sedation, via femoral access. The tip of a micro catheter was placed in the HCC's feeding artery/arteries and drug-eluting beads with a size of 300 to 500 μm were injected. The treatment was ended when complete stasis of intratumoral arteries (as seen on subtraction angiography) was reached or when the maximum doxorubicin dose of 150 mg had been given. When the maximum dose was reached without complete stasis, particles not saturated with doxorubicin were injected until complete embolization.

3.1.4 Perfusion CT examination

CT examinations were performed before and after the DEB-TACE treatment. A second-generation 64-slice dual-source CT was used to examine all patients (SOMATOM Definition Flash, Siemens Healthcare, Forchheim, Germany). Perfusion CT protocols are detailed in Table 8. The markers of regions of interest were placed in the portal vein and kidney cortex manually [12] in order to be able to discriminate arterial from portal venous perfusion. Perfusion CT examinations were performed before and shortly after the DEB-TACE treatment (mean interval of 12 ± 24.74 hours, range 1–48

hours). In case of repeated DEB-TACE treatments, only the first DEB-TACE was included in this study. To avoid eventual data clustering, only the largest HCC was included when a patient had multiple lesions.

Table 8. CT liver perfusion protocols in study I.

Parameter	Protocol A (n = 10)	Protocol B (n = 14)
Scan width (cm)	14.8–18.2	26.5–34.3
Scan delay (s)	8	8
Number of scans	26–30	18
Time resolution (ms)	75	75
Total examination time (s)	45	32
Slice acquisition (mm)	128 x 0.6*	192 x 0.6*
Tube voltage (kV)	80	80
Tube current (mAs)	175	180
Dose length product (mGy × cm) °	2,520	1,931
Effective dose estimation (mSv)	37	33.3
Contrast media dosage (mL)	50–60	50–60
Flow rate (mL/s)	6	6
Contrast media	Iomeron®, Bracco Imaging SpA, Milan, Italy	Iomeron®, Bracco Imaging SpA, Milan, Italy
Iodine concentration (mg/ml)	300	300

*Using the z-flying focal spot; °conversion factor k = 0.015 mSv/(mGy × cm).

Routine follow-up examinations (CT or MRI) performed to assess the treatments were not modified by the study protocol.

The response was assessed according to EASL criteria. Patients were also subdivided into complete responders (no viable tumor after treatment) and incomplete responders (remaining viable tumor after treatment), and the latter included patients with partial

response, stable disease, and progressive disease as well as those patients who needed to undergo further DEB-TACE treatment.

Quantitative perfusion analysis was performed in one center. ALP (mL/min/100 mL), PLP (mL/min/100 mL), and HPI (ALP/(ALP + PLP in %) maps were automatically calculated by the software Syngo® (CT Body Perfusion, VB10A, Siemens). Perfusion analyses were performed by two independent radiologists each with four years of experience in abdominal radiology. The circular region of interest marker was placed manually on the ALP map. The software copied the region of interest in the same position on the PLP and HPI maps. ALP, PLP, and HPI were calculated for each region of interest obtained, and the percentage difference from pre- to post-treatment values ((post-value – pre-value)/pre-value × 100) were calculated for each parameter, i.e. ΔALP, ΔPLP, and ΔHPI.

3.2 PAPER II

This was an open, dual center prospective study conducted at Uppsala University Hospital and Karolinska University Hospital. Patients admitted to Uppsala University Hospital were treated with TAI of doxorubicin-in-lipiodol emulsion without the use of any embolic agent, while patients treated at Karolinska University Hospital received DEB-TACE treatment using drug-eluting beads saturated with doxorubicin. The study was designed to detect a two-fold difference in the main parameter, AUC 0–7 days, for which a minimum of 11 patients in each group was needed [43].

3.2.1 Inclusion criteria

HCC eligible for TAI or TACE, creatinine ≤ 115 μmol/L, bilirubin ≤ 35 μmol/L, albumin ≥ 28 g/L, white blood cells ≥ 1.5×10^9/L, and INR ≤ 1.7.

3.2.2 Exclusion criteria

Non-measurable or non-assessable HCC, portal vein thrombosis (but not thrombosis of a segment branch of the portal vein), extra-hepatic cancer involvement, contraindications to arteriography or to doxorubicin or other anthracyclines, ascites grades 2 or 3, pregnancy, any systemic or local infections (with the exception of HIV responsive to therapy, hepatitis B virus, or hepatitis C virus), prior treatment with doxorubicin during the last three months, and prior TAI or TACE treatment.

3.2.3 Study events

Each patient was planned for four visits. Table 9 details the events taking place at each visit.

Table 9. Events tacking place at each patient visit in study II.

VISIT	EVENT
1	• Blood sampling and baseline CT or MRI if last examination is older than four weeks
2	• Endovascular treatment with the assigned drug-delivery system. Blood sampling for pharmacokinetic analysis and safety monitoring • Collecting urine (up to 24 h) for pharmacokinetic analysis
3	• Blood sampling for pharmacokinetic analysis and safety monitoring
4	• Follow-up CT or MRI

3.2.4 Preparation of the drug-delivery system

TAI

In this arm, one syringe contained the 3 mL aqueous doxorubicin solution, that is, 2.56 mL of iohexol (Omnipaque® 300 mg I/mL; GE Healthcare, Stockholm, Sweden), which, in addition to being a contrast agent, acts as a densifier to stabilize the emulsion [34], and 50 mg doxorubicin hydrochloride (Adriamycin®; Pfizer Inc., New York, NY) in 0.44 mL of sterile water. The other syringe contained 10 mL of lipiodol (Lipiodol Ultra Fluide®, Guerbet, Aulnay-sous-Bois, France). The syringe contents were mixed manually by pumping the contents from one syringe to the other 10−15 times to get a homogenous doxorubicin-in-lipiodol emulsion.

DEB-TACE

The particles (the drug-eluting beads) were saturated with doxorubicin by mixing 2 mL of particles with 75 mL of doxorubicin aqueous solution (2 mg/mL; Teva Parenteral Medicines Inc., Irvine, California) to reach a doxorubicin concentration of 37.5 mg/mL embolic agent. The suspension of particles saturated with doxorubicin was delivered to the intervention suite as a 5 mL suspension of 2 mL particles and 3 mL supernatant. A mixture of the suspension consisting of particles, normal saline solution, and contrast media (Visipaque 270 mg/mL) at a ratio of 1:2:3 mL was injected into the tumors' feeding vessel(s).

3.2.5 The endovascular procedures

The TAI and TACE procedures were performed under fluoroscopic guidance. A 5 Charrière sheath was placed in the right common femoral artery. Catheterization of the coeliac trunk and the hepatic artery was carried out. The selectivity was chosen as lobar for TAI and superselective for DEB-TACE. Catheterization was performed with either a 4 Charrière catheter or an additional micro catheter. A 7 Charrière sheath was placed in the femoral vein, and a 5 Charrière catheter was then placed through the slightly coarser sheath with its pigtail in the orifice from the hepatic vein to the vena cava. Local

blood sampling was carried out via this pigtail catheter. The sheath in the femoral vein enabled access to the iliac vein for systemic blood sampling during the 6 hours after treatment while the catheter was still in place. The drug-delivery system (the doxorubicin-in-lipiodol emulsion or the suspension of drug-eluting beads saturated with doxorubicin) was administered slowly by hand until complete embolization was reached or until the intended maximum dose was infused (50 mg doxorubicin for TAI and 150 mg for DEB-TACE). The individual doxorubicin dose given by TAI or DEB-TACE was recorded. The choice of size of the drug-eluting beads/agent was based on the size of the vessels in the tumor in each patient. If the maximum dose of doxorubicin was reached in DEB-TACE but not complete stasis, particles without doxorubicin were injected (bland embolization) until complete stasis was accomplished. No additional embolization materials were used in the TAI treatment.

3.2.6 Blood and urine sampling

Samples were collected from a peripheral vein before treatment. Blood samples were collected every10 min (TAI) and 15 min (DEB-TACE) after infusion start until the end of the infusion of the drug-delivery system. After that, samples were collected from the vena cava (via the catheter inserted via the left femoral vein with its pigtail adjacent to the orifices of the liver veins) and from the peripheral vein (femoral vein) at 0, 5, 15, and 30 min and 1, 2, and 6 h. The pigtail catheter inserted via the femoral vein was extracted after 6 h. Additional samples were collected from peripheral veins in the patient's arm at 24 h and again 5–7 days after the treatment. Blood samples were collected in ethylenediaminetetraacetic acid-containing Vacutainers® (4 mL, BD Biosciences, Franklin Lakes, NJ) and centrifuged (10 min, 18°C, 3600 × *g*). The plasma was thereafter transferred to dark polypropylene tubes before storage at −20°C until analysis. Urine was collected directly after the treatment and over the course of the next 24 h. The urine was weighed, and aliquots were collected in dark polypropylene tubes and stored at −20°C until analysis. Because doxorubicin is photosensitive, all samples were protected from light.

3.2.7 Drug analysis and pharmacokinetics

A Waters Acquity UPLC® system coupled to a Quattro Ultima Pt tandem quadrupole mass spectrometer (UPLC−MS/MS, Waters Corporation, Milford, MA) was used for the drug analysis (doxorubicin and its metabolite doxorubicinol). Noncompartmental analysis using Phoenix WinNonLin 6.3 was performed to assess concentration–time profiles for doxorubicin and doxorubicinol. Because the pharmacokinetics of doxorubicin is linear [76], the in vivo pharmacokinetic parameters – AUC and maximum concentration (Cmax) for TAI and DEB-TACE – were dose-normalized (to make the parameters comparable) according to the equation:

$$Dose - normalized\ parameter = \left(\frac{Individual\ parameter}{Individual\ dose}\right) X100$$

3.2.8 Adverse events

Expected serious adverse events requiring additional treatment included liver failure, hepatic abscess formation, and cholecystitis or other life-threatening events. The expected doxorubicin-related events from these were alopecia and renal failure. The expected TAI/TACE-related events included post-embolization syndrome (nausea, pain, and fever), alopecia, renal failure, and liver failure. Monitored blood tests included serum bilirubin, C-reactive protein (CRP), alanine amino transferase (ALAT), aspartate amino transferase (ASAT), alkaline phosphatase, prothrombin-INR, blood count, serum albumin, serum creatinine, blood urea, blood sodium, and blood potassium.

3.2.9 Response assessment

For the TAI group, an MRI (using a routine clinical liver protocol) was conducted 4 weeks after the endovascular treatment. For the DEB-TACE group, a CT (using a routine clinical liver protocol) was conducted 4 weeks after the endovascular treatment. Response was assessed according to the mRECIST criteria.

3.3 PAPER III

This was a retrospective, observational, single center study conducted at Karolinska University Hospital in a real-life setting to compare survival and adverse events of cTACE with DEB-TACE. Patients treated with either cTACE or DEB-TACE during the period January 2004–January 2013, were included. A search for ICD-10 code C220 (liver cell carcinoma) and the intervention registry code PCT20 (transarterial chemoembolization) in our database was conducted. Patients who had received both treatments as well as those who underwent local ablation or liver resection post TACE were excluded from the analysis of overall survival. Survival days were counted from the time of the first TACE treatment until death or censoring. Reasons for censoring were liver transplantation or end of follow-up (28 Nov. 2016).

Only the adverse events after the first TACE were evaluated. Patients with inadequate documentation or incomplete procedures were excluded from the analysis. The patient charts were searched for abdominal pain, nausea and vomiting, fever, fatigue, bleeding at the access site, infection, ascites, bilirubin increase, hepatic encephalopathy, and liver abscess within one week after each treatment. Table 10 lists necessary definitions of some of the retrieved data.

The factors of age (years), sex (male), BMI (kg/m^2), diabetes, liver function, tumor characteristics, and given treatment (cTACE or DEB-TACE) were studied in the univariate analysis.

Factors that had a significant effect on survival were included in a multivariate analysis together with factors known or suspected to affect mortality (sorafenib treatment, bilobar disease, extrahepatic spread of HCC, macroscopic vessel growth, increased ECOG score, increased Child–Pugh class, and alcohol overconsumption).

Table 10. Definitions of some data that was extracted from patients' records

DATA	Definition
Smoking	Current or former smoker / never smoked
Body mass index	Calculated as weight [kg] / height^2 [m]
Alcohol overconsumption	1. A diagnosis of alcoholic liver disease or statement of alcohol overconsumption in patient charts 2. An alcohol intake >30 grams per day (or 14 units per week) for males or >20 grams per day (or 10 units per week) for females
Alcohol moderate consumption	An intake below overconsumption but ≥ one unit per week
No alcohol consumption	Neither moderate nor overconsumption.
Diabetes mellitus type 2	A registered diagnosis in patient charts or a registered prescription of any anti-diabetic medication
Hypertension	A registered diagnosis in patient charts or a registered prescription of any anti-hypertensive medication
Cardiovascular disease	A registered diagnosis (ischemic heart disease, cardiac myopathy, stroke, heart failure, arrhythmia, and coronary valve disease) but not hypertension alone
Liver cirrhosis	A registered diagnosis (biopsy verified, based on elastography >15 kPa) or based on imaging findings

3.3.1 The endovascular procedure

Doxorubicin-in-lipiodol emulsion was injected superselectively or segmentally until a high grade of stasis or a maximum dose of 50 mg doxorubicin was reached when performing cTACE. Then a suspension of contrast and fragments of Gelfoam sponge (with a size of aproximatly1 millimeter) were injected to increase the degree of stasis. DEB-TACE was performed superselectively as a rule, otherwise it was performed segmentally. The DEB-TACE treatment was stopped when either complete stasis or a maximum doxorubicin dose of 150 mg was reached. When the maximum doxorubicin dose was reached but not complete stasis, particles (unsaturated with doxorubicin) of larger size were injected to reach complete embolization.

3.3.2 Response assessment

CT scans (using a routine clinical liver protocol) were carried out 4 weeks after each treatment, and the response was assessed according to WHO, EASL or mRECIST criteria depending on the preference of the evaluating radiologist at the time of treatment. The follow-up CT was presented at a multi-disciplinary conference where the decision to continue follow-up or to offer the patient a new DEB-TACE was made.

3.4 PAPER IV

This was a randomized controlled trial conducted at Karolinska University Hospital. Patients were randomized at 1:1 ratio to receive either TAE using polyvinyl alcohol particles as an embolic agent (Biocompatibles UK Ltd, Surrey, United Kingdom) or DEB-TACE using the same embolic agent saturated with doxorubicin.

3.4.1 Inclusion criteria

Adults of both genders with biopsy-verified RCC less than 7 cm in diameter and eligible for nephron-sparing surgery or radical nephrectomy.

3.4.2 Exclusion criteria

Known hypersensitivity to doxorubicin, pregnancy, recent myocardial infarction (within the last 6 months), glomerular filtration rate < 45 mL/min, and intratumoral arteriovenous shunts detected on subtraction angiography performed before superselective embolization of the vessels feeding the tumors.

3.4.3 Determination of the size of the embolic agent (drug-eluting beads)

Because the particles used in DEB-TACE are available in different sizes, a reproducible methodology to determine the size of the particles for each tumor was needed. Figure 9 illustrates how the size of the particles used was determined.

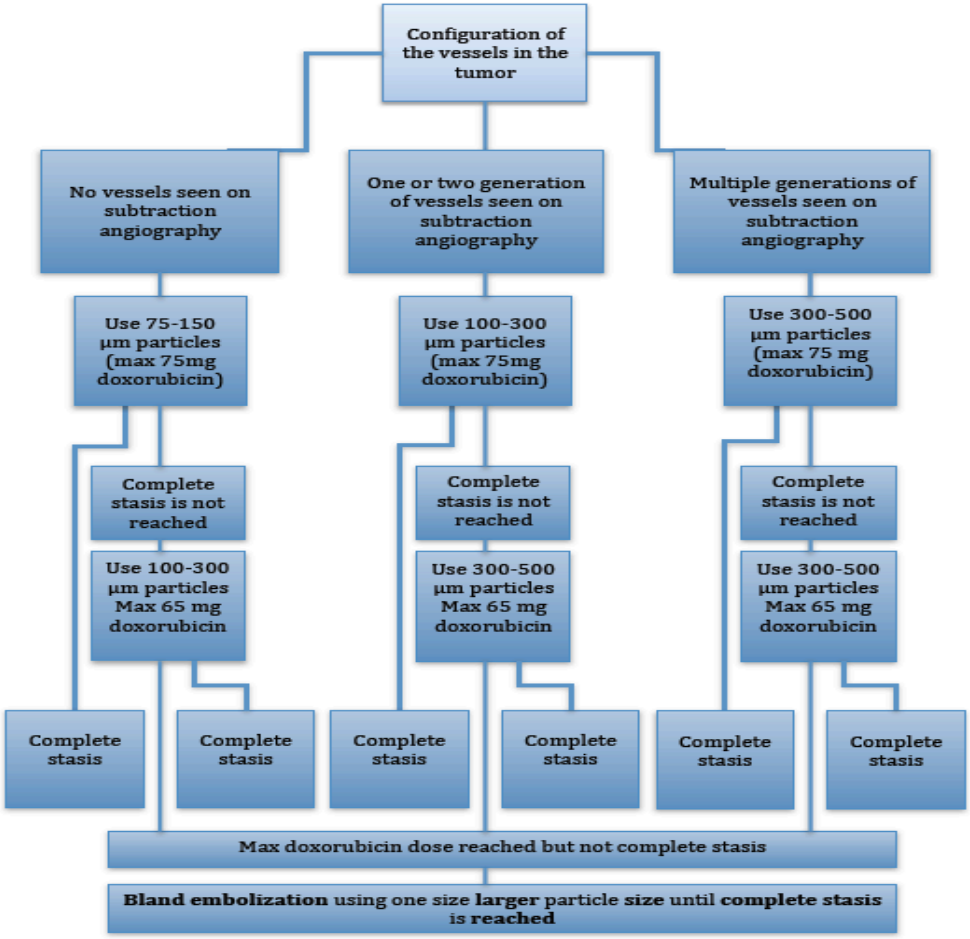

Figure 9. A diagram showing how the size of the particles was determined in study IV.

3.4.4 The endovascular procedure

A 5 Charrière sheath was placed in the right femoral artery after administration of 1% Lidocaine® (10 mg/mL; AstraZeneca AB, Södertälje, Sweden). A 5 Charrière Contra 2 catheter (Boston Scientific Limited, Galway, Ireland) was used to catheterize the renal artery under fluoroscopic guidance (figure 10). Intratumoral arteriovenous shunts were excluded by manual injection of 10–20 mL iodixanol (Visipaque® 270 mg/mL; GE Healthcare, Oslo, Norway). The vessel feeding the tumor was catheterized using a 2.7 Charrière microcatheter (Boston Scientific Limited, Cork, Ireland) (figure 11). A mixture of the suspension of particles, normal saline solution, and contrast media (Visipaque 270 mg/mL) at a ratio of 1:2:3 mL was injected under fluoroscopic guidance until complete stasis in the feeding vessels was obtained or when a maximum dose of 140 mg doxorubicin was reached. If the maximum doxorubicin dose was reached without complete stasis, bland embolization using particles that were one size larger was performed until complete stasis was reached.

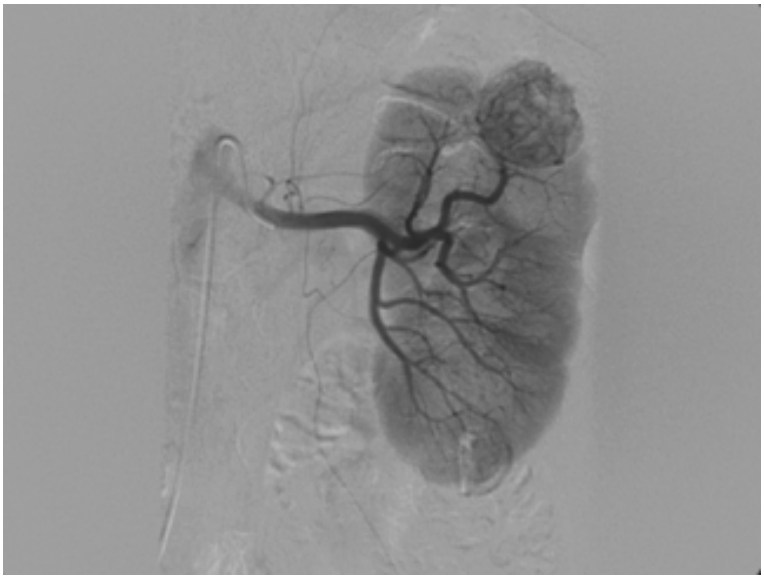

Figure 10. A subtraction angiography image showing the placement of the guiding catheter in the renal artery and contrast enhancement in the left kidney. Notice the round RCC laterally in the upper pole and its feeding vessel.

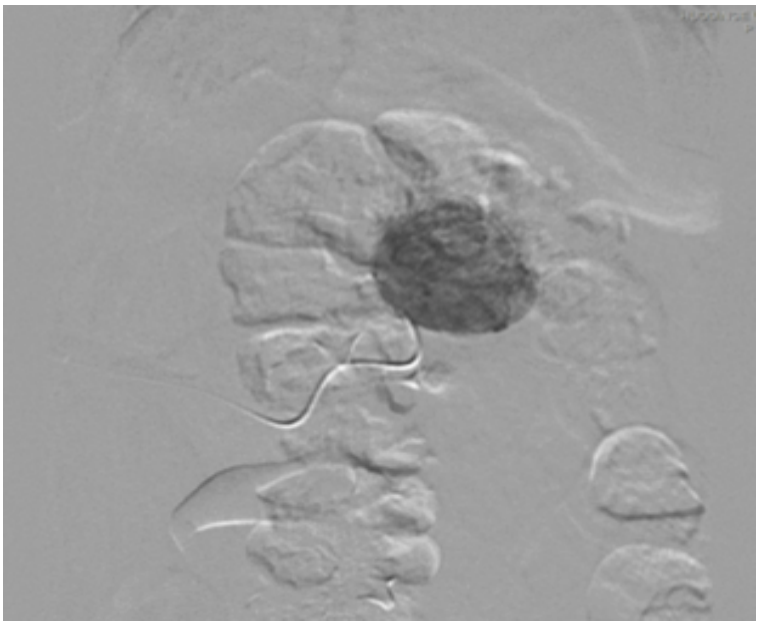

Figure 11. A subtraction angiography image showing the placement of the microcatheter in the tumor's feeding vessel and contrast enhancement only in the tumor.

3.4.5 Safety assessment

All patients were observed for one day after endovascular treatment. Vital signs and routine blood tests (blood hemoglobin, plasma potassium, plasma sodium, CRP, leukocyte count, and plasma creatinine) were assessed before and after endovascular treatment, before follow-up CT, and before surgical resection.

Prothrombin time, INR, and platelet count were assessed before endovascular treatment. Pain was evaluated in the intervention suite according to the visual analog scale (VAS) after endovascular treatment. During the patient´s stay at the urology ward, pain was also assessed when the patient complained of pain and every half hour after analgesia administration until pain relief was reached. Complications were evaluated according to the Society of Interventional Radiology (SIR) classification system [77].

3.4.6 Response assessment

The CT scan performed before treatment was considered as a baseline CT examination. The response was evaluated according to mRECIST on CT 4–6 weeks after the treatments. Necrosis in the excised tumor was also evaluated by light microscopy using the same measuring methodology used for mRECIST. Because the observation time was short (4 weeks), the commonly used term "stable disease" was replaced by the term "no response". The terms "complete response", "partial response", and "progressive disease" were used unaltered. A dual-source 64-row SOMATOM Definition Flash (Siemens Healthcare, Forchheim, Germany) or a 64-row GE LightSpeed VCT XT (GE Healthcare, Milwaukee, Wisconsin) CT scanner was used for all CT examinations. Follow-up CT scans included non-contrast phase, late arterial phase, parenchymal phase, and excretory phase imaging. The startup time for the phase with contrast media was determined by using a bolus-tracking technique that places the marker for the triggering region of interest in the abdominal aorta. The startup time for the late arterial phase was set at a 20-second post-threshold value of 160 HU. An additional 50 seconds

was added for parenchymal phase imaging. Excretory phase imaging was conducted 460 seconds after parenchymal phase imaging. After surgery, all surgical specimens were immediately fixed and embedded in paraffin wax. The specimens were reviewed according to a routine protocol using light microscopy. Necrosis was quantified histopathologically similarly to the mRECIST measurements by dividing the largest remaining viable tumor diameter by the largest tumor diameter multiplied by 100. The remaining viable tumor mass was marked with a pen under the light microscope, as was the pseudo capsule of the tumor, enabling diameters to be measured.

3.4.7 Surgery

Nephron-sparing surgery or nephrectomy was performed laparoscopically or with open surgery depending on the anatomical characteristics of the tumor and the preference of the operating surgeon.

3.5 STATISTICS

Data were tested for normal distribution using the Shapiro–Wilk test. Data were expressed as means ± SD or medians ± ranges. Intraclass correlation coefficients (ICCs) were used for determining the inter-reader agreement. ICC below 0.69 was defined as poor, between 0.70 and 0.79 as fair, between 0.80 and 0.89 as good, and above 0.9 as excellent. Bland–Altman analysis was used to assess the inter-reader agreement. Fisher's exact test (binomial ordinal values), chi^2-tests (polynomial ordinal values), or independent samples t-tests (numeric values) were used to test for significant differences. The Mann–Whitney U-test was used to test for significant differences when data were not normally distributed. The Wilcoxon signed rank test was used for non-parametric data. Student´s t-test was used to test for significant difference when data were normally distributed. Spearman correlation for ordinal variables was performed in order to assess relations between parameters. Receiver operation characteristics (ROC) analysis with area-under the curve (AUC) calculation was performed to estimate the accuracy of the quantitative assessment.

Cox regression models were used to estimate hazard ratios for overall survival. Kaplan–Meier statistics were used to calculate survival curves, which were tested by log-rank test for significant differences in paper III. IBM SPSS Statistics for Mac Version 22; release 22.0.0.1 (IBM Corp®, Armonk, New York) and IBM SPSS Statistics, Version 23.0 (SPSS Inc.®, Chicago, IL) were used for all calculations.

4 RESULTS

4.1 PAPER I

Out of 24 included patients, 21 (88%) had one HCC treated by DEB-TACE. The other 3 patients (12%) had two HCCs treated at the same session, of which only the largest HCC was included in the analysis. The mean size of the included HCC before and after DEB-TACE was 27 ± 11.9 mm (range 14–55 mm).

Routine follow-up examinations to assess the treatments' effects were performed in 22 patients using multi-phase contrast-enhanced CT (n = 12) or MRI (n = 10), and 2 patients were lost to follow up. The mean imaging follow-up time was 47 ± 22 days (range, 24–90 days).

Evaluation according to EASL criteria showed 7 patients with complete response (29%), 9 patients with partial response (38%), and 6 patients with progressive disease (35%). Regarding presence or absence of response, 7 were classified as complete responders (29%) and 15 as incomplete responders (63%). Interreader agreement was fair to excellent (ICC, 0.716–0.942).

Monitoring of blood perfusion of HCC before DEB-TACE treatment for both pre-treated and non-treated lesions showed that;

- Previously DEB-TACE–treated lesions showed a mean ALP of 33.5, PLP of 29.9, and HPI of 63.49, whereas non-treated lesions (lesions never treated in the past) showed a mean ALP of 43.77, PLP of 11.5, and HPI of 82.09.
- PLP of pre-treated lesions (n = 12) was significantly higher ($p = 0.01$) than non-treated lesions (n = 20), whereas no significant difference was seen in ALP or HPI.

Comparing blood perfusion of HCCs before DEB-TACE to after DEB-TACE

- There was a significant increase in PLP (+17%) and a significant decrease in ALP (−53%) and HPI (−25%) after DEB-TACE treatment (each $p < 0.05$).

Outcome prediction

- None of the perfusion parameters before DEB-TACE showed a significant correlation with EASL response grades.
- No significant difference in PLP was seen for non-treated lesions only (mean 17.5 vs. 8.9 mL/min/100mL; $p = 0.19$). All other parameters were similar between non-treated and treated (all, $p > 0.05$).
- PLP before DEB-TACE treatment was significantly higher in complete responders (mean 24.6 vs. 9.3 mL/min/100mL; $p < 0.05$) for all lesions. ROC analysis (figure 12) showed an AUC for PLP for predicting treatment response of 0.762 (95% confidence interval; 0.535–0.989). Using a cutoff HPI value of 43%, we found a sensitivity of 100% and specificity of 66% for predicting response to DEB-TACE.

Early DEB-TACE response assessment

- Both HPI (r = 0.48; $p = 0.02$) and ΔALP (r = 0.45; $p = 0.04$) early after DEB-TACE correlated significantly with EASL response grades (complete response, partial response, stable disease, and progressive disease).
- HPI after DEB-TACE was significantly lower in complete responders (33% vs. non-complete responders 52%; $p < 0.05$). ROC analysis showed an AUC for HPI in detection of incomplete response of 0.795 (95% confidence interval: 0.597–0.994). To obtain a 100% sensitivity for detection of incomplete response, a cut-off HPI of 51% or higher was necessary, resulting in a specificity of 60%.

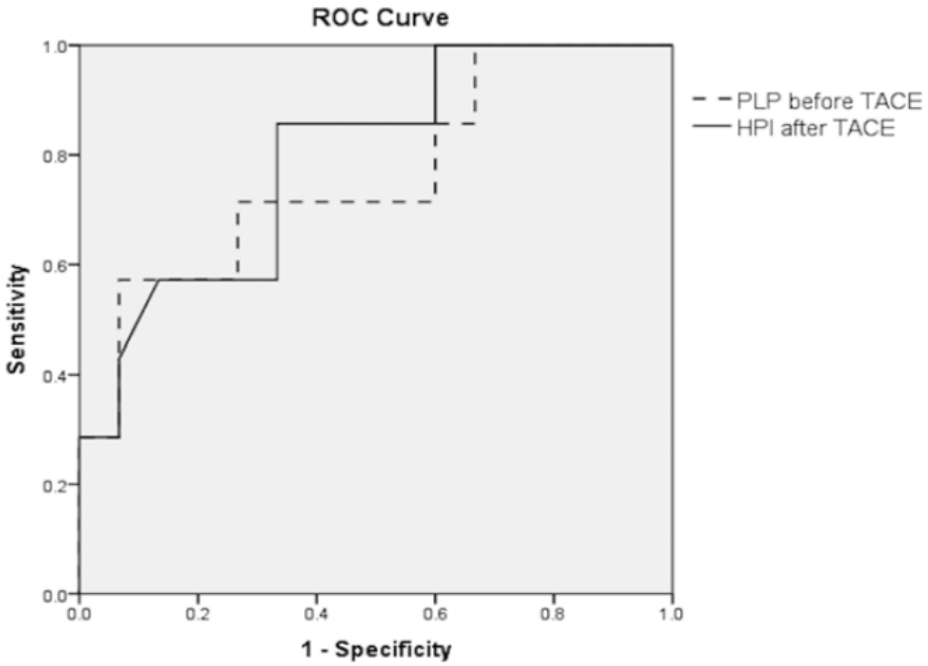

Figure 12. Receiver operating characteristic (ROC) analysis of PLP before DEB-TACE for prediction of treatment response and HPI after DEB-TACE for detection of residual tumor.

4.2 PAPER II

Thirteen patients received TAI (11 men and 2 women; mean age 69 years, range 55–83 years; and mean tumor size 62 mm, range 30–130 mm) and 12 patients received DEB-TACE (9 men and 3 women, mean age 73 years, range 53–85 years, and mean tumor size 36 mm, range 6–65 mm). Two patients in the TAI group were withdrawn because of severe liver failure after the treatment (one patient died). One patient from the same group did not attend the follow-up MRI, leaving 9 patients for response assessment in the TAI group. One patient in the DEB-TACE group did not attend the CT follow-up leaving 11 patients for response assessment. For the pharmacokinetic analysis, 11 patients from each group were included (figure 13).

All patients in the TAI group received 50 mg doxorubicin. The mean infusion time was 10 min (range 2.0–50 min). The terminal half-life (5–7 days) of doxorubicin was similar to that of doxorubicinol, which was 54 ± 8 h. Doxorubicin had a 2-fold longer half-life after 5–7 days in the DEB-TACE group.

Patients in the DEB-TACE group received different doses of doxorubicin depending on the size and the vascularity of the treated tumor. The mean administered dose was 83 mg (range 22.5−150 mg). The mean infusion time was 13 min (range 3.0–34 min). The terminal half-life at 5−7 days was slightly shorter for doxorubicin than for doxorubicinol (99 ± 99 h and 120 ± 53 h, respectively).

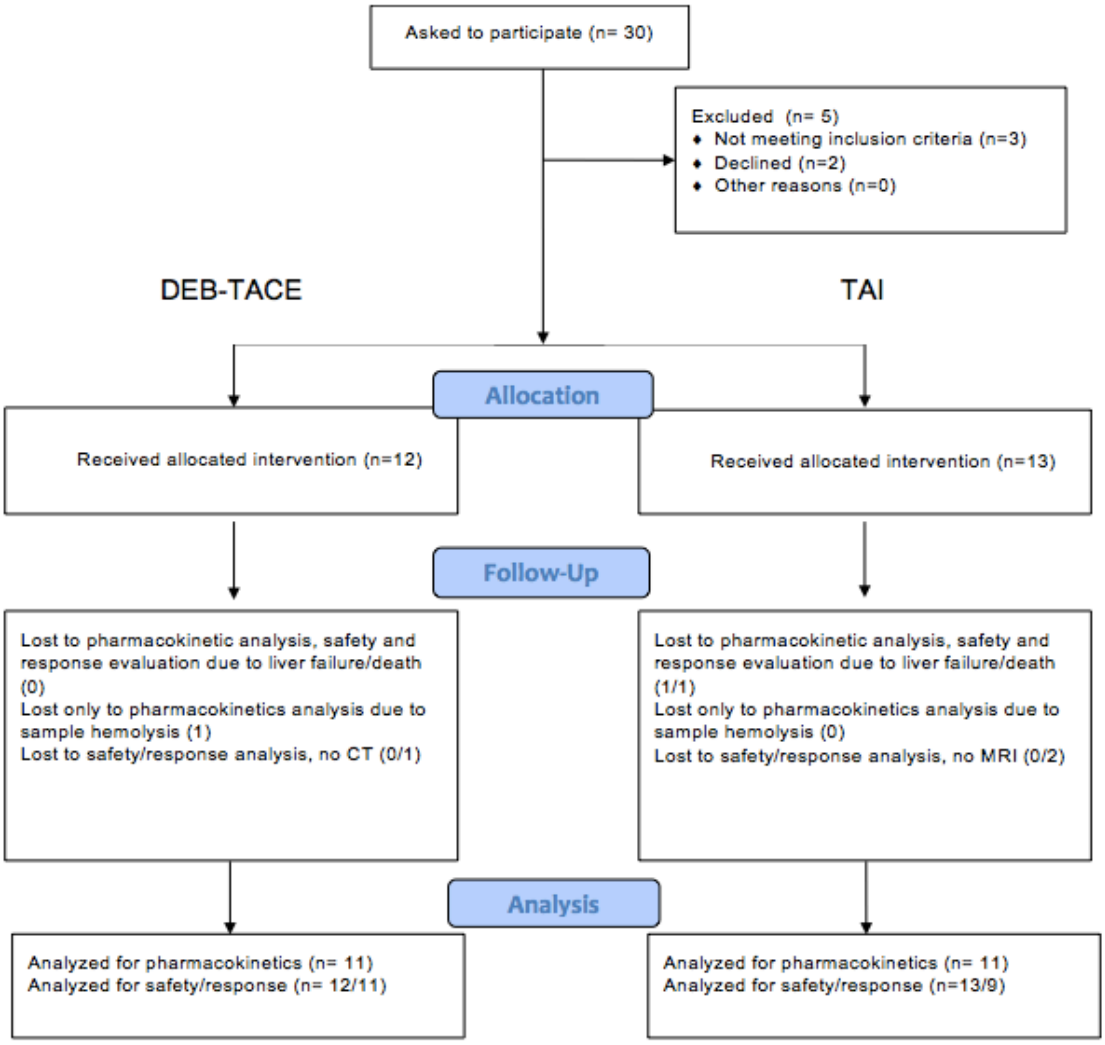

Figure 13. A diagram showing the flow of patients through study II.

The plasma concentration–time profiles and pharmacokinetic parameters for TAI and DEB-TACE were compared after normalizing. The maximum plasma doxorubicin concentration for TAI was 8-fold higher than for DEB-TACE (960 vs. 110 ng/mL,

respectively, $p < 0.001$). The mean AUC (0–24 h) values were 4-fold higher in TAI compared to DEB-TACE (540 vs. 140 h·ng/mL, $p < 0.0001$).

AUC (0−5 days) for both doxorubicin and doxorubicinol was significantly higher with TAI as was AUC (0–7 days) ($p < 0.05$, $p < 0.01$ respectively). AUC (0−7 days) was higher, although not significantly, in the TAI group.

Analysis of samples obtained from the vena cava at the level of the orifices of the hepatic veins showed that the plasma exposure to doxorubicin and doxorubicinol was higher in TAI compared to DEB-TACE (630 vs. 140 h·ng/mL and 130 vs. 15 h·ng/mL, respectively, $p < 0.0001$). The individual ratios of local to systemic plasma doxorubicin concentrations directly after administration (time 0) were 1.57 ± 0.63 and 1.21 ± 0.14 in the TAI and DEB-TACE groups, respectively. This difference lessened with time and stabilized at 15–30 min to around 1.1–1.2 in both groups. The remaining dose of doxorubicin remaining in the liver after 6 h was about 1.8 times higher in DEB-TACE ($p < 0.001$).

4.2.1 Safety

There were no statistically significant differences regarding the monitored safety parameters between the treatment groups. In some patients, the ASAT and ALAT concentrations were more elevated at 24 h post-treatment in the TAI group compared to the DEB-TACE group. However, after 5−7 days the elevated ASAT and ALAT concentrations were approaching their baselines again. The increase in CRP did not differ between the two groups.

In the TAI group, four patients out of 13 (31%) suffered from treatment-induced postembolization syndrome. Two patients (15%) suffered from liver failure, and one of them (8%) suffered later from circulatory arrest. These events led to patient withdrawal from the study. Other documented events were rectal bleeding (n = 1, 8%), bleeding from the venous access site (site for catheter placement) (n = 1, 8%), confusion (n = 1, 8%), and headache (n = 1, 8%). No other events were reported in this group.

In the DEB-TACE group, four patients (33%) developed postembolization syndrome (either demanding or not demanding treatment), one patient (8%) had small gas bubbles in the treated lesion, which were considered to be due to infection, two patients (17%) had back pain, and one patient (8%) had a urinary tract infection. No other events were reported in this group.

4.2.2 Response assessment

Response to treatment was assessed using CT or MRI before and at 4–6 weeks after the endovascular treatment (TAI or DEB-TACE) according to the mRECIST criteria.
Tumor responses are presented in Table 11. The overall response (CR and PR combined) was 91% (n = 10/11) for DEB-TACE and 67% (n = 6/9) for TAI.

Table 11. Treatment Responses According to mRECIST

mRECIST	TAI	DEB-TACE
PD	0 (0 %)	1 (9.1%)
SD	3 (33 %)	0 (0%)
PR	5 (56 %)	8 (73 %)
CR	1 (11 %)	2 (18 %)

PD=progressive disease, SD= stable disease, PR= partial response, CR= complete response.

4.3 PAPER III

The flow of patients through this study is shown in figure 14. The database search revealed 215 patients treated with cTACE or DEB-TACE between January 1st, 2004, and January 15th, 2013. Thirteen patients were excluded due to inadequate documentation or incomplete treatment. Adverse effects were evaluated in 202 patients after the first treatment. The overall survival analysis was performed on 179 patients because another 14 patients were excluded from this analysis because of receiving both treatments (during the period the center switched from cTACE to DEB-TACE). Another 9 patients were excluded due to surgical resection or thermal ablation after cTACE or DEB-TACE. Median follow-up was 7.1 years (range 3.8-12.1).

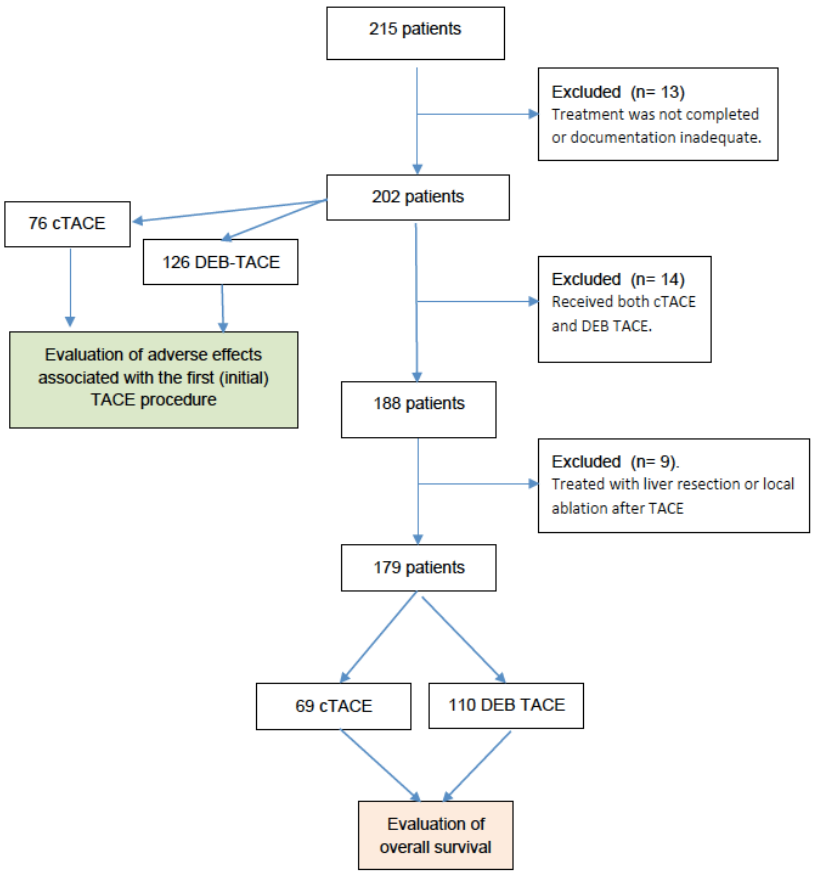

Figure 14. A chart showing the enrolment of patients through study III. Adverse events were evaluated after the first TACE procedure on 202 patients, and overall survival was calculated for 179 patients who received either cTACE or DEB-TACE treatment, respectively.

Patients in the DEB-TACE group were older and had a higher number of tumors ($p<0.05$) compared to those in the cTACE group. There was a larger proportion of patients having BCLC-class C in the in the cTACE group, partially due to macrovascular invasion or extrahepatic spread, compared to the DEB-TACE group ($p<0.05$). Underlying etiologic diagnosis, presence of liver cirrhosis, Child-Pugh class and ECOG performance status did not differ significantly between the two groups.

As for the treatment characteristics of the patient cohort evaluated for survival (n=179), the mean time from diagnosis to start of TACE treatment was 5 days shorter, and the number of treatment sessions was higher, in the DEB-TACE group ($p<0.05$). Pre-TACE ablation was more common in the cTACE group compared with the DEB-TACE group.

The adverse effects (abdominal pain, nausea and vomiting, fever, and fatigue) were significantly less common in patients treated with DEB-TACE compared to cTACE ($p < 0.05$). However, there was no significant difference in the frequency of serious complications such as intra abdominal bleeding, sepsis, ascites, or jaundice ($p = 0.15$). Bleeding from the puncture site (the vascular access) was less common in the DEB-TACE group ($p < 0.05$). Ascites, increase in bilirubin, infection, encephalopathy, and post-treatment abscess were similarly frequent after the endovascular treatments in the two groups.

Median overall survival did not differ significantly between the two treatments (17.1 months and 19.9 months for cTACE and DEB-TACE group respectively).

Total tumor size and increased number of tumors predicted decreased overall survival in the univariate analysis. In the multivariate analysis, adjusted hazard ratios demonstrated that only tumor size, and portal vein thrombosis were associated with decreased overall survival while sorafenib treatment after TACE with increased overall survival.

Figure 15 demonstrates similar all-cause mortality for the first five years of follow-up between patients treated with DEB-TACE as compared to cTACE (log-rank test p= 0.33).

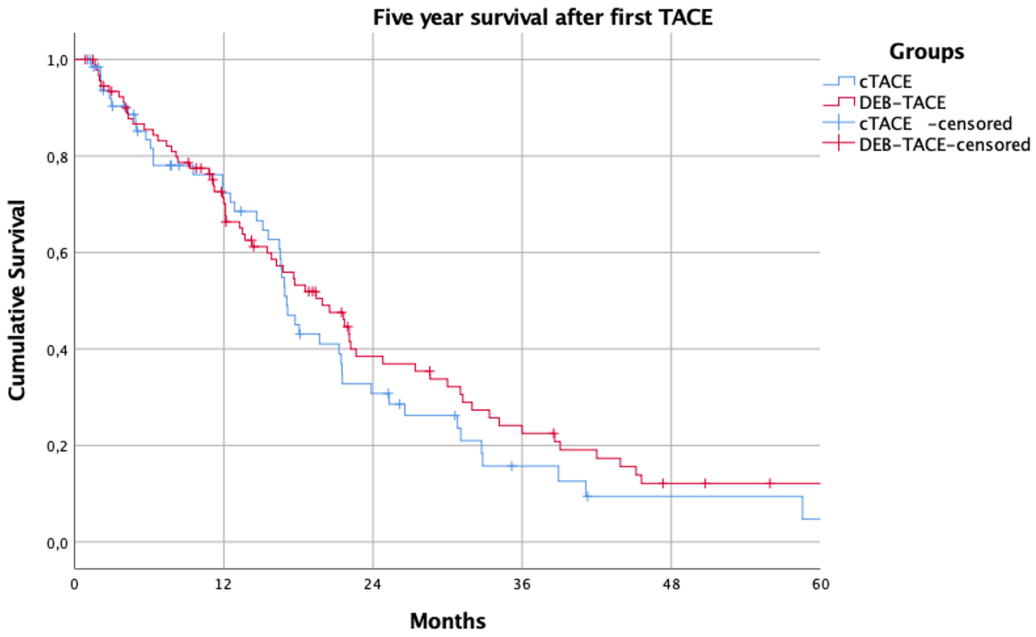

Figure 15. Kaplan–Meier survival curve for the first 5 years separated into cTACE and DEB-TACE groups (log rank p = 0.33).

4.4 PAPER IV

Between 2012 and 2015, 12 patients (5 men and 7 women, age 66 years ± 9.8) were recruited. Mean tumor size was 3.2 cm ± 0.62. Figure 16 shows the flow of enrollment.

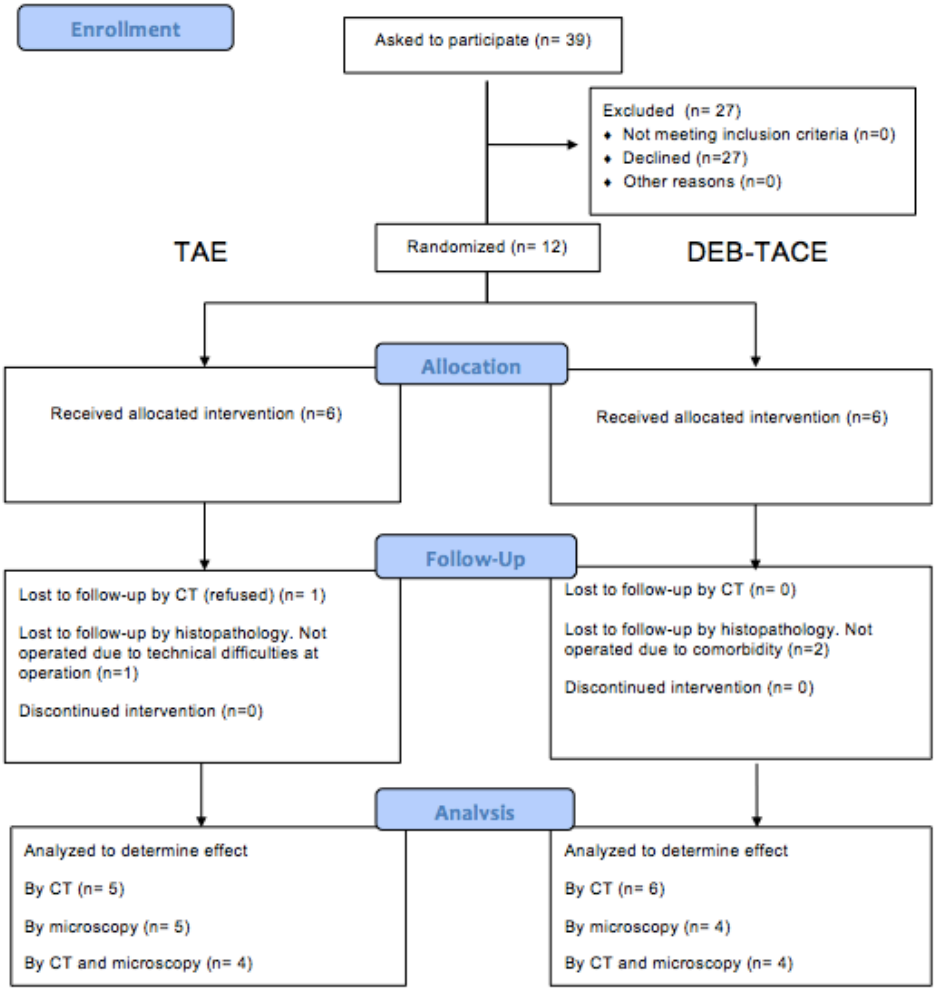

Figure 16. A diagram showing the flow of patients through study IV.

There was no significant difference between the DEB-TACE group and the TAE group in age ($p = 0.17$) or tumor size ($p = 0.52$). Only one tumor was Fuhrman grade 1, and all other tumors were Fuhrman grade 2. Subtraction angiography showed no intratumoral arteriovenous shunts. All patients were treated superselectively until complete stasis was reached. Patients in both arms were treated with particles 100–300 µm in size except for one patient in the DEB-TACE arm who was treated by 100–300 µm particles in the tumors' main feeding artery and 75–150 µm particles in a polar artery. The same patient was treated again, on request and outside the protocol, because of aggravated Parkinson disease that made the patient unfit for either surgery or ablation. This patient was identified as 11a for the first DEB-TACE and as 11b for the second treatment.

4.4.1 Pain assessment

In the TAI group one patient reported pain at the puncture site in the right groin (VAS 4) during closer device deployment in the intervention suite. All other patients reported

discomfort at the puncture site graded VAS 1 or 2. Two patients developed flank pain during the first 24 hours after allocated treatment and while still on the urology ward, both graded as VAS 3. All patients in this group were discharged the day after TAE treatment.

In the DEB-TACE group, all patients reported discomfort at the puncture site graded VAS 1 or 2. Three patients in this group developed flank pain during the first 24 hours after allocated treatment, graded as VAS 3, 5, and 6. Patient 11 had nausea and transient fever (38 C°) after the second DEB-TACE procedure. All patients in this group were discharged the day after the endovascular treatment.

4.4.2 Post treatment blood tests

There was a significant increase in leukocyte count ($p = 0.01$) and a non significant elevation of CRP in the DEB-TACE group. An initial plasma creatinine elevation from 65 to 80 μmol/L was observed in patient 11 after the second DEB-TACE. Patient 11's plasma creatinine had normalized 4 weeks later.

All blood tests were normal after the allocated treatment, before follow-up CT, and before surgery. No patient had major complications according to SIR classification of complications.

4.4.3 Surgery

In the DEB-TACE group, Patient 11 was disqualified for surgery (as previously mentioned). Patient 7 declined surgery and chose surveillance after being informed that a complete response was observed on CT evaluation. Local tumor recurrence was identified on CT in this patient 26 months later, and the patient was scheduled for operation. Therefore 10 patients out of 12 underwent surgical exploration. Of these, eight had nephron-sparing surgery and one had nephrectomy. One patient in the TAE group did not have the tumor removed (abandoned surgery due to multiple peritoneal metastases from a gastrointestinal stromal tumor, which complicated the surgery).

4.4.4 Response assessment

Patient 2 in the TAE group declined to undergo CT evaluation after endovascular treatment. Patient 11 did not proceed to surgery due to deterioration in performance status and received, on request, a second DEB-TACE. In this patient, necrosis increased from 70% to 100% after the second DEB-TACE procedure. Because the second DEB-TACE was outside the study protocol, the necrosis assessment was excluded from all statistics. Infarcted renal tissue was observed adjacent to the treated tumor on follow-up CT in all patients (figure 17 and 18). These infarcted areas were seen on angiography sharing blood supply with the tumors.

Tables 12 and 13 show the results of CT evaluation according to mRECIST and the percentage of necrosis seen on CT and on microscopy. CT showed that patients in the DEB-TACE group (n = 6) had significantly ($p = 0.018$) higher degree of necrosis compared to TAE (n = 5). The histopathologic evaluation showed similar results ($p = 0.016$). Percentage of necrosis seen on microscopy correlated significantly ($p = 0.0005$) with the radiological findings ($n^1 = n^2 = 4$).

Table 12. Patient Characteristics, Tumor Size, and Results in the TAE group

Participant	Gender	Age (years)	Tumor size (cm)	Biopsy report	mRECIST	Necrosis shown on CT (%)	Necrosis shown on microscopy (%)	Size of DEB(µm)	ECOG
2	M	55	3.0	RCC F2	-	-	0	100-300	1
3	M	69	3.0	RCC F2	NR	0	0	100-300	2
4	F	48	3.0	RCC F2	PR	77	50	100-300	1
5	F	74	3.0	RCC F1	NR	0	20	100-300	1
8	F	68	3.5	RCC F2	NR	0	-	100-300	2
9	M	64	3.0	RCC F2	PR	70	60	100-300	0

Abbreviations: M = male, F = female, RCC F = Renal Cell Carcinoma Fuhrman, mRECIST = modified Response Evaluation Criteria in Solid Tumors. PR = partial response, NR = no response. ECOG= Eastern Cooperative Oncology Group score for performance status.

Table 13. Patient Characteristics, Tumor Size, and Results in the DEB-TACE group

Participant	Gender	Age (Years)	Tumor size (cm)	Biopsy report	mRECIST	Necrosis shown on CT (%)	Necrosis shown on microscopy (%)	Size of DEB(µm)	ECOG
1	M	62	2.0	RCC F2	CR	100	95	100-300	1
6	F	52	3.0	RCC F1	PR	90	95	100-300	1
7	F	77	3.5	RCC F2	CR	100	-	100-300	3
10	M	69	3.0	RCC F2	PR	80	80	100-300	1
11a	F	77	4.5	RCC F2	PR	70	-	75-150, 100-300	3
11b	F	77	4.0	RCC F2	CR	100	-	75-150, 100-300	3
12	F	74	4.0	RCC F2	PR	90	80	100-300	2

Abbreviations: 11a = participant 11's first TACE, 11b = Participant 11's second TACE, M = male, F = female, RCC F = Renal Cell Carcinoma Fuhrman, mRECIST = modified Response Evaluation Criteria in Solid Tumors, CR = complete response, PR = partial response. ECOG = Eastern Cooperative Oncology Group score for performance status.

The necrotic areas seen on microscopy were too small to be outlined by a pen and were scattered on the specimen slides from patient 3; therefore, the maximum diameter of the tumor was unchanged and the percentage of necrosis was zero when evaluated by microscopy. For similar reasons, the RCC in patient 5 had an unchanged diameter after TAE, whereas microscopy showed 20% necrosis. On the other hand, even when no

remaining tumor region could be detected on CT, some isolated individual viable tumor cells could be identified using light microscopy.

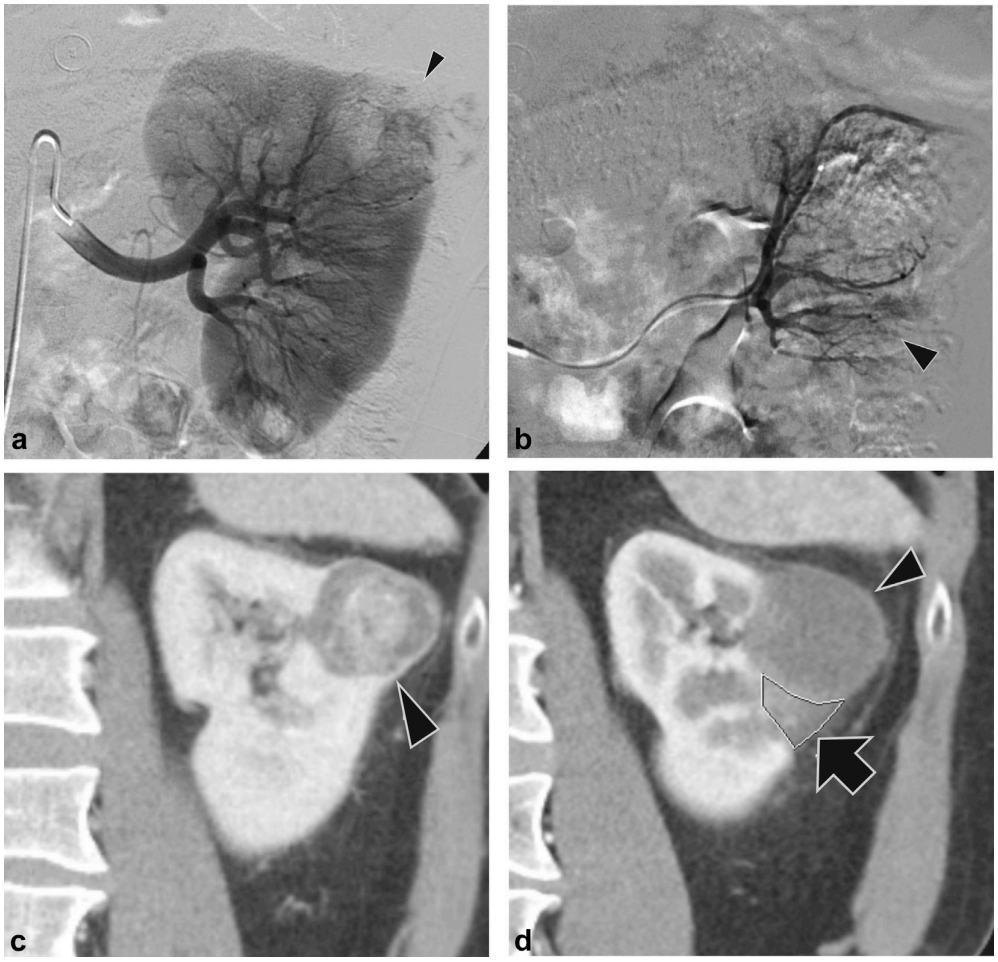

Figure 17. Images of RCC treated by TACE in patient 12. (a) Subtraction angiography image obtained after selective catheterization of the left renal artery showed contrast-filled intratumoral vessels (arrowhead) before TACE. (b) Subtraction angiography image obtained during TACE showed no contrast-filled vessels in the center of the tumor owing to ongoing embolization. The area adjacent to the RCC (arrowhead) shares its blood supply with the tumor. (c) CT image obtained before TACE showed contrast enhancement in the RCC (arrowhead). (d) CT image obtained 4 weeks after TACE showed no contrast enhancement in the RCC (arrowhead). Infarcted renal tissue (arrow) is adjacent to the treated tumor.

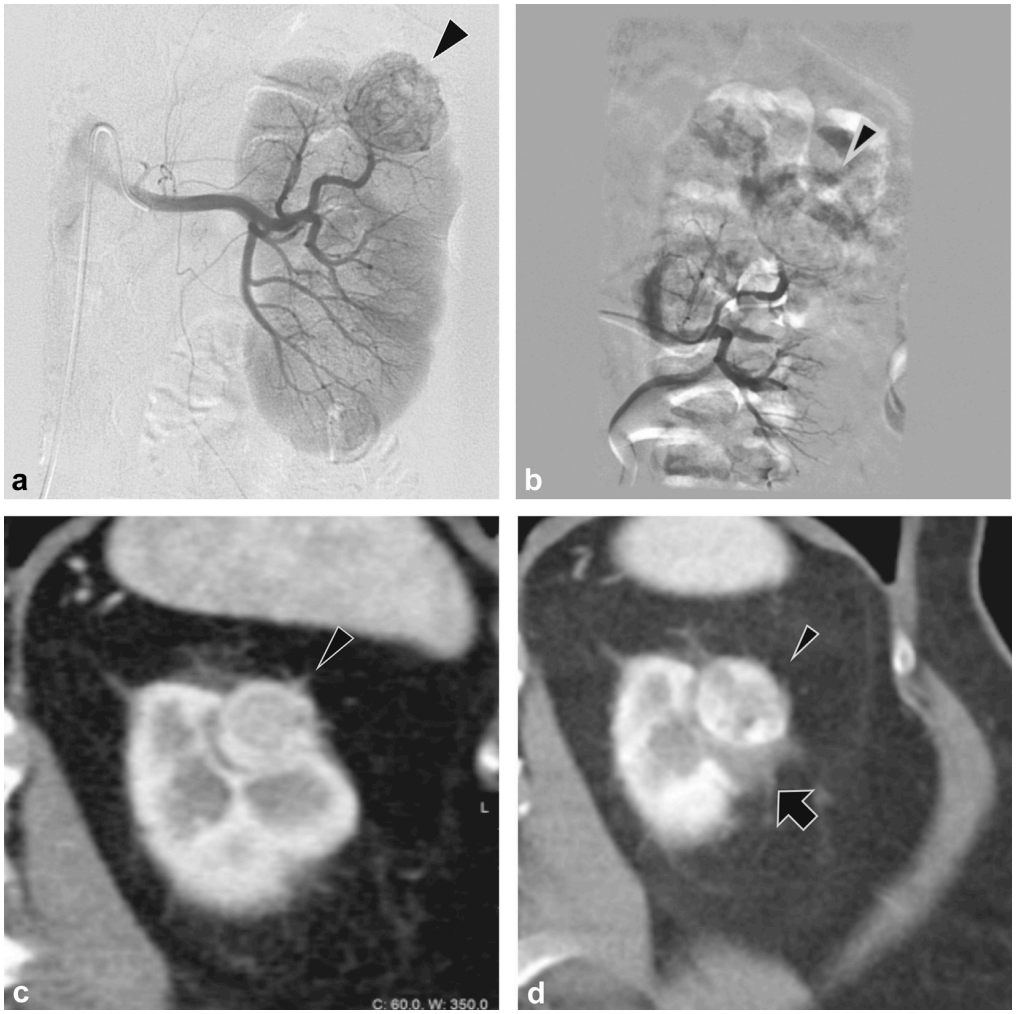

Figure 18. Images of RCC treated by TAE in patient 5. (a) Subtraction angiography image obtained after selective catheterization of the left renal artery showed contrast-filled intratumoral vessels (arrowhead) before TAE. (b) Subtraction angiography image obtained during TAE showed no contrast-filled vessels in the tumor (arrowhead) owing to ongoing embolization. (c) CT image obtained before TAE showed contrast enhancement in RCC (arrowhead). (d) CT image obtained 4 weeks after TAE showed persistent contrast enhancement in RCC (arrowhead). Infarcted renal tissue (arrow) is adjacent to the treated tumor.

5 DISCUSSION

The results of this project suggest that DEB-TACE is an effective and well-tolerated treatment for unresectable HCC. Monitoring the changes in HCC hemodynamics after DEB-TACE and studying the drug-delivery performance of the drug-eluting embolic agent might have provided answers to questions asked or discussed by interventionists performing DEB-TACE. DEB-TACE has been tried for the first time to treat RCC and the results seem promising.

5.1 PAPER I

Previous perfusion CT studies have shown that HCC is characterized by a high arterial and low portal perfusion[25]. The results of this study confirm that HCC has a high arterialization before DEB-TACE. The mean HPI in previously treated HCC was lower than in non-treated (63% and 82% respectively). One of the interesting findings that was shown by this study was that the HPI in previously treated lesions was lower not due to decreased in ALP but due to an increase in PLP in these lesions. This might lead to the assumption that portal supply can help the HCC to survive a DEB-TACE procedure.

A measurable trace of ALP was detected in most cases directly after DEB-TACE although the fluoroscopy and subtraction angiography had shown complete stasis. The question of whether this was caused by revascularization (reperfusion) or was due to the inaccuracy of fluoroscopy and subtraction angiography merits further studies with a different design.

The very low arterial blood flow shown by perfusion CT after DEB-TACE might have the advantage of allowing the doxorubicin released from the particles to perfuse into the tumor tissue leading to the extensive tumor necrosis obtained after DEB-TACE [78]. The decrease in ALP occurred together with an increase of PLP early after DEB-TACE and might have been due to changes in pressure gradient, i.e. lower arterial input might allow an increase of the portal input, which was suppressed by the high arterial input before DEB-TACE. Researchers have found that neovascularization progresses while portal venules diminish during HCC carcinogenesis [79].

In established HCC, portal vessels are believed to be absent or to be responsible for venous drainage in the presence of a fibrous tumor capsule [80]. Therefore, the increase in PLP might be explained either by blood perfused from surrounding sinusoids toward the tumor [81] or by reversed blood flow from preserved portal venules, which previously (prior to DEB-TACE) drained the HCC.

Using perfusion parameters before DEB-TACE and the %-difference in perfusion parameters (before and after the treatment) did not enable the prediction of complete tumor necrosis, which is the aim of the DEB-TACE treatment and is the most important parameter for treatment efficacy. However, to the best of our knowledge our study was the first to demonstrate that perfusion CT performed early after the DEB-TACE treatment might allow early assessment of treatment response.

The limited number of recruited patients and the lack of survival data are two important factors that have to be acknowledged. Further, larger studies are needed to refine the

proposed PLP and HPI cut-off values that can be used for outcome prediction and early response assessment. Different interventional radiologists performed the DEB-TACE procedures in this dual center study, but both centers used the same standards when applying DEB-TACE. The fact that performing perfusion CT exposes the patient to higher radiation dose limits this technique to oncological use in elderly patients.

5.2 PAPER II

As described in the introduction section, there are two clinically used drug-delivery systems of doxorubicin, doxorubicin-in-lipiodol emulsion and drug-eluting beads saturated with doxorubicin. To the best of our knowledge, this study was the first study to monitor and compare their pharmacokinetics. It was shown that doxorubicin is released faster and exposes a larger liver volume in TAI using doxorubicin-in-lipiodol emulsion than in DEB-TACE using drug-eluting beads. This fast release and the exposure of a larger liver volume led to more and faster enzymatic conversion to doxorubicinol.

Like many other studies dealing with drug pharmacokinetics, this study contained a limited number of participants; therefore, effect and safety data should be interpreted with caution. The 2-fold longer half-lives after 5–7 days for doxorubicin when given in DEB-TACE was due to a slower drug release rate from particles than from doxorubicin-in-lipiodol emulsion. The similar half-life after 5–7 days for doxorubicin and doxorubicinol clearly indicates that the pharmacokinetics of doxorubicinol is limited by its formation rate because it is a metabolite of doxorubicin.

The mean dose-normalized AUC values in the TAI group in this study were 3.3 to 4.9-fold lower compared to historic data presented by Johnson and colleagues [39] despite the fact that they used a similar dose of doxorubicin. The difference in AUC might be explained by differences in the physical stability of the emulsions because Johnson and colleagues used an emulsion that contained a 2:5 volume ratio of lipiodol and aqueous doxorubicin solution with normal saline, while we used an emulsion with a 3.3:1 volume ratio that has been reported to be more stable[31, 39, 76, 82]. In other words, our study confirmed that the emulsion we used was more stable.

The particles used to treat the DEB-TACE group in this study (size range 70−700 μm) generated a mean dose-normalized Cmax and AUC of doxorubicin that were consistent with data previously reported for DEB-TACE 500−700 μm [38]. However, Varela and co-workers reported a lower AUC0−7 days for doxorubicin of 662.6 ± 417.6 min·ng/mL from DEB-TACE 500−700 μm following a mean dose of 128 mg [37]. Varela's AUC value is 65 times lower than our dose-normalized mean (AUC0−7d 43,200 min·ng/mL), a disagreement that can not be explained by the dose difference (100 mg vs. 128 mg). It might be that an accidental error was made in the units for their AUC. If their result were in h·ng/mL but reported by mistake as min·ng/mL, then their AUC values would be similar to ours.

It is important to mention that the doxorubicin release from the drug-eluting particles is not homogeneous, partly because of the differences in the sizes of particles used in different patients, and partly because embolization prevented us from giving a predetermined doxorubicin dose. Researchers have found that smaller drug-eluting beads allow higher doses and that both correlate with higher plasma exposure of doxorubicin and as a result higher exposure to doxorubicinol [31]. A higher doxorubicin release rate obtained from smaller particles, which was observed in vitro, was explained

by the shorter diffusion pathway (i.e. particle diameter) combined with the larger total surface area (higher number of smaller particles than the number of larger particles needed to reach stasis) than a larger drug-eluting bead [83].

The local doxorubicinol-to-doxorubicin AUC ratio was 2-fold higher following TAI treatment than following DEB-TACE treatment ($p < 0.001$). This difference in ratio between the two formulations might be explained by a difference in their distribution of doxorubicin because the emulsion is injected into the lobe artery and spread over the whole lobe. The local bioavailability values for doxorubicin obtained after 6 h suggest that 51% of the injected doxorubicin reached the blood stream versus only 12% for the same period in DEB-TACE. This finding supports that the particle saturated with doxorubicin has a more localized and slower drug release compared to the doxorubicin-in-lipiodol emulsion. The static environment caused by embolization is likely to restrict a large portion of doxorubicin from reaching the blood stream. This might explain why the adverse effects are milder after DEB-TACE and why two patients in the TAI arm suffered from liver failure after TAI but there were no such events after DEB-TACE.

In the TAI group, two patients suffered from serious liver failure and one of them died, while no patient suffered from sever adverse effects in the DEB-TACE group. This might also be related to the injection of the emulsion in the lobe artery (right and left hepatic artery) resulting in distributing the doxorubicin-containing emulsion in a larger liver volume and thereby affecting the liver function, while in DEB-TACE the injection of the suspension is done superselectively.

The results of this study with its limited number of participants suggest that the overall response (%) after DEB-TACE treatment was higher but not statistically significant. A larger study population is needed to compare the response between these two treatments.

There are other limitations with this study, including differences in the degree of embolization achieved, the sizes of the particles applied, the selectivity of the placement of the catheter for administration, the intrahepatic distribution of the drug-delivery system, the local drug concentrations (not normalized), the release rate of doxorubicin from the delivery device, and the size of the target lesion(s). All of these factors have an impact on tumor response after these two therapies, but their relative importance is unknown. However, these limitations are also valid for any assessment of clinical outcome for this treatment in other studies.

However, these limitations are also valid for any assessment of clinical outcome for this treatment in other studies.

5.3 PAPER III

The results from this study showed that in a real-life setting, patients with HCC tolerated DEB-TACE better than cTACE. The reason behind this is most probably the lower plasma doxorubicin level and the smaller AUC after DEB-TACE compared to after cTACE[37, 84].

The major strength of this study was the long follow-up (median 7.1 years), which enabled comparison of survival after the DEB-TACE and cTACE treatments. Another strength was the real-life setting, which enabled the evaluation of these two methods in clinical practice. There are two earlier studies with similar follow-up and cohort size as

ours, one from Germany [85] and one from Taiwan [86], both had smaller study populations and demonstrated similar effects for the two treatments on survival. However, median survival differed between these two studies (14–17 months versus 37 months, respectively) regardless of which treatment was given. This variation in survival between the studies reflects the variation in selection criteria in different regions in the world, the variation in underlying HCC etiologies in patients, and the heterogeneity of the patient groups [87]. Very strict selection criteria have been shown to increase survival after TACE to up to 4 years [88]. In our study we had a mixed cohort (BCLC B and C), which probably influenced the survival.

The lack of randomization between the two treatment regimens and the fact that the two groups were not comparable in all aspects limited this study. Another limitation was due to changes in clinical factors such as CT protocols and improved diagnostic methodology over time because the two cohorts were treated over two different periods (2004–2008 vs. 2009–2012). However, having similar clinical routines, similar referral policies, and having the same staff at the angiography unit are factors that might have reduced the effect of time. We were not able to compare tumor response after the two treatments because arterial enhancement from residual viable tumor mass might be masked by the deposited radiopaque lipiodol at post-treatment CT.

We cannot exclude bias due to improved skills and administrative routines owing to increased patient volumes and training. This would speak in favor of DEB-TACE, but despite that we could not demonstrate a significantly improved survival by DEB-TACE over cTACE in this real-life setting. Thus, the major observed improvement for our patients after the introduction of DEB-TACE was the decreased frequency of adverse events in conjunction with the treatment episode.

5.4 PAPER IV

Doxorubicin is known to be ineffective as a systemic chemotherapy for RCC[75]. The significantly more extensive tumor necrosis observed in this study after DEB-TACE compared to after TAE was likely caused by combining the chemotherapeutic effect of doxorubicin with embolization. The randomized design and the similarity in tumor size, catheter positioning, embolic endpoint, and particle size in the two treatment groups, rendered other explanations unlikely.

The significant correlation between the histopathological evaluation (on microscopy) and the radiological evaluation (on CT) shown by this study suggests that CT can be used to evaluate the grade of necrosis in RCC after DEB-TACE. However, studies with larger populations, a greater range in tumor size, and with longer follow-up are needed to validate this aspect more thoroughly and to assess the effect of palliative use of DEB-TACE on survival in patients with RCC.

Researchers have used transarterial alcohol injection to treat RCC, but that treatment has been shown to be painful, to require general anesthesia, and to require a mean length of hospital stay of approximately 4 days [72]. The highest VAS scored in this study after DEB-TACE was 6 and no patient was hospitalized longer than one day. The tumors treated by DEB-TACE in this study had similar vascularization. All patients except for patient 11 were treated using particles in the size of 100–300 μm. Patient 11 was treated by particles sized 75–150 μm that were injected into a separate vessel supplying a small portion of the tumor, whereas the rest of the tumor was treated also

by particles sized 100–300 μm. This made it impossible to evaluate the effect of the particle size on the outcome.

No intratumoral arteriovenous shunts were identified in the treated tumors in this study, such shunts must be considered as an absolute contraindication to DEB-TACE because the particles would pass the shunt to reach the venous side and make their way to vessels in other organs e.g. the heart and the lungs.

One patient had concomitant Parkinson disease that worsened during the study. The patient´s participation in this study delayed the operation and the patient became no longer eligible for surgery. Unfortunately, it was not possible to predict this drawback at the time of inclusion. The deterioration in Parkinson disease was not considered a reason to terminate the study, although the consequence was major for the patient.

Sporadic viable tumor cells were identified on microscopy even in cases where complete response was observed on CT. This fact implies a very high risk of tumor recurrence after DEB-TACE and limits this procedure to palliation. This conclusion is supported by the fact that tumor recurrence was observed after 26 months in patient 7 and after 14 months in patient 11 because the tumors in these two patients were observed instead of removed surgically. Therefore, it is imperative to clearly state that thermal ablation is a curative treatment in inoperable patients with RCC and should always be considered first.

Although a statistically significant difference in outcome was observed between the two groups, the small population size limits this study and the results must be interpreted with caution. The size of the particles and the degree of stasis are two parameters that were determined by the interventionist, introducing a potential operator variation that was not controlled for. In patient 3, the CT evaluation showed no necrosis after TAI whereas microscopy revealed small necrotic areas that did not change the maximum diameter of the tumor after the treatment, hence the necrosis grade was scored 0%. The grade of tumor necrosis in patient 5 was also scored 0% when evaluated by CT whereas microscopy revealed 20% necrosis. Therefore, the cytoreductive effect of TAE might have been underestimated on CT.

6 CONCLUSIONS

6.1 GENERAL CONCLUSIONS

DEB-TACE modifies the perfusion and vascularization of HCC. The drug-eluting embolic agent used in DEB-TACE has a more controlled and prolonged release of doxorubicin. DEB-TACE is a reliable treatment for unresectable HCC, and it might be superior to TAI and to cTACE by virtue of causing fewer and milder adverse effects. DEB-TACE seems to be more effective than TAE in treating RCC and can be used as a palliative treatment.

6.2 SPECIFIC CONCLUSIONS

Paper I

High arterial and low portal-venous perfusion of HCCs early after DEB-TACE indicates an incomplete response. Portal perfusion of HCCs was significantly higher in treated HCCs compared to non-treated ($p = 0.01$).

Paper II

Doxorubicin-in-lipiodol emulsion releases doxorubicin faster compared to drug-eluting beads in HCC patients and provides more extensive local and systemic exposure to both doxorubicin and doxorubicinol. Drug-eluting beads' release is more controlled than a doxorubicin-in-lipiodol emulsion.

Paper III

In a real-life setting, the overall survival did not differ between cTACE and DEB-TACE. Adverse effects were milder in the DEB-TACE group, and the post-treatment complication rate was similar.

Paper IV

Treatment with DEB-TACE and TAE was feasible and safe for treating localized RCC. DEB-TACE had a significantly more potent cytoreductive effect on treated RCCs compared to TAE as observed on CT examination and light microscopy. However, viable tumor cells were detected by microscopy even when CT showed total tumor necrosis, a fact that limits the use of DEB-TACE to palliation.

7 ACKNOWLEDGEMENTS

My thanks, appreciation, and gratefulness to my supervisor **Professor Rimma Axelsson** who not only introduced me to the world of scientific research, but also believed in me and in my ideas.

My gratitude to my co-supervisors **Associate Professor Torkel Brismar** and **Associate Professor Per Stål**, for their extensive effort and invaluable knowledge, experience and time.

My sincere appreciation and gratefulness to:

Professor Peter Aspelin, who will always be my idol, for his valuable effort, advice, and support.

Laszlo Mihocsa and **Helena Ivanics**, who introduced me to the field of interventional radiology, taught and supported me.

Carl Fredrik Engström, **Mikael Åström**, **Klaus Lange**, **Martin Delle**, **Maria Vinell**, **Jan Engström**, **Bo Kalin**, all interventional radiologists whom I have learned incredibly a lot from.

Johana Hakumäki, Director of Imaging and Physiology at Karolinska University Hospital; **Katarina Fredriksson**, Head of abdominal radiology; and **Maria Kristoffersen Wiberg**, Former head of the Radiology Department at Karolinksa University Hospital in Huddinge for their support and help to make this project possible.

The intervention team at Karolinska University Hospital, site Huddinge: **Agneta Åström, Bariq Al-amiry, Anita Hällgren, Anneth Mihocsa, Eva Jonsson, Alexanda Lotak. Galina Netkacheva, Beata Samok, Tove Törner, Subeda Osman, Susanne Haglund, Rinne´ Hallberg, Erika Lagtun, Helena Johansson, Emma Eriksson (Vivi-Ann), Sonja Munoz** and **Marie Mäkelä** for their valuable help, support, competence, and professionalism.

My mentor **Logman Hanare**, for his support.

Elsa Lilienberg, Ilse Dubbeldore, Rickard Nyman, Lars Lundell, Ove Gustafsson, Ramin Ghaffarpour, and **Mikael Fischer** for great collaboration and lots of hard work.

Johan Teiler not only as a co-author, but also as a colleague and friend who carried out many of my duties during my absence from our clinic.

Anders Svensson for his valuable explanations, discussions, and advice.

All co-authors and especially **Marquez, H. P., Haubenreisser, H., Mathew, R. P., Alkadhi, H., Henzler, T., Ebeling Barbier, C., Norén, A., Duraj, F., Hedeland, M., Bondesson, U., Sjögren, E., Lennernäs, H., Haji M., Seth E., Wahlin S.,** and **Bozoki, B.** for productive collaborations and discussions.

Stefan Gabrielson, Nikos Kartalis, Aristeidis Grigoriadis, Louiza Loizou, and **Katharina Brehmer** for their advice and support.

All my friends and colleagues at the Department of Radiology.

And to my family.

8 REFERENCES

1. Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. CA: a cancer journal for clinicians, 61(2), 69-90.
2. Galle, P. R., Forner, A., Llovet, J. M., Mazzaferro, V., Piscaglia, F., Raoul, et al. (2018). EASL clinical practice guidelines: management of hepatocellular carcinoma. *Journal of hepatology*.
3. Saar, B., & Kellner‐Weldon, F. (2008). Radiological diagnosis of hepatocellular carcinoma. Liver International, 28(2), 189-199.
4. Llovet, J. M., Brú, C., & Bruix, J. (1999). Prognosis of hepatocellular carcinoma: the BCLC staging classification. In Seminars in liver disease (Vol. 19, No. 03, pp. 329-338). © 1999 by Thieme Medical Publishers, Inc.
5. Trevisani, F., D'Intino, P. E., Grazi, G. L., Caraceni, P., Gasbarrini, A., Colantoni, A., et al. (1996). Clinical and pathologic features of hepatocellular carcinoma in young and older Italian patients. Cancer: Interdisciplinary International Journal of the American Cancer Society, 77(11), 2223-2232.
6. Bialecki, E. S., & Di Bisceglie, A. M. (2005). Diagnosis of hepatocellular carcinoma. Hpb, 7(1), 26-34).
7. Bargellini, I., Battaglia, V., Bozzi, E., Lauretti, D. L., Lorenzoni, G., & Bartolozzi, C. (2014). Radiological diagnosis of hepatocellular carcinoma. Journal of hepatocellular carcinoma, 1, 137).
8. Vilana, R., Forner, A., Bianchi, L., García-Criado, Á., Rimola, J., de Lope, C. R., et al. (2010). Intrahepatic peripheral cholangiocarcinoma in cirrhosis patients may display a vascular pattern similar to hepatocellular carcinoma on contrast-enhanced ultrasound. *Hepatology*, *51*(6), 2020-2029.
9. Cuenod, C. A., Leconte, I., Siauve, N., Resten, A., Dromain, C., Poulet, B., et al. (2001). Early changes in liver perfusion caused by occult metastases in rats: detection with quantitative CT. Radiology, 218(2), 556-561.
10. Sahani, D. V., Holalkere, N. S., Mueller, P. R., & Zhu, A. X. (2007). Advanced hepatocellular carcinoma: CT perfusion of liver and tumor tissue—initial experience. Radiology, 243(3), 736-743.
11. Fischer, M. A., Leidner, B., Kartalis, N., Svensson, A., Aspelin, P., Albiin, N., et al. (2014). Time-resolved computed tomography of the liver: retrospective, multi-phase image reconstruction derived from volumetric perfusion imaging. European radiology, 24(1), 151-161.
12. Fischer, M. A., Brehmer, K., Svensson, A., Aspelin, P., & Brismar, T. B. (2016). Renal versus splenic maximum slope based perfusion CT modelling in patients with portal-hypertension. European radiology, 26(11), 4030-4036.
13. Yang, Z. F., & Poon, R. T. (2008). Vascular changes in hepatocellular carcinoma. The Anatomical Record, 291(6), 721-734.
14. Fischer, M. A., Kartalis, N., Grigoriadis, A., Loizou, L., Stål, P., Leidner, B., et al. (2015). Perfusion computed tomography for detection of hepatocellular carcinoma in patients with liver cirrhosis. European radiology, 25(11), 3123-3132).
15. Kim, S. H., Kamaya, A., & Willmann, J. K. (2014). CT perfusion of the liver: principles and applications in oncology. Radiology, 272(2), 322-344.
16. Wang, J. H., Wang, C. C., Hung, C. H., Chen, C. L., & Lu, S. N. (2012). Survival comparison between surgical resection and radiofrequency ablation for patients in BCLC very early/early stage hepatocellular carcinoma. Journal of hepatology, 56(2), 412-418.
17. Clark, T., Maximin, S., Meier, J., Pokharel, S., & Bhargava, P. (2015). Hepatocellular carcinoma: review of epidemiology, screening, imaging diagnosis, response assessment, and treatment. Current problems in diagnostic radiology, 44(6), 479-486
18. Llovet, J. M., Schwartz, M., & Mazzaferro, V. (2005, May). Resection and liver transplantation for hepatocellular carcinoma. In Seminars in liver disease (Vol. 25, No. 02, pp. 181-200). Copyright© 2005 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.
19. Mazzaferro, V., Bhoori, S., Sposito, C., Bongini, M., Langer, M., Miceli, R., & Mariani, L. (2011). Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence‐based analysis of 15 years of experience. Liver transplantation, 17(S2), S44-S57.
20. Jansen, M. C., Van Hillegersberg, R., Chamuleau, R. A. F. M., Van Delden, O. M., Gouma, D. J., & Van Gulik, T. M. (2005). Outcome of regional and local ablative therapies for hepatocellular carcinoma: a collective review. European journal of surgical oncology, 31(4), 331-347.

21. Zimmerman, A., Grand, D., & Charpentier, K. P. (2017). Irreversible electroporation of hepatocellular carcinoma: patient selection and perspectives. Journal of hepatocellular carcinoma, 4, 49.
22. Dhanasekaran, R., Kooby, D. A., Staley, C. A., Kauh, J. S., Khanna, V., & Kim, H. S. (2010). Comparison of conventional transarterial chemoembolization (TACE) and chemoembolization with doxorubicin drug eluting beads (DEB) for unresectable hepatocelluar carcinoma (HCC). Journal of surgical oncology, 101(6), 476-480.
23. Yao, F. Y., Kerlan, R. K., Hirose, R., Davern, T. J., Bass, N. M., Feng, S., et al. (2008). Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. Hepatology, 48(3), 819-827.
24. Graziadei, I. W., Sandmueller, H., Waldenberger, P., Koenigsrainer, A., Nachbaur, K., Jaschke, W., et al. (2003). Chemoembolization followed by liver transplantation for hepatocellular carcinoma impedes tumor progression while on the waiting list and leads to excellent outcome. Liver Transplantation, 9(6), 557-563.
25. Malagari, K., Chatzimichael, K., Alexopoulou, E., Kelekis, A., Hall, B., Dourakis, S., et al. (2008). Transarterial chemoembolization of unresectable hepatocellular carcinoma with drug eluting beads: results of an open-label study of 62 patients. Cardiovascular and interventional radiology, 31(2), 269-280.
26. Terzi, E., Golfieri, R., Piscaglia, F., Galassi, M., Dazzi, A., Leoni, S., et al. (2012). Response rate and clinical outcome of HCC after first and repeated cTACE performed "on demand". Journal of hepatology, 57(6), 1258-1267.
27. Idée, J. M., & Guiu, B. (2013). Use of Lipiodol as a drug-delivery system for transcatheter arterial chemoembolization of hepatocellular carcinoma: a review. Critical reviews in oncology/hematology, 88(3), 530-549.
28. Özcan, M. M., & Atalay, Ç. (2006). Determination of seed and oil properties of some poppy (Papaver somniferum L.) varieties. Grasas y aceites, 57(2), 169-174.
29. Kan, Z.; Wright, K.; Wallace, S. Ethiodized oil emulsions in hepatic microcirculation: in vivo microscopy in animal models. Academic radiology 1997, 4, (4), 275-82.
30. Tancredi, T.; McCuskey, P. A.; Kan, Z.; Wallace, S. Changes in rat liver microcirculation after experimental hepatic arterial embolization: comparison of different embolic agents. Radiology 1999, 211, (1), 177-81.
31. Dubbelboer, I. R., Lilienberg, E., Ahnfelt, E., Sjögren, E., Axén, N., & Lennernäs, H. (2014). Treatment of intermediate stage hepatocellular carcinoma: a review of intrahepatic doxorubicin drug-delivery systems. Therapeutic delivery, 5(4), 447-466.
32. Zeng, Q., Li, Y., Chen, Y., Ouyang, Y., He, X., & Zhang, H. (2004). Gigantic cavernous hemangioma of the liver treated by intra-arterial embolization with pingyangmycin-lipiodol emulsion: a multi-center study. Cardiovascular and interventional radiology, 27(5), 481-485.
33. Lilienberg, E., Ebeling Barbier, C., Nyman, R., Hedeland, M., Bondesson, U., Axén, N., & Lennernäs, H. (2013). Investigation of hepatobiliary disposition of doxorubicin following intrahepatic delivery of different dosage forms. Molecular pharmaceutics, 11(1), 131-144.
34. Lencioni, R., De Baere, T., Burrel, M., Caridi, J. G., Lammer, J., Malagari, K., et al. (2012). Transcatheter treatment of hepatocellular carcinoma with Doxorubicin-loaded DC Bead (DEBDOX): technical recommendations. Cardiovascular and interventional radiology, 35(5), 980-985.
35. Lewis, A. L., & Holden, R. R. (2011). DC Bead embolic drug-eluting bead: clinical application in the locoregional treatment of tumours. Expert opinion on drug delivery, 8(2), 153-169.
36. Biondi, M., Fusco, S., Lewis, A. L., & Netti, P. A. (2013). Investigation of the mechanisms governing doxorubicin and irinotecan release from drug-eluting beads: mathematical modeling and experimental verification. Journal of Materials Science: Materials in Medicine, 24(10), 2359-2370.
37. Varela, M., Real, M. I., Burrel, M., Forner, A., Sala, M., Brunet, M., et al. (2007). Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. Journal of hepatology, 46(3), 474-481.
38. Poon, R. T., Tso, W. K., Pang, R. W., Ng, K. K., Woo, R., Tai, K. S., & Fan, S. T. (2007). A phase I/II trial of chemoembolization for hepatocellular carcinoma using a novel intra-arterial drug-eluting bead. Clinical Gastroenterology and Hepatology, 5(9), 1100-1108.
39. Johnson, P. J., Kalayci, C., Dobbs, N., Raby, N., Metivier, E. M., Summers, L., et al. (1991). Pharmacokinetics and toxicity of intraarterial adriamycin for hepatocellular carcinoma: effect of coadministration of lipiodol. Journal of hepatology, 13(1), 120-127.
40. Llovet, J. M., Ricci, S., Mazzaferro, V., Hilgard, P., Gane, E., Blanc, J. F., et al. (2008). Sorafenib in advanced hepatocellular carcinoma. New England journal of medicine, 359(4), 378-390.

41. Brown, D. B., Gould, J. E., Gervais, D. A., Goldberg, S. N., Murthy, R., Millward, S. F., et al. (2009). Transcatheter therapy for hepatic malignancy: standardization of terminology and reporting criteria. Journal of Vascular and Interventional Radiology, 20(7), S425-S434.
42. Pleguezuelo, M., Marelli, L., Misseri, M., Germani, G., Calvaruso, V., Xiruochakis, E., et al. (2008). TACE versus TAE as therapy for hepatocellular carcinoma. Expert review of anticancer therapy, 8(10), 1623-1641.
43. Malagari, K., Pomoni, M., Kelekis, A., Pomoni, A., Dourakis, S., Spyridopoulos, T., et al. (2010). Prospective randomized comparison of chemoembolization with doxorubicin-eluting beads and bland embolization with BeadBlock for hepatocellular carcinoma. Cardiovascular and interventional radiology, 33(3), 541-551.
44. Raoul, J., Guyader, D., Bretagne, J., Heautot, J., Duvauferrier, R., Bourguet, P., et al. (1997). Prospective randomized trial of chemoembolization versus intra‐arterial injection of 131I‐labeled–iodized oil in the treatment of hepatocellular carcinoma. Hepatology, 26(5), 1156-1161.
45. Sangro, B., & Salem, R. (2014, November). Transarterial chemoembolization and radioembolization. In Seminars in liver disease (Vol. 34, No. 04, pp. 435-443). Thieme Medical Publishers.
46. Riaz, A., Gates, V. L., Atassi, B., Lewandowski, R. J., Mulcahy, M. F., Ryu, R. K., et al. (2011). Radiation segmentectomy: a novel approach to increase safety and efficacy of radioembolization. International Journal of Radiation Oncology• Biology• Physics, 79(1), 163-171.
47. Ljungberg, B., Campbell, S. C., Cho, H. Y., Jacqmin, D., Lee, J. E., Weikert, S., & Kiemeney, L. A. (2011). The epidemiology of renal cell carcinoma. European urology, 60(4), 615-621.
48. Gupta, K., Miller, J. D., Li, J. Z., Russell, M. W., & Charbonneau, C. (2008). Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer treatment reviews, 34(3), 193-205.
49. Chow, W. H., Devesa, S. S., Warren, J. L., & Fraumeni Jr, J. F. (1999). Rising incidence of renal cell cancer in the United States. Jama, 281(17), 1628-1631.
50. Patard, J. J., Leray, E., Rodriguez, A., Rioux-Leclercq, N., Guillé, F., & Lobel, B. (2003). Correlation between symptom graduation, tumor characteristics and survival in renal cell carcinoma. European urology, 44(2), 226-232.
51. Kim, H. L., Belldegrun, A. S., Freitas, D. G., Bui, M. H., Han, K. R., Dorey, F. J., & Figlin, R. A. (2003). Paraneoplastic signs and symptoms of renal cell carcinoma: implications for prognosis. The Journal of urology, 170(5), 1742-1746.
52. Park, J. W., Jo, M. K., & Lee, H. M. (2009). Significance of 18F‐fluorodeoxyglucose positron‐emission tomography/computed tomography for the postoperative surveillance of advanced renal cell carcinoma. BJU international, 103(5), 615-619.
53. Ljungberg, B., Bensalah, K., Canfield, S., Dabestani, S., Hofmann, F., Hora, M., et al. (2015). EAU guidelines on renal cell carcinoma: 2014 update. European urology, 67(5), 913-924.
54. Volpe, A., Finelli, A., Gill, I. S., Jewett, M. A., Martignoni, G., Polascik, T. J., et al. (2012). Rationale for percutaneous biopsy and histologic characterisation of renal tumours. European urology, 62(3), 491-504.
55. Bielsa, O., Lloreta, J., & Gelabert-Mas, A. (1998). Cystic renal cell carcinoma: pathological features, survival and implications for treatment. British journal of urology, 82(1), 16-20.
56. Kim, S. P., Alt, A. L., Weight, C. J., Costello, B. A., Cheville, J. C., Lohse, C., et al. (2011). Independent validation of the 2010 American Joint Committee on Cancer TNM classification for renal cell carcinoma: results from a large, single institution cohort. The Journal of urology, 185(6), 2035-2039.
57. Okhunov, Z., Shapiro, E. Y., Moreira, D. M., Lipsky, M. J., Hillelsohn, J., Badani, K., et al. (2012). RENAL nephrometry score accurately predicts complications following laparoscopic renal cryoablation. The Journal of urology, 188(5), 1796-1800.
58. Joniau, S., Eeckt, K. V., Srirangam, S. J., & Van Poppel, H. (2009). Outcome of nephron‐sparing surgery for T1b renal cell carcinoma. BJU international, 103(10), 1344-1348.
59. Lane, B. R., & Novick, A. C. (2007). Nephron‐sparing surgery. BJU international, 99(5b), 1245-1250.
60. Gratzke, C., Seitz, M., Bayrle, F., Schlenker, B., Bastian, P. J., Haseke, N., et al. (2009). Quality of life and perioperative outcomes after retroperitoneoscopic radical nephrectomy (RN), open RN and nephron‐sparing surgery in patients with renal cell carcinoma. BJU international, 104(4), 470-475.
61. Stern, J. M., Svatek, R., Park, S., Hermann, M., Lotan, Y., Sagalowsky, A. I., & Cadeddu, J. A. (2007). Intermediate comparison of partial nephrectomy and radiofrequency ablation for clinical T1a renal tumours. BJU international, 100(2), 287-290.

62. Van Poppel, H., Da Pozzo, L., Albrecht, W., Matveev, V., Bono, A., Borkowski, A., et al. (2011). A prospective, randomised EORTC intergroup phase 3 study comparing the oncologic outcome of elective nephron-sparing surgery and radical nephrectomy for low-stage renal cell carcinoma. European urology, 59(4), 543-552.
63. Sun, M., Becker, A., Tian, Z., Roghmann, F., Abdollah, F., Larouche, A., et al. (2014). Management of localized kidney cancer: calculating cancer-specific mortality and competing risks of death for surgery and nonsurgical management. European urology, 65(1), 235-241.
64. Van Poppel, H., & Joniau, S. (2007). Is surveillance an option for the treatment of small renal masses?. European urology, 52(5), 1323-1330.
65. Aron, M., & Gill, I. S. (2007). Minimally invasive nephron-sparing surgery (MINSS) for renal tumours: Part II: probe ablative therapy. European urology, 51(2), 348-357.
66. Ljungberg, B., Cowan, N. C., Hanbury, D. C., Hora, M., Kuczyk, M. A., Merseburger, A. S., et al. (2010). EAU guidelines on renal cell carcinoma: the 2010 update. European urology, 58(3), 398-406.
67. Clark, T. W., Millward, S. F., Gervais, D. A., Goldberg, S. N., Grassi, C. J., Kinney, T. B., et al. (2009). Reporting standards for percutaneous thermal ablation of renal cell carcinoma. Journal of vascular and interventional radiology: JVIR, 20(7 Suppl), S409.
68. Ogan, K., Jacomides, L., Dolmatch, B. L., Rivera, F. J., Dellaria, M. F., Josephs, S. C., & Cadeddu, J. A. (2002). Percutaneous radiofrequency ablation of renal tumors: technique, limitations, and morbidity. Urology, 60(6), 954-958.
69. Schwartz, M. J., Smith, E. B., Trost, D. W., & Vaughan Jr, E. D. (2007). Renal artery embolization: clinical indications and experience from over 100 cases. BJU international, 99(4), 881-886.
70. Li, D., Pua, B. B., & Madoff, D. C. (2014, March). Role of embolization in the treatment of renal masses. In Seminars in interventional radiology (Vol. 31, No. 1, p. 70). Thieme Medical Publishers.
71. Sauk, S., & Zuckerman, D. A. (2011, December). Renal artery embolization. In Seminars in interventional radiology (Vol. 28, No. 4, p. 396). Thieme Medical Publishers.
72. Munro, N. P., Woodhams, S., Nawrocki, J. D., Fletcher, M. S., & Thomas, P. J. (2003). The role of transarterial embolization in the treatment of renal cell carcinoma. BJU international, 92(3), 240-244.
73. Albiges, L., Choueiri, T., Escudier, B., Galsky, M., George, D., Hofmann, F., et al. (2015). A systematic review of sequencing and combinations of systemic therapy in metastatic renal cancer. European urology, 67(1), 100-110.
74. Anselmo Da Costa, I., Rausch, S., Kruck, S., Todenhöfer, T., Stenzl, A., & Bedke, J. (2017). Immunotherapeutic strategies for the treatment of renal cell carcinoma: Where will we go?. Expert review of anticancer therapy, 17(4), 357-368.
75. Haas, N. B., Lin, X., Manola, J., Pins, M., Liu, G., McDermott, D., et al. (2012). A phase II trial of doxorubicin and gemcitabine in renal cell carcinoma with sarcomatoid features: ECOG 8802. Medical Oncology, 29(2), 761-767.
76. Choi, J. W., Cho, H. J., Park, J. H., Baek, S. Y., Chung, J. W., Kim, D. D., & Kim, H. C. (2014). Comparison of drug release and pharmacokinetics after transarterial chemoembolization using diverse lipiodol emulsions and drug-eluting beads. PloS one, 9(12), e115898.
77. Sacks, D. (2003). Society of Interventional Radiology clinical practice guidelines. In Journal of Vascular and Interventional Radiology.
78. Meyer, T., Kirkwood, A., Roughton, M., Beare, S., Tsochatzis, E., Yu, D., et al. (2013). A randomised phase II/III trial of 3-weekly cisplatin-based sequential transarterial chemoembolisation vs embolisation alone for hepatocellular carcinoma. British journal of cancer, 108(6), 1252.
79. Choi, J. Y., Lee, J. M., & Sirlin, C. B. (2014). CT and MR imaging diagnosis and staging of hepatocellular carcinoma: part I. Development, growth, and spread: key pathologic and imaging aspects. Radiology, 272(3), 635-654.
80. Kitao, A., Zen, Y., Matsui, O., Gabata, T., & Nakanuma, Y. (2009). Hepatocarcinogenesis: multistep changes of drainage vessels at CT during arterial portography and hepatic arteriography—radiologic-pathologic correlation. Radiology, 252(2), 605-614.
81. Miyayama, S., Matsui, O., Zen, Y., Yamashiro, M., Hattori, Y., Orito, N., et al. (2011). Portal blood supply to locally progressed hepatocellular carcinoma after transcatheter arterial chemoembolization: observation on CT during arterial portography. Hepatology Research, 41(9), 853-866.
82. Shin, S. W. (2009). The current practice of transarterial chemoembolization for the treatment of hepatocellular carcinoma. Korean journal of radiology, 10(5), 425-434.
83. Lewis, A. L., Gonzalez, M. V., Lloyd, A. W., Hall, B., Tang, Y., Willis, S. L., et al. (2006). DC bead: in vitro characterization of a drug-delivery device for transarterial chemoembolization. Journal of vascular and interventional radiology, 17(2), 335-342.

84. van Malenstein, H., Maleux, G., Vandecaveye, V., Heye, S., Laleman, W., van Pelt, J., et al. (2011). A randomized phase II study of drug-eluting beads versus transarterial chemoembolization for unresectable hepatocellular carcinoma. Oncology Research and Treatment, 34(7), 368-376.
85. Kloeckner, R., Weinmann, A., Prinz, F., dos Santos, D. P., Ruckes, C., Dueber, C., & Pitton, M. B. (2015). Conventional transarterial chemoembolization versus drug-eluting bead transarterial chemoembolization for the treatment of hepatocellular carcinoma. BMC cancer, 15(1), 465.
86. Liu, Y. S., Lin, C. Y., Chuang, M. T., Tsai, Y. S., Wang, C. K., & Ou, M. C. (2018). Five-year outcome of conventional and drug-eluting transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. BMC gastroenterology, 18(1), 124.
87. Fako, V., & Wang, X. W. (2017). The status of transarterial chemoembolization treatment in the era of precision oncology. Hepatic oncology, 4(2), 55-63.
88. Burrel, M., Reig, M., Forner, A., Barrufet, M., de Lope, C. R., Tremosini, S., et al. (2012). Survival of patients with hepatocellular carcinoma treated by transarterial chemoembolisation (TACE) using Drug Eluting Beads. Implications for clinical practice and trial design. Journal of hepatology, 56(6), 1330-1335.