LIPIODOL ACCUMULATION PATTERN AS IMAGING BIOMARKER OF TUMORAL RESPONSE AFTER CONVENTIONAL TRANSARTERIAL CHEMOEMBOLIZATION AND SURVIVAL OUTCOME IN HEPATOCELLULAR CARCINOMA PATIENTS

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DISCLAIMER

I declare that this dissertation records the results of the study performed by me and that it is of my own composition.

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(MOHD YADIE SYAZWAN BIN AZIZI)

Date:

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

AFP	Alpha-Fetoprotein
BCLC	Barcelona Clinic Liver Cancer
CLT	Cadaveric liver transplant
CPS	Child-Pugh Score
cTACE	Conventional transarterial chemoembolization
DEB-TACE	Drug-eluting beads transarterial chemoembolization
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HUSM	Hospital Universiti Sains Malaysia
LDLT	Living donor liver transplant
PEI	Percutaneous ethanol injection
PS	Performance status
RECIST	Response evaluation criteria in solid tumors
SIRT	Selective internal radiotherapy
TACE	Transarterial chemoembolization
WHO	World Health Organization

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ABSTRAK

Latar belakang: TACE adalah rawatan pilihan untuk karsinoma hepatoselular yang tidak dapat dibedah dan prosedur yang berjaya akan meningkatkan kadar kelangsungan hidup pesakit. Liputan antitumoral yang baik pada tumor hati yang disasarkan adalah diperlukan untuk menghasilkan nekrosis tumor yang baik dan menghasilkan kesan terapi yang baik. TACE dengan menggunakan campuran antikanser dan minyak beriodine (Lipiodol) dapat memberikan gambaran keseluruhan mengenai tahap pengumpulan dan pengekalan ubat di dalam tumor yang disasarkan yang di kenal pasti pada pemeriksaan CT-scan yang berikutnya, sekaligus dapat meramalkan hasil rawatan. Kajian ini bertujuan untuk mengetahui hubungan antara pola akumulasi lipiodol dan tindak balas tumor yang disasarkan terhadap rawatan yang diberikan, dan kadar kelangsungan hidup keseluruhan pesakit HCC.

Metod: Kajian rekod retrospektif ini dilakukan dari tahun 2013 hingga 2020 pada pesakit yang menerima TACE dengan menggunakan ubat antikanser dan Lipiodol di Hospital Universiti Sains Malaysia, yang memenuhi kriteria inklusi dan pengecualian. Corak pengumpulan lipiodol diperhatikan kira-kira selepas enam minggu selepas TACE pada imbasan CT susulan dan kemudian dikelaskan kepada 4 corak pengumpulan; corak 4, pengumpulan lengkap; corak 3, kuat (> 75% daripada jumlah tumor); corak 2, sederhana (<75% daripada jumlah tumor); dan corak 1 - pengumpulan rendah. Penilaian tindak balas tumor dilakukan mengikut kriteria mRECIST. Ujian Chi-Square atau Fischer Exact dan analisis ujian regresi logistik berganda digunakan untuk menentukan hubungan antara corak pengumpulan lipiodol dan tindak balas tumor terhadap rawatan. Ujian analisis kelangsungan hidup (analisis Kaplan-Meier) digunakan untuk menentukan hubungan antara corak akumulasi dan keseluruhan kelangsungan hidup pesakit yang menerima TACE. Ujian Regresi Bahaya Berkadar Sederhana dan Berbilang Cox

digunakan untuk mengkaji faktor-faktor lain yang berkaitan dengan kelangsungan hidup keseluruhan.

Hasil: Sebanyak 38 subjek diperoleh dalam kedua-dua kumpulan BCLC tahap B (n = 33) dan tahap BCLC C (n = 10). Pada tahap BCLC B, 18% (n = 7) berada dalam akumulasi lengkap, 26% (n = 10) dalam pengumpulan intensif, 16% (n = 6) dalam akumulasi sederhana dan 13% (n = 5) dalam corak akumulasi rendah. Ujian tepat Fisher untuk subjek BCLC tahap B menunjukkan hubungan yang signifikan antara corak akumulasi lipiodol dan tindak balas tumor dengan nilai Fisher Tepat 27.025 (p <0.001). Ujian Spearman-rho melaporkan hubungan signifikan corak pengumpulan lipiodol dan tindak balas tumor dengan magnitud 0,84 dalam kumpulan ini. Pada tahap BCLC C tidak ada pola akumulasi lengkap yang diamati, 5% (n = 2) berada dalam akumulasi intensif, 11% (n = 4) berada dalam akumulasi sederhana dan 11% (n = 4) berada dalam pola akumulasi rendah. Ujian tepat Fisher untuk subjek di BCLC Tahap C menunjukkan tidak ada hubungan yang signifikan antara corak pengumpulan lipiodol dan tindak balas tumor dengan nilai Tepat Fisher 2.281 (p> 0.05). Analisis kelangsungan hidup menunjukkan bahawa jumlah kes yang lebih tinggi dapat bertahan pada 1 tahun dan 3 tahun sepenuhnya (85.7% pada 1 tahun dan 17.1% pada 3 tahun) dan sengit (88.9% pada 1 tahun dan 38.1% pada 3 tahun) kumpulan pengumpulan lipiodol pada kumpulan BCLC tahap B berbanding dengan corak pengumpulan yang lain. Survival median dalam kumpulan BCLC B untuk setiap kumpulan adalah 26 bulan (lengkap), 30 bulan (intens), 9 bulan (sederhana) dan 16 bulan (rendah). Pada kumpulan BCLC C di mana hanya corak akumulasi sederhana dan rendah yang diperhatikan di atas satu tahun, dengan kadar survival 1 tahun adalah 50% (sederhana) dan 25% (rendah) dengan tidak ada yang bertahan pada tiga dan lima tahun.Masa survival median untuk kumpulan BCLC tahap C adalah enam bulan (intens), empat bulan (sederhana), dan lapan bulan (rendah). Analisis regresi cox sederhana dan berganda

menunjukkan bahawa bilangan kumpulan nodul hati dan jumlah prosedur TACE yang dilakukan adalah antara faktor kematian prognostik yang signifikan dalam HCC. Pesakit yang mempunyai 5-9 nodul hati mempunyai risiko kematian 12.1 kali lebih tinggi berbanding dengan kumpulan pesakit dengan 1-4 nodul hati (HR: 12.1, 95% CI: 1.17 - 124.57). Pesakit yang menerima satu prosedur TACE mengalami penurunan risiko kematian sebanyak 0,57 (HR: 0,565, 95% CI: 0,393 - 0,812). Walaupun analisis regresi tambahan tidak melaporkan pengaruh signifikan pola akumulasi lipiodol pada perkembangan penyakit, analisis korelasi melaporkan korelasi positif sederhana antara pola akumulasi lipiodol dan perkembangan penyakit (r_s (36) = 0,796, $p < 0,001$).

Kesimpulan: Pengumpulan lipiodol pada tumor hati dapat dinilai menggunakan ciri pengimejan garis dasar kuantitatif dan ini menunjukkan korelasi yang signifikan dengan tindak balas tumor terhadap rawatan dan mempengaruhi hasil kelangsungan hidup pesakit. Kajian kami mengesahkan penemuan kajian sebelumnya dan mengesahkan sifat unik dan fungsi Lipiodol sebagai agen biomarker khusus tumor, pembawa ubat, dan pengimejan untuk merawat pesakit HCC.

Kata kunci: TACE, lipiodol, pola retensi lipiodol, karsinoma hepatoselular.

ABSTRACT

Background: TACE is the locoregional treatment of choice for unresectable hepatocellular carcinoma, and a successful procedure would improve the survival rate of the patient. Good antitumoral coverage in the targeted liver tumor is necessary to produce good tumoral necrosis and results in a good therapeutic effect. TACE by using a mixture of anticancer and iodized oil (Lipiodol) may provide an overview of the degree of accumulation and retention within the targeted tumor on subsequent CT-scans follow up, thus predicting the outcome of the treatment. This study aimed to determine the correlation between the pattern of accumulation pattern of lipiodol and the targeted tumoral response toward the treatment given and the overall survival rate of HCC patients.

Methods: This retrospective record review was done from 2013 until 2020 in patients who received TACE with anticancer and Lipiodol in Hospital Universiti Sains Malaysia, who are fulfilling inclusion and exclusion criteria. Lipiodol accumulation pattern is observed approximately after six weeks post-TACE on the follow-up CT scans and later is classified into 4 accumulation patterns; pattern 4, complete accumulation; pattern 3, intense (>75% of tumor volume); pattern 2, moderate (<75% of tumor volume); and pattern 1 – low accumulation. Evaluation of the tumoral response was done according to the mRECIST criteria. Chi-Square or Fischer Exact test and multiple logistic regression test analysis was used to determine the association between the lipiodol accumulation pattern and the tumor response towards the treatment. A survival analysis test (Kaplan-Meier analysis) was used to determine the association between the accumulation pattern and the overall survivability of the patient who received TACE. Simple and Multiple Cox Proportional Hazard Regression tests were used to study other associated factors affecting overall survivability.

Results: A total of data from 38 subjects were obtained in both BCLC stage B (*n*=33) and BCLC stage C (*n*=10) groups. In BCLC stage B, 18% (*n*=7) were in complete accumulation, 26% (*n*=10) in intense accumulation, 16% (*n*=6) in moderate accumulation and 13% (*n*=5) in low accumulation pattern. Fisher's exact test for BCLC stage B subjects showed significant association between lipiodol accumulation pattern and tumor response with Fisher's Exact value of 27.025 ($p<0.001$). Spearman-rho test reports a significant association of lipiodol accumulation pattern and tumor response with a magnitude of 0.84 in this group. In BCLC stage C no complete accumulation pattern was observed, 5% (*n*=2) were in intense accumulation, 11% (*n*=4) were in moderate accumulation and 11% (*n*=4) were in low accumulation pattern. The Fisher's exact test for subjects in BCLC Stage C showed no significant association between lipiodol accumulation pattern and tumor response with Fisher's Exact value 2.281 ($p>0.05$). Survival analysis shows higher proportion of cases survived at 1-year and 3-year in complete (85.7% at 1-year and 17.1% at 3-year) and intense (88.9% at 1-year and 38.1% at 3-year) lipiodol accumulation group in BCLC stage B group as compared to other accumulation patterns. The median survival time in BCLC stage B for each group were 26 months (complete), 30 months (intense), 9 months (moderate) and 16 months (low). In BCLC stage C group where only moderate and low accumulation pattern were observed above one year, with 1-year survival rate was 50% (moderate) and 25% (low) with none survive at three and five years. The median survival time for BCLC stage C group were six months (intense), four months (moderate), and eight months (low). Simple and Multiple cox regression analysis revealed that the number of liver nodules group and number of TACE procedures done were among significant prognostic factor of death in HCC. Patients that have 5-9 liver nodules had a 12.1 times higher risk of death as compared to the group of patients with 1-4 liver nodules (HR: 12.1, 95% CI: 1.17 – 124.57). Patients that received one TACE procedure are expected to have a decrease in risk of death by 0.57 (HR: 0.565, 95% CI: 0.393 – 0.812). Though

additional regression analysis did not report a significant influence of lipiodol accumulation pattern on disease progression, correlation analysis reported a moderate positive correlation between lipiodol accumulation pattern and disease progression ($r_s(36) = 0.796$, $p < 0.001$).

Conclusion: Lipiodol deposition in liver tumors can be evaluated using quantitative baseline imaging characteristics and it shows significant correlation with tumor response toward the treatment and influence the survival outcome of the patients. Our study confirms the findings of previous studies and validates the unique properties and function of Lipiodol as a tumor-specific, drug-carrying, and imaging biomarker agent to treat HCC patients.

Keywords: TACE, Lipiodol, Lipiodol accumulation, hepatocellular carcinoma.

CHAPTER 1: BACKGROUND

1.1 Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of death globally. World Health Organization (WHO) has recorded that HCC is the fifth most common cancer in men and ninth in women with 554,000 and 228,000 cases reported, respectively (*WHO, World health statistics 2014*). In Malaysia, liver cancer is in the top 10 most common cancers diagnosed (Ferlay J et al., 2015). High prevalence of the main aetiologies, Hepatitis B viral (HBV) infection and hepatitis C viral (HCV) infection, are among the top three causes of cancer in Asia. The annual mortality rate from liver cancer in Malaysia is 6.1% in the year 2013, which showed an increment of 42.8% since 1990, an average of 1.9% increment a year (IHME, Forecast Package, CIA, NHS, Wikidata, The WorldBank, and Wikipedia. Liver-Cancer-in-Malaysia. 2017). HBV remained the major cause of HCC in Malaysia (Norsa'adah et al., 2013).

The guideline for the management of HCC has been published by both the American Association for the Study of Liver Disease and European Associations for the Liver Study (Marrero.J et al., 2018, Galle.P et al., 2018). Both of the guidelines are based on the stratification of patients according to the Barcelona Clinic Liver Cancer (BCLC) classification, which provides a proper combination of tumor stage and liver function parameters for the disease stratification and treatment (Llovet JM. et al, 1999, Forner A. et al, 2010). It classifies patients into five distinct prognostic categories with different first-line treatments according to the tumor burden, liver function (by assessing Child-Pugh score), and their performance status.

According to BCLC guidelines, patients with early HCC (stage 0 or early stage A without other associated disease), surgical resection, and orthotopic liver transplantation have curative potential, but fewer than 20% of patients are suitable candidates. The rest of the patients in these groups are subjected to interventional therapies. Available interventional therapies include percutaneous ablation, transarterial embolization or chemoembolization (TACE), and transarterial radioembolization. Such therapies show good control of disease progression and, in some cases, increase the patient's eligibility for curative treatment (Molla N et al., 2014).

Meanwhile, for unresectable HCC, especially patients with BCLC stage B, TACE is a treatment of choice. It is widely used for patients with large or multiple tumors. Even among the patients with resectable tumors, some may have other comorbidities from chronic liver diseases, which could result in contraindication for resection. These patients could also undergo TACE. There are a few methods of performing TACE. One of the methods is using Lipiodol.

Lipiodol (also known as ethiodized oil) is an oil-based iodinated contrast medium that comprises a combination of iodine and ethyl esters of poppy seed oil. The iodine is intercalated into the constituent fatty acid resulting in a mixture of iodestearic and stearic-acid derived esters. It has multiple uses in the diagnostic radiology field, such as hysterosalpingography, lymphography, and dacryocystography. It is also used for the treatment of HCC in conventional TACE (cTACE) or also known as Lipiodol TACE. When Lipiodol is infused intra-arterially, it has a unique property of selective uptake and retention within the liver tumor, specifically the hyperarterialized one. Lipiodol and anticancer drug mixture, followed by particle embolization using gelatine sponge, has been demonstrated to improve the pharmacokinetics of the drug and tumor response. The radioopacity of Lipiodol also helps in monitoring treatment delivery due to its retention within the tumor. Therefore it serves as an imaging biomarker post-treatment used to assess the tumor response (De Baere T. et al., 2016). Post-

treatment response is usually assessed by using computed tomography (CT)-scan. A few studies have shown that tumor response with necrosis is proportional to the fraction of tumor volume opacified by oil. Complete oil retention demonstrates an improved survival rate among the patients who received cTACE (Takayasu K. et al., 2000; Kim DY et al., 2012; El Khaddari S et al., 2012). However, these studies are performed on the earlier BCLC stage A and B populations. In contrast, cTACE's efficacy in the advanced group of HCC, such as in BCLC group C has remained unclear.

There is also a recent transition to use non-Lipiodol drug-eluting bead or resin microspheres (DEB-TACE) for the TACE procedure (Hu et al., 2017; Cheng et al., 2018). These embolic microspheres can sequester chemotherapeutic agents and release them in controlled mode over one week. Such delivery had shown more sustained tumor-selective drug delivery and permanent embolization. Previous studies have shown no significant difference in overall survivability, the median number of procedures, and overall tumor response between cTACE and DEB-TACE. However, a significant increase in global hepatic damage, biliary injuries, and intrahepatic biloma is observed in the DEB-TACE procedure (Mounier A. et al, 2016). The study stipulated that the side effect is largely contributed to increased hepatic arterial flow in advanced cirrhotic disease, among other factors. However, it is also highlighted that the drug efficacy and non-selective drug delivery to the nontumoral liver parenchyma are unable to be fully assessed due to lack of bioimaging marker in post-TACE treatment in DEB-TACE cases.

In the Interventional Radiology Unit of Hospital Universiti Sains Malaysia (HUSM), both cTACE and DEB-TACE are performed. The Patient's financial status significantly influenced the patient selection due to DEB-TACE being more expensive than the cTACE. In this study, we would like to focus on cTACE due to the ability to detect Lipiodol deposition in the follow-up CT scan. Although new advancement is done using novel radioopaque DEB

particles where embolized territory following treatment could be visible on follow-up CT (Reicher et.al, 2019), it is currently unavailable for usage in our center; therefore, it is excluded. The study would also include patients in BCLC stage C in whom the TACE is done as a part of the palliative or disease-modifying therapy in our center. It is done, with or without additional systemic therapy, such as Sorafenib, a multi-tyrosine kinase inhibitor. Sorafenib has been shown to be effective in first line with regorafenib is good second line therapy in case of radiological progression of the disease (Galle P.R et al.,2018). Very recently, Lenvatinib, demonstrated non-inferior survival to Sorafenib in an open-label, multicentre, non-inferiority, randomised trial (Kudo et al., 2018). It is also recommended in first-line therapy for HCC patients with well-preserved liver function (Child-Pugh A), good performance status and with advanced tumor (BCLC stage C). However, no effective second line option after Lenvatinib has been explored (Galle P.R et al.,2018).

The study would highlight the usage of lipiodol as both embolic agent as well as a biomarker. With this study, the importance of having a bioimaging marker used to assess tumoral coverage would be highlighted. The biomarker's ability to predict tumor response, and its outcome in overall survival will be mentioned. By doing so, we could improve their treatment algorithm and justify the usage of the therapy in our patients.

Problem Statement.

The efficacy of drug delivery and tumor response are essential features of any therapy follow-up. The usage of mRECIST criteria in diagnosing tumor response based on the enhancing pattern has some benefits, but also its efficacy in the hypovascular tumor could be questioned. Therefore, there is a place for a new imaging biomarker in the assessment of the tumor response and efficacy. Lipiodol is believed to able to play this role. The degree of lipiodol accumulation in post-TACE assessment could be used as a marker for anticancer drug deposition, as mentioned above. However, there is a lack of data to support this theory adequately, especially in the BCLC stage C patients.

1.2 Objectives

1.2.1 General:

To determine the association between the degree of lipiodol accumulation pattern among patients receiving cTACE in HUSM with tumor response after the patient's treatment and overall survivability in both BCLC stage B and C groups.

1.2.2 Specific:

1. To determine the pattern of lipiodol accumulation post-treatment among HCC patients who received cTACE treatment in both BCLC stage B and C groups in HUSM.
2. To determine the association between the lipiodol accumulation pattern and the tumor response towards the treatment according to RECIST criteria in

patients who received cTACE treatment in both BCLC stage B and C groups in HUSM.

3. To determine the association between the lipiodol accumulation pattern with the overall survivability among patients who received cTACE treatment in both BCLC stage B and C groups in HUSM.
4. To determine the association of prognostic factors such as age, tumor size, number of liver nodules, Child-Pugh score, AFP level, and number of repeated TACE and the overall survivability of both BCLC stage B and C group patient who received TACE in HUSM.

1.3 Hypothesis

Null hypothesis 1: There is no Lipiodol accumulation pattern observable in patients receiving cTACE treatment on post-treatment CT assessment.

Null hypothesis 2: There is no association between lipiodol accumulation pattern and tumor response toward the anticancer drugs given in both BCLC stage B and C groups.

Null hypothesis 3: There is no association between the lipiodol accumulation pattern and the overall survivability among HCC patients who received cTACE.

Null hypothesis 4: There is no association between age, tumor size, number of liver nodules, Child-Pugh score, AFP level, and number of repeated TACE and the overall survivability of both BCLC stage B and C groups patient who received TACE in HUSM.

1.4 Research Question(s).

1. What are the lipiodol accumulation patterns that will be observed in the patients receiving cTACE in HUSM?

   Hypothesis: The patient will have all four different types of lipiodol accumulation patterns, which are complete, intense, moderate, and low types.

2. Is there any association between the accumulation pattern of lipiodol within the tumor and the tumor response after the cTACE treatment?

   Hypothesis: A higher degree of lipiodol retention intratumorally will be associated with better tumoral necrosis, thus indicating a positive tumor response after cTACE.

3. What is the correlation between the degree of lipiodol retention intratumorally with the patient's overall survivability after receiving the treatment? Hypothesis: A higher degree of lipiodol retention is associated with higher overall survivability.

CHAPTER 2: LITERATURE REVIEW

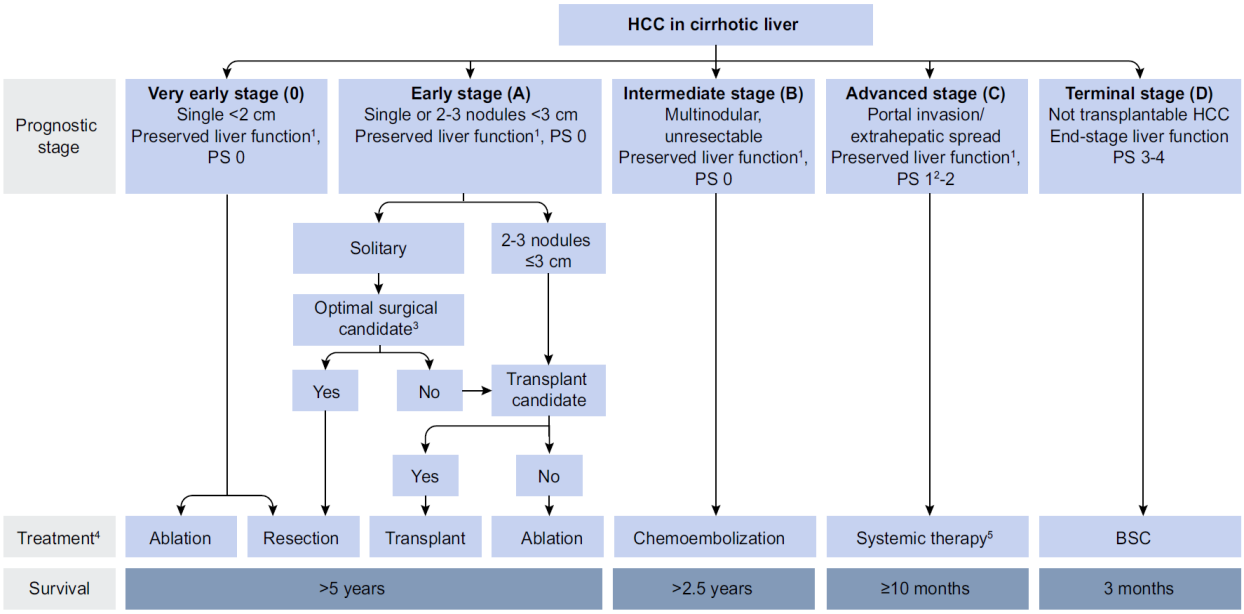
2.1 Imaging-based diagnosis of HCC

Imaging plays an essential part in the diagnosis of HCC. Dynamic imaging interpretation results in a typical diagnostic pattern for this disease (Bruix J. et al., 2001). Its diagnosis relies on the vascular derangement that occurred during hepatic carcinogenesis. Contrast-enhanced imaging is necessary, and the diagnosis is based on dynamic lesion appearance in different vascular phases such as plain, arterial, porto-venous, and delayed phase. Typical hallmarks are hypervascularity in the late arterial phase (arterial phase enhancement) and washout on portal venous or delayed phases (Matsuo O et al., 2011). The major HCC diagnostic guidelines recommended the usage of multiphasic CT and MRI. Meanwhile, there is moderate evidence for the recommendation of contrast-enhanced ultrasound usage in HCC diagnosis (Vilana R. et al., 2010).

2.2 Staging systems, outcome prediction, and treatment allocation

Prognostic assessment is made after diagnosis has been established. The classification is made to establish prognosis and enable proper treatment option selection for each candidate. Unlike most other solid tumors, HCC patients face two life-threatening conditions: rapidly progressing cancer tissues and underlying liver cirrhosis, which further complicates prognostic assessment (Forner A. et al., 2018). Several staging systems have been built to provide clinical HCC classification. Both EASL and AASLD consensus groups on the liver study are endorsing BCLC classification due to its incorporation of prognostic variables, which include tumor status, liver function, and health performance status together with treatment-dependent variables from cohort studies and randomized control trials (Cabibbo G. et al., 2010). Patients with HCC are divided into five stages (0, A, B, C, and D) (EASL, 2018). Prognosis prediction is defined by factors related to tumor status (size, number, vascular invasion, nodal

involvement, presence of distant metastasis), liver function, and health status (EASL, 2018). Health status is determined according to the Eastern Cooperative Oncology Group (ECOG) Performance Status. Treatment allocation is based on dependant variables such as bilirubin level, portal hypertension, or the presence of symptoms based on ECOG assessment (EASL, 2018).



Modified BCLC staging systems and treatment strategy (EASL 2018).

TACE is the first line of treatment of choice for the intermediate HCC (BCLC stage B). The median survival of the untreated patient in this group is 16 months or 49% at two years (Llovet JM. et al., 2003). The current intermediate HCC definition includes a broad range of patients according to liver function and tumor burden. Some of the proposals classify large solitary HCC lesions bigger than 5cm with expansive growth as an intermediate stage, one of which is also being used in this study (Bolondi L. et al., 2012, Hucke F. et al., 2014). TACE is also used in patients with early-stage HCC (BCLC stage A) as a bridge to liver transplantation

or when liver transplantation, hepatic resection, and image-guided ablation are not possible (Perkins, 2007).

In advanced HCC (BCLC stage C), patients usually have a cancer-related symptom, macrovascular invasion, or extrahepatic spread (lymph node involvement or distant metastases). This group bears a poorer prognosis with survival times of 6-8 months (Cabibbo G. et al., 2010). Sorafenib, a multi-tyrosine kinase inhibitor, shows promising survival benefits in this group's randomized control trials (Llovet *et al.*, 2008). TACE in this group is more commonly given with Sorafenib combination, which shows a demonstrable effect in delaying tumor progression.

2.3 Transarterial chemoembolization (TACE).

TACE is recommended as first-line therapy in intermediate-stage disease, as mentioned previously. HCC exhibits intense arterial neo-angiogenic activity during its progression. Intra-arterial infusion of cytotoxic agent and followed by embolization of the tumor's feeding vessels will result in a strong cytotoxic and ischaemic effect targeted to the tumor. This is due to tumoral tissue is fed by arterial inflow, unlike the surrounding parenchyma, which receives the majority of inflow through the portal system (Raoul *et al.*, 2011). The most common drugs used during TACE, either as single agents or in combination regimens, are doxorubicin, cisplatin, or miriplatin (De Baere *et al*., 2016). Super-selective chemoembolization is recommended to increase treatment efficacy. A super selective embolization is done by advancing the catheter into the specific targeted tumoral feeder's vessel rather than a selective chemoembolization where embolization is done on the distal arterial branches in which tumoral feeder vessels arising from. Identification of tumoral feeder is crucial to target the tumor better and obtain complete necrosis. This can be done before the procedure using the arterial phase

of pre-treatment contrast-enhanced CT, or it is also can be done using digital subtracted angiography (DSA) during the procedure (Ronot *et al.*, 2016).

2.4 Lipiodol retention and accumulation pattern.

Multiple studies have been done to investigate the prognostic value of lipiodol retention in HCC patients (Poyanli A. *et al.*, 2001, Takayasu K. et al., 2001, Woo SH *et al.*, 2014, Ping Y, *et al.,* 2016, Cheng SC. *et al.,* 2016). Some of the studies showed that the poor retention of lipiodol within the tumor leads to lower treatment efficacy with a higher risk of liver damage due to dispersion of the anti-tumor drugs to the nontumoral liver parenchyma (Woo SH *et al.* 2014, Ping Y, *et al.,* 2016, Cheng SC. *et al.,* 2016). TACE was also found to be comparable with hepatic resection in compactly retained lipiodol within the tumor in terms of survival rate and tumor recurrence in the early-stage group (Lee HS. *et al.,* 2002).

According to Yang P et al., the lipiodol accumulation pattern is classified into four types in their study in 2016, which is also provides the basis for this study (Yang P. *et al*., 2016). They categorized the lipiodol accumulation pattern into complete, intense (>75% of tumor volume), moderate (<75% of tumor volume), and low type (Figure 1). Dense and complete accumulation of lipiodol in the tumor could suggest a sufficient tumor necrotizing efficacy. Meanwhile, poor and incomplete accumulation indicates insufficient treatment, thus shows a poor progression-free rate regardless of whether the tumoral blood supply is good or poor (Matsuo N et al. 1992, Cheng SC et al., 2016).

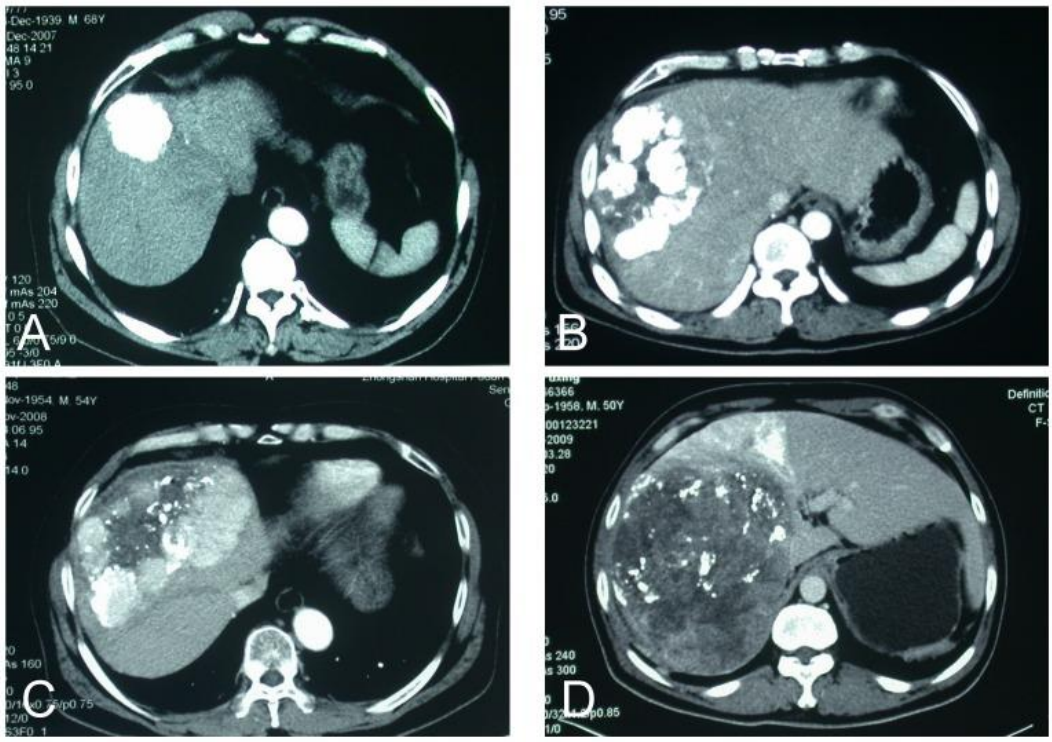


Figure 1. Post TACE lipiodol accumulation pattern types; (A) Complete (B) Intense (C) Moderate (D) Low.

2.5 TACE repetition.

An aggressive schedule of TACE repetition (i.e., TACE every two months) might induce liver failure in an unacceptable proportion of patients (N Engl J Med 1995). According to EASL, TACE should not be repeated when substantial necrosis is not achieved after two rounds of treatment or follow-up treatment fail to induce marked necrosis at sites that have progressed after an initial tumor response (EASL 2018). Besides, TACE should not be repeated upon untreatable progression, defined as tumor progression associated with a clinical profile that prevents re-treatment. This includes extensive liver involvement, extrahepatic metastasis

or vascular invasion, impaired liver function, and deranged performance status (Forner A. *et al.*, 2014). Thus, it is important to identify the patient who may not be responding well to the TACE for an early alternative combination treatment strategy instead of waiting for another 2 or 3 cycles of treatment, which may cause more adverse effects to the patient (Bolondi *et al.*, 2012, EASL 2018)

2.6 Response rate assessment.

Tumor response is typically measured according to (RECIST) or the modified Response Evaluation Criteria In Solid Tumors (mRECIST). The following definition is used to determine the overall target lesion response at a given baseline timepoint. In this study, the assessment is based on mRECIST criteria as per EASL guideline:

Complete response = disappearance of any intratumoral arterial enhancement in all typical intrahepatic target lesions AND disappearance of all atypical intrahepatic target lesions and extrahepatic target lesions. Nodal lesions with short-axis diameters regressed to <1 cm are considered normal.

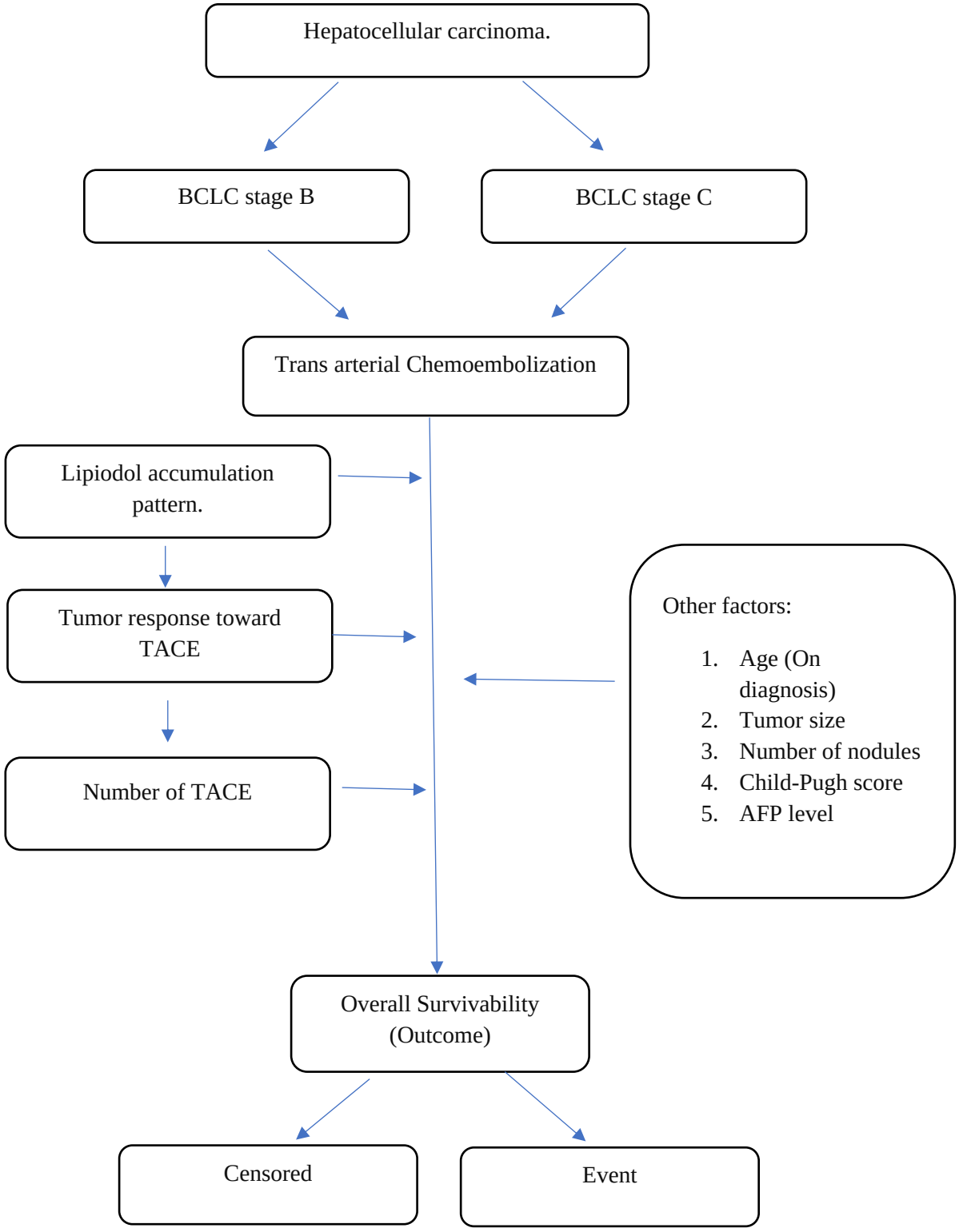
Partial response = at least a 30% decrease in the sum of diameters of the target lesions (including viable tumor diameters for typical intrahepatic target lesions and short axis diameters for nodal lesions), taking as reference the baseline sum of the longest diameters.

Progressive disease = at least a 20% increase AND an absolute increase of at least 5 mm in the sum of diameters of the target lesions (including viable tumor diameters for typical intrahepatic target lesions and short axis diameters for nodal lesions), taking as reference the nadir sum of diameters recorded since baseline.

Stable disease = neither sufficient decrease to qualify for PR nor sufficient increase to qualify for PD.

Multiphasic enhanced CT or MRI is used to assess response after resection, and for this study, after loco-regional therapies. Follow-up strategies for the management of the detected tumoral residual or recurrence after each treatment are planned after the scan (EASL 2018).

2.7 Conceptual framework.



2.8 Rationale of the study

TACE is the first-line treatment for intermediate HCC patients, with multiple studies showing significant improvement in disease control and overall survivability in both cTACE and DEB-TACE (de Baere *et al.*, 2016). A few studies demonstrated the importance of having a compact accumulation of the Lipiodol into the tumor to ensure complete tumoral necrosis. It could be achieved in the very early and early-stage with a scarce report of advantage in the advanced group (Yang *et al.*, 2016). Unfortunately, the major recommendation guidelines for managing HCC patients do not include complete lipiodol uptake as a therapeutic aim. Moreover, with DEB-TACE alternative treatment, the disadvantage of lacking bioimaging markers for optimal drug retention assessment post-procedure is seldom stressed in literature. This study will stress the importance of achieving good lipiodol retention as a bioimaging marker and its correlation with the tumor response and overall survival among early (BCLC stage B) and advanced (BCLC stage C) patients in HUSM. Furthermore, the study will divide the accumulation pattern into four groups which are Low, Moderate, Intense, and Complete, to further stratified its significance in each group where the previous studies had only divided it into two or three groups. This study will also add further significance in recognizing good lipiodol accumulation as a therapeutic aim in the future.

CHAPTER 3: METHODOLOGY

3.1 Study design.

Retrospective record review study of HCC patients who had received TACE from 2013 until 2020 in Radiology department of HUSM.

3.2 Study Location and Duration

The assessment and data collection will be made in the Radiology Department, HUSM. The data collection study will be conducted from July 2020 until December 2020.

3.3 Study Population

i. Reference Population: Patients of HUSM.

ii. Source population: Patients who has been diagnosed with HCC

iii. Target population: Patients who underwent cTACE by using anticancer drugs and Lipiodol

3.4 Sampling technique

Convenience sampling in which all patients fulfilled the inclusion and exclusion criteria will be included.

3.5 Inclusion criteria

1. All HCC patients underwent cTACE by using Lipiodol with a combination of the chemotherapeutic agent and gelatine sponge.
2. Relevant medical and imaging records of the patient are available.
3. Proper regular follow-up between 5-6 weeks post-TACE.

3.6 Exclusion criteria

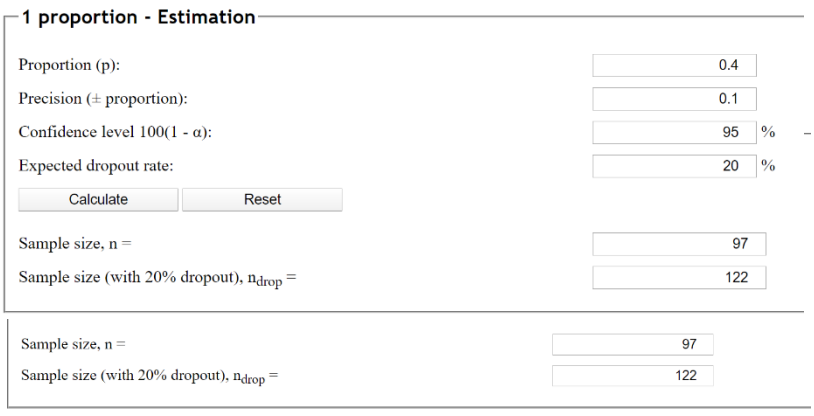
1. Patients who underwent a combination of treatment (i.e., TACE + Radiofrequency ablation or microwave ablation, TACE + SIRT, etc.).
2. Patients that underwent two or more succession of cTACEs without multiphasic CT liver done in between.

3.7 Sample Size Calculation.

The sample size for each objective of the study was determined as follows:

Objective 1: To determine the pattern of lipiodol accumulation post-treatment among HCC patients who received cTACE treatment in both BCLC stage B and C groups in HUSM

For objective 1, the sample size will be calculated using a single proportion estimation (Ariffin, 2020),



Pattern	Proportion	Precision	α	n	n + 20%	reference
Intense	0.40	0.1	0.05	93	117	Yang P et al. (2016)
Moderate	0.50	0.1	0.05	97	122	Yang P et al. (2016)

Objective 2: To determine the association between the lipiodol accumulation pattern and the tumor response towards the treatment according to RECIST criteria in patients who received cTACE treatment in both BCLC stage B and C groups in HUSM.

For objective 2, the sample size will be calculated using two proportion estimation (Ariffin, 2020), based on the formula below

2 proportions - Hypothesis Testing

Proportion in control (p_0):	0.6	
Proportion in case (p_1):	0.2	
Significance level (α):	0.05	Two-tailed
Power (1 - β):	80	%
Expected dropout rate:	20	%
Calculate		
Reset		
Sample size, n =	23	
Sample size (with 20% dropout), n_{drop} =	29	

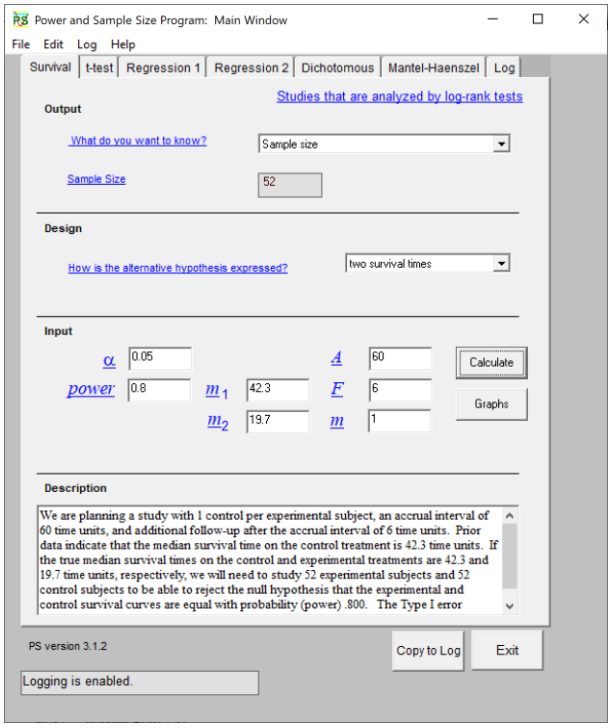
Variables	P_0	P_1	α	β	n	n + 20%	reference
Lipiodol Accumulation Pattern	0.6	0.2	0.05	0.20	23	29	Chen CS et al. 2016

[P_0 – the proportion of intense accumulation pattern among those who had a good tumor response toward TACE,

P_1 – the proportion of intense accumulation pattern among those who had poor tumor response toward TACE [expert opinion])

Objective 3: To determine the association between the lipiodol accumulation pattern with the overall survivability among patients who received cTACE treatment in both BCLC stage B and C groups in HUSM.

For objective 3, the sample size will be calculated using survival analysis (PS Software, 2018)



Variable	Median Survival Time of Group 1 (months)	Median Survival Time of Group 2 (months)	Accrual Time	Follow Up	n	n + 20%	reference
Accumulation Pattern	42.3	19.7	60	6	52	62	Yang P et al. (2016)
$\alpha = 0.05$ Power $(1 - \beta) = 80\%$							

[m1 – median survival time for group 1 – intense pattern, m2 – moderate pattern]

Objective 4: To study the association of other factors such as age, tumor size, number of liver nodules, Child-Pugh score, AFP level, and number of repeated TACE that may be affecting the overall survivability of both BCLC stage B and C group patient who received TACE.

For objective 4, the sample size will be calculated using survival analysis (PS Software, 2018)

Variable	Median Survival Time of Group 1 (months)	Median Survival Time of Group 2 (months)	n	n + 20%	reference
Age	31.5	18.5	94	113	Yang P et al. (2016)
Tumor Size	36.3	18.5	61	73	
No. of nodule	26.6	23.1	1338	1366	
Child's Pugh Score	31.5	12.1	27	32	
AFP level	31.9	13.8	36	43	
No. of TACE	32.9	23.1	228	274	
Accrual Time = 0 months Follow Up time = 60 months $\alpha = 0.05$ power $(1 - \beta) = 0.80$					

Based on the sample size calculation for objectives 1, 2, 3, and 4, the minimum sample size that needs to achieve the objective was 274 patients and 1338 number of nodules. However, only approximately 150 subjects' preliminary data are available for the study in this retrospective review. Post-hoc power will be recalculated at the end of the study to ascertain the power of the study.

3.8 Research tool

1. General Electronic Centricity™ Open PACS AI Solution, U.S.A.
2. Artis Interventional Angiography System – Artis Zee (Bi-Plane), Siemens, Germany.
3. SOMATOM Definition AS 128 – Lines Multislice Computed Tomography System, Siemens, Germany.
4. Nio Color 3MP LED (MDNC-3421), BARCO Medical Display System, Belgium.
5. Radiology clinic HCC census.
6. Patient's medical record from Unit Rekod HUSM.
7. Assessment form (Appendix 1).

3.9 Operational Definition

Child-Pugh Score	:	A scoring system to measure the severity of chronic liver disease inclusive of liver cirrhosis which is composed of total bilirubin, serum albumin, INR, presence of ascites, or hepatic encephalopathy.
Radioopaque	:	Dense structure that resist the passage of the x-rays and appears as light or white in a radiographic image.
Liver cirrhosis	:	Common endpoint of chronic liver disease which cause hepatocellular necrosis.
Cytotoxic	:	Substance or process which results in cell damage or death.

Contrast enhancement	:	Method of exaggerating the visible difference between the adjacent structure on imaging by administering contrast media/agents.
Censored	:	Subject who does not experience the event of interest during the study period and still alive at study closure or defaulted follow up.
Survival time	:	Number of months from date of treatment to the date the patient died or the date of study closure for patients who survived.
Accrual time	:	The time from which the patient who received TACE in 2010-2020 had the first treatment and will be followed up until 60 months or when the patient died.

3.10 Data collection

The subject's information by each observer was recorded in the data collection sheet (Appendix 1) and labelled with a serial number to maintain the subject's privacy. The information is obtained from the Radiology Clinic's HCC census, patient's old records, and Radiology Information System (RIS). The patient's images are reviewed using GE Healthcare Picture archiving and communication system (PACS).

3.11 Image analysis

Image analysis was done via standard BARCO Nio Color 3MP MDNC-3421. The initial tumor is identified in the initial scan, and the pattern of enhancement of the tumor is recorded. The degree of enhancement of the tumor is classified into three-points scale: grade 0, no enhancement of the tumor; grade 1, partial or poor enhancement of the tumor and grade 2, good enhancement of the tumor. Interobserver discrepancies in image interpretation will be solved by consensus.