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Multimodal treatment of HPB cancer

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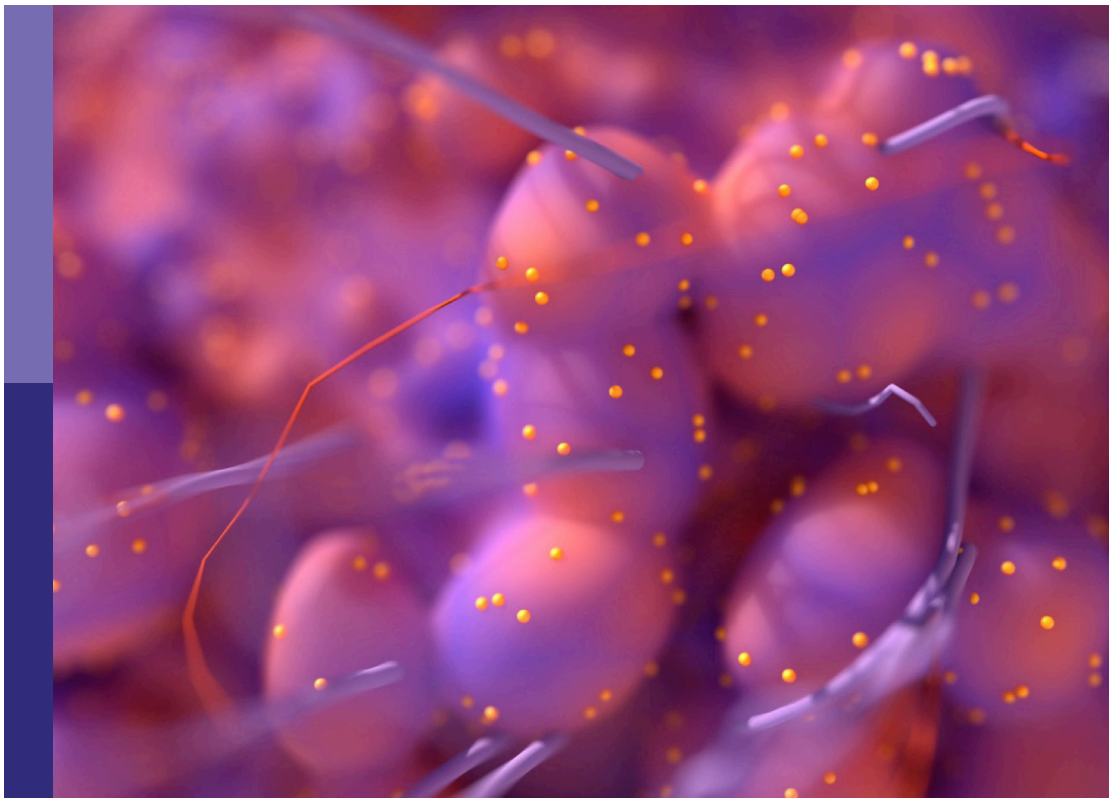

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Multimodal treatment of HPB cancer

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BRIEF RESEARCH REPORT
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A Patient With Stage III Locally Advanced Pancreatic Adenocarcinoma Treated With Intra-Arterial Infusion FOLFIRINOX: Impressive Tumoral Response and Death due to *Legionella pneumophila* Infection: A Unique Case Report

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Patients affected by pancreatic ductal adenocarcinoma (PDAC) have very poor prognosis, whereby at a follow-up of 5 years, the mortality rate is very similar to the incidence rate. Globally, around 10% of patients are amenable to radical surgery at the time of diagnosis, which represents the only chance of cure or long-term survival for these patients. Almost 40% of patients with PDAC show locally advanced pancreatic cancer (LAPC). LAPC is not a metastatic disease, although it is not amenable to radical surgery. For these patients, systemic induction chemotherapy with intravenous FOLFIRINOX (5-fluorouracil, folic acid, irinotecan, oxaliplatin) regimen is administered, with the aim of conversion to surgery, although the conversion rate remains low, at approximately 10% to 15%. Pancreatic arterial chemotherapy has been explored to overcome the intrinsic tumor pancreatic resistance to systemic chemotherapy, where an intra-arterial port-a-cath is placed by means of interventional oncology techniques under angiographic guidance in the operating theater. Here, we treated a patient with an intra-arterially modified FOLFIRINOX regimen. Three courses were administered, and the patient experienced no adverse events. At the end of the third course, the patient rapidly developed lung failure due to nosocomial *Legionella pneumophila* infection, despite the impressive pathological tumor response shown in the autopsy report. This is a first and unique report that demonstrates that pancreatic intra-arterial FOLFIRINOX can be safe and efficacious. We believe that this preliminary result will be confirmed in the next patients to be enrolled and that it provides a glimmer of hope for patients with this lethal disease.

Keywords: pancreatic cancer, pancreatic arterial infusion, FOLFIRINOX, arterial port-a-cath, loco-regional treatment

INTRODUCTION

Patients affected by pancreatic ductal adenocarcinoma (PDAC) have very poor prognosis, with the mortality rate very similar to the incidence rate at the follow-up of 5 years (1, 2). Globally, around 10% of patients are amenable to radical surgery at the time of diagnosis, which represents the only chance of cure or long-term survival for these patients (3).

Almost 40% of patients with PDAC show locally advanced pancreatic cancer (LAPC) (3), and although this is not a metastatic disease, it is not amenable to radical surgery. This is due to T4 presentation that involves infiltration of the celiac artery, the superior celiac artery, and/or the venous splenic–portal–mesenteric axis, without or with regional lymph node involvement, indicative of stage III according to the TNM classification (8th edition) (4). For these patients, systemic induction chemotherapy with an intravenous (IV) FOLFIRINOX (5-fluorouracil, folic acid, irinotecan, oxaliplatin) regimen is administered, with the aim of conversion to surgery, although with a low conversion rate (10%–15%) (5–7). This low rate is due to several biological and pathological characteristics, among which abundant desmoplastic tissue is a relevant barrier to chemotherapy penetration into the tissue microenvironment (8–17).

To overcome the low chemotherapy efficacy, several studies have explored the possibility to administer intra-arterial (IA) pancreatic infusion chemotherapy through interventional radiology techniques, in particular with gemcitabine, platinum salts, or 5-fluorouracil. These studies have obtained very interesting results in terms of safety, tolerability, and response rates, with low systemic toxicity (18–24). IA infusion chemotherapy has been mainly evaluated in liver colorectal cancer metastases and hepato-biliary cancers, and it is performed in specialized centers with interventional radiology and loco-regional oncological medical expertise in a multidisciplinary team organization (14, 25–27).

The biological background of IA pancreatic infusion therapy is to realize high intra-tumoral concentrations of the chemotherapeutic agents with the longest intra-tumor times, for prolonged drug bioavailability and low systemic toxicity (19). To administer IA chemotherapy, a port-a-cath device is placed into the artery using interventional radiology techniques (21).

CASE PRESENTATION

First Hospitalization

A 68-year-old woman suffering from recurrent upper abdominal pain in the previous 3 months was hospitalized on December 14, 2020, at the Interventional and Medical Oncology Unit of the National Cancer Institute, Giovanni Paolo II of Bari (Bari, Italy). She had no history of smoking, alcohol consumption, diabetes, known chronic pancreatitis, obesity, or familial cancer. She had a 10-year history of rheumatoid arthritis that was initially treated with prednisone and methotrexate, and then in the previous 2 years, etanercept had been added to obtain better control of the rheumatic disease. For her recent history, she had not shown any vomiting, jaundice, weight loss, or loss of appetite.

Physical examination showed multiple, non-adherent, palpable lateral-cervical, and axillary lymph nodes (maximum diameter, 1 cm). Some swelling of the fingers and wrist joints was also seen. At the time of hospital admission, the patient had undergone a thorax-abdomen computed tomography (CT) scan (October 13, 2020) that had shown a hypodense nodule at the head of the pancreas, with the maximum diameter of 26 mm and an upper abdomen nuclear magnetic resonance (NMR) (October 29, 2020) that had confirmed the pancreatic lesion with a maximum diameter of 25 mm. She also showed carcinoma embryonic antigen and carbohydrate antigen (CA-19.9) in the normal range, with a performance status according to the European Cooperative Oncology Group (ECOG) of 0 to 1.

During this initial hospitalization, the patient underwent ultrasound endoscopy (December 15, 2020), which showed an irregular hypoechogenic head pancreatic nodule with maximum dimensions of 32 mm × 24 mm, and vascular encasement of the gastroduodenal artery and the whole of the superior mesenteric vein. A fine needle biopsy was performed with a gauge 19 needle. A positron emission tomography/CT scan (December 17, 2020) demonstrated a large area of deoxy-glucose uptake in the pancreas (standardized uptake value, 8.4). A total body CT scan (December 18, 2020) showed a lesion at the head of the pancreas with dimensions of 31 mm × 28 mm, with superior mesenteric vein involvement (**Figure 1**).

Further NMR imaging of the upper abdomen (December 22, 2020) confirmed a hypo-vascular tumor with dimensions of 30 mm × 30 mm for the head and the uncinate process of the pancreas. The tumor had infiltrated the superior mesenteric vein, which had resulted in reductions in the circumferences of the portal vein (>180°) and the superior mesenteric artery (<180°). A reduction in the circumference of the inferior vena cava was also seen, with no signs of vascular wall infiltration. Concomitant CA-19.9 assessment indicated an increased value of 163.8 U/ml.

The patient was discharged while awaiting the pathological diagnosis.

Second Hospitalization

The pathological diagnosis was complete in January 2021, and it revealed a PDAC associated with necrotic and inflammatory phenomena (**Figure 2**). Based on the clinical and pathological diagnoses, the patient was again hospitalized at the Interventional and Oncology Unit of the National Cancer Institute Giovanni Paolo II of Bari.

An updated total body CT scan (January 18, 2021) demonstrated the presence of the known tumor, with 30 mm × 30 mm maximum dimensions and a retro-dilated pancreatic Wirsung duct (diameter, 5 mm). The tumor had further infiltrated the superior mesenteric vein, which had resulted in increased reduction of the circumference (>180°) and also involved the portal vein.

Evaluation of all of the radiology examinations of the PDAC of the patient resulted in its staging as IIIB, according to the TNM classification (8th edition), which was not amenable to radical surgical resection. Due to the low efficacy of systemic FOLFIRINOX as an induction therapy, the patient was offered

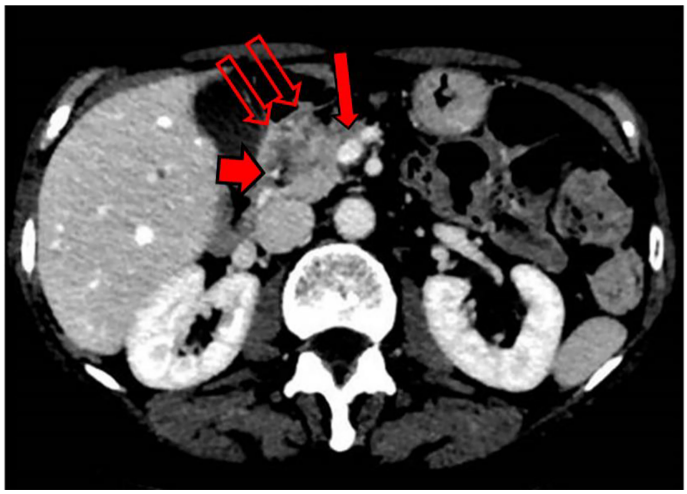

FIGURE 1 | Abdomen CT scan. Single thin arrow, the head pancreatic tumor; short broad arrow, tumor involvement of the superior duodenal-pancreatic artery; twin open arrows, tumor involvement of the portal vein near to the spleno-portal axis.

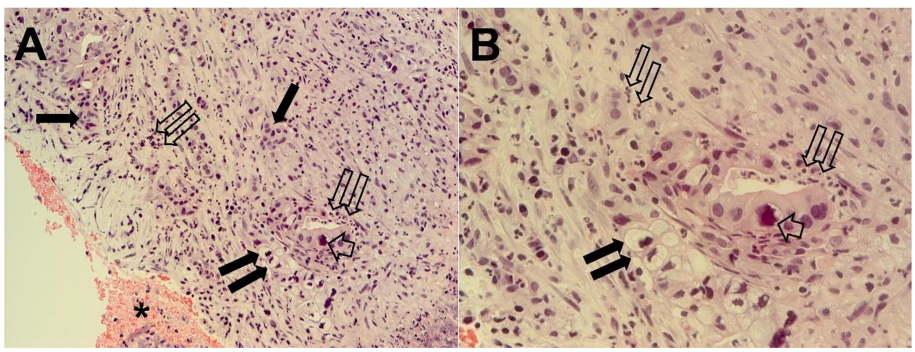

FIGURE 2 | Tumor pancreatic tissue section from ecoendoscopy and needle biopsy cyto included. Tumor cells are organized in differentiated adenomorphic structures or in more poorly differentiated cords, embedded in abundant stroma that are rich in inflammatory cells. Necrotic areas are also evident. Single thin arrows, clusters of tumor cells; short broad arrow, a large nucleus; twin open arrows, inflammatory cells; twin filled arrows, cord of mucous cells with large vacuole and hyperchromic nucleus. The same microscopy field is shown at magnifications of ×200 **(A)** and ×400 **(B)**.

enrolment in a phase II experimental protocol ongoing at the Institute. The patient accepted the experimental therapy and provided written informed consent to participate in the study.

Experimental Clinical Study

This experimental clinical protocol was approved in 2020 by the local Ethical Committee of the National Cancer Institute Giovanni Paolo II of Bari (ID N° 948). The study was designed to treat patients with LAPC.

In summary, the study involves infusion of FOLFIRINOX *via* the pancreatic IA route, with the following modified schedule administered on day 1: oxaliplatin 85 mg/m^2 over 2 h; leucovorin 400 mg/m^2 over 2 h; irinotecan 130 mg/m^2 over 90 min; and fluorouracil 2,400 mg/m^2 as a continuous infusion over 46 h (i.e., starting on day 1). The use of bolus 5-fluorouracil administration was avoided in this schedule (28). Each cycle was for 14 days.

The infusions were carried out using an electromechanical pump to overcome the pressure of the arterial system. To avoid endothelial arterial damage, dexamethasone 8 mg was administered *via* the pancreatic artery before the start of the chemotherapy, and then after the infusion of 5-fluorouracil. Systemic premedication was administered IV with palonosetron 0.25 mg, chlorphenamine 10 mg, and omeprazole 40 mg.

The main inclusion trial criteria were as follows: pathological diagnosis of PDAC; inoperable stage III LAPC; arterial and/or venous vascular encasement by the tumor; ECOG performance status 0 to 1; age, 18 to 80 years; American Society of Anesthesiologists classification from 1 to 3; adequate hematological parameters (hemoglobin ≥9 g/dl; absolute neutrophil count ≥1,500/mm^3; platelet count ≥100,000/mm^3); prothrombin time with an international normal ratio ≤1.5 times

the upper limit of normal; adequate renal function (serum creatinine, ≤1.5 times the upper limit of normal; creatinine clearance calculated by Cockroft–Gault formula, ≤30 ml/min); adequate hepatic function (aspartate amino transferase, aspartate alanine transferase levels, ≤2.5 times the upper limits of normal; bilirubin, ≤2.5 mg/dl); and written informed consent.

The main exclusion criteria were for metastatic disease, ascites, infected tumor, other previous or concomitant malignant tumors, pregnancy, high risk for no cardiac surgery, presence of metallic stent, HIV, HBV, and HCV infection.

As previously described, the patient was enrolled into this protocol.

Interventional Technical Procedure

During this second hospitalization period for the patient (January 19, 2021), an IA port-a-cath device was implanted. Briefly, in the angio-CT operating theater, under general anesthesia, and using ultrasonic guidance, avascular sheath (6 Fr; Introducer II Standard Kit A; Terumo Radifocus) was inserted into the right femoral artery; this was passed into the aorta artery and then introduced into the celiac axis. Next, microcatheters (2.7/2.4-Fr coaxial microcatheter system; Terumo Progreat) were introduced into the gastroduodenal artery, respectively superior mesenteric artery and splenic artery. All arterial rami were non-pancreatic directed, and the superior and inferior pancreatic duodenal arteries were embolized using magnetic spiral devices (Helix 3D detachable coil system; Axium). This vascular modification was performed to avoid the escape of blood from the pancreas, and in this way the pancreatic arterial system was all sustained by the splenic artery and the ramus from which it originated, including the main great pancreatic artery, which together with the caudal pancreatic artery was anastomosed with the transversal pancreatic artery. Subsequently, the tip of the hydrophilic diagnostic catheter (4 Fr; Glidecath angiographic catheter; Radifocus) was placed at the level of the splenic hilum, and then the hydrophilic guide was inserted into this, to allow the hydrophilic diagnostic catheter to be removed and the definitive placement of the polyurethane catheter (6.5 Fr; PolyFlow polyurethane single-lumen portal; Smiths Medical, Minneapolis, MN, USA) for the IA FOLFIRINOX infusion. At the end of the technical procedure, a selective splenic arterial examination was performed (**Figure 3**).

PATIENT TREATMENT

First Cycle of IA FOLFIRINOX Infusion

After the interventional procedure, the patient was monitored for several days, with no complications or adverse effects observed. The first cycle of IA FOLFIRINOX was then started on January 26, 2021.

Immediately before starting this IA therapy, the trans-port-a-cath angiography demonstrated the patency of the remodeled pancreatic arterial system. Then, the IA FOLFIRINOX was administered and was seen to be very well tolerated, without side effects and without any hematological grade toxicity.

The patient was discharged from the hospital.

Second Cycle of IA FOLFIRINOX Infusion, as an Outpatient

The second cycle was administered on February 9, 2021, and again before the therapy, the trans-port-a-cath angiography demonstrated the patency of the pancreatic arterial system. The ECOG performance status of the patient remained at 0 to 1.

Over the following 2weeks, the patient felt good and showed no clinical side effects or hematological toxicity.

Third and Last Hospitalization

The trans-port-a-cath angiography before the third cycle of the therapy on February 24, 2021, showed thrombosis of the great pancreatic artery. Due to this complication, the patient was

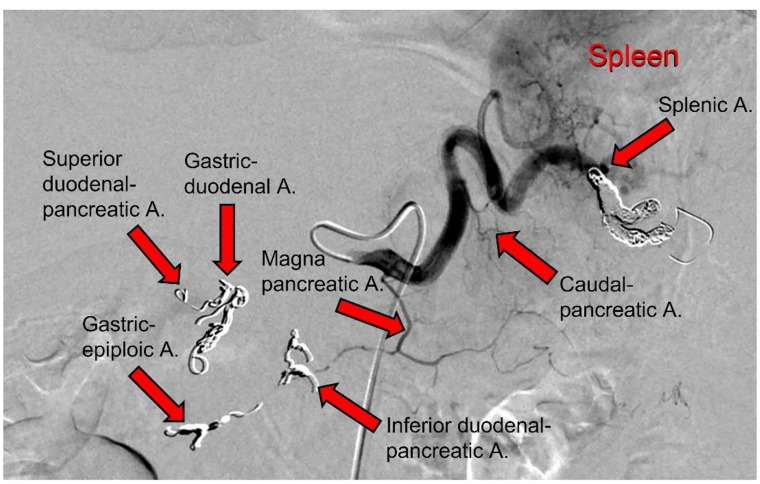

FIGURE 3 | Angiographic scan following injection of contrast medium through the splenic artery after the completion of the technical procedure. The following arteries were embolized, as indicated (arrows): gastric-duodenal a.; superior duodenal-pancreatic a.; gastric-epiploic a.; inferior duodenal-pancreatic a.; and splenica.

hospitalized at the Interventional and Medical Oncology Unit of the National Cancer Institute, Giovanni Paolo II of Bari. She was immediately administered trans-port-a-cat artery thrombolytics, with continuous infusions of urokinase and both IA and IV dexamethasone. The urokinase and corticosteroid infusions were continued, and on February 27, 2021, the trans-port-a-cath angiography indicated partial resolution of the pancreatic artery thrombus. The urokinase and corticosteroid infusions were prolonged, until they were stopped on March 1, 2021, when the trans-port-a-cath angiography indicated the disappearance of the arterial thrombus.

CA-19.9 assessment (March 3, 2021) showed a value of 543.4 U/ml. CT scan evaluation (March 8, 2021) demonstrated a partial response according to the RECIST evaluation criteria (20). This was confirmed by NMR abdomen examination (March 10, 2021), with ECOG performance status well maintained (0-1). On the same day, the trans-port-a-cath angiography confirmed the patency of the arterial pancreatic system, and the third cycle of IA FOLFIRINOX was started.

Unexpectedly, the patient complained of malaise on March 11, 2021, with tachypnea and fast heartbeat. Diagnosis of paroxysmal atrial fibrillation was made, and regression was obtained within 2 h through IV infusion of amiodarone. In addition, on pulmonary auscultation, snores and gasps were evident. Blood gas analysis showed a very low oxygen pressure of 47.7 mmHg. A thorax CT scan demonstrated bilateral pleural effusion, bilateral pulmonary thickening, and ground-glass areas with initial aspects of parenchymal consolidation. Based on radiological findings, the diagnosis of bilateral pneumonia of probable bacterial etiology was made. Oxygen therapy (3 l/min) was added. After infectious disease consultation, broad-spectrum antibiotic therapy was started with piperacillin plus tazobactam 4.5 g IV three times a day, clarithromycin 500 μg IV two times a day, sulfamethoxazole plus trimethoprim 1,920 mg IV three times a day, and ganciclovir 280 mg IV two times a day. Blood cultures and urinary antigenemia for *Pneumococcus* and *Legionella pneumophila* were performed. The patient also underwent bronchoscopy, and the broncho-alveolar lavage fluid obtained was used to investigate cytomegalovirus, SARS-CoV-2 coronavirus, pneumocystis carinii, and common bacteria and fungi.

The result of the urinary *Legionella pneumophila* antigenemia was available on March 12, 2021, at 3:00 p.m., and it was positive. At the same time, the serum search for IgG and IgM toward *Legionella* showed values of 1 (negative) and 40 (positive), respectively. The report of the infectious disease was forwarded to the Italian Health Authorities. Over the day, the patient showed hyperthermia of 38°C.

The continuous IA infusion of 5-fluorouracil was stopped on March 13, 2021. CA-19.9 assessment showed a lower value of 323 U/ml. During the day the patient worsened and showed marked asthenia and dyspnea. Blood gas analysis showed an oxygen pressure of 57.3 mmHg, and oxygen therapy (6 l/min) was applied using a mask (Venturi) over several hours. This provided increased blood oxygen pressure from 80 to 85 mmHg. After resuscitation consultation, continuous positive airway pressure ventilation replaced the oxygen therapy by mask, which obtained an improved blood oxygen pressure of 92 mmHg. In the late evening, the patient worsened again, and blood gas analysis showed a severe hypoxemic blood condition and acute respiratory acidosis.

During the night (March 14, 2021, 12:05 a.m.), the patient was transferred to the post-operatory Intensive Therapy Unit at the National Cancer Institute, Giovanni Paolo II of Bari. The patient was intubated, but at 01:30 a.m., the patient died due to irreversible respiratory failure.

AUTOPSY REPORT

In the subsequent autopsy, the macroscopic pancreas observation indicated head volume reduction with complete tumor mass disappearance (**Figure 4**).

Histopathological examination of the pancreatic tissue showed complete pathological response for the PDAC, with extensive regressive phenomena of the malignancy associated with an interstitial fibrosclerosis reaction circumscribing the regressed tumoral tissue (**Figure 5**). Here, it is very important to underline that the complete histopathological evaluation of the non-cancerous pancreatic tissue did not show any signs of hemorrhagic–necrotic pancreatitis, which demonstrated that the IA chemotherapy had been safe and that it was not a factor in the induction of pancreatitis. With specific reference to the lung examination, there was a yellow-white color and an increased consistency at palpation particularly on the parietal surface of the right upper lobe (**Figure 6A**). Finally, the pulmonary level showed clear histopathological changes due to severe bacterial *Legionella pneumophila* infection (**Figure 6B**).

DISCUSSION

Pancreatic ductal adenocarcinoma is the seventh cause of cancer incidence and the fourth cause of cancer deaths in the world (1). This is due to several factors, which include late diagnosis, the intrinsic aggressive biology of pancreatic cancer, and the characteristic desmoplastic microenvironment of the tumor. At a follow-up of 5years, only 10% of patients remain alive (2, 3). This poor prognosis is due to several factors: first, the diagnosis is late in about half of the cases due to a lack of symptoms; and secondly, in the metastatic stage, patient survival ranges from 7 to 11 months with nab-paclitaxel plus gemcitabine or FOLFIRINOX regimens (5, 29).

The only chance to achieve long-term survival for these patients is radical surgery in non-metastatic disease. Globally, surgically treated patients with stage III PDAC plus adjuvant therapy have a median survival of approximately 2 years (30). Here, for patients with ECOG performance status 0 to 1, the FOLFIRINOX schedule is used, while for those with ECOG performance status 0 to 2, the nab-paclitaxel–gemcitabine schedule can be used (31).

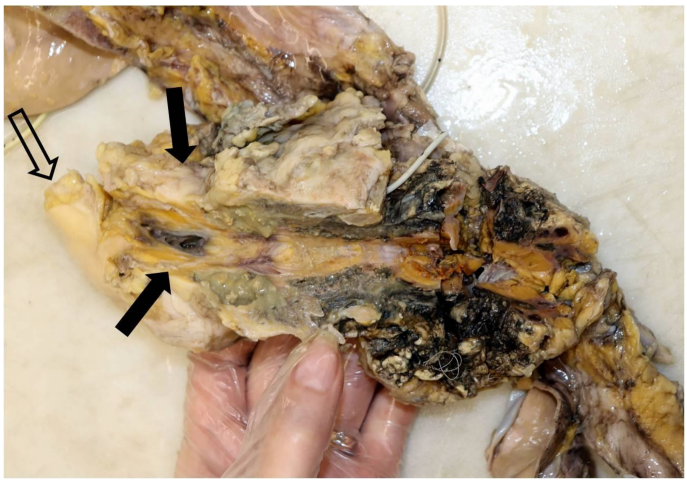

FIGURE 4 | Macroscopic examination of the pancreas, sectioned longitudinally. Single arrows, head of the pancreas, with no visible tumor lesion; twin open arrows, duodenum.

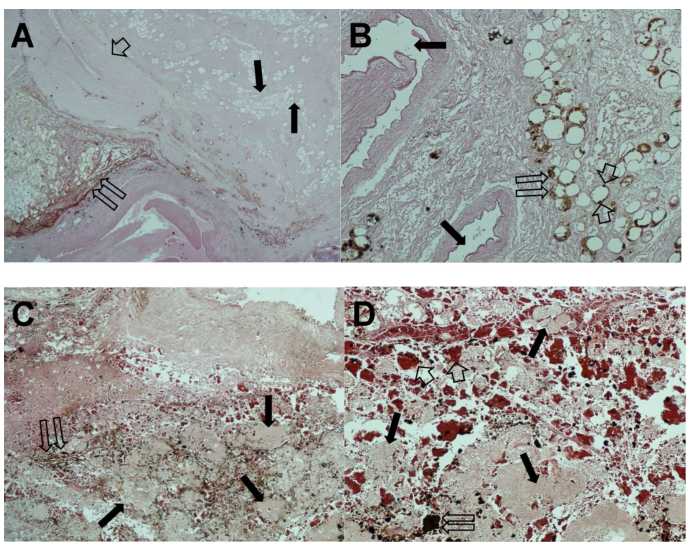

FIGURE 5 | Hematoxylin and eosin staining of pancreatic tissue sections. **(A)** Twin open arrows, necrotic tumor area; single thin arrows, fibrous and adipose replacement; short broad arrow, extensive fibrotic area (magnification, ×100). **(B)** Twin open arrows, residual neoplastic glands with regressive phenomena; single thin arrows, major pancreatic ducts; short broad arrows, single pycnotic nuclei (magnification, ×200). **(C)** The important regressive phenomena. Twin open arrows, hemosiderin deposits; single thin arrows, necrotic tumor area (magnification, ×100). **(D)** Twin open arrows, hemosiderin deposit; single thin arrows, necrotic tumor area; short broad arrows indicate hemosiderin extravasation (magnification, ×200).

Due to the important role of surgery in the therapy of LAPC, the subset of patients with stage III LAPC should be treated upfront with induction chemotherapy, with the aim of downstaging the disease (32, 33). Again, IV FOLFIRINOX or nab-paclitaxel plus gemcitabine are used in the induction setting, although only 10% to 15% conversion rate is obtained, which for induction chemoradiotherapy is a very poor result (6).

To overcome this low response rate to systemic IV chemotherapy, administration of IA pancreatic chemotherapy has been proposed (34–36). The main advantage of IA infusion chemotherapy over IV chemotherapy is that the first-pass effects of the therapy are applied directly to the tumor microenvironment, which produces elevated drug concentrations with greater bioavailability of chemotherapy and few systemic side effects (21).

From a general point of view, the main clinical studies of IA infusion chemotherapy have been performed for liver metastases from colorectal and breast cancers, and primary hepatobiliary tumors (14, 25, 26). These clinical reports have indicated that IA

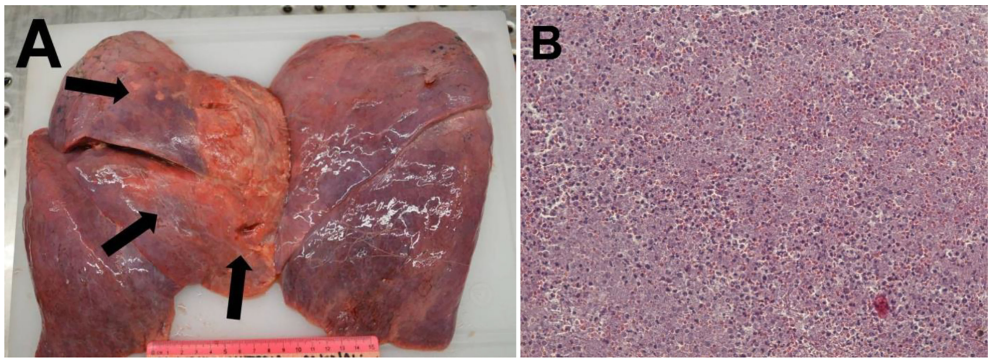

FIGURE 6 | **(A)** Macroscopic examination of the lung. Single thin arrows, surface of the right upper lobe with a yellow-white color. **(B)** Hematoxylin and eosin staining. The completely subverted parenchyma structure with hypoarated or hepatized-like tissue. The alveoli were filled with inflammatory cells, including lymphomonocytes and granulocytes. Red blood cell infiltration is also evident. Features of emphysema can be seen at the periphery of the section, with breaking of the alveolar walls and fusion of the contained spaces.

infusion chemotherapy is safe and can provide very encouraging results.

With specific regard to PDAC, the rationale to use IA infusion chemotherapy is even greater considering that the bioavailability of the chemotherapeutic agents near the cancer cells is very low (3). This is due to two main aspects: first, the slender and sparse anatomical pancreatic vascularization, although angiogenic rebound has been demonstrated in PDAC (8, 12); secondly, the abundant dense desmoplastic stromal tissue that characterizes the microenvironment of PDAC, which is a true barrier that is impenetrable to the drugs. Obtaining elevated concentrations of chemotherapeutic agents in the tumor microenvironment might be the prerequisite for the penetration of the drugs into the desmoplastic stromal cancer tissue, to finally arrive near to, and get into, the tumor cells (16).

In the two last decades, it has been demonstrated that IA infusion chemotherapy is feasible and useful in locally and advanced disease using the following drugs: gemcitabine, gemcitabine plus cisplatin, gemcitabine plus oxaliplatin, gemcitabine plus 5-fluorouracil, and epirubicin plus cisplatin (18–23, 35–37). A meta-analysis by Liu et al. (38) indicated the advantages of IA pancreatic infusion chemotherapy over systemic IV chemotherapy (38).

To the best of our knowledge, no report on IA infusion of FOLFIRINOX or modified FOLFIRINOX schedules has been published. Here, for the first time, we treated a patient with LADC with three cycles of modified FOLFIRINOX therapy, after which the CT and NMR evaluations demonstrated radiological tumor response. However, the patient died from acute respiratory insufficiency due to acquired nosocomial *Legionella pneumophila* infection.

Based on the autopsy report and the histopathological report on the non-tumoral pancreatic tissue and the tumoral pancreatic tissue, an impressive pathological complete response had been obtained. On the other hand, no histopathological acute inflammation and acute necrotic hemorrhagic pancreatic reaction was seen for the normal tissue, which demonstrated that this IA FOLFIRINOX infusion therapy was not dangerous for the normal pancreatic tissue.

From a clinical point of view, we would underline the absence of side effects due to the therapy over the three cycles of IA pancreatic infusion, with no hematological toxicities following therapy administration. The particularity of this clinical case is due to the death of the patient due to the indicated nosocomial acquired *Legionella pneumophila* infection, despite the documented pathological tumor response.

This clinical trial is ongoing; however, we feel the need to divulgate this unexpected impressive complete tumor response to the medical scientific community. Finally, we believe that this preliminary result will be confirmed in the next patients to be enrolled, which should thus provide a glimmer of hope for patients with this lethal disease.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the IRCCS Istituto Tumori "Giovanni Paolo II" Bari, Italy. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization, CD and GR. Methodology, GR, SS, and CD. Software, PM, Validation, GR and CD. Formal analysis, GR, CL and SS. Investigation, CD, GR and MP. Resources, CL and VF. Data curation, GR and CL. Writing original draft preparation,

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CEUS and CT/MRI LI-RADS in Association With Serum Biomarkers for Differentiation of Combined Hepatocellular-Cholangiocarcinoma From Hepatocellular Carcinoma

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Background: Combined Hepatocellular-cholangiocarcinoma (cHCC-CCAs) are with both unambiguously differentiated hepatocellular and biliary components. cHCC-CCAs show various imaging features similar to hepatocellular carcinoma (HCCs) and intrahepatic cholangiocarcinoma (ICCs), which makes the differential diagnosis between them challenging. The accurate diagnosis of cHCC-CCAs is of great importance in selecting treatment methods and performing patient management.

Purpose: To investigate the diagnostic efficacy of CEUS and CT/MRI LI-RADS in association with tumor biomarkers for differentiation of cHCC-CCAs from HCCs.

Methods: A total of 54 cHCC-CCAs and 55 HCCs in two centers were retrospectively collected. The diagnostic criteria for cHCC-CCAs if one or more of the following conditions were satisfied: (1) arterial phase hyperenhancement (APHE) on CEUS and LR-M on CT/MRI; (2) LR-5 on both CEUS and CT/MRI with elevated carbohydrate antigen 19-9 (CA19-9); (3) LR-M on both CEUS and CT/MRI with elevated alphafetoprotein (AFP). The sensitivity, specificity, accuracy and area under the receiver operating characteristic curve (AUC) were calculated.

Results: The rates of APHE and Rim-APHE on CEUS in cHCC-CCAs were 81.5% and 9.3%, respectively. The rate of early and marked washout on CEUS in cHCC-CCAs were 59.3% and 27.8%, respectively. 64.8% and 25.9% of cHCC-CCAs showed APHE and Rim-APHE on CT/MRI, respectively. 46.3% and 35.2% of cHCC-CCAs showed washout and delay enhancement on CT/MRI, respectively. The kappa value of LI-RADS categories of cHCC-CCAs on CEUS and CT/MRI was 0.319 (P=0.008). The sensitivity, specificity, accuracy and AUC of the aforementioned diagnostic criteria for cHCC-CCAs were 64.8%, 84.4%, 76.1% and 0.746, respectively.

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Conclusion: The combination of the CEUS and CT/MRI LI-RADS with serum tumor markers shows promising diagnostic performance of cHCC-CCAs.

Keywords: liver imaging reporting and data system, contrast-enhanced ultrasound, contrast-enhanced magnetic resonance imaging, contrast-enhanced computed tomography, combined hepatocellular-cholangiocarcinoma

INTRODUCTION

Combined Hepatocellular-cholangiocarcinoma (cHCC-CCAs) comprise a minority (2.0%-5.0%) of primary hepatic malignancies (1). Tumors with both unambiguously differentiated hepatocellular and biliary components are defined as cHCC-CCAs, based on the 2019 World Health Organization classification (1). The origin, biological behavior, treatment method and prognosis of cHCC-CCAs differ from HCCs and intrahepatic cholangiocarcinoma (ICCs), the first and second common primary hepatic malignancies (2, 3). Liver resection may be the optimal treatment method for cHCC-CCAs, as pointed out by recent studies (4, 5). Thus, accurate diagnosis of cHCC-CCAs is of great importance in selecting treatment methods and performing patient management. In the past ten years, the pre-treatment diagnosis of cHCC-CCAs may sometimes be ignored by clinicians due to its low probability. Recently, knowledge for this specific type of tumor accumulates through clinical practice and is widely reported, which makes the pre-treatment diagnosis of cHCC-CCAs by contrast-enhanced imaging modalities a frontier of medical imaging (6–10).

cHCC-CCAs show various imaging features similar to HCCs and ICCs, which makes the differential diagnosis between them challenging. Recently, combining contrast-enhanced imaging and biomarkers to diagnose cHCC-CCAs shows promising potential for differentiating cHCC-CCAs from HCCs and ICCs (8, 11, 12). However, the diagnostic performance of mono-modality contrast-enhanced imaging with biomarkers for cHCC-CCAs is still unsatisfactory. As recently reported, the sensitivities for cHCC-CCAs reported in two studies (11, 12) were 32.5% and 50%, respectively, far from meeting clinical requirements.

In order to standardize the enhanced imaging for focal liver lesions, The American College of Radiology (ACR) published LI-RADS for CT/MRI and CEUS (13, 14). LI-RADS classifies liver lesions based on the size and imaging features and provides corresponding clinical management strategies. Compared with traditional enhanced imaging diagnosis, LI-RADS defines the image features and classifies lesions more definitely and detailly. The LR-M category of LI-RADS aims to differentiate HCCs from other non-HCC malignancies, which may be used as a reference for the diagnosis of cHCC-CCAs.

Previously, either enhancement patterns or LI-RADS combined with biomarkers were used as diagnostic criteria for cHCC-CCAs (11, 12). Usually, mono-modality was included in the criteria. The combination of multi-modality imaging methods in the differential diagnosis of cHCC-CCAs has not been mentioned before. We notice that ICCs may demonstrate different enhancement patterns on CEUS and CT/MRI due to the biliary components and their different principles of enhanced imaging modalities (15, 16). Inspired by the aforementioned facts, we infer in this study that cHCC-CCAs can also show inconsistent enhancement patterns and be classified into different LI-RADS categories on CEUS and CT/MRI, which may provide a practically useful way for the diagnosis of cHCC-CCAs.

Therefore, we aim to combine the CEUS and CT/MRI LI-RADS with tumor biomarkers to differentiate cHCC-CCAs from HCCs and investigate the diagnostic efficacy of the new criteria.

MATERIALS AND METHODS

The study was approved by the research ethics board. Pathologically confirmed cHCC-CCAs in two centers between 2013 and 2021 were retrospectively collected in this study. Inclusion criteria were (1) patients with pathologically confirmed cHCC-CCAs, (2) patients with high risk for HCCs, (3) patients with pre-treatment CEUS and contrast enhanced CT/MRI within 1 month, and (4) patients with the examination of alphafetoprotein (AFP) and carbohydrate antigen 19-9 (CA19-9) levels before treatment. We randomly selected HCCs in the same period as the time of cHCC-CCAs collection to satisfy a 1:1 proportion. A total of 54 cHCC-CCAs and 55 HCCs patients were collected.

CEUS Examination

Patients underwent B-mode ultrasound and CEUS examination by an ultrasound system, such as EPICQ 7 (Philips Medical Solutions) and SIEMENS 3000 (Siemens Healthineers), equipped with an abdominal convex transducer (frequency range of 2.0-5.0 MHz). For the CEUS examination, 1.2 to 2.0 mL contrast agent (SonoVue, Bracco) was injected intravenously and flushed with 5 mL of 0.9% saline solution. The imaging timer was started immediately upon completion of injection. The target lesion was observed for 4 to 6 minutes and then the images was stored.

Contrast-Enhanced CT/MRI Examinations

Dynamic contrast enhanced CT scanning was performed by Somatom Definition Flash dual-energy CT (Siemens Medical Solutions). The contrast agent, Iohexol (350mgl/ml, Beilu Pharmaceutical Co., Ltd) at a dosage of 1.2 ml/kg body weight and a flow rate of 3.5 ml/s, was injected with a pressure injector *via* the median cubital vein. The hepatic arterial phase imaging acquisition started at about 25 s to 35 s after the initiation of contrast injection. The portal venous phase imaging acquisition started at about 50 s to 70 s after the initiation of contrast injection, and the late phase was at about 180 s after the initiation of contrast injection.

Contrast-enhanced MRI scans were performed according to each institution's protocol for focal liver lesions. MR imaging was performed with Siemens Magnetom Verio 3.0T magnetic resonance unit (Siemens Medical Solutions). Liver MR imaging protocol consisted of in-phase and opposed-phase T1 weighted imaging, FSE T2-weighted imaging with fat suppression and diffused weighted imaging. Gadoxetic acid (Primovist; Bayer Healthcare) was used as the contrast agent for EOB-DTPA enhanced imaging (EOB-MRI). Ultravist was used as the contrast agent for extracellular contrast agent MRI (ECA-MRI). Arterial, portal venous and delay (or transitional) phase images were acquired at delay times of 15 s to 18 s, 50 s to 60 s and 180 s after contrast injection using Volumetric Interpolated Breath-hold Examination (VIBE) sequence (TR/TE/FA, 4.2/1.5/9, 300×400 matrix). For EOB-MRI, Hepatobiliary phase imaging was completed 20 minutes after the contrast injection.

Image Analysis

All observers were blinded to pathology and other imaging results. One observer (J. D. with more than 12 years of experience in liver CEUS) reviewed the CEUS images of liver nodules and assigned a category to each nodule based on CEUS LI-RADS (2017 version) (14). The observers determined the presence or absence of the following features based on definitions proposed by CEUS LI-RADS (2017 version): (1) size, (2) arterial phase hyperenhancement (APHE), (3) mild or late washout, (4) ancillary features, including definite growth, nodule-in-nodule and mosaic architecture. The criterion for CEUS LR-M was lesions with Rim-APHE or early washout or marked washout. One radiologist (X. Z. with more than 15 years of experience in CT/MRI) reviewed the CT/MRI images of lesions and classified the lesion into the corresponding category based on CT/MRI LI-RADS (2018 version) (13). The observers determined the presence or absence of the following features based on definitions proposed by CT/MRI LI-RADS (2018 version): (1) size, (2) APHE, (3) washout appearance according to the type of MRI (conventional washout was defined as hypointensity on the PVP or DP on ECA-MRI or hypointensity on the PVP on EOB-MRI), (4) enhancing "capsule", (5) threshold growth, (6)ancillary features, including restricted diffusion, mild-moderate T2 hyperintensity, corona enhancement, transitional phase hypointensity, hepatobiliary phase hypointensity, nodule-in-nodule and mosaic architecture. According to the diagnostic algorithm of ACR LI-RADS, lesions in LR-1 are defined as definitely benign lesions, LR-2, benign lesions, LR-3, the intermediate probability of malignancies, LR-4, probably HCCs, LR-5, definitely HCCs, LR-TIV, definite tumors in vein and LR-M, probably or definitely but not HCC-specific malignancies. Some uncommon HCCs and most of the non-HCC malignancies can be classified into the LR-M. Thus, LR-M can differentiate HCCs from other malignancies.

The diagnostic criteria for cHCC-CCAs if one or more of the following conditions were satisfied: (1) APHE on CEUS and CT/MRI LR-M; (2) CEUS LR-5 and CT/MRI LR-5 with elevated CA19-9; (3) CEUS LR-M and CT/MRI LR-M with elevated AFP.

Statistical Analysis

Quantitative data were expressed as the mean ± standard deviation. Qualitative data were presented as numbers and percentages. Differences in quantitative variables were tested by the independent sample t-test. Comparison of the categorical variables was performed by the χ^2 test or Fisher's. CEUS and CT/MRI LI-RADS for each lesion was assessed by Cohen's kappa. The area under the receiver operating characteristic curve (AUC) was used to analyze the performance of the diagnostic criteria. The sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) for cHCC-CCAs were calculated. A P value < 0.05 indicated a significant difference. Statistical analyses were performed using the SPSS software, version 22.0 (SPSS Inc.,).

RESULTS

Clinical Data of Patients

A total of 54 patients with cHCC-CCAs and 55 ones with HCCs were included in this study. No significant differences in sex, age, etiology, tumor size and tumor markers were observed between the two groups ($P > 0.05$). There were more patients with liver cirrhosis in the HCCs group than that in the cHCC-CCAs group. In addition, the percentage of patients in the HCCs group undergoing ultrasound-guided biopsy was higher than that for the cHCC-CCAs group. The clinical characteristics of patients in the two groups were shown in **Table 1**. In the group of cHCC-CCAs, 15 patients underwent ECA-MRI and 11 patients underwent EOB-MRI. In the group of HCCs, only one patient underwent MRI with extracellular agents and 29 patients underwent EOB-MRI.

Imaging Features of cHCC-CCAs and HCCs on CEUS and CT/MRI

A total of 81.5%, 9.3% and 9.3% lesions in the cHCC-CCAs group and 94.5%, 1.8% and 3.6% lesions in the HCCs group showed APHE, Rim-APHE and non-APHE in the arterial phase ($\chi^2 = 4.610$, P=0.1), respectively. Early washout, marked washout, delay and mild washout and non-washout were observed in 59.3%, 27.8%, 37.0% and 1.9% of cHCC-CCAs, and 14.5%, 7.3%, 72.7% and 7.3% of HCCs, respectively. Early washout and marked washout were more frequent in cHCC-CCAs than that of HCCs ($\chi^2 = 28.339$, P<0.001) (**Table 2**).

64.8%, 25.9% and 9.3% of cHCC-CCAs showed APHE, Rim-APHE, and non-APHE in the arterial phase, while the percentages of HCCs with these imaging features were 94.5%, 0, and 5.5%, respectively ($\chi^2 = 17.814$, P<0.001). Hypo-enhancement, delayed enhancement and iso- or hyper-enhancement in the portal and delay phases of CT/MRI were observed in 46.3%, 35.2% and 18.5% of cHCC-CCAs, and 96.4%, 0 and 3.6% of HCCs, respectively. Statistical significance of image features in the portal and delay phases was observed between the two groups ($\chi^2 = 34.378$, P<0.001) (**Table 3**).

TABLE 1 | Clinical characteristics of patients in the cHCC-CCAs and HCCs groups.

	cHCC-CCAs	HCCs	*P*
Sex (Male/Female)	43/11	45/10	0.772
Age	58.7 ± 9.6	57.2 ± 9.9	0.406
Etiology (Hepatitis B virus/Hepatitis C virus/Others)	44/4/6	46/3/6	0.915
Liver cirrhosis (Yes/No)	39/15	49/6	0.026
Tumor size on CEUS (cm)	4.42 ± 2.49	3.95 ± 2.23	0.306
Tumor size on CT/MRI (cm)	4.37 ± 2.46	3.72 ± 2.17	0.149
Pathological specimen (liver resection/ultrasound guided biopsy)	44/10	28/27	0.001
AFP (>15ng/ml/≤15 ng/ml)	35/19	31/24	0.367
CA199 (>39ng/ml/≤39 ng/ml)	19/35	13/42	0.186

TABLE 2 | Contrast enhancement patterns of cHCC-CCAs and HCCs on CEUS.

	cHCC-CCAs	HCCs
Arterial phase		
-APHE	44	52
-Rim APHE	5	1
-Non APHE	5	2
Portal and delay phases		
-Early washout	32	8
-Marked washout	15	4
-Delay and mild washout	20	40
-No washout	1	4

TABLE 3 | Contrast enhancement patterns of cHCC-CCAs and HCCs on CT/MRI.

	cHCC-CCAs	HCCs
Arterial Phase		
-APHE	35	52
-Rim APHE	14	0
-Non APHE	5	3
Portal and delay phases		
Hypo-enhancement	25	53
Delayed enhancement	19	0
Iso- or hyper enhancement	10	2

LI-RADS Categorizations of cHCC-CCAs on CEUS and CT/MRI

5.6%, 37.0% and 57.4% of cHCC-CCAs were categorized to LR-4, LR-5 and LR-M by CEUS, while 3.7%, 35.2% and 61.1% of cHCC-CCAs were categorized to LR-4, LR-5 and LR-M by CT/MRI, respectively. The Kappa value of the intermodality classifications on CEUS and CT/MRI LI-RADS for cHCC-CCAs was 0.319, *P*=0.008 (**Table 4**).

For HCCs, 1.8%, 9.1%, 69.1% and 20% lesions on CEUS, 1.8%, 16.4%, 81.8% and 0 on CT/MRI were categorized to LR-3, LR-4, LR-5 and LR-M, respectively. The Kappa value of the intermodality classifications on CEUS and CT/MRI LI-RADS for HCCs was 0.003 (*P*=0.968) (**Table 5**).

Diagnostic Performance of CEUS and CT/MRI LI-RADS in Association With Serum Biomarkers for the Diagnosis of cHCC-CCAs

We provided three diagnostic criteria, mentioned above, for cHCC-CCAs from HCCs. 35 cHCC-CCAs and 7 HCCs met at least one of the three criteria mentioned above (**Figures 1, 2**). 25 cHCC-CCAs showed APHE on CEUS and were in CT/MRI LR-M;

TABLE 4 | LI-RADS categorizations of cHCC-CCAs by CEUS and CT/MRI.

CT/MRI	CEUS			Total
	LR-4	LR-5	LR-M	
LR-4	0	1	2	3
LR-5	2	11	7	20
LR-M	0	7	24	31
Total	2	19	33	54

TABLE 5 | LI-RADS categorizations of HCCs by CEUS and CT/MRI.

CT/MRI	CEUS				Total
	LR-3	LR-4	LR-5	LR-M	
LR-3	0	0	1	0	1
LR-4	0	1	6	2	9
LR-5	1	4	31	9	44
LR-M	0	0	0	0	0
Total	1	5	38	11	55

(2) 6 cHCC-CCAs were in CEUS LR-5 and CT/MRI LR-5 with elevated CA19-9; (3) 15 cHCC-CCAs were in CEUS LR-M and CT/MRI LR-M with elevated AFP. 7 HCCs were in CEUS LR-5 and CT/MRI LR-5 with elevated CA19-9.The sensitivity, specificity, accuracy, PPV and NPV of the criteria for the diagnosis of cHCC-CCAs were 64.8%, 84.4%, 76.1%, 87.6% and 71.6%, respectively. The AUC was 0.746. (**Figure 3**).

DISCUSSION

cHCC-CCAs is a subtype of primary liver cancer with a low incidence compared with HCCs and ICCs (17). cHCC-CCAs can present imaging features similar to both HCCs and ICCs, which makes its differential diagnosis challenging. In this study, we show that CEUS and CT/MRI LI-RADS, presented by ACR, in association with serum biomarkers for differentiating cHCC-CCAs from HCCs, has significant diagnostic performance and can provide a diagnostic reference in clinical practice.

HCCs and ICCs are easy to diagnose based on the typical enhancement patterns on CT/MRI and CEUS (18, 19). However, the enhancement patterns of cHCC-CCAs are affected by the proportions of HCC- or ICC-like histologic components, leading to a significant barrier for the diagnosis of cHCC-CCAs (20). Recently, several studies focused on the combination of contrast-enhanced patterns and serum biomarkers to diagnose cHCC-CCAs due to the lack of typical enhanced patterns (8, 11, 12). The diagnostic criteria for cHCC-CCAs mentioned by Li et al. (11) and Huang et al. (12) include lesions with typical imaging features of HCCs and elevated CA19-9, lesions with typical imaging features of ICCs and elevated AFP, and lesions with typical imaging features of HCCs or ICCs with both elevated CA19-9 and AFP. Li et al. (11) showed a promising sensitivity in the diagnosis of cHCC-CCAs when using as the diagnostic criterion the simultaneous elevation of AFP and CA19-9, or different diagnostic results from tumor markers and CEUS (51.1%), and contrast-enhanced CT (53.5%). These results indicated that almost half of cHCC-CCAs were misdiagnosed even if the combination of imaging features and tumor markers were adopted as the diagnostic criteria.

ACR developed CT/MRI and CEUS LI-RADS to standardize categorization for liver lesions in high-risk patients and improve communication of clinicians by classifying the lesions into LR-1 to LR-5, LR-M and LR-TIV. Among the seven classes, LR-5 shows a high PPV and specificity for HCCs, which provides a reference for physicians in clinical decision-making (13, 14). Almost all the previous studies reported that the PPVs of both CEUS and CT/MRI LR-5 for HCCs were above 95% (21–23).

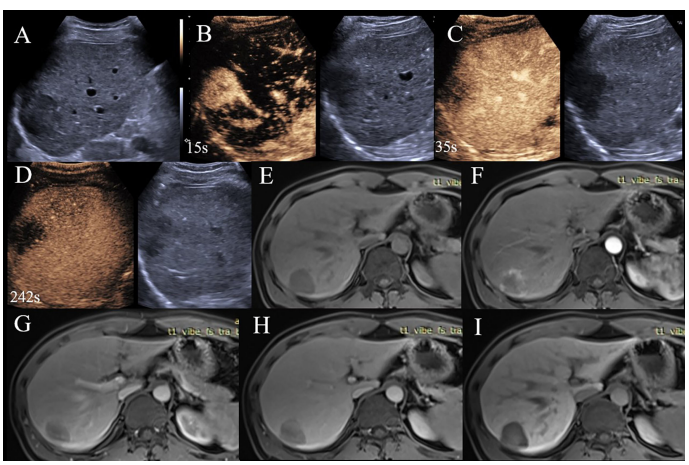

FIGURE 1 | A 36-year-old man with HBV related liver cirrhosis. Serologic data indicated AFP of 167 ng/ml and CA19-9 of 13.5 ng/ml. **(A)** A hypo echoic lesion located under the liver capsule with the size of 4.1×3.8cm. **(B)** The lesion displayed APHE on CEUE; **(C)** Early washout was observed at 35s after injection of contrast agent; **(D)** Washout was observed on delay phase, the lesion was categorized as CEUS LR-M. **(E)** The lesion displayed hypointensity on EOB-MRI; **(F)** Rim-APHE was observed on EOB-MRI; **(G, H)** Delayed enhancement was also observed on portal and transitional phases; **(I)** The lesion showed hypointensity on hepatobiliary phase, the lesion was EOB MRI LR-M. The final diagnosis was cHCC-CCA.

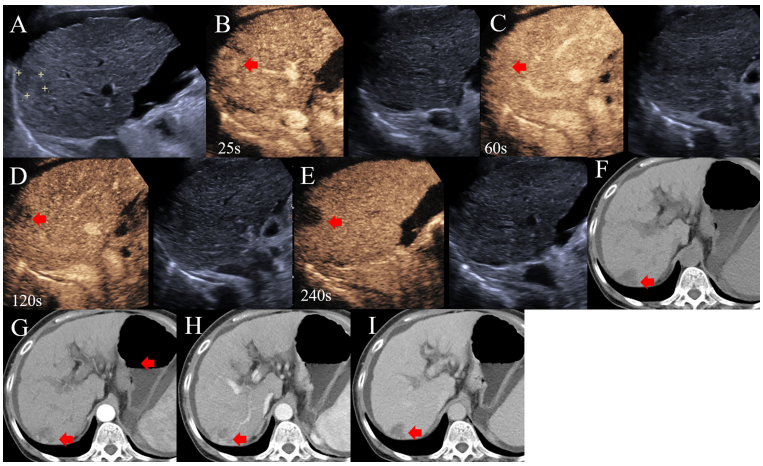

FIGURE 2 | A 68-year-old woman with HBV related liver cirrhosis and elevated AFP (1210ng/ml) and CA19-9 (43.03 ng/ml). **(A)** A hypo-echoic lesion with a size of 1.9cm was observed by US. **(B)** The lesion showed APHE on CEUS; **(C)** without washout 1 min after injection of contrast agent; **(D)** Delay and mild washout was observed 2 min after injection; **(E)** The lesion appeared punched-out 4 min after injection of contrast agent. The lesion was categorized to CEUS LR-5. **(F)** A hypo-intensive lesion was found on CT; **(G, H)** The heterogeneous enhancement was observed on the arterial and portal phases of contrast enhanced CT; **(I)** The lesion was hypo-intensive on the delay phase and classified into CT LR-5. The final diagnosis was cHCC-CCAs, as confirmed by pathology.

Thus, CEUS and CT/MRI LR-5 can be used as diagnostic criteria for HCCs. Therefore, LI-RADS provides a possibility for the differential diagnosis of cHCC-CCAs and HCCs (24–26). Using either CEUS LR-5 with elevated CA19-9, CEUS LR-M with elevated AFP or CEUS LR-5/LR-M with both elevated CA19-9 and AFP, as the diagnostic criteria for cHCC-CCAs, the AUC, sensitivity and specificity were 0.649, 40.0% and 89.9%, respectively (27). This result preliminarily demonstrated the diagnostic value of LI-RADS combined with tumor markers for cHCC-CCAs.

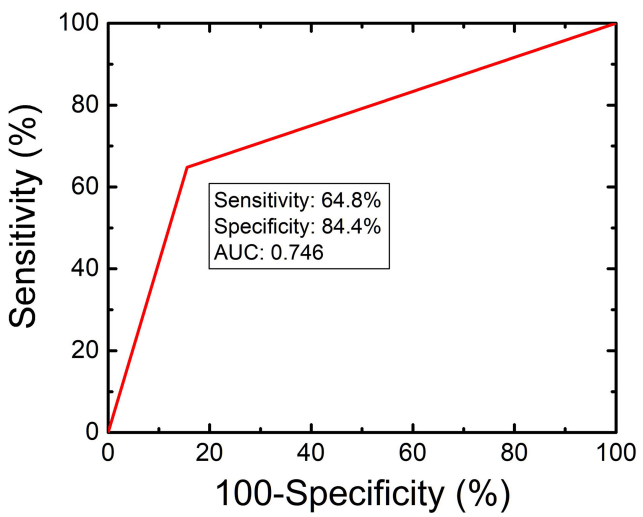

FIGURE 3 | Diagnostic performance of CEUS and CT/MRI LI-RADS in association with serum biomarkers for the diagnosis of cHCC-CCAs.

Although imaging features and elevated tumor markers attract attention in the diagnosis of cHCC-CCAs, the possible indication of the discordance between contrast-enhanced patterns in CEUS and CT/MRI was ignored. ICCs, which show "wash-in and washout" on CEUS and "Rim-APHE and delayed enhancement" on CT/MRI, have inconsistent contrast enhanced patterns in CEUS and CECT/MRI due to the different imaging principles. This discordance between contrast-enhanced patterns provided critical imaging information in the diagnosis of ICCs (15, 16). cHCC-CCAs have the same histologic components as ICCs. We, therefore, hypothesize that the discordance between contrast-enhanced patterns of CEUS and CT/MRI may be an indication for cHCC-CCAs.

In our study, we compared the major imaging features of cHCC-CCAs and HCCs on CEUS and CT/MRI. The results reveal that most cHCC-CCAs and HCCs showed APHE on CEUS without a statistical significance. The frequencies of marked washout and early washout in cHCC-CCAs, however, were higher than those of HCCs, which were consistent with a previous study (27). On CT/MRI, the frequencies of Rim-APHE and delayed enhancement in cHCC-CCAs were higher than those of HCCs, respectively. For the LI-RADS categorization, most of cHCC-CCAs were classified to CT/MRI and CEUS LR-5 or LR-M. The Kappa value of the inter-modality of the classifications by CEUS and CT/MRI LI-RADS for cHCC-CCAs was 0.319, indicative of a significant inconsistency between the two imaging methods. Most of the HCCs, on the contrary, were categorized to LR-5 both in CEUS and CT/MRI LI-RADS.

We propose new diagnostic criteria for cHCC-CCAs. as mentioned above, based on the combination of the different diagnostic results from the enhancement pattern on CEUS and CT/MRI and tumor markers. The result suggests that our new

diagnostic criteria have a good performance for cHCC-CCAs. Yang et al. (27) presented that "CEUS LR-M with elevated AFP" can be one of the diagnostic criteria for cHCC-CCAs. However, several studies found that 50% to 75% of lesions in CEUS LR-M were HCCs (23, 28), which was usually accompanied by an elevation of AFP. Thus, it can be inferred that the criterion "CEUS LR-M with elevated AFP" may lead to misdiagnosis. In the present study, we used the "CEUS LR-M and CT/MRI LR-M with elevated AFP" instead of "CEUS LR-M with elevated AFP" as a diagnostic criterion. Our choice is based on the fact that few HCCs can be categorized as both CT/MRI and CEUS LR-M.

There are a few limitations of our study. First, we included HCCs but not ICCs in the control group. Second, the inter-reader agreement between CEUS and CT/MRI LI-RADS was not explored.

In conclusion, most of cHCC-CCAs were categorized to LR-5 and LR-M by both CEUS and CT/MRI LI-RADS. By combining CEUS and CT/MRI LI-RADS in association with serum biomarkers we presented new criteria for the diagnosis of cHCC-CCAs. The results show that the new diagnostic algorithm shows a prior diagnostic performance. We believe the diagnostic criteria shown in this study can be used to help clinical decision-making.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

YZ and SY designed the study and wrote the manuscript. YZ, SY, LZ, XZ, ML, and JD collected the data. KY and XJ supervised the findings of this study. All authors contributed to the article and approved the submitted version.

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Neoadjuvant Treatment in Resectable Pancreatic Cancer. Is It Time for Pushing on It?

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Pancreatic resection still represents the only curative option for patients affected by pancreatic ductal adenocarcinoma (PDAC). However, the association with modern chemotherapy regimens is a key factor in improving the inauspicious oncological outcome. The benefit of neoadjuvant treatment (NAT) for borderline resectable/locally advanced PDAC has been demonstrated; this evidence raises the question of whether even resectable PDAC should undergo NAT rather than upfront surgery. NAT may avoid futile surgery because of undetected distant metastases or aggressive tumor biology, providing more effective systemic control of the disease, which is hampered when adjuvant chemotherapy is delayed or precluded. However, recent data show controversial results regarding the efficacy and safety of NAT in resectable PDAC compared to upfront surgery. Although several prospective studies and meta-analyses indicate better oncologic outcomes after NAT, there are some biases, such as the methodological approaches used to capture the events of interest, which could make these results hardly reproducible. For instance, per-protocol studies, considering only the postoperative outcomes, tend to overestimate the performance of NAT by excluding patients who will never be suitable for surgery due to the development of chemotoxicity or tumor progression. To draw reliable conclusions, the studies should capture the events of interest of both strategies (NAT/upfront surgery) from the time of allocation to a specific treatment in an intention-to-treat fashion. This critical review highlights the current literature data concerning the use of NAT in resectable PDAC, summarizing the results of high-quality studies and focusing on the methodological issues of the most recent pieces of evidence.

Keywords: pancreatic adenocarcinoma, resectable pancreatic adenocarcinoma, neoadjuvant treatment, chemotherapy, upfront surgery

Abbreviations: NAT, Neoadjuvant treatment; UFS, Up-front surgery; RCT, randomized controlled trials; OS, Overall survival; DFS, Disease-free survival; PDAC, Pancreatic ductal adenocarcinoma; RPDAC, Resectable pancreatic ductal adenocarcinoma; NCCN, National Comprehensive Cancer Network; ITT, intention-to-treat; PP, per-protocol.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the fourth and fifth most common cause of cancer deaths in the USA and Europe, respectively (1, 2). The incidence of pancreatic ductal adenocarcinoma has risen rapidly. By 2030, PDAC is expected to become the second most prevalent cause of death by cancer after lung cancer (3). Although surgery represents the only potentially curative treatment for PDAC, only 20% of patients are candidates for surgery because of the presence of distant metastasis or major vessel involvement at the time of the diagnosis (4). Based on the well-known radiological classification of PDAC, the National Comprehensive Cancer Network (NCCN) defines as resectable PDAC (RPDAC) tumors that do not show any contact with arteries (celiac axis, superior mesenteric artery, or common hepatic artery) or veins (the superior mesenteric vein or portal vein). If venous contact is present, this must involve ≤180° of the vessel circumference without any vein contour irregularity to qualify the tumor as resectable. Based on this radiological classification, the classification of PDAC has been standardized worldwide (5).

Neoadjuvant treatment (NAT) in PDAC is currently recommended by the International Guidelines for patients with borderline resectable or locally advanced disease, considering these neoadjuvant protocols as an induction therapy (6). In this subgroup of patients with advanced stage disease due to vascular involvement at the time of the diagnosis, the delivery of NAT takes over the task of testing the biological behavior of the tumor, decreasing the incidence of explorative surgery and downstaging disease in patients to achieve surgical resectability (7, 8). Thanks to the development of new effective chemotherapeutic protocols, namely gemcitabine and nab-paclitaxel (Abraxane) or leucovorin, 5-fluorouracil, irinotecan, oxaliplatin (FOLFIRINOX), postoperative oncologic outcomes of borderline resectable and even locally advanced PDAC have steadily improved and they are now comparable to those of patients with RPDAC at the time of the first diagnosis (9–12).

NEADJIUVANT TREATMENTS IN RESECTABLE PDAC: LIGHTS AND SHADOWS

In the last two decades, up-front surgery (UFS) has not substantially changed the overall (OS) and disease-free survival (DFS) of patients with RPDAC, despite the consistent development of adjuvant therapy (AT). The presence of undetected micrometastases at the time of surgery together with the biological aggressiveness of the tumor itself are the main reasons for slipping into early tumor recurrence (13, 14). Based on clinical evidence, many experts have suggested that PDAC, even in its early-stage, should be considered as a systemic disease that could potentially benefit from NAT (15–19). The recent NCCN Guidelines Version 1.2021 recommended NAT not only in cases of borderline resectable pancreatic cancer but also in high-risk resectable PDAC (based on radiological findings, elevated CA 19-9, large tumors, large regional lymph nodes, excessive weight loss, and extreme pain) (**Figure 1**). However, evidence on the benefits of NAT in RPDAC is still weak, so in daily clinical practice, upfront-surgery followed by adjuvant chemotherapy is still recommended as the standard treatment in cases of PDAC judged as "resectable" (4, 6). Although this management is currently performed in clinical practice, many concerns still remain as a large proportion of resected patients develop early recurrence, nullifying the potential advantages of the UFS (20). Besides, pancreatic resection is still a high-risk procedure, and nearly 50% of resected patients eventually fail to receive adjuvant therapy due to post-operative complications or reduced performance status. These possible downsides of surgery strengthen the concept that NAT might be given to patients with RPDAC to detect aggressive disease by preventing futile surgical procedures, treat the potential hidden micrometastases, achieve a higher R0 resection rate, and deliver systemic therapy in all cases (21). Once the diagnosis of RPDAC is established, the choice of surgery as first-line treatment is no longer so obvious, as NAT might be considered as well.

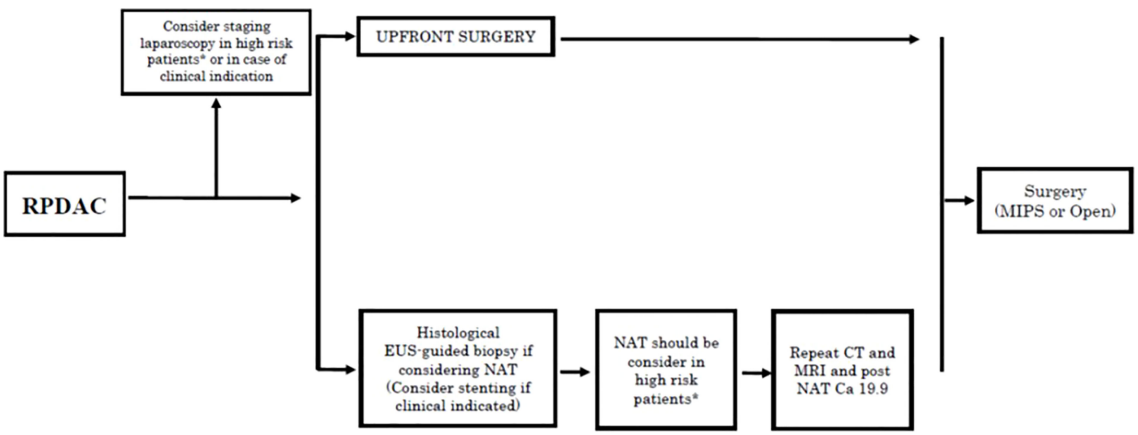

FIGURE 1 | Treatment algorithms for resectable pancreatic cancer reported in the NCCN Guidelines Version 1.2021. (6). RPDC, Resectable pancreatic ductal adenocarcinoma; NAT, neoadjuvant treatments; EUS, endoscopic ultrasound; CT, computed tomography; MRI, Magnetic resonance; MIPS, minimally invasive pancreatic surgery. *High risk patients: Patiens with high risk features in terms of radiological (large primary tumor, large lymphonodes suspected for metastatic) and/or biological findings (Ca 19.9 > 500 U/ml in case of absence of biliary obstruction and/or cholangitis, extreme irradiated pain, excessive weight loss).

Although studies have shown that NAT tends to improve the OS of patients with resectable PDAC, most of them were limited by the low level of evidence (retrospective cohort studies/case series), the small sample size, and older chemotherapy regimens used (22–24). Considering that several randomized controlled trials (RCT) failed to demonstrate a clear advantage in OS or did not provide the results in the specific subgroup of resectable patients, it has been necessary for researchers to rely on systematic reviews that pool the existing evidence (18, 25). Several meta-analyses recently reported favorable results regarding NAT in RPDAC, in terms of long-term survival and R0 resection rate. However, most of these studies were either flawed by substantial heterogeneity in terms of definition of resectability, chemo-radiotherapic regimens administered, or did not distinguish the results of resectable from borderline resectable PDAC. Moreover, we should consider that, when compared to those who underwent upfront-surgery, 30% of the patients who received NAT dropped-out from a surgical treatment program, and therefore did not receive any curative therapy (26–29).

Indeed, NAT in RPDAC patients may be related to potential drawbacks, such as the onset of jaundice, disease progression, and chemotherapy-related toxicity, leading to drop-out of the patient from the surgical plan (30). Theoretically, studies based on intention-to-treat (ITT) analysis may address these issues. In fact, ITT analysis considers the events of interest from the time of diagnosis and not from the time of surgical treatment.

A critical review should be performed even of those studies designed with an ITT perspective, to avoid misleading results produced by substantial methodological bias. For instance, in the recent meta-analysis reported by Versteijne et al. that showed a significant improvement in ITT-OS for RPDAC treated by NAT, several single-arm studies were included, which represents a significant reporting bias (31). In another recent meta-analysis by Van Dam et al., although the strict selection criteria (only RCT included) and the ITT methodology were used, the results focused mostly on borderline resectable tumors (32). As the role and true effectiveness of NAT in RPDAC remain unclear, in this critical review, we aimed to assess the benefits of NAT in patients with RPDAC compared with the standard practice, represented by upfront surgery. To minimize the selection bias, we decided to set the following inclusion criteria:

- Highest level of evidence studies:
  - RCT
  - The most recent metanalyses (2019–2021).
- Clear report of results of NAT in RPDAC (excludes those pooling together results of RPDAC and borderline resectable PDAC)
- ITT-based analysis
- Clear comparison between NAT and UFS for RPDAC.

KEY STUDIES INVESTIGATING NAT EFFECTIVENESS IN RPDAC

Among papers analyzing the benefits of NAT in patients affected by RPDAC, eight reports matched the criteria to be considered valuable for this critical review (18, 24, 27, 33–37) (**Table 1**). These studies indicate contentious results on the advantages of NAT for RPDAC, especially in terms of OS and DFS. Conversely, wider agreement was found when looking at the resection rate and pathologic parameters (i.e., R0 rate and lymph node metastasis rate).

Patients Survival

In a recent meta-analysis reported by Pan et al., 17 studies investigating the effectiveness of NAT for PDAC from 2011 to 2018 were included; however, only 9 of them focused specifically on RPDAC, while the others combined results obtained from studies on both RPDAC and border-line resectable PDAC (27). The per-protocol (PP) analysis (outcome observed after curative surgery) showed better OS for patients who underwent NAT (HR, 0.75 [95% CI, 0.63–0.89], I2 = 0%), but this finding was not confirmed in the ITT-pooled analysis, which showed comparable results between the NAT and the UPS group (HR, 1.02 [95% CI,

TABLE 1 | Summary of the key studies assessing NAT effectiveness in RPDAC.

Authors	Year	Country	Study design	No. of patients	OS		DFS	RR	Pathological parameters	
					PP	ITT			R0	LN0
Golcher et al. (18)	2015	Germany	RCT*	73*	=	=	=	=	=	=
Casadei et al. (37)	2015	Italy	RCT*	38*	NR	=	NR	<NAT	=	=
Reni et al. (24)	2018	Italy	RCT**	93	>NAT	>NAT	NR	=	>NAT	>NAT
Unno et al. (34)	2019	Japan	RCT°	360	>NAT	>NAT	NR	=	>NAT	>NAT
Lee et al. (35)	2019	Korea	Meta-analysis	9691	>NAT	=	=	<NAT	>NAT	>NAT
Pan et al. (27)	2020	China	Meta-analysis	2286	>NAT	=	>NAT	<NAT	>NAT	>NAT
Ye et al. (36)	2020	China	Meta-analysis	9773	NR	=	=	<NAT	>NAT	>NAT
Versteijne et al. (33)	2020	Netherlands	RCT	246	NR	=	>NAT	=	=	>NAT

NAT, neoadjuvant treatments; OS, overall survival; DFS, disease-free survival; PP, per-protocol analysis; ITT, intention-to-treat analysis; RR, resection rate; R0, negative margin; LN0, negative metastatic lymph nodes; >NAT, advantage in NAT patients; NR, not reported; =, comparable results between NAT and UFS.

**Concluded earlier due to the slow recruitment.*

***Due to the modifications in the standard of care for adjuvant therapy regimens, phase 3 of the PACT-15 was suspended.*

°Preliminary results presented at the 2019 ASCO Gastrointestinal Cancers Symposium.

0.85–1.22], I2 = 26.5%). Of note, although in this study RPDAC patients undergoing NAT presented a trend toward better DFS and a lower recurrence rate than those of the UFS group, this finding failed to achieve statistical significance (DFS: HR = 0.80, P = 0.137; recurrence rate: OR = 0.77, P = 0.131). Among the studies analyzed in this systematic review, Golcher et al. published in 2015 the first RCT on NAT for RPDAC, reporting comparable results to UFS in terms of OS and DFS (18). The study was stopped earlier than planned due to the slow recruitment (only 73 patients were recruited between 2003 and 2009) and the chemotherapy regimens used look outdated nowadays, making these results unreliable. Similarly, Casadei et al. in their RCT published in 2015 reported comparable OS between NAT and UFS; however, as with the aforementioned trial, this study was concluded earlier due to the difficulty in recruiting patients (only 38 were eventually recruited) (37) and the old chemotherapy regimens used represent a possible limitation again, as gemcitabine alone is actually outdated in favor of FOLFIRINOX or gemcitabine and Abraxane regimens.

In the same way, Lee et al. in their systematic review compared the OS of the two treatment strategies (NAT *vs* UFS) in RPDAC patients by stratifying the results according to the analytic methods (ITT *or* PP) (35). Interestingly, the authors performed a sensitivity analysis to investigate the sources of heterogeneity, making this report one of the most reliable from a methodological perspective. In the studies reviewed until 2018, as already reported by Pan et al., 12 PP analysis papers showed that NAT brought a survival benefit over UFS (HR 0.72, 95% CI 0.68–0.76, P <0.001), whereas the 7 studies conducted with ITT methodology did not show any statistical difference (HR 0.96, 95% CI 0.82–1.12, P = 0.610). When considering only patients in whom the anticancer therapy was effectively delivered (before or after surgery), PP-OS appeared significantly improved in the NAT strategy (HR 0.82, 95% CI 0.71–0.93, P = 0.003). However, from an ITT perspective, 36.3% of the patients in the NAT treatment strategy eventually failed to undergo surgery versus 17.3% of those who were deemed to have UFS, probably due to a significant increase in the so-called pre-surgical "attrition rate" in the NAT group. Attrition in surgery is defined as loss to follow-up secondary to self-discharge, inability to complete the therapeutic plan due to poor compliance or deterioration of the physical condition. When considering only patients who completed both surgery and chemotherapy, NAT showed a PP-OS advantage over UFS.

The PREOPANC, a Dutch randomized phase III trial of 16 centers, enrolling 246 patients with resectable or borderline resectable pancreatic cancer, was the first RCT to utilize preoperative chemoradiotherapy (33). In this study, the results obtained were substantially in keeping with the other studies previously described, with comparable OS in the ITT analysis. However, the application of the protocol used in this trial, namely, the use of single-agent gemcitabine adjuvant therapy, appears somehow outdated currently. Moreover, the median OS in the UFS group was better than expected (14 instead of 11 months), which might be related to a substantial drop-out of high-risk patients ("presurgical triage"), as reported by the authors. The PREOPANC trial, as well as previous studies, when considering resectable patients only, did not demonstrate a significant change in OS and DFS of RPDAC patients; in contrast, a trend toward better survival was observed for the UPS arm. However, the advantages found in the R0 rate and positive lymph node rates might support NAT in RPDAC.

To date, only 2 studies have reported an advantage of NAT compared to UFS in terms of OS. Reni et al. (PACT-15) published in 2018 the results of a randomized, open-label, phase 2–3 trial: the trial had strict selection criteria and it was structured into three arms: two arms included patients undergoing UFS with two different adjuvant treatments, while the third arm included patients who received NAT (24). Median OS was 38.2 months (27.3–49.1) for patients randomly assigned to the NAT arm, and 20.4 (95% CI 14.6–25.8) and 26.4 months (95% CI 15.8–26.7) for patients randomly assigned to the 2 UFS groups. However, as mentioned by the authors, during phase 2 of the trial, the standard-of-care for adjuvant therapy changed and new chemotherapy regimens, which are apparently more active or based on more robust evidence than the PEXG regimen (second arm), were available only for the metastatic disease setting. Therefore, the authors decided to not proceed with phase 3 of the trial. Moreover, the sample size of each study arm was about 1/3 of the required population needed to statistically demonstrate the OS advantage of NAT over UFS.

Lastly, the Prep-02/JSAP05 is a Japanese randomized multi-institutional phase II/III trial that compared NAT using gemcitabine and S-1 (NAC-GS) with upfront surgery for patients with RPDAC (34). As a matter of fact, this study is the first multiinstitutional Phase III trial showing that NAT leads to significant advantages in terms of OS in patients with RPDAC in ITT analysis, with the preliminary results being presented at the 2019 ASCO Gastrointestinal Cancers Symposium. Unlike the previous papers reviewed, this study reported a median OS of 36.7 months in the NAT group and 26.6 months in the UFS group (p = 0.015; HR: 0.72; 95% CI: 0.55–0.94); patients in the NAT arm were treated with different therapeutic protocols with a longer duration of systemic therapy than those in the UFS arm, and these preliminary results have not been confirmed in a thorough report yet. Unfortunately, no significant conclusions can be drawn from the aforementioned preliminary results yet. Indeed, after more than three years since this report, no study has been published, raising some doubts about the completion of the trial itself.

Resection Rate and Pathologic Parameters

Among the secondary outcomes, the two meta-analyses reviewed showed concordant results in terms of resection rate that was significantly lower in RPDAC patients undergoing NAT (27, 35). Noticeably, this finding was confirmed in the systematic review of Ye et al. that was mostly focused on these parameters: a significantly lower resection rate was observed in the NAT compared with the UFS group (OR = 2.18, 95% CI 1.41–3.37, P = 0.0004, I2 = 43%) regardless of the treatment protocols used. The authors concluded that NAT in patients with RPDAC may jeopardize the opportunity for surgical resection (36).

In the PREOPANC trial, the resection rate was 62% in the NAT arm and 72% in the UFS arm; however, this finding failed to reach statistical significance (P = .058). The Prep-02/JSAP05 and the PACT-15 trial did not show any difference in the resection rate, but the need for stronger evidence on this issue was recommended (24, 33, 34). A lower resection rate may not necessarily represent a downside of NAT; for some authors, NAT could in fact triage patients who would not benefit from surgery.

Concerning pathologic parameters, there is some evidence in all studies that a higher R0 resection rate and a lower rate of metastatic lymph nodes were recorded in NAT compared to UFS. For instance, recently, in the meta-analysis reported by Xu et al., patients who underwent NAT presented an increased R0 resection rate for RPDAC (OR = 1.59, 95% CI = 1.41–1.80) (38). However, when analyzing from an ITT perspective, this result failed to reach significance (OR = 1.45, 95% CI = 0.91–2.30). Notably, we decided to exclude this study from our review because the ITT methodology was assessed for one parameter only (R0 rate), thus failing to meet the inclusion criteria set in this review.

PRESENT EVIDENCES AND FUTURE PERSPECTIVES

In this critical review, we aimed to reduce potential methodological biases of the available studies by evaluating the highest quality papers and the most recent systematic reviews reporting data on the use of NAT in RPDAC. Furthermore, we considered only studies based on ITT analysis instead of PP methodology because we strongly believe that ITT is the only analytic method able to capture and analyze all the events of interest (i.e., radio-chemotoxicity, unsuitability for surgery after NAT) from the diagnosis, thus demonstrating the real harms and benefits of new oncological approaches.

Nowadays, whereas there is robust evidence to support the systematic use of NAT in borderline resectable tumors, we are far from achieving a definitive agreement on the opportunity to offer NAT as the first-line treatment to all patients with RPDAC. The RCT published so far, comparing the two above-mentioned strategies, failed to demonstrate with statistical significance the advantage of NAT in terms of OS and DFS in patients with RPDAC (18, 24, 33, 37). The results of another Japanese RCT that seems to show improved survival in patients who underwent NAT for RPDAC have not been published in full yet, thus raising some doubts about the good completion of the trial (34).

In favor of NAT for RPDAC, there could be the feeling that the drop-out from surgery, which is higher when NAT is performed, should not be considered a missed chance of cure but an opportunity for sparing futile high-risk surgery. However, this assumption needs clear conformation based on evidence. Alternatively, a proportion of resectable patients could miss the chance of radical surgery due to the pre-surgical "attrition" and the disease progression during NAT. Furthermore, for patients with high bilirubin levels at the time of the diagnosis, there might be a considerable delay in starting the chemotherapy, as not all biliary stenting procedures achieve an immediate effect.

We believe that the definition of resectability based on technical features only (absence of tumor vascular involvement) does not capture those patients for whom NAT can have a strong rationale and that studies should pobably be more focused on high-risk resectable cancers with high levels of serum CA 19-9 or evidence of lymph node involvement.

In the future, the choice of the best multimodal treatment of RPDAC should probably be based on the biological behavior of the tumor rather than on the loco-regional staging of the tumor, which currently represents the cornerstone of the decision-making process with regard to first-line treatment. More effective and individualized systemic therapeutic regimens will probably stem from a better knowledge of clinic-pathological prognostic factors such as molecular profiling and novel biomarkers (39).

AUTHOR CONTRIBUTIONS

MV and ABC conceived the paper. MV, ABC, and RR wrote the manuscript. FM, AV, GC, EDB, and DN contributed critical revision of the manuscript for important intellectual content. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.914203/full#supplementary-material

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Imaging and Clinicopathological Features of Acinar Cell Carcinoma

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Background: Acinar cell carcinoma (ACC) is a rare pancreatic epithelial malignancy that poses a significant threat. However, there are few related clinical studies. The present study aimed to analyze the imaging and pathological features of ACC to provide a reference for better diagnosis and treatment planning.

Methods: Thirty-nine with ACC, referred to Qianfoshan Hospital, Qilu Hospital and Provincial Hospital in Shandong Province from December 2012 to December 2020, were enrolled. Their imaging and clinicopathological features were analyzed. They were followed up for 1 year, and Cox regression was used to analyze the factors affecting patient prognosis.

Results: ACC was more common in the middle-aged and elderly and peaked at approximately 60 years. The clinical manifestations of the patients were mostly flatulence and upper abdomen pain. The tumor was located in the head of the pancreas in 19 cases, with an average size of 5.8 cm. We found nerve invasion and liver metastasis in one case each. 8 patients showed irregular amorphous tumor calcification on plain computed tomography and 5 showed high and low signals on T1- and T2-weighted images, respectively. Immunohistochemistry revealed 100.0% positive rates for CK, β-catenin, and Ki-67. Thirty-three patients underwent surgical resection, and the 2-year overall mortality rate was 25.6%. Cox analysis revealed that smoking was an independent risk factor affecting patient prognosis.

Conclusion: An in-depth understanding of the imaging and clinicopathological features of ACC is conducive to better diagnosis and treatment planning for ACC and subsequent improvement in patient prognosis.

Keywords: acinar cell carcinoma, imaging features, clinicopathological features, treatment, prognosis

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INTRODUCTION

Acinar cell carcinoma (ACC) is a rare pancreatic epithelial malignancy derived mainly from pancreatic acinar cells and terminal branches of the pancreatic duct, accounting for approximately 1% of all pancreatic tumors (1). The hallmark pathological feature of ACC is its exocrine function and its potent capacity to invade and metastasize (2), which makes treatment of ACC more difficult than that of other pancreatic tumors and leads to an extremely pessimistic prognosis (3). The 5-year

mortality rate of patients with ACC exceeds 50%, and its lethality ranks among the highest among all malignancies (4). However, due to the rarity of ACC and its different morphological characteristics, research on ACC is not homogeneous at home and abroad, and its diagnosis remains controversial (5). In clinical practice, ACC can only be confirmed by surgery or biopsy, which has a great hidden peril for its treatment (6).

Although ACC falls into the category of pancreatic tumors, its pathological manifestations differ from those of conventional pancreatic cancer. Therefore, comprehensive clinical practice guidelines are needed for its differentiation (7). Currently, imaging remains one of the best methods with high accuracy for early diagnosis of tumors (8). ACC, due to acinar secretion, shows substantial cystic changes on imaging (9), which may be the key to early diagnosis of ACC. Confronted with the deficiency in the current clinical research on ACC, further understanding of ACC-related lesions is the basis for improving the diagnosis rate and ensuring patients' life and health. In this study, the imaging and pathological features of ACC patients confirmed by pathology in our hospital were analyzed, with an aim of improving the clinical awareness regarding ACC and providing a reference for better diagnosis and treatment planning.

MATERIALS AND METHODS

Patient Information

Thirty-nine patients with ACC, admitted to Qianfoshan Hospital、Qilu Hospital and Provincial Hospital in Shandong Province between December 2012 and December 2020, were selected for this retrospective analysis. Of them, 23 patients had undergone computed tomography (CT) and magnetic resonance imaging (MRI) examinations and 16 had undergone only CT examination. The study design was approved by the institutional ethics committee.

Eligibility Criteria

Patients aged > 18 years, diagnosed with ACC by surgery or pathological puncture, and having complete case data, were enrolled. In contrast, those with multiple tumors, cardiovascular and cerebrovascular diseases, autoimmune deficiency, organ dysfunction, or history of surgery, radiotherapy, and chemotherapy were excluded. In addition, pregnant or lactating patients and those who received antibiotic treatment within half a year before admission or had a life expectancy < 1 month were also excluded.

METHODS

CT Examination

15 patients underwent CT using the following GE Discovery CT750HD scan parameters: voltage, 120 kV; current, 105-524mA; layer thickness, 5 mm; and spacing, 1 mm. 15 patients underwent CT using the following GE Discovery CT750 HD and Philips Brilliance iCT.The remaining 9 patients underwent CT using the following Toshiba Aquilion ONE 320 Slice CT scan parameters: voltage, 120 KV; current, 120mA; slice thickness, 5 mm; pitch, 0.7; rotation time, 0.5 s; and scanning time, 8.6 s. The scanning range for all patients was from the parietal septum to the level of the anterior superior iliac spine. The plain and contrast-enhanced CT scans were reconstructed by 1.25 mm and transmitted to the PACS software. After plain scanning, 15patients were injected with 2mL/kg of iopromide contrast agent (Bayer, Germany) at a rate of 2.5-4 mL/s, 24 patients underwent forearm vein injection of 80 ml iohexol (350 mgI/ml) with a 2.8ml/s injection rate, and 10 ml physiological saline was injected at the same rate; arterial, portal, and delayed phase scans were performed at 30, 65, and 120 s after injection, respectively.

MRI Examination

14 of the 39 patients underwent MRI. Using 3.0T vero MR scanner (Simens, Vero, Germany) with the body coil; axial T1WI repetition time, 1000 ms; echo time, 5.6ms; T2WI repetition time, 1400 ms; echo time, 92 ms; T2WI repetition time of fat pressing, 4820 ms; echo time, 83 ms; field of view, 400 mm×400 mm; matrix 320×320, collected twice; slice thickness, 5 mm; and spacing 1 mm. 9 patients using 3.0T vero MR scanner (GE,HDX TWINSP); axial T1WI repetition time, 800; echo time, 6.9 ms; T2WI repetition time of fat pressing, 4500 ms; echo time, 85ms; Field of view (FOV), 380mm×380 mm; matrix 320×320, collected twice; slice thickness, 5 mm; and spacing 1 mm.

Pathological Examination

Immunohistochemical staining was performed on tumor tissue sections of patients to examine indexes, including those for CK, CK-7, CK-19, Synaptophysin, β-catenin, vimentin, and Ki-67.

Evaluation Criterion

The primary endpoints were lesion site and morphology, tumor diameter line, tumor capsule condition, fat encapsulation, bleeding and calcification, cystic degeneration (necrosis), and plain and enhanced CT values of solid components of the lesion. All images were evaluated by two senior radiographers, and the consensus reached by them was considered the examination result. The histopathological results were evaluated by senior pathologists in our hospital, and the histological sections were staged according to the American Joint Committee on Cancer staging system.

Follow-Up for Prognosis

All patients were followed up for 1 year through hospital reexamination.

Statistical Methods

The statistical software used for data analysis and processing was SPSS22.0. Categorical variables are expressed as percentages and compared using the chi-square test. Continuous variables are presented as mean ± standard deviation, and the comparison was made by independent sample t-test, as well as one-way ANOVA and LSD *post hoc* test. Cox regression analysis was used to

determine the related influencing factors. Differences were considered statistically significant when P<0.05.

RESULTS

Pathological Changes

Tumor location: The tumor was located in the head of the pancreas in 48.7% (n=19) of the cases, in the body of the pancreas in 25.6% (n=10) and in the tail of the pancreas in 25.6% (n=10).

Size: According to the imaging results, the tumor size ranged from 1.48 to 13.2 cm, with an average of 5.77 cm.

Capsule: 28 cases with an intact capsule showed focal invasion of peripheral pancreatic tissue, while in 11 cases, the lesion broke through the capsule without a clear boundary or complete capsule.

Morphology: The tumors were round or quasi-round in 31 cases and lobulated or irregular in 8 cases.

Central density: It was observed that the tumors in 79.49% (n=31) patients had varying degrees of central low density on enhanced CT images with a density of <50%, and the tumors in 20.51% (n=8) patients were cystic lesions.

Other pathological changes: We observed nerve invasion in 1 case and liver metastasis in 1 case, without vascular invasion or lymph node metastasis (**Figure 1**).

Clinical Manifestations

The clinical manifestations of the patients were mostly flatulence and pain in the upper abdomen. Further, 17.95% (n=7) patients had jaundice and 12.82% (n=5) felt a significant mass pressing the abdomen. Tumor marker examination showed that CEA and CA199 levels were all within the normal range, while elevated AFP levels were found in 12.82% (n=5) patients. None of the patients developed joint disease or subcutaneous fat necrosis.

Calcification and Bleeding

Of the patients, 20.51% (n=8) showed irregular amorphous tumor calcification on plain CT scans, and 12.82% (n=5) showed high signal on T1-weighted images and low signal on T2-weighted images. A total 20.51% (n=8) patients showed an

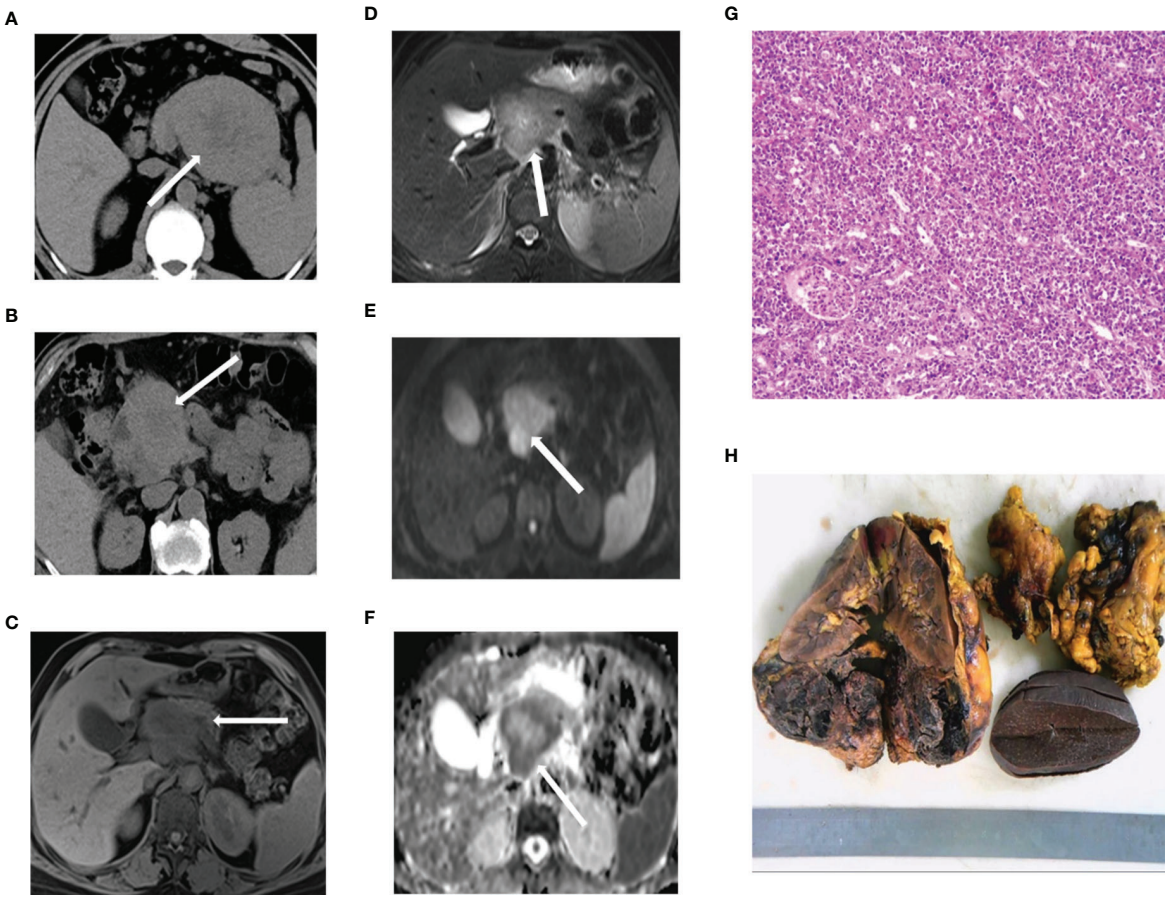

FIGURE 1 | Preliminary computed tomography and magnetic resonance imaging findings **(A)** ACC in the tail of the pancreas. **(B)** ACC in the head of the pancreas. **(C–F)** ACC in a 66-year-old man. T1-weighted **(C)** and T2-weighted **(D)** images reveal a well-defined cystic lesion in the pancreatic head indicated with an arrow. Diffusion-weighted imaging **(E)** shows the area of high signal, and ADC **(F)** reveals, on the contrary. **(G)** Immunohistochemical staining of the tumor cells. **(H)** This specimen is of a 7.5-cm, well-encapsulated mass obtained from the pancreatic tail through the Whipple resection. ACC, acinar cell carcinoma.

unevenly high signal on T1 and T2 weighted images but no high density on plain CT scans (**Figure 2**).

Dynamic Enhanced Scanning

Arterial CT and MRI results showed uneven enhancement for all lesions except a cystic tumor, the enhancement of which was lower than that of the surrounding normal pancreas, and no enhancement was observed in the cystic necrosis area (**Figure 3**).

Duct Distribution

Among the 19 patients with pancreatic head tumors, 26.31% (n=5) showed mild dilatation of the bile duct tree and main pancreatic duct and 52.63% (n=10) showed mild dilatation (**Figure 4**).

Surgical Treatment and Tumor Metastasis

A total of 84.61% (n=33) patients underwent surgical resection after admission, including 20 patients who underwent pancreaticoduodenectomy and 13 patients who underwent distal pancreatectomy. A total of 17.95% (n=7) patients developed lymph node metastasis, including 2 case of liver invasion, 4 cases of duodenal invasion, and 1 case of bile duct invasion. All patients received palliative chemotherapy (8 to 12 cycles of capecitabine combined with oxaliplatin (XELOX) after admission.

Immunohistochemical Results

Immunohistochemistry revealed 100.0% positive rates for CK, β-catenin, and Ki-67. The positive rates were 82.1% for both Synaptophysin and Vimentin, 53.8% for CK-7, and 33.3% for CK-19 in patients with ACC (**Figure 5**).

Prognostic Follow-Up

34 of the 39 patients were successfully followed up, of which 10 patients died, with a 2-year overall mortality rate of 25.6%. COX analysis revealed that smoking was an independent risk factor affecting the prognosis of patients with ACC (P<0.05) (**Table 1**).

DISCUSSION

ACC, an extremely rare type of pancreatic cancer, is highly harmful and has a dire patient prognosis, albeit with a low incidence rate (10, 11). ACC mainly manifests as pancreatic lesions. When tumors invade the surrounding organs, large space-occupying lesions can be formed in metastatic organs, which may easily lead to misdiagnosis as other neoplastic diseases during preoperative imaging (12). Second, the early clinical manifestations of ACC are usually abdominal pain and bloating, without other pathological functional changes, which is

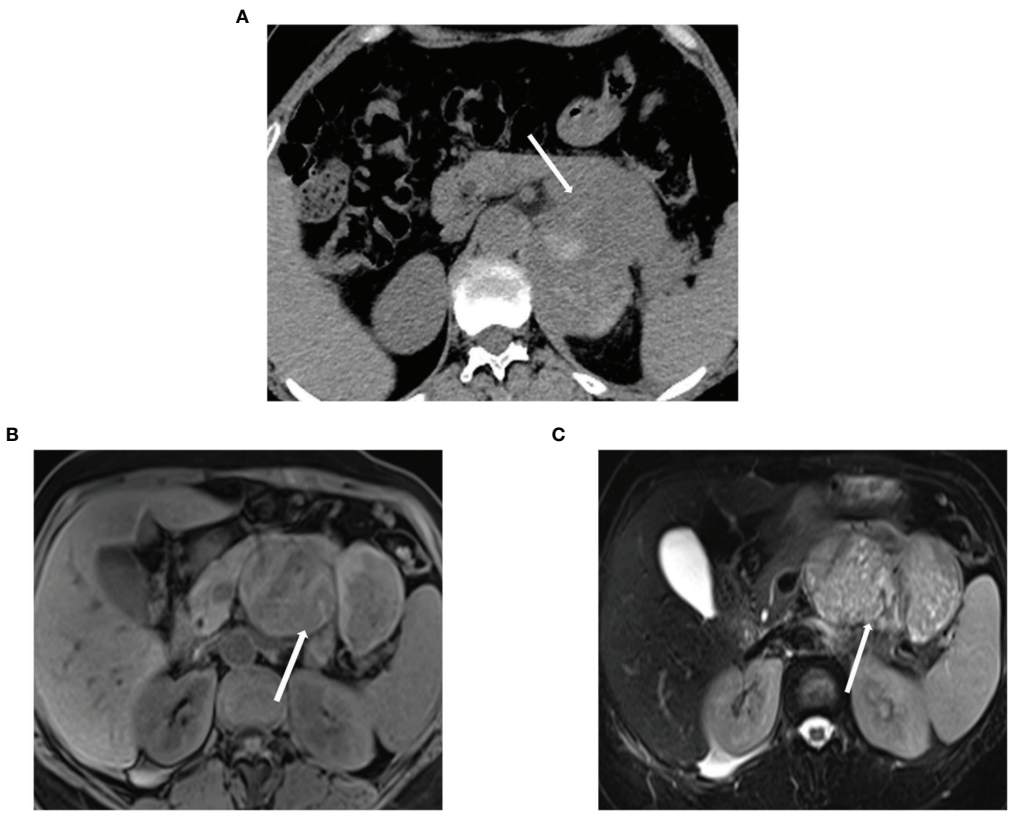

FIGURE 2 | Calcification, bleeding, and necrosis **(A)** ACC in a 67-year-old woman. Unenhanced CT image reveals a pancreatic mass with irregular punctual calcifications (arrow). **(B, C)** T1-weighted and T2-weighted images reveal the tumor with the cystic necrosis (arrow). ACC, acinar cell carcinoma.

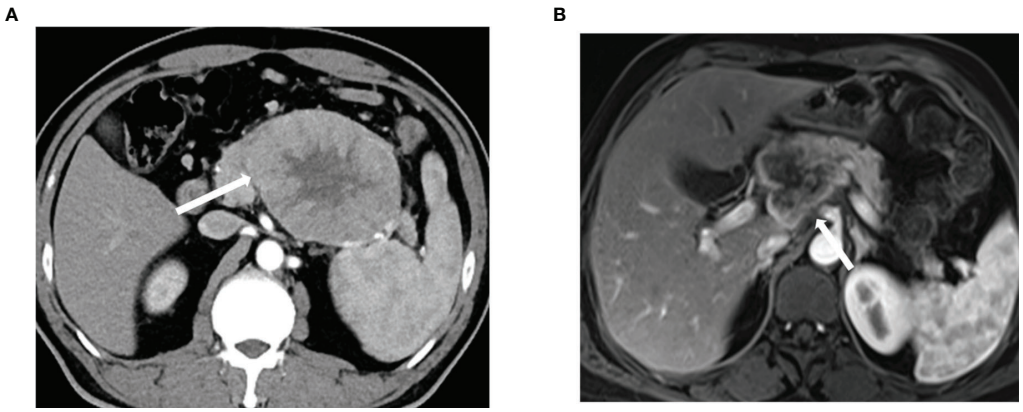

FIGURE 3 | Biphasic contrast enhanced computed tomography and magnetic resonance imaging of ACC **(A)** Venous enhancement is heterogeneous. **(B)** There is uneven enhancement of lesions in the portal arterial phase. ACC, acinar cell carcinoma.

one of the key reasons ACC is initially ignored (13). Improving the clinical examination protocol of ACC is imperative to ensure patient safety and improve prognosis. In this study, the imaging and clinicopathological features of ACC were preliminarily analyzed, and the results are as follows.

Clinical Features of ACC

According to the clinical data of the study participants, ACC is more common in the middle-aged and elderly, and peaks at about 60 years, which is consistent with previous findings (14) and can thus prove the accuracy of our experiment. In addition, male patients are slightly more than female patients. There is no obvious familial inheritance, but the disease may be connected with a history of pancreatic diseases. Abdominal pain is the main clinical manifestation, with no other remarkable clinical symptoms. Routine tumor marker examination shows no significant changes in CEA and CA199 levels. In addition, according to the literature reports, CA125 is closely related to ACC progression, elevation of CA125 in CAA is common clinically, but it is also associated with digestive tumors. Further studies are required to confirm the relationship between them.

Pathological Features of ACC

Compared with other pancreatic tumors, ACC usually presents as larger tumors with expansive growth which and clearer

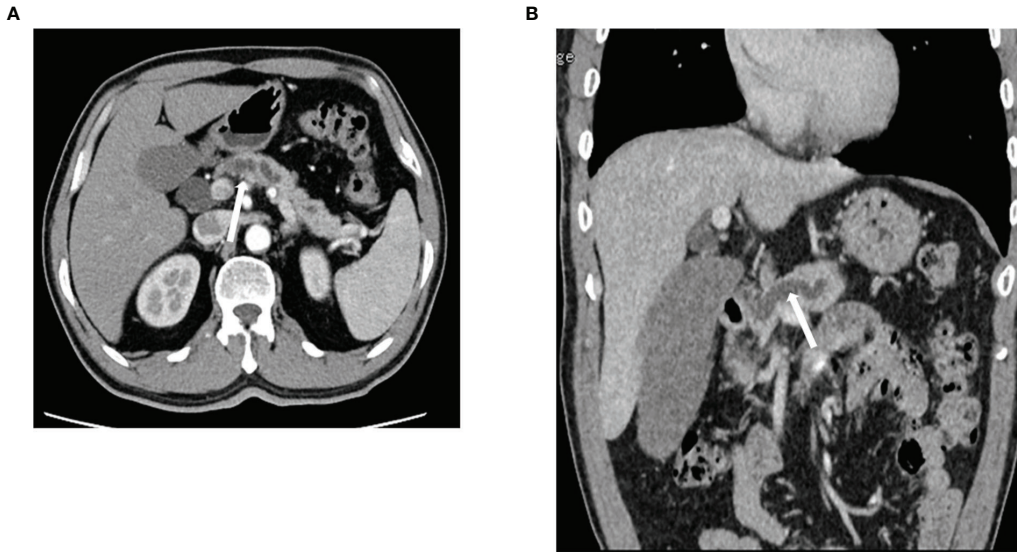

FIGURE 4 | Expansion of the pancreatic duct. ACC in a 63-year-old man **(A, B)** Contrast-enhanced computed tomography reveals ductal dilatation. ACC, acinar cell carcinoma.

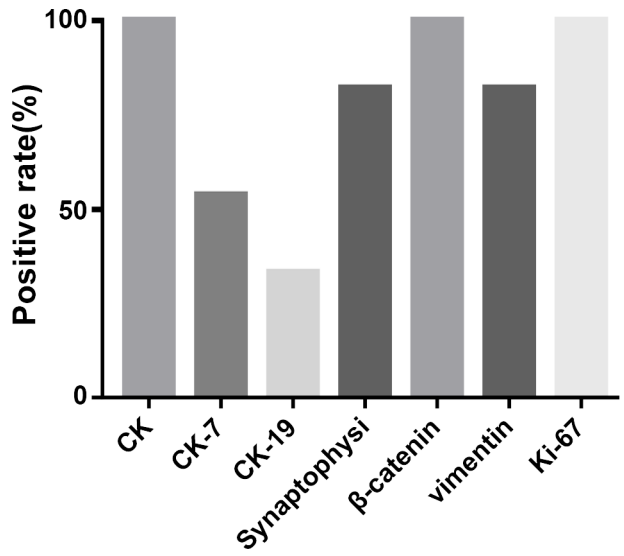

FIGURE 5 | Immunohistochemical results.

margins at onset. The tumor capsule is mostly solid. Cystic degeneration, necrosis, and hemorrhage are commonly observed at the center of large lesions. Microscopically, ACC presents a relatively dense cellular structure separated by fibrous stroma; the cells have abundant cytoplasm, visible proeosinophilic granules, and show rapid mitosis. It has been suggested that immunohistochemical labeling of pancreatic enzyme products could improve diagnosis of ACC (15), which may be related to our findings. However, Chou et al. observed that ACC-labeled trypsin was positive and chymotrypsin and lipase were also highly sensitive (16), which is consistent with the results of this study. In addition, ACC shows an obvious partial endocrine differentiation tendency, and some lesions are positive for chromogranin and synaptophysin markers.

Imaging Features of ACC

The shape of the ACC is mostly irregular and grows along the long axis of the pancreas. It compresses the surrounding tissues and causes collagen fibers to proliferate to form a pseudocapsule with relatively clear boundaries. However, due to the slow growth of tumors and lack of neurophilic sites, most tumors lack blood supply, and the arterial phase enhancement is lower than that of the pancreatic tissue. However, the tumor contains almost all sinusoids, showing progressive enhancement. The higher the tumor differentiation, the smaller the volume, the more uniform the blood supply, and lesser the area of necrosis. Both CT and MRI manifestations show a uniform honey degree, while T1WI and T2WI show slightly higher signals, and the enhancement presents a gradual increase. The tumor is poorly differentiated, large, and abundant calcification, hemorrhage, and necrosis can be seen when the blood supply is unbalanced. CT and MRI show mixed density and signals. The larger the range of hemorrhagic necrosis, the more uneven the enhancement. MRI is more effective for the inspection of intratumoral hemorrhage, and CT is more effective for the inspection of calcification. According to the results of this experiment, we can summarize the imaging features of ACC as follows: 1. Larger mass. The average lesion size was 5.8 cm. It was <2 cm in only one case, and the maximum size observed was 22 cm. This is consistent with the findings of previous studies (17). 2. The disease may occur anywhere in the pancreas, most frequently in the head of the pancreas. 3. The lesion has clear boundaries. Contrast-enhanced scanning shows linear enhancement of the capsule in some lesions, but in most of them it is incomplete, with local invasion of adjacent tissues. The capsule can be seen in most lesions, which is also consistent with previous research results (18). 4. Rare calcification. 5. The tumors mainly have solid components: with different proportions of low-density regions, this area was not significantly enhanced and indicated hemorrhage or necrotic cystic degeneration. 6. ACC is mostly a hypovascular lesion (19), the enhancement degree of which is lower than that of the adjacent normal pancreatic parenchyma in each phase, and the arterial phase enhancement is greater than that of the normal pancreatic parenchyma.

Differential Diagnosis of ACC

Combining our results with those of previous studies, we summarize the differential diagnosis of ACC. ACC has larger lesions, sharper margins, and earlier enhancement peaks than pancreatic ductal adenocarcinoma, the most common form of pancreatic cancer. The tissue structure of ACC is similar to that

TABLE 1 | Cox analysis of factors influencing the prognosis of patients with acinar cell carcinoma.

	Univariate analysis			Multivariate analysis		
	RR	95% CI	P	RR	95% CI	P
Age	1.608	0.842-2.942	0.124	–	–	–
BMI (kg/cm^2)	1.184	0.642-2.542	0.541	–	–	–
Gender	2.962	1.242-4.842	0.321	–	–	–
Family medical history	0.658	0.242-4.523	0.207	–	–	–
Smoking	2.064	1.421-3.604	0.012	2.354	1.242-4.641	0.004
Past pancreatic diseases	1.608	0.842-2.942	0.124	–	–	–
Clinical presentations	1.184	0.642-2.542	0.541	–	–	–
Lesion size	3.542	0.684-8.612	0.292	–	–	–

BMI, body mass index; CI, confidence interval; RR, P.

of pancreatic neuroendocrine tumor (20), with consistent positive rates of some labeled proteins (21). However, pancreatic neuroendocrine tumors are characterized by cystic degeneration and necrosis accompanied by hemorrhage, and most cases have a malignant tendency, ill-defined boundaries, dilation of the pancreatic duct, and metastasis (22). The clinical manifestations of solid pseudopapillary tumors are quite different. Solid pseudopapilloma of the pancreas is more common in young and middle-aged women, especially in the tail of the pancreas (23); although the capsule is equally common, calcification is also significant (24).

Prognostic Factors Affecting ACC

As we all know, the meaning of independent risk factors is that the larger the lesion, the higher the risk of death for the patient's prognosis. If RR<1, it is a protective factor (that is, the larger the value, the lower the patient's prognostic risk). This suggests that the risk of death in our patients was mainly affected by disease progression. In other words, early diagnosis of ACC can effectively improve the prognosis and survival rate of patients, which was the focus of this study.

Limitations of This Study

First, to ensure the uniformity, we followed up all patients for one year. However, this prevented evaluation of the relationship between imaging findings and the long-term prognosis of patients. Therefore, a longer follow-up investigation is necessary. Moreover, since ACC is relatively rare, its clinical treatment requires improvement. Third, it is unclear if different treatment modalities are key to determining the prognosis of patients. Last, but not least, because ACC is rare, MRI multifunctional imaging was not available earlier. In addition, this study did not explore the characteristic changes, including lipase hypersecretion syndrome, multiple subcutaneous fat necrosis, and eosinophilia. To ensure that the results were representative, the patient data included typical imaging manifestations of solid pancreatic cystic lesions. This also resulted in a sample size of only 39, which was too small to evaluate the aforementioned characteristic changes. In this study, only 23 patients underwent MRI. Therefore, in future, a combination of preoperative diagnosis, pathology, and MRI must be used for ACC diagnosis. We will continue to deepen the relevant research on ACC to obtain more accurate findings for clinical reference. As an important immunohistochemical data, BCL-10 was not included in this manuscript due to the limitations of objective reasons. We realized that BCL-10 combined with imaging examination might provide more diagnostic information, which provided a direction for our future research.

In summary, ACC of the pancreas is a rare tumor. MRI and CT are complementary imaging methods. CT is sensitive to central calcifications. MRI is superior for observing the relationship between lesions and normal tissues, components of lesions, internal hemorrhage of tumors, and ductal dilatation, among other factors. Despite the rarity of this disease, further imaging and pathology based studies should be performed to determine the clinical findings and treatment outcomes of this disease.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Adjuvant Sorafenib Following Radiofrequency Ablation for Early-Stage Recurrent Hepatocellular Carcinoma With Microvascular Invasion at the Initial Hepatectomy

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Background: The efficacy of radiofrequency ablation (RFA) for patients with early-stage recurrent hepatocellular carcinoma (HCC) with microvascular invasion (MVI) at the initial hepatectomy is limited. Our study aimed to explore whether adjuvant sorafenib following RFA could improve the situation.

Methods: We retrospectively included 211 patients with early-stage (tumor number of ≤3 and tumor size of 2–5 cm) recurrent HCC with MVI at the initial hepatectomy who underwent adjuvant sorafenib following RFA or RFA alone in 13 centers from June 2013 to June 2020. In the combination group, sorafenib of 400 mg twice daily was administered within 7 days after RFA. Overall survival (OS) and recurrence-free survival (RFS) were compared. Subgroup analysis based on MVI grade was performed. MVI grade was based on the practice guidelines for the pathological diagnosis of HCC and included M1 (≤5 MVI sites, all located within adjacent peritumoral liver tissues 0–1 cm away from the tumor margin) and M2 (>5 MVI sites, or any MVI site located within adjacent peritumoral liver tissues > 1 cm away from the tumor margin).

Results: A total of 103 patients received the combination therapy and 108 patients received RFA alone. The combination therapy provided better survival than RFA alone (median RFS: 17.7 vs. 13.1 months, $P < 0.001$; median OS: 32.0 vs. 25.0 months, $P = 0.002$). Multivariable analysis revealed that treatment allocation was an independent prognostic factor. On subgroup analysis, the combination therapy provided better survival than RFA alone in patients with M1 along with either a tumor size of 3–5 cm, tumor number of two to three, or alpha-fetoprotein (AFP) > 400 μg/L, and in those with M2 along with either a tumor size of 2–3 cm, one recurrent tumor, or AFP ≤ 400 μg/L.

Conclusions: Adjuvant sorafenib following RFA was associated with better survival than RFA alone in patients with early-stage recurrent HCC with MVI at the initial hepatectomy. Moreover, MVI grade could guide the application of adjuvant sorafenib.

Keywords: recurrent hepatocellular carcinoma, microvascular invasion, sorafenib, radiofrequency ablation, adjuvant therapy

INTRODUCTION

Nearly 70% of patients with early-stage hepatocellular carcinoma (HCC) develop recurrence within 5 years following hepatectomy (1). Repeated hepatectomy and salvage liver transplantation are effective treatments for HCC recurrence (2). However, the wide application of these two strategies is limited due to poor liver functional reserve following initial hepatectomy and liver donor shortage for transplantation.

Radiofrequency ablation (RFA) has shown similar survival outcomes to repeated hepatectomy in treating early-stage recurrent HCC following hepatectomy (3). However, RFA presented worse survival than repeated hepatectomy in patients with aggressive recurrent HCC, including those with a tumor size greater than 3 cm (3–5), an alpha-fetoprotein (AFP) level greater than 200 μg/L (3), and who relapsed within 2 years following initial resection (5). Therefore, it is significant to enhance the efficacy of RFA in patients with aggressive early-stage recurrent HCC.

Microvascular invasion (MVI) is associated with poor tumor differentiation, aggressive behavior, and worse survival outcomes in recurrent HCC (6). Previous studies have investigated RFA for patients with early-stage recurrent HCC with MVI at the initial hepatectomy (6–8). These studies integrated repeated hepatectomy and RFA as one curative group. The survival outcomes of the curative treatments were limited, even inferior to transarterial chemoembolization (TACE) (8). Therefore, more effort should be made to enhance the efficacy of RFA in patients with early-stage recurrent HCC with MVI at the initial hepatectomy.

Sorafenib was once the first-line systemic therapy for advanced HCC (9, 10). Several studies have shown the combination of sorafenib, and RFA is associated with a lower incidence of post-RFA recurrence and better survival than RFA alone in treating primary or recurrent HCC (11–13), indicating the important role of sorafenib in enhancing the efficacy of RFA. For instance, Feng et al. evaluated the efficacy of combined sorafenib and RFA in 64 patients with HCC at Barcelona Clinic Liver Cancer group (BCLC) stage 0–B1, of which 48 were recurrent, and sorafenib was administered after RFA in 54 patients. The combination therapy exhibited a 4-year overall survival (OS) rate of 50.3%, significantly better than 30.9% in the RFA-alone group (11). Moreover, as an angiogenesis inhibitor, sorafenib has exhibited significant survival benefit as an adjuvant therapy following curative hepatectomy in patients with MVI-positive HCC (14, 15). Nevertheless, there has been no published evidence on applying sorafenib following RFA in patients with early-stage recurrent HCC with MVI at the initial hepatectomy.

Therefore, our study aimed to determine the role of adjuvant sorafenib following RFA in patients with early-stage recurrent HCC with MVI at the initial hepatectomy, with an attempt to improve the present situation of applying RFA in patients with high-risk early-stage recurrent HCC.

Abbreviations: HCC, hepatocellular carcinoma; RFA, radiofrequency ablation; AFP, alpha-fetoprotein; MVI, microvascular invasion; TACE, transarterial chemoembolization; BCLC, Barcelona Clinic Liver Cancer group; OS, overall survival; ECOG, Eastern Cooperative Oncology Group; CEUS, contrast-enhanced ultrasound; CECT, contrast-enhanced computed tomography; RFS, recurrence-free survival; HBsAg, hepatitis B surface antigen; PLT, platelet; ALB, albumin; ALT, alanine aminotransferase; TBIL, total bilirubin; HR, hazard ratio; CI, confidence interval.

MATERIALS AND METHODS

Study Design and Patients

This is a retrospective multicentric study conducted in 13 medical centers in China, namely Anhui Provincial Hospital, Beijing Cancer Hospital, the First and the Third Department of Shanghai Eastern Hepatobiliary Surgery Hospital, Fudan Zhongshan Hospital, Cancer Center of Sun Yat-sen University, Bethune First Hospital of Jilin University, Tianjin Medical University Cancer Hospital, Xijing Hospital, Cancer Hospital Chinese Academy of Medical Sciences, The First Affiliated Hospital of Zhejiang University, The First Affiliated Hospital of Zhengzhou University, and the Southwest Hospital of AMU. The study was approved by all the Ethics Committees of the individual centers, and it conformed to the standards of the Declaration of Helsinki. Informed consent was waived because of the retrospective design of the study.

From June 2013 to June 2020, 21,912 consecutive patients were diagnosed with intrahepatic recurrences after R0 liver resection for HCC according to the non-invasive criteria of the American Association for the Study of Liver Diseases (16). HCC with MVI positivity was diagnosed in the resected liver specimens in 1,312 patients. In each institution, MVI at the first resection was confirmed by two experienced pathologists in hepatology over 5 years. The inclusion criteria were as follows: (1) age between 18 and 75 years; (2) first intrahepatic recurrence after R0 hepatectomy; (3) early-stage recurrent HCC with tumor number of ≤3 and tumor size of 2–5 cm; (4) absence of macrovascular invasion or extrahepatic metastasis; (5) Child-Pugh Class A-B; (6); adequate hematologic and renal function as previously described (17); (7); an Eastern Cooperative Oncology Group (ECOG) performance score of 0; and (8) the duration of sorafenib treatment was at least 3 months in the combination group. Patients with a history of another malignancy, associated severe organic dysfunction, or previous or concomitant systemic

anti-cancer treatments were excluded. The therapeutic selection between the combination therapy and RFA alone was made by a multidisciplinary team consisting of specialists from hepatic surgery, interventional radiology, and oncology, based on tumor characteristics and liver function, as well as patients' willingness. For example, patients with high-risk factors for recurrence including larger tumor size or more tumor lesions may be recommended to receive the combined therapy, whereas patients with earlier tumor stage of primary HCC or worse liver function may be recommended to receive RFA alone.

RFA Procedure and Sorafenib Administration

In each institution, percutaneous RFA was performed by two interventional clinicians with over 10 years of RFA experience under real-time ultrasound guidance as previously reported (18). Treatment was performed under moderate sedation and local anesthesia. A commercially available Cool-tipTM RFA system (Valleylab, Boulder, CO, USA) with a needle of 3-cm active tip length was used. The needle was inserted into the tumor under ultrasound guidance, aiming to generate an ablative zone covering an area larger than 1 cm around the tumor. The number of needle punctures and ablation points was determined by tumor size. The multiple-overlapping technique was applied for each tumor. The needle tract was ablated at the end of the procedure to prevent bleeding and tumor seeding. Technical success of ablation was evaluated by contrast-enhanced ultrasound (CEUS) 1 month after RFA. If residual unablated tumor was detected, then additional RFA was performed.

For patients who received the combination of sorafenib and RFA, sorafenib was administered orally at a dosage of 400 mg twice daily. The drug was administered within 7 days following RFA based on the liver function status. For limited toxicity, the administration regimen was modified to 200 mg twice daily or 400 mg on alternate days, but the drug was discontinued if severe toxicity occurred.

Follow-Up

Routine contrast-enhanced computed tomography (CECT) and CEUS were performed 4 weeks after RFA to assess treatment effectiveness. The patients were then followed-up once every 3 months for the first 2 years and once every 6 months thereafter. At each follow-up visit, clinical evaluation, CEUS, liver function tests, and AFP were performed. CECT or magnetic resonance imaging was performed once every 6 months. Chest CT and bone scintigraphy were performed when extrahepatic metastasis was clinically suspected. When local tumor progression and intrahepatic or extrahepatic recurrence were diagnosed, patients were offered treatments, which included repeated hepatectomy, RFA, TACE, sorafenib (only in the RFA-alone group), levatinib, apatinib, immunotherapy, or the best supportive care according to the number and size of recurrent tumors and liver function.

Outcomes

Adverse events were evaluated by the National Cancer Institute Common Toxicity Criteria Grading version 4.0. Severe adverse events (grade ≥3) were defined as clinical events requiring additional therapeutic interventions or prolonged hospitalization (19). OS was defined as the time interval between the initial diagnosis of recurrent HCC and the date of death or the last follow-up. Recurrence-free survival (RFS) was defined as the time interval between the initial diagnosis of recurrent HCC and the date of HCC re-recurrence or the last follow-up. The study was censored on December 31, 2020.

Statistical Analysis

Continuous variables were presented as mean ± SD. Categorical variables were presented as numbers and percentages. Difference test was conducted using t-test for continuous variables and χ^2 test or Fisher's exact test for categorical variables. Patients' characteristics, including age, sex, hepatitis B surface antigen (HBsAg), tumor size, tumor number, platelet (PLT), albumin (ALB), alanine aminotransferase (ALT), total bilirubin (TBIL), prothrombin activity, AFP, BCLC stage of primary HCC, interval of recurrence from initial treatment, initial hepatic resection type, antiviral treatment for hepatitis B, and MVI grade of primary HCC were analyzed by univariable and multivariable Cox proportional hazard regression models to identify potential survival predictors. Of note, MVI grade was based on the practice guidelines for the pathological diagnosis of primary liver cancer (20). M1 represents low-risk with MVI of ≤5 sites and all located within adjacent peritumoral liver tissues 0–1 cm away from the tumor margin, and M2 stands for high-risk with MVI of >5 sites, or any MVI site located within adjacent peritumoral liver tissues > 1 cm away from the tumor margin. Survival curves were generated by the Kaplan–Meier method and compared by the log-rank test. To elaborate the role of MVI grade in the treatment of recurrent HCC, subgroup analysis based on significant survival predictors was performed in patients with different MVI grades. Statistical analysis was conducted using SPSS software (version 20.0, SPSS Inc., Chicago, IL). All tests were two-sided, and $P < 0.05$ indicated statistical significance.

RESULTS

Patient Characteristics

The flow chart of patient enrollment was shown in **Figure 1**. We finally enrolled 103 patients in the combined RFA and sorafenib group (mean age, 54 ± 6 years; 86 men) and 108 patients in RFA-alone group (mean age, 53 ± 9 years; 94 men). The baseline characteristics were summarized in **Table 1**. All the listed variables were comparable between the two groups (all $P > 0.05$).

Efficacy

The mean ± SD follow-up time was 39.3 ± 12.1 months for the combination group and 38.4 ± 12.6 months for RFA-alone group. The numbers of patients who received modification, discontinuation, and withdrawal of sorafenib in the combination group were 72, 7, and 3, respectively. Survival analysis revealed that the combination therapy provided better survival than RFA alone (median RFS: 17.7 vs. 13.1 months, $P <$

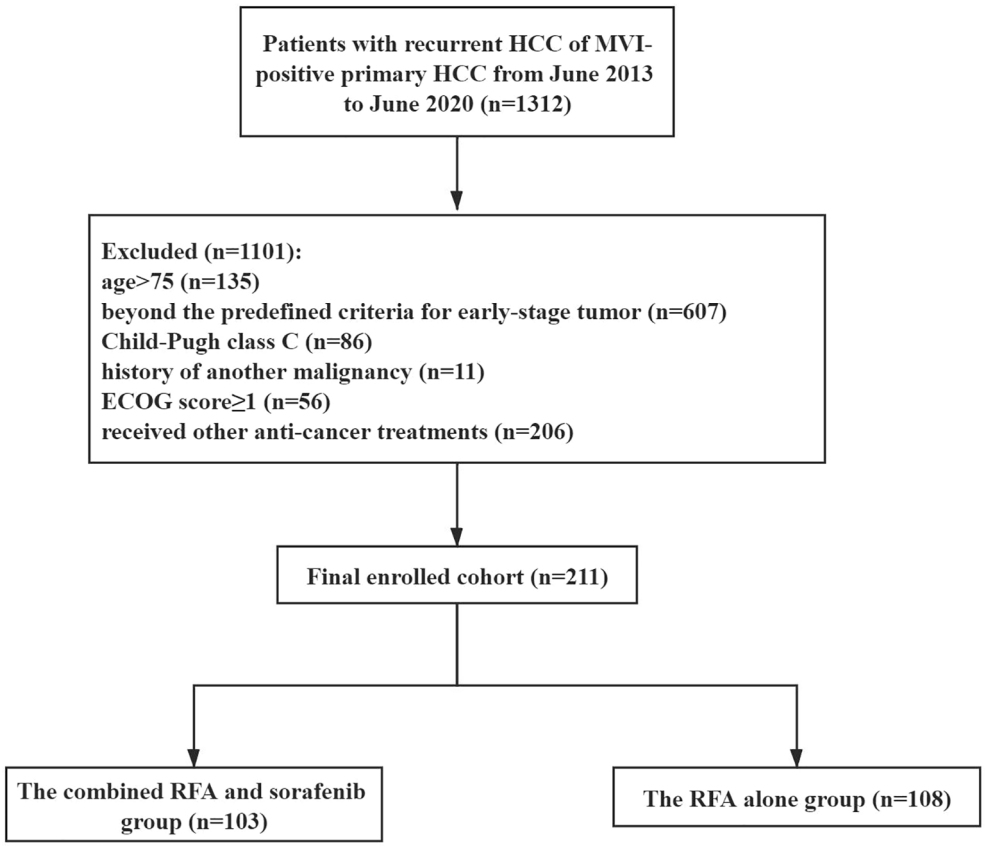

FIGURE 1 | The flow chart of patient enrollment. HCC, hepatocellular carcinoma; MVI, microvascular invasion; ECOG, Eastern Cooperative Oncology Group; RFA, radiofrequency ablation.

TABLE 1 | Baseline characteristics of the two treatment groups.

Variable	RFA-Sorafenib (n = 103)	RFA (n = 108)	*P*-value
Age (year) (range)	54 ± 6	53 ± 9	0.139
Sex (man/woman)	86 (83.5%) /17 (16.5%)	94 (87.0%) /14 (13.0%)	0.468
HBsAg (+/–)	95 (92.2%) /8 (7.8%)	102 (94.4%) /6 (5.6%)	0.519
Tumor size (cm) (2–3/3–5)	50 (48.5%) /53 (51.5%)	51 (47.2%) /57 (52.8%)	0.848
Tumor number (1/2–3)	63 (61.2%) /40 (38.8%)	57 (52.8%) /51 (47.2%)	0.219
PLT ($\times10^9$/L)	102.7 ± 35.6	112.0 ± 25.7	0.095
ALB (g/L)	35.5 ± 2.1	35.3 ± 3.2	0.875
ALT (U/L)	31.2 ± 6.8	29.3 ± 14.6	0.101
TBIL (μmol/L)	9.8 ± 4.7	8.9 ± 6.5	0.561
Prothrombin activity (%)	89.6 ± 15.6	91.6 ± 13.2	0.382
AFP (μg/L) (≤ 400/>400)	62 (60.2%) /41 (39.8%)	65 (60.2%) /43 (39.8%)	0.999
Tumor stage of primary HCC (BCLC A/B)	84 (81.6%) /19 (18.4%)	96 (88.9%) /12 (11.1%)	0.132
Interval of recurrence from initial treatment (year)			0.649
≤1	54 (52.4%)	60 (55.6%)	
>1	49 (47.6%)	48 (44.4%)	
Initial hepatic resection type			0.403
One segment	64 (62.1%)	61 (56.5%)	
More than one segments	39 (37.9%)	47 (43.5%)	
Antiviral treatment for hepatitis B (yes/no)	72 (69.9%) /31 (30.1%)	80 (74.1%) /28 (25.9%)	0.500
MVI grade (M1/M2)	59 (57.3%) /44 (42.7%)	59 (54.6%) /49 (45.4%)	0.698

RFA, radiofrequency ablation; HBsAg, hepatitis B surface antigen; PLT, platelet; ALB, albumin; ALT, alanine aminotransferase; TBIL, total bilirubin; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer group; MVI, microvascular invasion.

0.001; median OS: 32.0 vs. 25.0 months, $P = 0.002$) (**Figures 2A, B**). In patients with M1, the RFS of the combination group was longer than that of RFA-alone group (median RFS: 18.7 vs. 14.0 months, $P = 0.013$) (**Supplemental Figure 1A**); however, the OS was similar between the two groups (median OS: 33.4 vs. 25.5 months, $P = 0.102$) (**Supplemental Figure 1B**). Meanwhile, in patients with M2, both RFS and OS of the combination group were superior to those of RFA-alone group (median RFS: 17.2 vs. 12.5 months, $P < 0.001$; median OS: 28.8 vs. 22.5 months, $P = 0.004$) (**Supplemental Figures 2A, B**).

Univariable and Multivariable Analysis

Univariable and multivariable analysis showed that tumor size [3–5 cm vs. 2–3 cm, hazard ratio (HR) =1.526, 95% confidence interval (CI): 1.140–2.044, $P = 0.005$], tumor number (2–3 vs. 1, HR =1.485, 95% CI: 1.092–2.011, $P = 0.015$], PLT (>100 × 10^9/L vs. ≤100 × 10^9/L, HR = 2.296, 95% CI: 1.151–4.582, $P = 0.018$], AFP (>400 μg/L vs. ≤400 μg/L, HR = 2.150, 95% CI: 1.587–2.911, $P < 0.001$), interval of recurrence from initial treatment (>1 years vs. ≤1 year, HR = 0.641, 95% CI: 0.465–0.883, $P = 0.006$), MVI grade (M2 vs. M1, HR = 1.695, 95% CI: 1.251–2.295, $P = 0.001$), and treatment allocation (RFA vs. combination therapy, HR =1.956, 95% CI: 1.439-2.658, $P < 0.001$) were independent prognostic factors of RFS, whereas tumor size (3–5 cm vs. 2–3 cm, HR = 1.715, 95% CI: 1.217–2.416, $P = 0.002$), tumor number (2–3 vs. 1, HR = 1.744, 95% CI: 1.181–2.590, $P = 0.004$), PLT (>100 × 10^9/L vs. ≤100 × 10^9/L, HR = 3.563, 95% CI: 1.665–7.625, $P = 0.001$), AFP (>400 μg/L vs. ≤400 μg/L, HR = 2.287, 95% CI: 1.615–3.238, $P < 0.001$), MVI grade (M2 vs. M1, HR = 1.623, 95% CI: 1.111–2.139, $P = 0.007$), and treatment allocation (RFA vs. combination therapy, HR = 1.636, 95% CI: 1.129–2.370, $P = 0.009$) were independent prognostic factors of OS (**Table 2**).

Subgroup Analysis

On the basis of significant survival predictors including tumor size, tumor number, and AFP, we performed subgroup analysis in patients with different MVI grades. The median survival of the combination group and RFA-alone group along with the HRs of the combination therapy in different subgroups is summarized in **Figures 3A, B**. The detailed survival curves were shown in **Supplemental Figures 3–14**.

In patients with M1, the survival rates were similar between the two treatment groups in the 2- to 3-cm subgroup (RFS, $P = 0.215$; OS, $P = 0.650$). In contrast, the combination therapy exhibited superior survival rates than RFA alone in the 3- to 5-cm subgroup (RFS, $P = 0.007$; OS, $P = 0.031$). For patients with one recurrent tumor, the survival rates were similar between the two treatment groups (RFS, $P = 0.185$; OS, $P = 0.596$). Meanwhile, for patients with two to three recurrent tumors, the combination group had better RFS and similar OS than RFA-alone group (RFS, $P = 0.013$; OS, $P = 0.052$). In the subgroup of AFP ≤400 μg/L, the survival rates were similar between the two treatment groups (RFS, $P = 0.180$; OS, $P = 0.335$). However, in the subgroup of AFP >400 μg/L, the combination group was superior to RFA-alone group in terms of both RFA and OS (RFS, $P < 0.001$; OS, $P = 0.003$).

In patients with M2, the combination therapy exhibited superior survival rates than RFA alone in the 2- to 3-cm subgroup (RFS, $P = 0.001$; OS, $P = 0.031$). In contrast, the survival rates were similar between the two treatment groups in the 3- to 5-cm subgroup (RFS, $P = 0.122$; OS, $P = 0.113$). For patients with one recurrent tumor, the combination group had better RFS and similar OS than RFA-alone group (RFS, $P = 0.001$; OS, $P = 0.094$). Meanwhile, for patients with two to three recurrent tumors, the survival rates were similar between the two treatment groups (RFS, $P = 0.174$; OS,

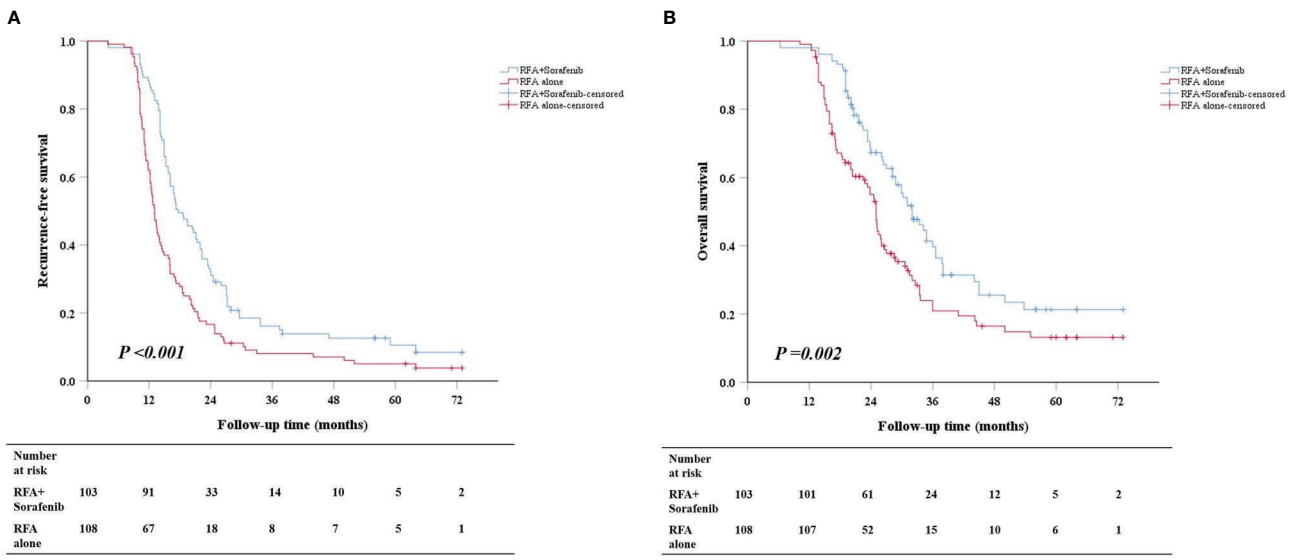

FIGURE 2 | Cumulative survival curves of RFS **(A)** and OS **(B)** between the combination group and RFA-alone group in the whole cohort. RFS, recurrence-free survival; OS, overall survival; RFA, radiofrequency ablation.

TABLE 2 | Univariable and multivariable analysis of prognostic factors.

Variables†	Recurrence-Free Survival						Overall Survival					
	Univariable Analysis			Multivariable Analysis			Univariable Analysis			Multivariable Analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age [≤ 60 years]	0.842	0.630–1.125	0.245				0.803	0.573–1.126	0.204			
Sex [man]	0.989	0.666–1.468	0.956				0.884	0.539–1.450	0.626			
HBsAg [–]	0.570	0.318–1.025	0.060				0.351	0.143–0.857	0.022*	0.432	0.173–1.081	0.079
Tumor size [2–3 cm]	1.461	1.099–1.942	0.009*	1.526	1.140–2.044	0.005*	1.544	1.108–2.150	0.010*	1.715	1.217–2.416	0.002*
Tumor number [1]	1.655	1.239–2.211	0.001*	1.485	1.092–2.011	0.015*	1.864	1.337–2.599	< 0.001*	1.744	1.181–2.590	0.004*
PLT [≤ 100 × 10^9/L]	2.245	1.139–4.423	0.019*	2.296	1.151–4.582	0.018*	3.223	1.562–6.652	0.002*	3.563	1.665–7.625	0.001*
ALB [≤ 35 g/L]	1.302	0.980–1.731	0.069				1.578	1.136–2.192	0.007*	0.863	0.529–1.410	0.557
ALT [≤ 40 U/L]	1.016	0.756–1.367	0.914				1.124	0.800–1.579	0.499			
TBIL [≤ 20.5 μmol/L]	1.422	1.041–1.941	0.027*	1.183	0.847–1.653	0.324	1.130	0.795–1.608	0.495			
Prothrombin activity [≤ 70%]	1.277	0.957–1.702	0.097				1.592	1.146–2.212	0.006*	1.579	0.966–2.580	0.068
AFP [≤ 400 μg/L]	1.886	1.413–2.516	< 0.001*	2.150	1.587–2.911	< 0.001*	2.172	1.559–3.027	< 0.001*	2.287	1.615–3.238	< 0.001*
Tumor stage of primary HCC [BCLC A]	1.105	0.748–1.633	0.615				1.150	0.736–1.798	0.539			
Interval of recurrence from initial treatment [≤ 1 year]	0.658	0.493–0.879	0.005*	0.641	0.465–0.883	0.006*	0.650	0.466–0.909	0.012*	0.728	0.497–1.068	0.104
Initial hepatic resection type [one segment]	1.076	0.807–1.434	0.618				1.347	0.970–1.871	0.076			
Antiviral treatment for hepatitis B [yes]	0.886	0.644–1.218	0.455				0.862	0.592–1.253	0.436			
MVI grade [M1]	1.512	1.136–2.012	0.005*	1.695	1.251–2.295	0.001*	1.528	1.099–2.126	0.012*	1.623	1.111–2.139	0.007*
Treatment allocation [combination therapy]	1.749	1.315–2.325	< 0.001*	1.956	1.439–2.658	< 0.001*	1.670	1.199–2.327	0.002*	1.636	1.129–2.370	0.009*

**Statistically significant at alpha = 0.05. †Data in square brackets is the reference.*
HR, hazard ratio; CI, confidence interval; HBsAg, hepatitis B surface antigen; PLT, platelet; ALB, albumin; ALT, alanine aminotransferase; TBIL, total bilirubin; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer group; MVI, microvascular invasion.

P = 0.080). In the subgroup of AFP ≤400 μg/L, the combination group was superior to RFA-alone group in terms of both RFA and OS (RFS, P = 0.004; OS, P = 0.045), whereas the survival rates were similar between the two treatment groups (RFS, P = 0.062; OS, P = 0.102) in the subgroup of AFP >400 μg/L.

Re-Recurrence and Treatment

On follow-up, the first re-recurrence occurred in 90 of 103 (87.4%) patients in the combination group, and 103 of 108 (95.4%) patients in RFA-alone group (P = 0.038). For the 90 patients with re-recurrence after combined treatment, further treatments aiming at cure were given to 31 patients (34.4%). In the 103 patients with re-recurrence after RFA, such treatments were given to 26 patients (25.2%). The recurrence patterns of re-recurrences were similar between the two groups (**Table 3**). The second and third re-recurrences and the therapies given were summarized in **Supplemental Figure 15**.

Adverse Events

No unexpected severe adverse events or treatment-related deaths occurred (**Table 4**). The common adverse events in the two groups were pain, pleural effusion, gastrointestinal bleeding, and fever. There were no significant differences between the two groups. In addition, adverse events that are likely attributable to sorafenib including hand–foot–skin reactions, diarrhea, hypertension, and alopecia were specifically seen in the combination group. These adverse events responded well to conservative treatments.

DISCUSSION

This multicentric study demonstrated that adjuvant sorafenib following RFA provided better survival than RFA alone in patients with early-stage recurrent HCC with MVI at the initial hepatectomy. Moreover, we also found that MVI grade could

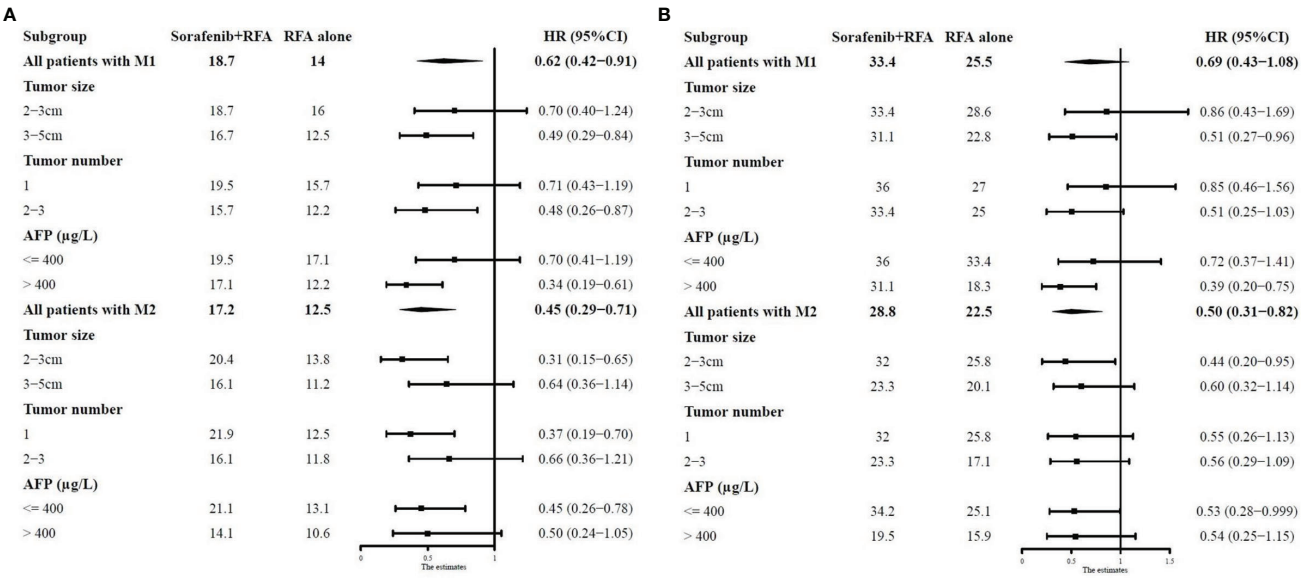

FIGURE 3 | Forest plots showing the median RFS **(A)** and OS **(B)** of the combination group and RFA-alone group along with the HRs of the combination therapy in different subgroups. RFS, recurrence-free survival; OS, overall survival; RFA, radiofrequency ablation; HR, hazard ratio; CI, confidence interval; AFP, alpha-fetoprotein.

guide the application of adjuvant sorafenib. In detail, for patients with M1, only patients with a tumor size of 3–5 cm, tumor number of two to three, or AFP >400 μg/L would benefit from the combination therapy, whereas for patients with M2, the combination therapy would be recommended in those with a tumor size of 2–3 cm, one recurrent tumor, or AFP ≤400 μg/L.

The survival advantage of combining RFA with sorafenib for patients with recurrent HCC with MVI at the initial hepatectomy is multifactorial. First, the heat-sink phenomenon compromises RFA-induced tumor necrosis and limits the effectiveness of RFA (21). Recurrent tumors that develop from HCC with MVI positivity are more likely to possess increased angiogenesis due to the aggressive behavior of the initial HCC (6). The anti-angiogenic effect of sorafenib can decrease microvascular density, reduce blood perfusion around the tumor, and thus cause less heat-sink effect, leading to enhanced zones of RFA-induced coagulative necrosis (22, 23). Second, sorafenib can inhibit epithelial–mesenchymal transition of HCC cells following insufficient ablation, thus slowing HCC progression (24). Furthermore, sorafenib can cause enhancement of macrophage number of T cells, thus contributing to delivering an anti-tumor effect on non–RFA-targeted tumor micrometastases (25). These effects of sorafenib probably contributed to the decrease in tumor re-recurrence after RFA and better survival of the combination therapy. Compared with the previous studies on the combination therapy (11–13), this study was a multicentric one and focused on patients with early-stage recurrent HCC with MVI at the initial hepatectomy, pioneering to determine the role of adjuvant sorafenib following RFA in patients with early-stage recurrent HCC with MVI at the initial hepatectomy. The ratios of M1 and M2 were similar to those reported previously (26). Notably, we found that MVI grade could guide the application of adjuvant sorafenib. On the whole, the combination therapy improved both RFS and OS in patients with M2, whereas OS was not improved in patients with M1. To be specific, for patients with M1, only patients with a tumor size of 3–5 cm, tumor number of two to three, or AFP >400 μg/L would benefit from the combination therapy, whereas for patients with M2, the combination therapy would be recommended in those with a tumor size of 2–3 cm, one recurrent tumor, or AFP ≤400 μg/L. M2 grade is associated with higher recurrence rate and worse survival than M1 grade in patients with HCC, possibly due to higher likelihood of residual tumor (26). Therefore, the aforementioned advantages of sorafenib could be fully taken in patients with M2. Likewise, larger tumor size, more tumors, and higher AFP level have also been documented as risk factors for HCC prognosis (27, 28). For patients with HCC with a tumor size of 3–5 cm, tumor number of

TABLE 3 | The recurrence pattern of re-recurrences in the two treatment groups.

Recurrence pattern	RFA-Sorafenib	RFA	*P*-value
First recurrence			1.000
Intrahepatic recurrence	87	98	
Extrahepatic recurrence	2	3	
Intrahepatic recurrence + Extrahepatic recurrence	1	2	
Second recurrence			1.000
Intrahepatic recurrence	18	18	
Extrahepatic recurrence	1	0	
Intrahepatic recurrence + Extrahepatic recurrence	2	2	
Third recurrence			0.682
Intrahepatic recurrence	6	3	
Extrahepatic recurrence	1	1	
Intrahepatic recurrence + Extrahepatic recurrence	0	1	

RFA, radiofrequency ablation.

TABLE 4 | Adverse events between the two treatment groups.

Variable	RFA-Sorafenib (n = 103) Grade 1–2/3–4 (%/%)	RFA (n = 108)	*P*-value
Pain	52/2 (50.5/1.9)	59/4 (54.6/3.7)	0.518
Pleural effusion	1/2 (1.0/1.9)	1/1 (0.9/0.9)	1.000
Gastrointestinal bleeding	1/2 (1.0/1.9)	1/1 (0.9/0.9)	1.000
Fever	19/2 (18.4/1.9)	22/0 (20.4/0)	0.233
Hand-foot skin reactions	27/10 (26.2/9.7)	0/0 (0/0)	–
Diarrhea	43/9 (41.7/8.7)	0/0 (0/0)	–
Hypertension	21/4 (20.4/3.9)	0/0 (0/0)	–
Alopecia	18/4 (17.5/3.9)	0/0 (0/0)	–
Nausea/vomiting	48/2 (46.6/1.9)	0/0 (0/0)	–
Fatigue	29/8 (28.2/7.8)	0/0 (0/0)	–
Dysphonia	6/1 (5.8/1.0)	0/0 (0/0)	–
Decreased appetite	45/9 (43.7/8.7)	0/0 (0/0)	–
Pyrexia	18/1 (17.5/1.0)	0/0 (0/0)	–
Rash	22/1 (21.4/1.0)	0/0 (0/0)	–
Weight decreased	19/1 (18.4/1.0)	0/0 (0/0)	–
Headache	8/1 (7.8/1.0)	0/0 (0/0)	–
ALT increased	40/11 (38.8/10.7)	0/0 (0/0)	–
Hyperbilirubinemia	19/2 (18.4/1.9)	0/0 (0/0)	–
Constipation	13/1 (12.6/1.0)	0/0 (0/0)	–
Oral mucositis	15/1 (14.6/1.0)	0/0 (0/0)	–

RFA, radiofrequency ablation; ALT, alanine aminotransferase.

two to three, or AFP >400 μg/L, it becomes difficult for RFA alone to reach at least 1 cm of safety margin beyond the tumor at every direction (29). Insufficient ablation zone can leave residual tumor in adjacent liver tissues, leading to early recurrence and poor prognosis (30). Therefore, it is necessary to apply adjuvant sorafenib to facilitate RFA in patients with M1 along with a tumor size of 3–5 cm, tumor number of two to three, or AFP >400 μg/L. We also found that the combination therapy was not beneficial in patients with M1 along with a tumor size of 2–3 cm, one recurrent tumor, or AFP ≤400 μg/L, probably because sorafenib was unable to be taken full advantage in this subpopulation. Likewise, the combination therapy may not be recommended for patients with M2 along with a tumor size of 3–5cm, tumor number of two to three, or AFP >400 μg/L because adjuvant sorafenib seemed inadequate to enhance the efficacy of RFA. Therefore, our study provided a hint that not all patients with recurrent HCC with MVI at the initial hepatectomy would benefit from the combination of sorafenib and RFA. Clinicians could apply the combination therapy in a meticulous and precise way with the assistance of MVI grade to avoid unnecessary healthcare burdens and delay in treatment for ineligible patients.

Univariable and multivariable analysis revealed that, in addition to MVI grade and treatment allocation, tumor size, tumor number, PLT, AFP, and interval of recurrence from initial treatment were also independent prognostic factors. Tumor size and number can reflect tumor burden and their prognostic role has been proved in Feng X's study investigating the role of RFA combined with sorafenib in patients with BCLC stage 0–B1 HCC (11). PLT can facilitate tumor proliferation and metastasis *via* activating the TGFβ/Smad pathway in cancer (31). It has been incorporated into several prognostic indices in predicting HCC survival (32–34). High AFP levels and a short interval of recurrence from initial treatment are associated with aggressiveness and worse survival of HCC (35), and they have been proved to be independent risk factors in patients with early-stage RHCC (3).

There are several limitations to this study. First, as with any retrospective studies, there are the risks of selection and confounding biases. Second, no biopsy was done to confirm recurrence and re-recurrence. However, the noninvasive diagnostic criteria have been shown to achieve high accuracies in many prospective studies (36, 37). Third, the majority of patients had hepatitis B infection in the current study; therefore, the application of this study may be limited in patients with HCC from other etiologies. Fourth, because the combination therapy was included in an aggressive and iterative multimodal management of additional re-recurrences, long-term OS should be evaluated in this context.

In conclusion, adjuvant sorafenib following RFA was associated with better survival than RFA alone in patients with early-stage recurrent HCC with MVI at the initial hepatectomy. Moreover, MVI grade could guide the application of adjuvant sorafenib. More solid evidence from large multicentric prospective studies is necessary to validate these findings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Anhui Provincial Hospital, the Ethics Committee of the Beijing Cancer Hospital, the Ethics Committee of the Shanghai Eastern Hepatobiliary Surgery Hospital, the Ethics Committee of the Fudan Zhongshan Hospital, the Ethics Committee of the Cancer Center of Sun Yat-

sen University, the Ethics Committee of the Bethune First Hospital of Jilin University, the Ethics Committee of the Tianjin Medical University Cancer Hospital, the Ethics Committee of the Xijing Hospital, the Ethics Committee of the Cancer Hospital Chinese Academy of Medical Sciences, the Ethics Committee of the First Affiliated Hospital of Zhejiang University, the Ethics Committee of the First Affiliated Hospital of Zhengzhou University, and the Ethics Committee of the Southwest Hospital of AMU. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

M-SC, YC, W-YL, and Z-WP contributed to conception and design of the study. YC and Y-JZ organized the data collection. MC-W and Y-JZ performed the statistical analysis. M-CW and Z-WP wrote the first draft of the manuscript. Y-JZ, M-SC, W-YL, and Z-WP wrote sections of the manuscript. All authors contributed to manuscript revision and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.868429/full#supplementary-material

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ORIGINAL RESEARCH
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Conversion Therapy of Large Unresectable Hepatocellular Carcinoma With Ipsilateral Portal Vein Tumor Thrombus Using Portal Vein Embolization Plus Transcatheter Arterial Chemoembolization

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Background: The study aimed to assess the safety and efficacy of conversion therapy with portal vein embolization (PVE) and transcatheter arterial chemoembolization (TACE) in patients with large unresectable hepatocellular carcinoma (HCC) and ipsilateral portal vein tumor thrombus (PVTT).

Methods: This retrospective study evaluated consecutive patients with initially large (≥5 cm) unresectable HCC with ipsilateral PVTT who underwent PVE + TACE at our center between June 2016 and September 2020 (Group A). Clinically equivalent patients from three centers who were receiving tyrosine kinase inhibitors (TKIs) + TACE (Group B) were included. The survival times were evaluated and compared between the two therapeutic groups.

Results: In Group A (n = 33), the median tumor diameter was 14 cm (range, 5–18 cm) and 19 (57.6%) patients underwent radical resection 18–95 days after PVE. Radical liver resection was not performed because of inadequate hypertrophy (n = 11), pulmonary metastasis (n = 1), lack of consent for surgery (n = 1), and the rupture of the HCC (n = 1). There were no patients who underwent radical resection in Group B (n = 64) (P = 0.000). The mean and median overall survival (OS) were 736.5 days and 425.0 days in Group A and 424.5 days and 344.0 days in Group B, respectively. Compared with TKIs + TACE, treatment with PVE + TACE prolonged OS (P = 0.023).

Conclusions: This study shows that conversion therapy was safe and effective in patients with initially large unresectable HCC with ipsilateral PVTT treated with PVE + TACE. Moreover, PVE + TACE conferred more favorable outcomes than treatment with TKIs + TACE.

Keywords: conversion therapy, initial unresectable, hepatocellular carcinoma (HCC), portal vein embolization (PVE), transcatheter arterial chemoembolization (TACE), tyrosine kinase inhibitors (TKIs)

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third cause of cancer-related deaths worldwide and the second cause in China (1, 2). HCC has a strong propensity to invade the adjacent vasculature (3). Portal vein tumor thrombosis (PVTT) is found in 44–62.2% of patients with HCC who have already lost the chance of radical resection; thus, PVTT is recognized as a major prognostic risk factor (4, 5). Even with the best supportive care, the overall survival (OS) of HCC patients with PVTT is only 2–4 months (6, 7). Many factors contribute to the poor prognosis of patients with portal vein invasion, such as more invasive tumor behavior, aggravation of portal hypertension, facilitation of tumor transfer throughout the liver parenchyma or distant metastasis, and decreased hepatopetal portal blood flow (8). The Barcelona Clinic Liver Cancer (BCLC) staging and management system, which is widely accepted and applied in western countries, classifies HCC patients with PVTT as having at least advanced HCC, and systemic therapy with tyrosine kinase inhibitors (TKIs) is recommended as the first-line therapy regardless of PVTT grades (9). Besides TKIs, more aggressive therapies have been used in the clinic, which may improve the prognosis of HCC patients with PVTT and prolong the survival time of patients, such as transarterial chemoembolization (TACE), radiotherapy, hepatic resection, liver transplantation, and various combination of therapies (10–13).

Hepatic resection remains the mainstay of the curative treatment of primary hepatic malignancies. Conversion therapy, which has become a topic of interest for treating advanced liver cancer, converts unresectable advanced HCC or potentially resectable HCC to resectable HCC (14). Conversion therapies for HCC mainly include systemic therapy (15), portal vein embolization (PVE), associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) (16), and TACE. PVE, for the first time reported by Makuuchi et al. (17), is a well-established procedure for inducing future liver remnant (FLR) hypertrophy. The indications for liver resection have been expanded by PVE, which can lower the risk of postoperative liver failure (18).

According to Cheng et al. (11), >90% of PVTT develops on the same side of the lobe of the main tumor, and the tumor thromboses progress in the portal vein with far-from-heart modes. Thus, a combination of PVE and TACE is a potential therapy option for large unresectable HCC with ipsilateral PVTT, in which surgery cannot be performed because of the small FLR and PVTT. This treatment protocol was based on the hypothesis that PVE not only induces FLR hypertrophy but also prevents PVTT from developing toward the main portal vein by mechanically embolizing the targeted portal vein. If a sufficient FLR is achieved and PVTT is successfully treated after PVE, radical surgery can be performed subsequently.

Here, we evaluated 33 patients with initially unresectable large (≥5 cm) HCC with ipsilateral PVTT who underwent PVE and TACE. The oncological results, including OS and progression-free survival (PFS), were compared with those of equivalent patients receiving TKIs + TACE. This study reports our experience with conversion therapy with PVE + TACE in an initially large unresectable HCC with ipsilateral PVTT. Additionally, we intend to evaluate the safety and efficacy of PVE and TACE in these sufferers. To our knowledge, this is the first study that focused on the conversion therapy of large unresectable HCC with ipsilateral PVTT using PVE + TACE.

MATERIALS AND METHODS

Patients

This retrospective study included (a) patients diagnosed with initially large unresectable HCC with ipsilateral PVTT who received PVE + TACE at one center from June 2016 to September 2020, and (b) equivalent patients who received TKIs + TACE at the same center and two other centers. This study was conducted in conformity to the principles of the Declaration of Helsinki. The ethics committee of our hospital authorized the study protocol and waived the need for informed consent.

The criteria for inclusion were as follows: 1) over 18 years of age; 2) histopathologically or radiologically diagnosed HCC on the basis of the American Association for the Study of Liver Diseases criteria; 3) the HCC was unresectable because of PVTT and small standardized FLR (sFLR) (sFLR ≤40% and >30% in patients with cirrhosis, while patients with normal livers generally need ≤30% and >25%); 4) the size of the dominant tumor was ≥5 cm, and the ipsilateral PVTT was of grade Vp1, Vp2, or Vp3 and >1 cm from the main portal vein—according to the Japanese grading system for tumor emboli (19); 5) presence of PVTT spreading from the branches of intrahepatic portal vein (defined as low-attenuation intraluminal filling defect verified by contrast-enhanced computed tomography or magnetic resonance imaging); 6) Child–Pugh class A or B; 7) no extrahepatic invasion; 8) an Eastern Cooperative Oncology Group performance status score of 2 or less; and 9) no contraindication to TACE, PVE, or TKIs. Patients were excluded if they met any of the following criterion: 1) the dominant tumor size of <5 cm or contralateral PVTT; 2) left or right portal vein invasion of <1 cm from the main portal vein or PVTT with Vp4 grade as per the Japanese grading system; 3) other concurrent malignancies; 4) previous therapy for PVTT; 5) received other treatments (including radiofrequency ablation, iodine-125 seed implantation, radiotherapy, etc.) except for the aforementioned treatment for PVTT during the study; 6) sFLR ≤30% in patients with cirrhosis, while sFLR was ≤25% in patients with normal livers, or 7) lost to follow-up.

FLR volume was measured directly by computed tomography and total estimated liver volume (TELV) was calculated from the formula: TELV = −794.41 + 1,267.28 × BSA (body surface, square meters). Then, sFLR was obtained based on the formula: sFLR = FLR/TELV.

Thirty-three patients received PVE + TACE (Group A). Concomitantly, 64 clinically equivalent patients received TKIs + TACE from three centers (Group B).

Volumetric Assessment

The IQQA-Liver software (EDDA Technology Inc., Princeton, NJ, USA) was used to reconstruct 3-dimensional images and to

measure liver volumes. A radical resection operation was considered safe and sufficient when the sFLR ratio was >40% in the cirrhotic liver or >30% in the normal liver.

TACE

The presence of tumor-feeding arteries was confirmed using digital subtraction angiography. Infusions of Lipiodol (5–20 ml), embolizing fluids, and/or microspheres were infused into the tumor-feeding arteries until the tumor blood flow slowed or stopped completely (**Figure 1**). Patients who underwent TACE were evaluated during follow-up every 4–6 weeks after the procedure. A repeat TACE was performed after confirming Child–Pugh class A or B status, the absence of any liver dysfunction (uncontrolled jaundice, intractable ascites, massive hematemesis, or severe hepatic encephalopathy), and a lesion that was not fully necrotic.

PVE Procedure

To decrease the risk of PVTT spreading throughout the liver parenchyma or distant metastasis, there are three announcements about PVE for HCC patients with ipsilateral PVTT. First, a contralateral approach is the necessary prerequisite. Second, movements during PVE must be gentle. Third, in the case of migration of PVTT, suction through a catheter should be avoided when the catheter is positioned in the portal vein with PVTT. The contralateral approach provides a more favorable orientation for catheter control and flow-guided distal embolization. Under ultrasound guidance, a suitable branch of the portal vein was punctured by a needle, and portography was performed. The targeted portal vein was embolized using n-butyl cyanoacrylate (NBCA) mixed with iodized oil (n = 19), polyvinyl alcohol (n = 14), gelfoam (n = 6), and/or steel coil (n = 30) (**Figure 2**). All procedures were

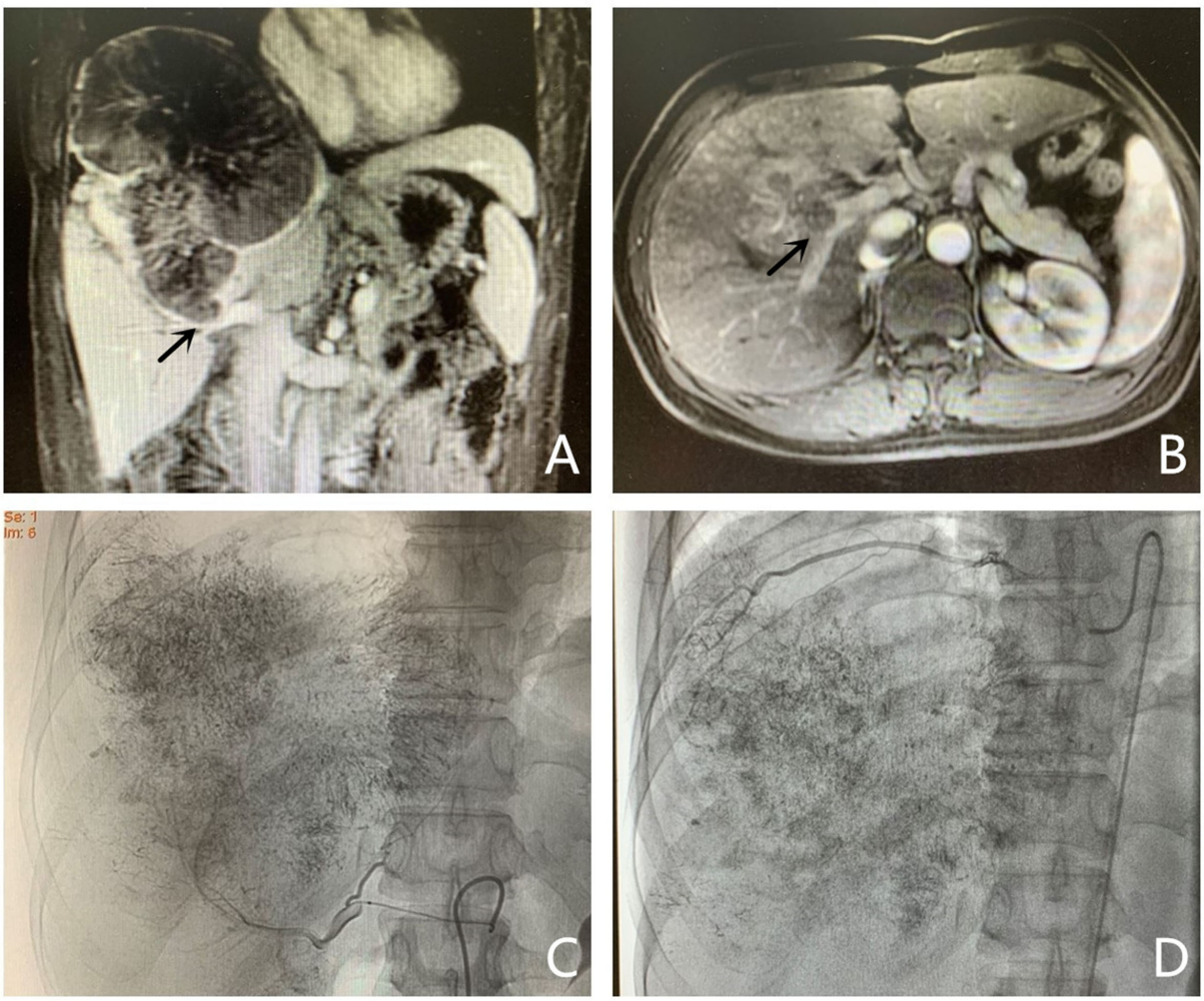

FIGURE 1 | Photographs acquired by magnetic resonance imaging (MRI) and transarterial chemoembolization implemented in a 44-year-old man (PVE + TACE group). **(A, B)** Hepatocellular carcinoma with ipsilateral PVTT, with an intraluminal filling defect which was a low-attenuation and spread from the intrahepatic portal vein branches (black arrow), was detected in the right lobe on enhanced abdominal MRI before therapy. **(C, D)** Images of transarterial chemoembolization. Tumor-feeding arteries are confirmed using digital subtraction angiography. Lipiodol, chemotherapeutic agents, and microspheres were injected into the right hepatic **(C)** and right phrenic arteries **(D)**.

performed by the same physician. According to a previous report (20), after the embolization, the head end of the catheter was retracted to the hepatic parenchyma. The following day, the catheter was removed.

Hepatectomy

The tumor was considered resectable if (1) R0 resection could be achieved with sufficient remnant liver volume and function, sFLR >40% in cirrhotic liver and >30% in normal liver, (2) liver function was Child–Pugh stage A or B, (3) there was no extrahepatic metastasis, and (4) no contraindications for hepatectomy existed.

TKI Therapy and Combination Therapy

TACE combined with TKIs was recommended for patients after assessing its clinical effects, potential adverse events, and costs. Sorafenib, lenvatinib, regorafenib, and apatinib were also recommended. If the recommendation was accepted, TKIs were administered 3–7 days after the first TACE procedure. Sorafenib was administered twice daily, at a dose of 0.4 g.

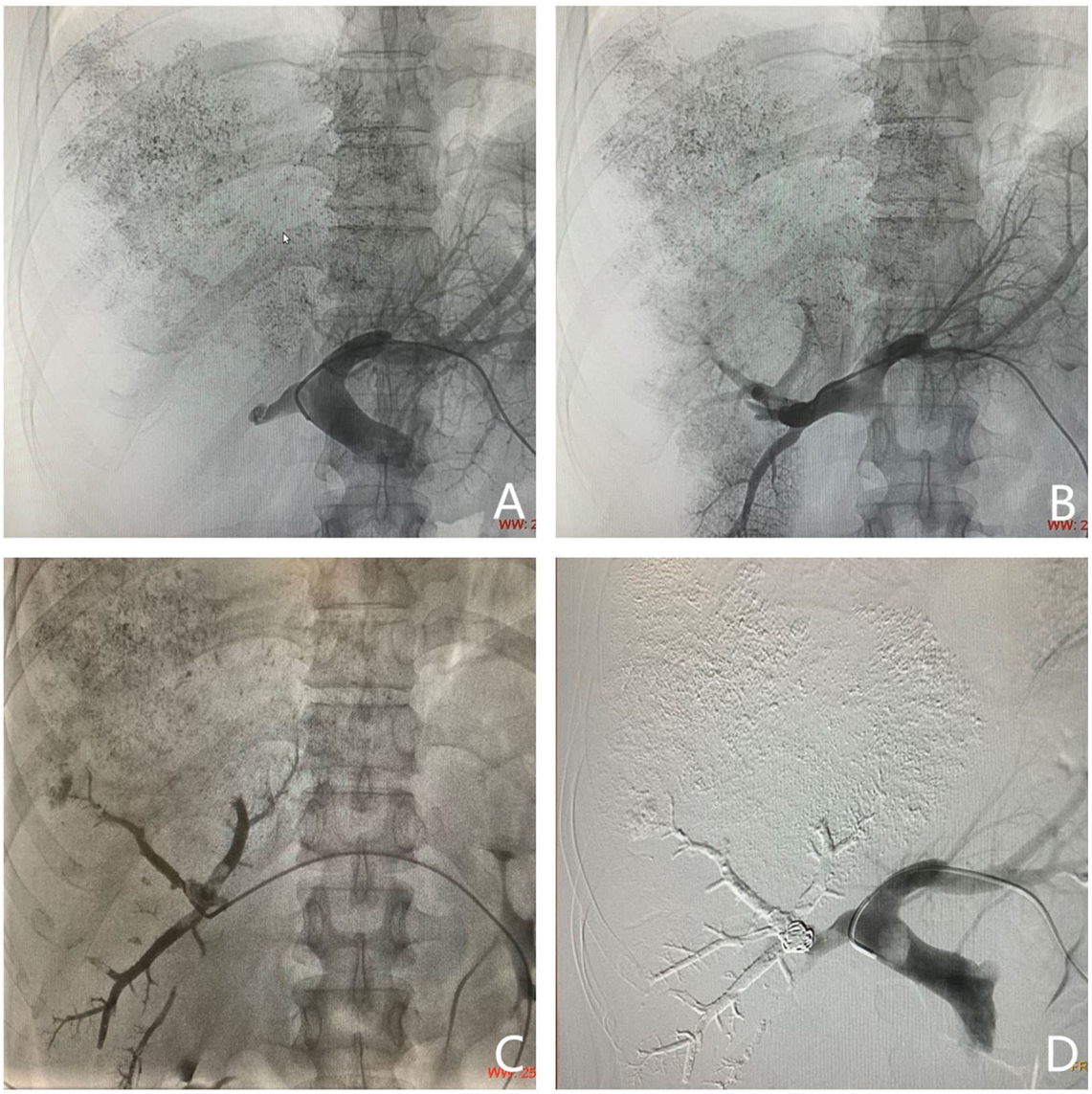

FIGURE 2 | Images of portal vein embolization performed in a 44-year-old man (same patient as in **Figure 1**). Puncturing a suitable branch of the portal vein with the guidance of ultrasound. **(A)** Portography with a catheter placed into the main portal vein. The right portal vein is not completely visible; it appears as a thrombosis in the right portal vein and matched the images acquired by magnetic resonance imaging. **(B)** Portography with a catheter placed into the right portal vein. **(C)** Image showing embolizing of the targeted portal vein. **(D)** Portography after embolizing of the right portal vein. The targeted portal vein was embolized using an NBCA/Lipiodol mixture and coils.

Regorafenib was administered at a dose of 0.16 g once daily. Apatinib was administered at a dose of 0.75 g once daily. Lenvatinib was administered at doses of 8 mg (less than 60 kg) or 12 mg (greater than or equal to 60 kg) once a day. The administration of TKIs was discontinued for three days leading up to the TACE procedure. Therapeutic administration was continued only after any effects of TACE (pyrexia, nausea, or vomiting) subsided. Drug reduction and interruption of drug-related adverse events were permitted.

Safety Assessment

Adverse events (AEs) were graded based on the Common Terminology Criteria for Adverse Events (version 5.0) and logged during follow-ups at intervals of 1–2 months.

Follow-Up and Assessment

The outpatient follow-up was conducted every 1–2 months. The censoring date was 5 May 2021. The OS and PFS of the two treatment groups were compared. The definition of OS was the interval from the first TACE session to death or last follow-up. As advanced HCC with PVTT progresses quickly, PVTT progression can induce portal hypertension and deterioration of liver function. The emergence of tumor spread, liver function deterioration, and esophageal and gastric variceal bleeding were indicators of disease progression. The definition of PFS was the interval from the day of the first TACE session to the occurrence of at least one of the aforementioned events or death.

Statistical Analysis

A Student's *t*-test was used to compare the continuous variables between the treatment category and baseline characteristics, and Fisher's exact or χ^2 test was employed to compare categorical variables. The Kaplan–Meier method was used to estimate survival curves, and the log-rank test was used to analyze differences. Using the Cox regression model, independent prognostic factors, which were correlated with OS identified by univariate analyses, were confirmed through multivariate analyses. SPSS, version 25.0 (SPSS Inc., Chicago, IL, USA), was employed for statistical analyses, with the statistical significance set at $P < 0.05$.

RESULTS

Patient Characteristics

A total of 357 patients underwent PVE. As shown in **Figure 3**, 152 patients did not have HCC, and 172 patients did not satisfy the enrollment criteria. Thirty-three patients received PVE + TACE (Group A), and 64 patients received TKIs + TACE from three centers (Group B). The baseline characteristics of the two treatment groups are shown in **Table 1**.

FLR Hypertrophy and Surgery Rate

In the PVE + TACE group, mean sFLR increased from 29.7% before PVE to 35.9% (P =0.000) after PVE, respectively. In Group A, 19 patients (57.6%) underwent radical resection through laparotomy 18–95 days after PVE (**Table 2**). In one patient, because of the rupture and bleeding of the large HCC, an emergency surgery was performed 5 days after PVE and 1 day after the first TACE procedure. Liver resection was not performed because of a small FLR in 11 patients, because of pulmonary metastasis in one patient, and because of lack of consent for surgery in one patient (**Figures 4**, **5**). In the TKIs + TACE group, no patient underwent radical resection (P =0.000).

Number of TACE Sessions

Patients in Group A had an average of 2.3 (range, 1–6) TACE sessions and those in Group B had an average of 2.44 sessions (range, 1–13; P = 0.70).

Treatment-related Complications

PVE-related postoperative reactions and TACE-related post-chemoembolization syndrome (pyrexia, nausea, emesis, loss of appetite, and abdominal pain) occurred in nearly all patients. All symptoms are alleviated within a few days of TACE or PVE.

The most common AEs were decreased albumin (16 patients, 48.5%), elevated aspartate transaminase (14 patients, 42.4%), and decreased platelet count (13 patients, 39.4%) in the PVE + TACE group. Grade 3 adverse events included decreased platelet count (two patients, 6.1%), elevated aspartate transaminase level (one patient, 3.0%), and elevated total bilirubin level (one patient, 3.0%). Grade 4 adverse events included rupture and bleeding of the large HCC (1 patient, 3.0%).

OS Analysis

The median follow-up times in Groups A and B were 777.0 and 805.0 days, respectively (p = 0.220). More patients died in Group B than in Group A during the follow-up period [53 (82.8%) vs. 19 (57.6%); P = 0.013]. The mean and median OS were 736.5 days [95% confidence interval (CI) 507.8–965.3 days] and 425.0 days (95% CI, 96.5–753.5 days) in Group A and 424.5 days (95% CI, 319.0–530.1 days) and 344.0 days (95% CI, 251.6–436.4 days) in Group B, respectively. There was a significant difference in OS between Groups B and A (P = 0.023) (**Figure 6A**). Compared with TKIs + TACE, the treatment consisting of PVE + TACE prolonged OS.

In Group A, 19 patients underwent radical resection 18–95 days after PVE (Group A_{Sur}), and 13 patients did not undergo radical surgery, and one patient underwent emergency surgery 1 day after the first TACE in Group A (Group A_{NoSur}). The mean and median OS for patients in Group A_{Sur} were 881.4 days (95% CI, 571.5–1191.3 days) and 684.0 days (95% CI, 0.0–1442.6 days), respectively. There was a significant difference in OS between Group B and Group A_{Sur} (P = 0.009) (**Figure 7A**). Compared with TKIs + TACE, treatment consisting of PVE + TACE + sequential radical resection prolonged OS.

The mean and median OS for patients in Group A_{NoSur} were 501.2 days (95% CI, 239.1–763.2 days) and 292.0 days (95% CI, 131.0–453.0 days), respectively. There was no significant difference in OS between Group B and Group A_{NoSur} (P = 0.610) (**Figure 8A**).

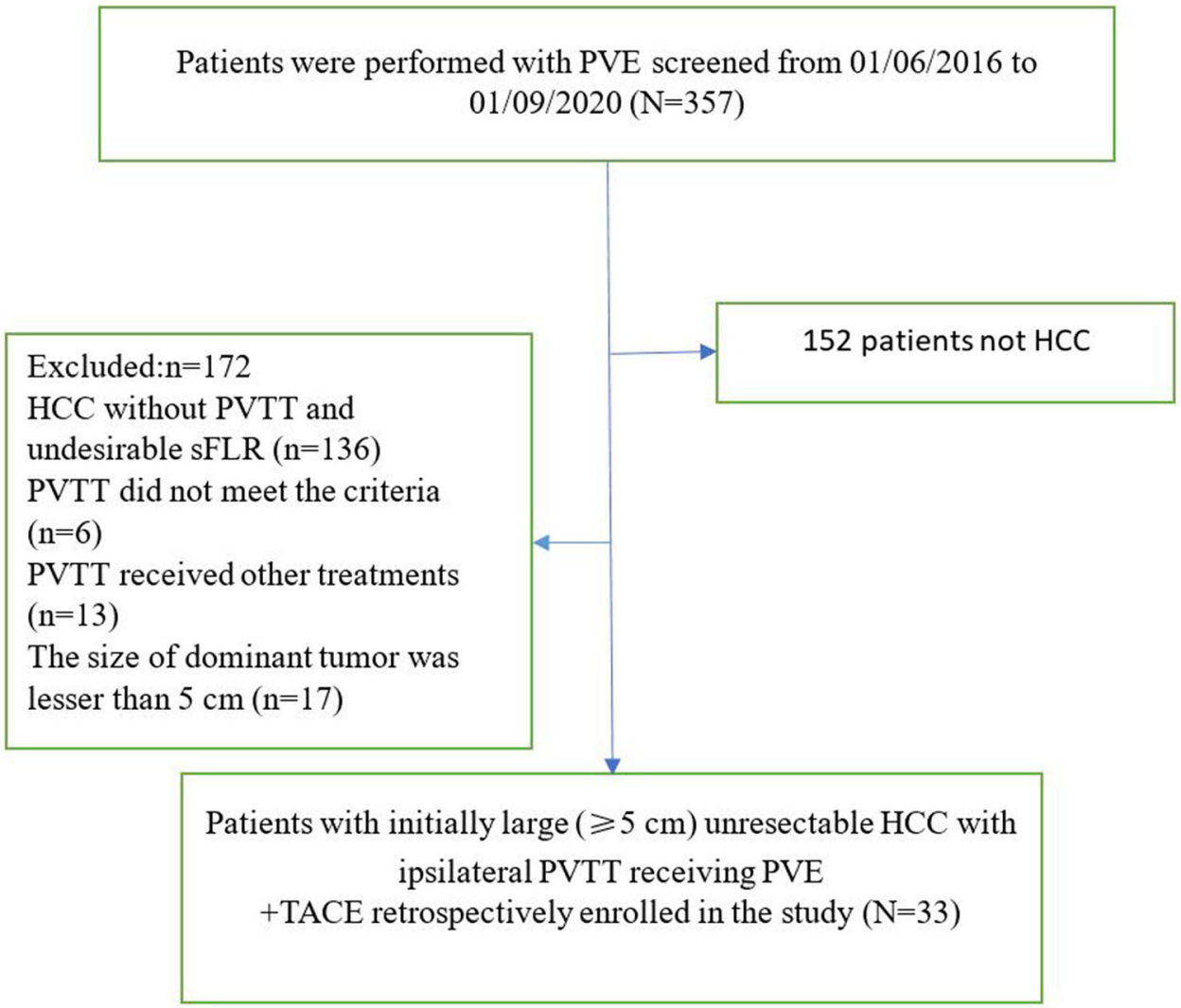

FIGURE 3 | The flow diagram of patients (PVE + TACE group). PVE, portal vein embolization; HCC, hepatocellular carcinoma; PVTT, portal vein tumor thrombus; sFLR, standized future liver remnant; TACE, transarterial chemoembolization.

PFS Analysis

The mean and median PFS were 449.6 days (95% CI, 271.8–627.3 days) and 188.0 days (95% CI, 94.8–281.2 days) in Group A and 255.2 days (95% CI, 175.9–334.5 days) and 197.0 days (95% CI, 99.9–294.1 days) in Group B, respectively. There was a significant difference in PFS between Group B and Group A (P = 0.047) (**Figure 6B**). Compared with TKIs + TACE, the treatment comprised of PVE + TACE prolonged PFS.

The mean and median PFS were 551.6 days (95% CI, 277.9–825.3 days) and 240.0 days (95% CI, 136.2–343.8 days), respectively, in Group A_{Sur}. There was a significant difference in PFS between Group B and Group A_{Sur} (P = 0.037) (**Figure 7B**). Compared with TKIs + TACE, treatment comprised of PVE + TACE + sequential radical resection prolonged PFS.

The mean and median PFS were 336.4 days (95% CI, 124.8–548.1 days) and 162.0 days (95% CI, 105.2–218.8 days) in Group A_{NoSur}, respectively. There was no significant difference in PFS between Group B and Group A_{NoSur} (P = 0.425) (**Figure 8B**).

Prognostic Factors for OS

The independent prognostic factors that contributed to OS were confirmed using the Cox regression model. Univariate analysis revealed that OS was correlated with treatment options (P = 0.025), maximum tumor diameter <10 cm (P = 0.017), and alpha-fetoprotein <400 (P = 0.015). Multivariate analysis was performed and maximum tumor diameter <10 cm [HR = 0.538 (95% CI, 0.325–0.890); P = 0.016] and treatment options (PVE + TACE) [HR = 0.582 (95% CI, 0.399–1.001); P = 0.050] were identified as independent prognostic factors for OS (**Table 3**).

TABLE 1 | Baseline demographic and clinical characteristics of patients with large unresectable HCC with ipsilateral PVTT.

Characteristics	Group A (N = 33)	Group B (N = 64)	p-value
Age, median (range), years	51 (43.5–60.5)	50.5 (43.25–57.75)	0.982
<65	29 (87.9)	59 (92.2)	0.484
≥65	4 (12.1)	5 (7.8)	
Sex (male/female)			0.356
Male	27 (81.8)	57 (89.1)	
Female	6 (18.2)	7 (10.9)	
Child–Pugh			0.157
A	32 (97)	55 (85.9)	
B	1 (3)	9 (14.1)	
Etiology			0.058
Hepatitis B	27 (81.8)	61 (95.3)	
Non-B	6 (18.2)	3 (4.7)	
Tumor number			0.110
Single	26 (78.8)	39 (65.4)	
Multiple	7 (21.2)	25 (34.6)	
HCC maximum diameter (cm)	14 (8.5–15.1)	11.7 (8.3–14.0)	0.138
<10	10 (30.3)	25 (39.1)	0.504
≥10	23 (69.7)	39 (60.9)	
AFP (ng/ml), median (Q1, Q3)	1417 (159.8, 31,114.5)	8,795 (169.2, 112,070.5)	0.289
<400	11 (33.3)	18 (28.1)	0.644
≥400	22 (66.7)	46 (71.9)	
DCP (mAU/ml), median (Q1, Q3)	8,055 (1,745.5, 74,646)	7,664.0 (2,095.0, 29,012)	0.127
<2,050	9 (27.3)	24 (37.5)	0.811
≥2,050	24 (72.7)	48 (62.5)	
PVTT			0.387
VP2	15 (45.5)	23 (69.2)	
VP3	18 (54.5)	41 (30.8)	
TKI			
Sorafenib	0	45	
Lenvatinib	0	15	
Apatinib	0	3	
Regorafenib	0	1	

Data are presented as n (%) or median (Q1, Q3). Q1 and Q3 are 25th percent and 75th percent of interquartile range.
Group A: PVE + TACE group; Group B: TKI +TACE.
PVE, portal vein embolization; TACE, transcatheter arterial chemoembolization; AFP, alpha-fetoprotein concentration; DCP, Des-gammacarboxy prothrombin; TKI, Tyrosine Kinase Inhibitor.

TABLE 2 | Characteristics of surgical and postoperative features.

Patient No.	Dominant tumor size, cm	Number of intrahepatic tumors	Japanese grading system	Days after PVE	Disease progression	Alive or die (OS)
1	5	1	Vp2	46	Yes	Die (1281 days)
2	17.3	1	Vp3	23	Yes	Die (677 days)
3	12	1	Vp3	64	Yes	Die (184 days)
4	11.8	1	Vp2	30	Yes	Die (249 days)
5	5.8	2	Vp3	39	Yes	Die (497 days)
6	16.5	1	Vp3	27	Yes	Die (50 days)
7	9	1	Vp2	29	Yes	Die (365 days)
8	12	1	Vp2	22	No	Alive
9	14	1	Vp2	18	Yes	Alive
10	9.3	1	Vp2	95	No	Alive
11	8	1	Vp2	26	No	Alive
12	14	1	Vp3	55	Yes	Alive
13	6.5	1	Vp2	41	Yes	Alive
14	14.7	1	Vp3	55	Yes	Die (200 days)
15	6	2	Vp3	66	Yes	Die (400 days)
16	18	1	Vp3	22	Yes	Alive
17	6	1	Vp3	20	No	Alive
18	7.4	2	Vp2	42	No	Alive
19	10.8	3	Vp1	35	Yes	Alive

PVE, portal vein embolization; OS, overall survival.

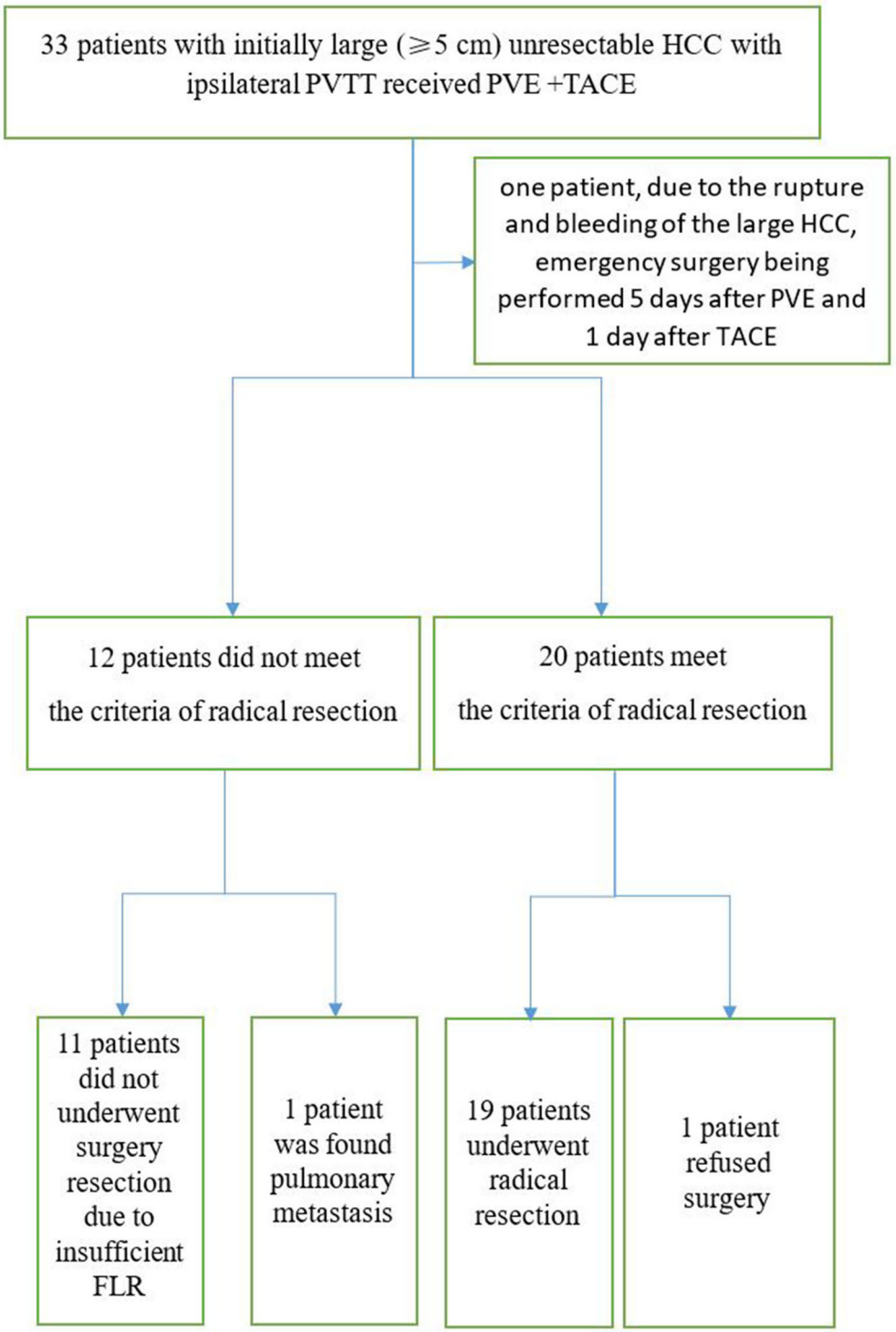

FIGURE 4 | Consolidated Standards of Reporting Trials diagram including all 33 patients who entered the study. PVE, Portal vein emboliaztion; PVTT, Portal vein tumor thrombus; TACE, Transarterial chemoembolization.

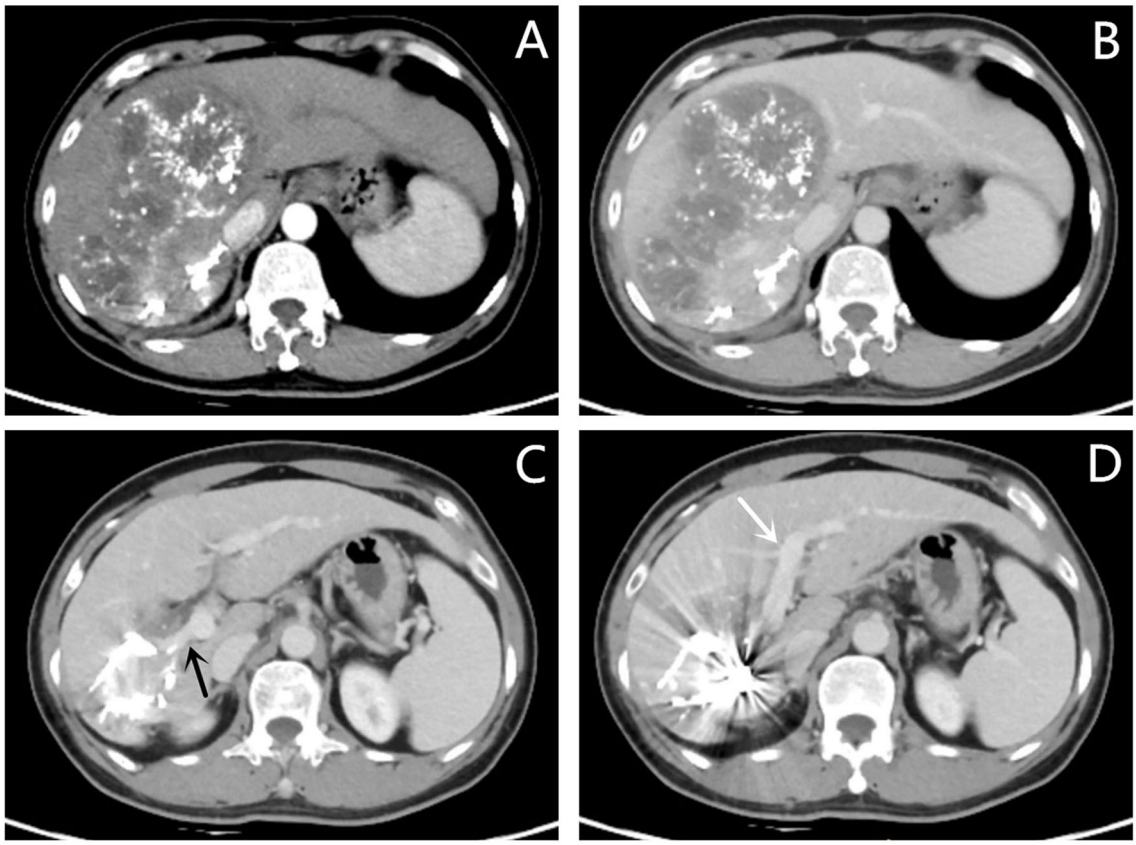

FIGURE 5 | Images of follow-up contrast-enhanced CT of the same patient as in **Figures 1**, **2** after PVE and three sessions of transarterial chemoembolization. **(A, B)** Hepatic arterial phase **(A)** and portal vein phase **(B)** showing that the lesion is fully necrotic. **(C)** Images of the portal vein phase showing a clear initial part of the right portal vein (black arrow). **(D)** Images of the portal vein phase showing a clear left portal vein (white arrow). Compared with **Figure 1**, the left lobe of the liver proliferated significantly after PVE.

Subsequent Treatment

Patients with tumor progression underwent subsequent treatment. In Group A, three patients had TKIs and programmed cell death protein-1 (PD-1) inhibitors added to their treatment regimen. In Group B, seven patients had a PD-1 inhibitor added to their primary treatment.

DISCUSSION

This retrospective study investigated and evaluated the safety and efficacy of conversion therapy with PVE + TACE in patients with an initially large unresectable HCC with ipsilateral PVTT. Additionally, survival time was compared with the equivalent patients from three centers who were receiving TKIs + TACE.

Compared with patients who received TACE with TKIs (Group B), those treated with TACE and PVE (Group A) had a longer OS (736.5 days vs 424.5 days; $P = 0.023$) and PFS (449.6 days vs 255.2 days; $P = 0.047$). In Group A, 19 (57.6%) patients underwent radical resection after PVE, and one patient had an opportunity to undergo radical resection but refused surgery. On evaluation of the group of patients in Group A that did not undergo radical resection (Group A_{NoSur}), we found that TACE with PVE in Group A_{NoSur} did not prolong OS (501.2 days vs. 424.5 days; $P = 0.610$) and PFS (336.4 days vs. 255.2 days; $P = 0.425$) compared with TACE and TKIs in Group B. However, compared with TACE and TKIs in Group B, TACE with PVE in Group A_{Sur} prolonged OS (881.4 days vs. 424.5 days; $P = 0.009$) and PFS (551.6 days vs 255.2 days; $P = 0.037$). It can be speculated that radical resection after PVE + TACE in Group A can prolong OS and PFS, but PVE + TACE alone without sequential radical resection cannot prolong OS and PFS compared with TKIs + TACE by mechanically embolizing the targeted portal vein. In other words, the possible reason for this finding was that radical resection, the opportunity for which was provided by PVE through inducing FLR hypertrophy, prolonged OS and PFS. The results suggest that PVE + TACE is a feasible conversion therapy for patients with initially large (>5 cm) unresectable HCC with PVTT to achieve successful resection with a potential long survival time.

Besides surgical resection, targeted therapy (sorafenib and lenvatinib as the first-line treatments; regorafenib, apatinib, cabozantinib, and ramucirumab as the second-line therapies); TACE; radiation therapy; and liver transplantation have been

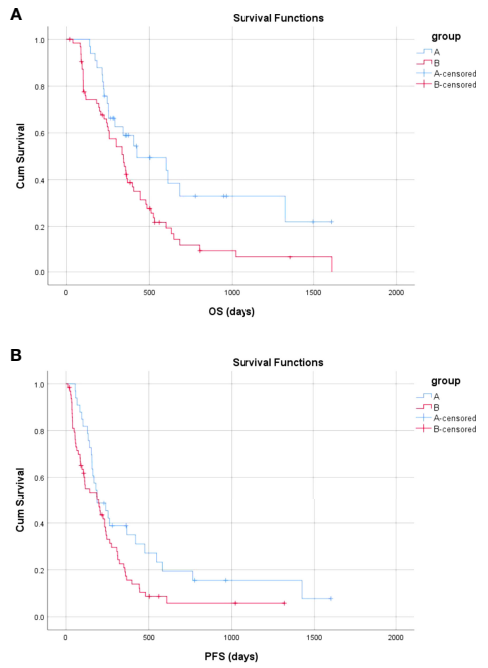

FIGURE 6 | Overall and progression-free survival in Group A (transarterial chemoembolization with portal vein embolization) and Group B (transarterial chemoembolization with molecular targeted therapy). **(A)** The overall survival in Group A and Group B **(B)** The progression-free survival in Group A and Group B.

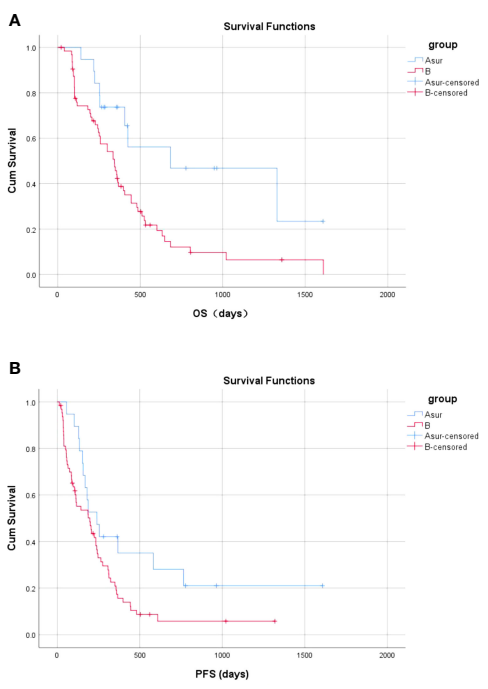

FIGURE 7 | Overall and progression-free survival between Group A_{Sur} and Group B **(A)** The OS in Group A_{Sur} and Group B **(B)** The PFS in the no-operative Group A_{Sur} and Group B.

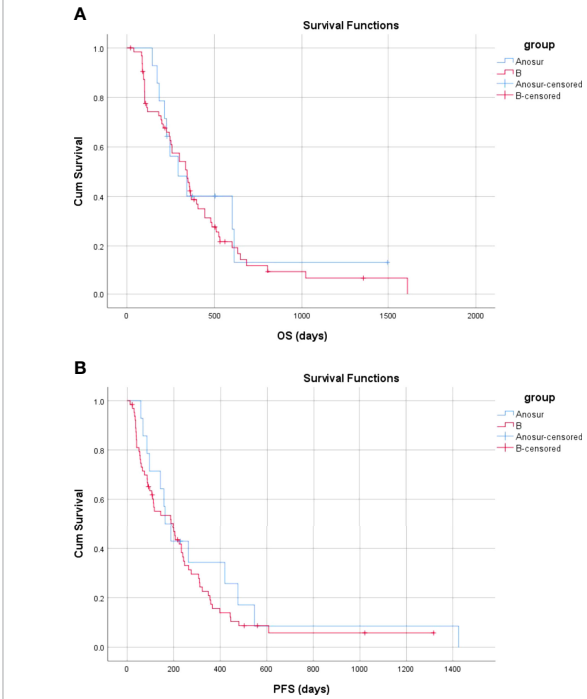

FIGURE 8 | Overall and progression-free survival between Group A_{NoSur} and Group B **(A)** The OS in Group A_{NoSur} and Group B **(B)** The PFS in the no operative Group A_{NoSur} and Group B.

recommended and practiced in HCC patients with PVTT. According to the Asia-Pacific region study, sorafenib monotherapy has only prolonged the survival time for 2–3 months (21). According to Jeong et al. (22), the real-world practical effect of sorafenib monotherapy in HCC with PVTT may be worse because of the selection bias and the median survival time was only 3.1 months. In addition, immunotherapy has developed greatly, and targeted therapy combined with immunotherapy might be a promising treatment strategy. Zhu et al. (15) reported 10 initially unresectable patients (15.9%) underwent R0 resection after TKI and anti-PD-1 antibody combinations, and one of them received PVE due to insufficient FLR. In HCC patients with PVTT, TACE alone had more survival benefit compared to supportive care, which has been confirmed by retrospective and prospective studies (23–25). However, the effect of TACE alone is still unsatisfactory. TACE combined with other therapies may gain a survival benefit and has been recommended as a new therapeutic strategy. With the aid of precision radiotherapy technology, external radiotherapy has been applied to more and more HCC patients with PVTT (26, 27). Compared with external radiotherapy, internal radiotherapy including iodine-125 seed implantation and transarterial radioembolization is more invasive. In comparison with hepatic resection, liver transplantation resolves the lesion and restores liver function simultaneously. Though the indication is expanding, in most

TABLE 3 | Univariate and multivariate analysis of predictors associated with overall survival in total cohort.

Variables	n	Univariate Analysis		Multivariate Analysis	
		Hazard Ratio	p-value	Hazard Ratio	p-value
Treatment option	33	0.547 (0.323–0.928)	0.025	0.582 (0.399–1.001)	0.050
PVE + TACE	64				
TKI + TACE					
Age, median , years	88	0.839 (0.398–1.767)	0.644		
<65	9				
≥65					
Sex (male/female)		0.706 (0.349–1.428)	0.333		
Male	13				
Female	84				
Child–Pugh		0.849 (0.598–1.206)	0.380		
A	87				
B	10				
Etiology		1.335 (0.484–3.678)	0.577		
Hepatitis B	88				
Non-B	9				
Tumor number		0.813 (0.501–1.319)	0.402		
Single	65				
Multiple	32				
HCC maximum diameter		0.543 (0.329–0.895)	0.017	0.538 (0.325–0.890)	0.016
<10 cm	35				
≥10 cm	62				
AFP (ng/ml)		0.502 (0.285–0.884)	0.015	0.572 (0.324–1.011)	0.055
<400	28				
≥400	69				
DCP (mAU/ml)		0.806 (0.471–1.380)	0.432		
<2,050	25				
≥2,050	72				
Portal vein invasion grade		1.100 (0.681–1.777)	0.697		
VP2	38				
VP3	59				

PVE, portal vein embolization; TACE, transcatheter arterial chemoembolization; TKI, Tyrosine Kinase Inhibitor; AFP, alpha-fetoprotein concentration; DCP, Des-gammacarboxy prothrombin.

research, PVTT is still considered as an absolute contraindication to liver transplantation (28–30).

Hepatic resection is the mainstay of curative treatment for patients with HCC (9). However, resecting HCC with macrovascular invasion is not recommended by the BCLC guidelines (5), while surgical resection is recommended by some Asian guidelines or consensus for patients with PVTT, and highly selected patients meeting these criteria acquire R0 resection (11). A large retrospective study indicated that liver resection could result in survival benefits as long as the PVTT is limited to Vp1–Vp3 (31). However, in this study, before treatment, the tumors were unresectable in all patients because of PVTT and insufficient FLR. Thus, sufficient FLR must be gained before performing curative resection. Besides PVE, ALPPS can also induce FLR hypertrophy (27). In theory, not a surgery-like ALPPS, PVE is an interventional, minimally invasive procedure, which could cause less damage and perioperative complications and is often more acceptable in advanced HCC patients. However, the safety and effectiveness of PVE and ALPPS in large HCC patients with ipsilateral PVTT should be studied by future research.

This study has some limitations. First, selection bias could not be eliminated in this study because of its retrospective character. Second, the number of samples in this study was small. Third, the type of TKI administered was not the same for all patients in Group B. Fourth, the follow-up period was relatively short. Fifth, with the advent of targeted therapy and immunotherapy, conversion therapy for HCC with ipsilateral PVTT in the experimental group excluded TKIs and anti-PD-1 antibodies. Prospective research with a large sample size is essential to verify the efficacy of conversion therapy with PVE, TACE, TKIs, and anti-PD-1 antibodies in HCC patients with ipsilateral PVTT.

Conclusively, the outcome of this study shows that conversion therapy with PVE + TACE could be a safe procedure for patients with large unresectable HCC with ipsilateral PVTT. Besides, the patients who underwent TACE + PVE had longer OS and PFS compared with TACE + TKIs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Eastern Hepatobiliary

Surgery Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CH collected related papers and drafted the manuscript. NG and YY participated in the design of the review. SC was responsible for the supervision of the work. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Induction Chemotherapy and Ablative Stereotactic Magnetic Resonance Image-Guided Adaptive Radiation Therapy for Inoperable Pancreas Cancer

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Background: Radiation therapy (RT) dose for inoperable pancreatic ductal adenocarcinoma (PDAC) has historically been non-ablative to avoid injuring gastrointestinal (GI) organs at risk (OARs). Accruing data suggest that dose escalation, in select patients, may significantly improve clinical outcomes. Early results of ablative stereotactic magnetic resonance image-guided adaptive radiation therapy (A-SMART) have been encouraging, although long-term outcomes are not well understood.

Methods: A single institution retrospective analysis was performed of inoperable non-metastatic PDAC patients who received induction chemotherapy then 5-fraction A-SMART on a 0.35T-MR Linac from 2018-2021.

Results: Sixty-two patients were evaluated with a median age of 66 years (range 35-91) and nearly all achieved Eastern Cooperative Oncology Group (ECOG) performance status 0-1 (96.8%). Locally advanced disease was common (72.6%), otherwise borderline resectable (22.6%), or medically inoperable (4.8%). All received induction chemotherapy for a median 4.2 months (range, 0.2-13.3) most commonly FOLFIRINOX (n=43; 69.4%). Median prescribed dose was 50 Gy (range 40-50); median biologically effective dose (BED_{10}) was 100 Gy_{10}. The median local control (LC), progression-free survival (PFS), and overall survival (OS) from diagnosis were not reached, 20 months, and 23 months,

respectively. Also, 2-year LC, PFS, and OS were 68.8%, 40.0%, and 45.5%, respectively. Acute and late grade 3+ toxicity rates were 4.8% and 4.8%, respectively.

Conclusions: To our knowledge, this is the largest series of induction chemotherapy followed by ablative 5-fraction SMART delivered on an MR Linac for inoperable PDAC. The potential for this novel treatment strategy is to achieve long-term LC and OS, compared to chemotherapy alone, and warrants prospective evaluation.

Keywords: pancreas cancer, ablative, radiotherapy, magnetic resonance image, chemotherapy

INTRODUCTION

The prognosis of patients with inoperable pancreatic ductal adenocarcinoma (PDAC) is dismal despite substantial efforts to meaningfully improve outcomes (1). Over the last decade, modest gains have been realized by intensifying chemotherapy although long-term local control (LC) and overall survival (OS) are rarely achieved (2–4). Conversely, significantly escalating radiation therapy (RT) to an ablative dose has not been considered feasible for most patients using conventional image guidance because of interfraction anatomic changes and uncertainty in assuring that dose to nearby organs at risk (OARs) is safe prior to delivering each fraction (5).

In recent years, the hypothesis that ablative radiation dose may improve long-term OS has garnered increasing attention (6–11). Stereotactic magnetic resonance image-guided adaptive radiation therapy (SMART) is particularly well suited for dose escalation, especially to mobile tumors in the abdomen and pelvis, because of its unique imaging and online adaptive replanning capabilities (9–13). A multi-institutional retrospective analysis by Rudra and colleagues demonstrated that dose escalation above a biologically effective dose (BED_{10}) >70 Gy_{10} using a 0.35 Tesla (T) magnetic resonance (MR)-guided cobalt-60 treatment machine was associated with significantly improved OS (9). Subsequent single institution experiences of ablative SMART (A-SMART) prescribed up to 50 Gy in 5 fractions (BED_{10} = 100 Gy_{10}) have also demonstrated minimal grade 3 or higher toxicity and favorable early efficacy (10, 14).

While these data are encouraging, there is a paucity of published outcomes of ablative RT for inoperable PDAC with extended follow-up. Therefore, we performed an updated analysis of our previously published institutional experience of A-SMART for inoperable PDAC (11).

MATERIALS AND METHODS

Patient Selection and Staging

After obtaining institutional review board (IRB) approval, we performed a single institution retrospective analysis of patients treated on the MRIdian Linac (ViewRay, Oakwood Village, OH) between 2018-2021 for non-metastatic PDAC.

Patients were staged with endoscopic ultrasound and computerized tomography (CT) scans. Most also had MR scans of the abdomen although positron emission tomography (PET) scans were not routinely used for initial staging. Resectability was determined according to the National Comprehensive Cancer Network (NCCN) criteria (15).

Only patients who received induction chemotherapy were included. A-SMART was considered if restaging studies showed no evidence of distant progression. There was not a maximum tumor size or minimum distance between gross tumor and gastrointestinal (GI) organs at risk (OARs) for patients to be offered A-SMART. As such, even patients with extensive abutment of gross disease and GI OARs were treated with A-SMART. Conversely, A-SMART was not offered if there was duodenal invasion by tumor as seen on endoscopic evaluation. No patient had prior abdominal RT. Patients were not routinely prescribed prophylactic proton pump inhibitors.

Radiation Therapy Planning and Delivery

Our treatment planning and delivery approach has been previously published (11). Simulation and treatment were done in the supine position typically with both arms down at the sides for comfort and reproducibility. Fiducial markers and intravenous/oral contrast were not used because gross disease and surrounding OARs could be distinctly visualized during treatment using continuous cine-MR imaging. Simulation included a 0.35 T mid-inspiration breath hold and balanced steady-state free precession sequence (TrueFISP) MR scan acquired over 17-25 seconds on the MRIdian Linac. This was followed by a simulation CT scan.

Target delineation and OAR segmentation were defined on the MR simulation scan, which was the primary scan for treatment planning. Contouring of GI OARs was done ensuring that the full thickness of the muscular wall in addition to the lumen of each structure was included. The gross tumor volume (GTV) included all visible tumor within the pancreas and any involved regional lymph nodes. After we gained confidence that ablative dose delivered to gross tumor alone was tolerated well, in late 2019 there was a systematic shift to routinely include a clinical target volume (CTV) that included a 5 mm isotropic margin around the GTV, proximal ~3 cm of the celiac axis (CA) and superior mesenteric artery (SMA) (**Figure 1**). Based on physician preference, the elective region was prescribed the same dose as the GTV (n=36; 61%) or a lower dose (33-35 Gy) in 5 fractions using a simultaneous integrated boost (SIB) (n=23; 39%). The planning target volume (PTV) was created through an isotropic 3 mm expansion of the GTV, or CTV if

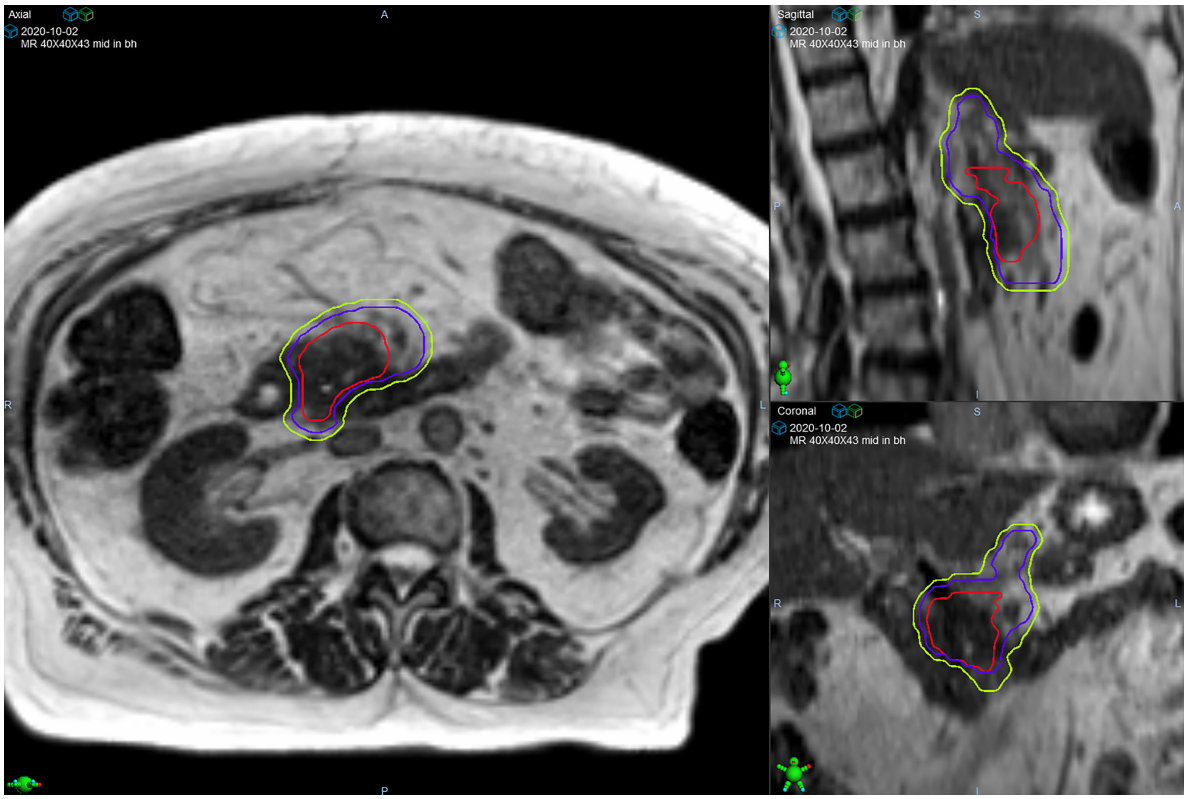

FIGURE 1 | Target volumes of a patient with pancreatic head/uncinate process lesion who was prescribed 50 Gy in 5 fractions. The gross tumor volume (red line) is surrounded by the clinical target volume (purple line) that includes the celiac axis and superior mesenteric artery. The planning target volume (green line) was created from a 3 mm expansion of the clinical target volume.

present, for all patients. The PTVs for the SIB approach were denoted as PTV50 and PTV33-35 to differentiate the ablative and lower dose levels. A 120-140% hotspot was intentionally delivered to as much of the GTV as possible. The highest priority for all delivered plans was to ensure that OAR constraints were met (stomach, duodenum, small bowel: V35 ≤0.5 cc, V40 ≤0.03 cc; large bowel: V38 ≤0.5 cc, V43 ≤0.03 cc; liver mean ≤15 Gy; kidneys mean ≤10 Gy; spinal cord V25 V25 ≤0.03 cc), even if this meant sacrificing target coverage (11). We used an isotoxicity planning approach to maximize target coverage, by which treatment plans were normalized to the nearest GI OAR.

All patients were treated with continuous cine-MR imaging and real-time tissue tracking with automatic beam gating. Prior to each daily treatment, the GTV was used to define the tracking region of interest (known as the "tracking structure") in the sagittal plane and treatment was automatically held when >3-5% was displaced >3 mm from its original position (i.e., outside of the "tracking boundary"). Mid-inspiration breath hold was preferred over free breathing respiratory gating to improve the duty cycle efficiency and decrease the time that the patient was required to be in the treatment machine. On-table adaptive replanning was performed if deemed medically necessary based on the predicted dose (i.e., the dose resulting from the initial plan recalculated on the anatomy of the day). The highest priority during both initial planning and adaptive re-planning was to ensure all OAR constraints were met, and then secondarily optimization of target volume coverage by the prescription dose.

Post-SMART Evaluation and Additional Therapy

Follow-up consisted of physical examination, CT scans (chest, abdomen, pelvis), and labs including CA19-9 at 4-6 weeks after SMART and otherwise at approximately 3-month intervals. We did not evaluate patients prior to 4 weeks because CA19-9 could potentially be transiently elevated from treatment rather than disease progression. PET/CT scans were not routinely ordered although were occasionally acquired to further investigate findings from CT and/or magnetic resonance image (MRI) scans. Treatment response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria. Toxicity outcomes were prospectively recorded at least once during SMART and then at each follow up visit using Common Terminology Criteria for Adverse Events (CTCAE) version 5.

Chemotherapy after SMART was given at the discretion of the treating medical oncologist, although in general was not

recommended unless there was concern for or definitive evidence of tumor progression based on radiographic findings and/or CA19-9 change. Patients were offered surgery based on multi-disciplinary tumor board discussion after SMART and this was intended to be done within 8 weeks after SMART, if possible.

Outcomes Assessment

LC was defined as absence of in-field treatment failure. Progression free survival (PFS) was defined as the time to local progression, distant progression, or death. OS was determined to be the time to death or otherwise last follow-up.

Common Terminology Criteria for Adverse Events (CTCAE version 5.0) was used to evaluate toxicity. Acute toxicity was considered to have occurred during or within 90 days from the beginning of SMART. Toxicity was prospectively recorded in the electronic medical record at the time of each clinic encounter.

Statistical Evaluation

The Research Electronic Data Capture system was used to collect and manage data. Median and range for continuous variables and frequencies and percentages for categorical variables were used for describing patient, tumor, and treatment characteristics. Clinical outcomes were evaluated using the Kaplan-Meier method. Treatment response was determined according to the Response Evaluation Criteria in Solid Tumors 1.1. Patients were censored at the date of last follow-up who were alive and did not experience tumor progression. The Kaplan-Meier method was used to determine estimated LC, PFS, and OS. A Cox proportional hazards model was used to evaluate prognostic factors of LC, PFS, and OS in univariate (UVA) and multivariate analyses (MVA). All variables with $P<0.10$ in the univariate analysis were entered in the multivariate model. Statistical significance was set at $P<0.05$. Statistical analysis was performed using SAS (version 9.4, SAS Institute, Cary, NC).

RESULTS

Patient, Tumor, and Treatment Characteristics

Sixty-two patients were evaluated (**Table 1**), most with tumors in the head of pancreas (n=55; 88.7%). Nearly all had Eastern Cooperative Oncology Group (ECOG) performance status 0-1 (n=60; 96.8%). The median largest tumor dimension after induction chemotherapy and prior to A-SMART was 3.8 cm (range, 1.5-6.9 cm). The majority had locally advanced disease (n=45; 72.6%) while borderline resectable (n=14; 22.6%) and resectable but medically inoperable (n=3; 4.8%) PDAC were less common.

Induction chemotherapy was given to all patients, most commonly FOLFIRINOX (n=43; 69.4%) or gemcitabine/nab-paclitaxel (n=15; 24.2%), for a median 4.2 months (range, 1.2-13.3 months). The median CA19-9 at diagnosis was 168.7 U/mL (range, 0.9-12,868.6 U/mL) that decreased after chemotherapy to a median 45.2 U/mL (range, 1-3686 U/mL).

TABLE 1 | Patient, tumor, and treatment characteristics.

Characteristic	N (range)
Total number of patients	62
Age (year), median	66 (35-91)
Gender	35 (59.3%)
Male	24 (40.7%)
Female	
ECOG performance status	60 (96.8%)
0-1	2 (3.2%)
2	
Histology	62 (100%)
Adenocarcinoma	
Tumor location	55 (88.7%)
Head	7 (11.3%)
Body/tail	
Largest tumor size (cm), median	3.8 (1.5-6.9)
Resectability	45 (72.6%)
Locally advanced	14 (22.6%)
Borderline resectable	3 (4.8%)
Resectable, medically inoperable	
Clinical T stage	1 (1.6%)
1	13 (21.0%)
2	9 (14.5%)
3	39 (62.9%)
4	
Clinical N stage	43 (69.4%)
0	18 (29.0%)
1	1 (1.6%)
2	
Clinical M stage	62 (100%)
0	
CA 19-9 (U/mL), median	168.7 (0.9-12,868.6)
Initial diagnosis	45.2 (1-3686)
Before SMART	
Induction chemotherapy regimen	43 (69.4%)
FOLFIRINOX	15 (24.2%)
Gemcitabine/nab-paclitaxel	4 (6.4%)
Gemcitabine	
Induction chemotherapy duration (months), median	4.2 (0.2-13.3)
Radiation dose	50 (40-50)
Total prescribed dose (Gy), median	5
Total prescribed fractions	
Elective volume coverage	50 (80.6%)
Yes	12 (19.4%)
No	
Post-SMART therapy	14 (22.6%)
Surgery	6 (9.7%)
Irreversible electroporation	32 (51.6%)
Chemotherapy	

ECOG, Eastern Cooperative Oncology Group; SMART, stereotactic magnetic resonance-guided adaptive radiation therapy; GTV, gross tumor volume.

The median prescribed radiation dose was 50 Gy (range, 40-50 Gy) delivered in 5 consecutive fractions. In our early experience a few patients were prescribed 40 Gy (n=2; 3.2%) or 45 Gy (n=5; 8.1%), and when we did not observe severe toxicity from these doses, we increased to 50 Gy (n=55; 88.7%) that since has been routine. The prescription dose was delivered to most of the target volumes on the initial plan created from the simulation day anatomy despite the proximity of GI OARs, and this coverage was similar across the adapted fractions while ensuring that all GI OAR constraints were met (**Table 2**).

TABLE 2 | Target volume coverage on the initial plan versus the on-table adaptive plans.

Target Volume	Initial plan dose (Gy)from simulation anatomy			On-table adaptive plan dose (Gy)from treatment day anatomy		
	Median	Mean ± SD	Range	Median	Mean ± SD	Range
GTV D_{90}	48.1	48.9 ± 5.3	36.6-60.5	48.4	48.6 ± 5.2	36.5-61.0
GTV D_{80}	52.0	52.0 ± 4.8	41.2-61.6	51.4	51.4 ± 4.6	40.6-61.5
CTV D_{90}	42.8	44.5 ± 6.7	30.1-56.0	44.9	44.2 ± 5.9	31.3-55.0
CTV D_{80}	49.9	48.8 ± 6.5	33.9-59.0	50.5	48.4 ± 5.5	33.8-56.9
PTV33-35 D_{90}	39.2	40.7 ± 6.6	24.0-53.0	39.3	39.7 ± 6.1	25.1-60.8
PTV33-35 D_{80}	44.7	45.2 ± 6.1	28.2-54.9	45.0	44.2 ± 5.6	29.7-63.3
PTV50 D_{90}	47.2	46.9 ± 5.0	33.2-55.4	46.2	45.8 ± 5.5	33.2-94.4
PTV50 D_{80}	50.0	49.4 ± 4.6	37.-56.5	48.7	48.2 ± 4.2	37.0-63.3

D_{90}, dose to 90% of the volume; D_{80}, dose to 80% of the volume; GTV, gross tumor volume; CTV, clinical target volume; PTV, planning target volume.

The median GTV D_{90} on the original versus adapted plans was 48.1 Gy and 48.4 Gy, respectively. The median PTV50 D_{90} on the original versus adapted plans was 47.2 Gy and 46.2 Gy, respectively.

Online adaptive replanning was performed for 5 fractions in nearly all patients (n=58; 93.5%) and was indicated because of predicted GI OAR constraint violations (**Figure 2**). Only 2 of our first patients were treated without adapted fractions; and both were prescribed 40 Gy to gross disease only.

Additional Therapy After A-SMART

Surgery was performed in 14 patients (22.6%) after a median 10.7 weeks (range, 5.6-44.1 weeks) from A-SMART, 10 (71.4%) with borderline resectable, and 4 (28.6%) locally advanced PDAC at initial diagnosis. Resection and reconstruction of the superior mesenteric vein/portal vein was done in 7 (50%) patients; none had resection of the CA or SMA. All received FOLFIRINOX (n=13; 92.9%) or gemcitabine/nab-paclitaxel (n=1; 1.6%), for a median 4.7 months (range, 1-8.1 months). The prescribed radiation dose was 40 Gy (n=1; 7.1%) or 50 Gy (n=13; 92.9%). Nearly all (n=12; 85.7%) had radiographic stable disease after A-SMART, yet all had significant histopathologic response in the primary lesion (1 ypT0, 11 ypT1, 2 ypT2) and 13 (92.9%) had negative lymph nodes. Thirteen (92.9%) achieved negative surgical margins.

Irreversible electroporation (IRE) was performed in 6 patients (9.7%) at a median 9.6 months (range, 2.3-29.0 months) after A-SMART. The most common indication was regional disease recurrence outside of the treatment field without distant progression (n=5); one patient did not have increasing CA19-9 or radiographic evidence of progressive disease although had stable disease by RECIST that was considered, by tumor board consensus to possibly represent an incomplete response to A-SMART.

Chemotherapy was typically not resumed after A-SMART unless there was radiographic evidence of disease progression and/or increasing CA19-9. As of the last follow-up date, 32 (51.6%) patients had not resumed chemotherapy.

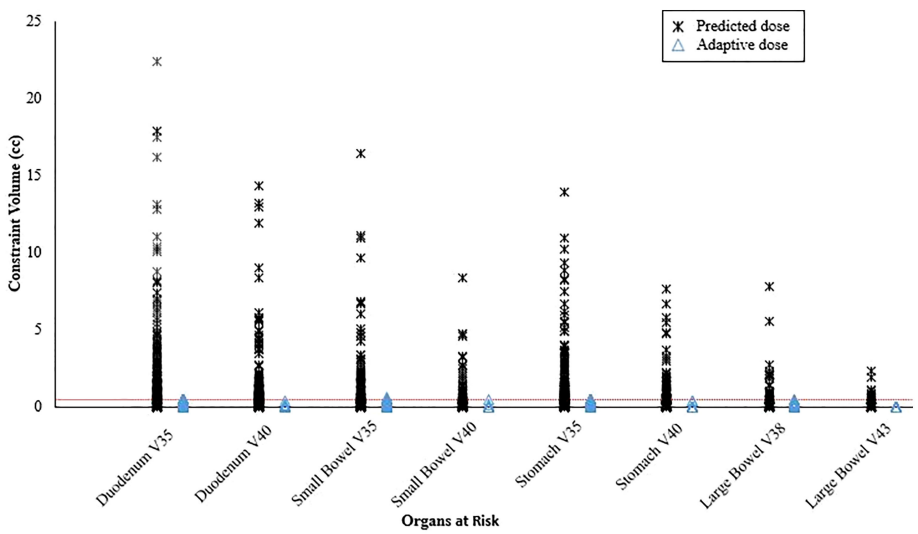

FIGURE 2 | Predicted organ at risk dose/volumes assuming the original plan was used on the day of treatment anatomy compared to on-table adaptive replanning that was able to ensure all dose constraints were met for every fraction. The horizontal dotted line represents the constraint volume of 0.5 cc.

Disease Control and Survival

Median follow-up for all patients was 18.6 months (interquartile range [IQR], 6.8-44.9 months) from diagnosis and 11.0 months (IQR, 1.5-36.0) from start of A-SMART. At the time of analysis, 23 patients (37.1%) were still alive.

Median LC from diagnosis was not reached. 1- and 2-year LC from diagnosis were 98.3% (IQR, 94.8-100%), and 87.7% (IQR, 77.0-98.3%), respectively (**Figure 3A**). Median PFS from diagnosis was 20 months (IQR, 17.0-25.0). 1- and 2-year PFS from diagnosis were 88.4% (IQR, 80.4-96.5%), and 40% (IQR, 25.8-54.2%), respectively (**Figure 3B**). Median OS from diagnosis was 23 months (IQR, 18.0-29.0). 1-year, and 2-year OS from diagnosis were 90.2% (IQR, 82.7-97.6%), and 45.5% (IQR, 31.5-59.5%), respectively (**Figure 3C**).

Median LC after A-SMART was not reached. 1- and 2-year LC after A-SMART were 98.2% (IQR, 79.8%-98.6%), and 68.8% (IQR, 45.9%-91.7%), respectively (**Figure 3D**). Median PFS after A-SMART was 12 months (IQR, 10.0-16.0). 1- and 2-year PFS after A-SMART were 49.0% (IQR, 35.1-62.95%), and 20.6% (IQR, 7.5-33.7%), respectively (**Figure 3E**). Median OS from A-SMART was 14 months (IQR, 11.0-22.0). 1-year, and 2-year OS from A-SMART were 53.8% (IQR, 40.3-67.4%), and 27.7% (IQR, 13.9-41.5%), respectively (**Figure 3F**).

The percentage CA19-9 change after induction chemotherapy and prior to A-SMART was the only significant prognostic factor for OS on multivariate analysis (hazard ratio 1.005; 95% confidence interval 1.001-1.009; P=0.008) (**Table 3**).

There was no statistically significant difference in LC from diagnosis based on surgery versus no surgery (not reached for both). Median PFS from diagnosis was shorter in patients who did not have surgery (18 vs. 35 months; P=0.06) due to more rapid distant progression; patients who had surgery had numerically higher median OS although the difference was not statistically significant (median 35 vs. 21 months; P=0.27).

Treatment-Related Toxicity

The delivery of ablative dose did not cause significant toxicity in most patients. Acute grade 3 toxicity (4.8%) included duodenal stenosis requiring stenting in 2 patients with tumor in the head of pancreas abutting the second/third part of the duodenum and one patient with abdominal pain lasting several hours after receiving the first fraction that resolved with medication and did not recur. There was no acute grade 4-5 toxicity. Late grade 3+ toxicity (4.8%) consisted of 2 patients with grade 3 GI bleed that resolved with transfusion. One patient's status post Whipple procedure 7 weeks after A-SMART, with an initially unremarkable postoperative course, died 6 weeks later due to a gastroduodenal artery bleed not definitely related to A-SMART (possible grade 5).

DISCUSSION

To our knowledge, this is the largest published experience of A-SMART delivered in 5 consecutive fractions for inoperable PDAC. Building on our initial clinical experience of 35 patients (11), the current analysis included 62 patients who all received induction chemotherapy and achieved median and 2-year OS from diagnosis of 23 months and 45.5%, respectively. These outcomes add to a small, yet growing, body of literature suggesting that radiation dose escalation could be associated with improved OS for patients with inoperable PDAC (7–11). Most recently, Reyngold and colleagues evaluated 119 locally advanced PDAC patients who received induction chemotherapy and ablative RT in 15 (19%) or 25 (81%) fractions delivered using CT guidance and median OS from diagnosis and RT were 26.8 and 18.4 months, respectively (8). While no study has prospectively compared outcomes based on prescribed dose for inoperable PDAC, LC, and OS after ablative RT are seemingly higher than what is has been reported after non-ablative dose (7, 16, 17). We must acknowledge the potential effect of evolving chemotherapy regimens on improving clinical outcomes including OS and, therefore, prospective evaluation is needed to better understand the impact of radiation dose escalation when delivered after contemporary multi-agent chemotherapy. However, outcomes from the recently published LAPC-1 trial that included FOLFIRINOX for 8 cycles then 40 Gy in 5 fractions suggest that there is a potential role for radiation dose escalation; 2-year LC from chemotherapy was ~60% (versus 87.7% in our study) and median OS was 15 months in unresected patients (versus 21 months in our study) (16).

Why might radiation dose escalation impact OS? About one-third of PDAC-related deaths are due to local progression (18), and it is by delaying or preventing these deaths through radiation dose intensification that long-term OS might be improved, at least for select patients. While a radiation dose response relationship with LC has been demonstrated (19), the modest improvement in LC achieved when using RT versus chemotherapy alone has not translated into improved OS as demonstrated in the LAP07 trial likely because non-ablative dose is not sufficient to achieve durable LC (20). Conversely, ablative radiation dose achieves excellent long-term LC as demonstrated in the current analysis where the median LC was not reached and 2-year LC from the start of A-SMART approached 70% despite some tumors measuring up to almost 7 cm. Similar outcomes have been reported in other recently published dose-escalated RT studies (8, 9). Of note, elective volume/nodal irradiation has increasingly been adopted as treatment for pancreas SBRT, including our institution; and recent data published by the Stanford group suggests that this at least improves PFS, although further evaluation is needed (21–23).

The emergence of MR guidance has led to a fundamental shift in how RT is delivered for some cancers (24). SMART provides superior soft tissue image quality, real-time continuous intrafraction cine-MRI, soft tissue tracking, and automatic beam gating, which are critical to ensuring that OAR constraints are met while delivering ablative dose, especially with ultra-hypofractionation, to most, if not all, of the target (11). In addition, an MR Linac enables rapid online adaptive replanning that allows for OAR constraints to be met and target volume coverage to be maximized despite interfraction anatomic changes by reoptimizing the original plan to account for the current day's anatomy (25). In the current analysis, we demonstrated that treating with the original plan would have violated at least one GI OAR constraint for nearly all fractions and that treating with an adaptive plan resulted in all constraints being met (**Figure 3**). SMART also seems to achieve safe dose escalation in only 5 fractions whereas a more fractionated course (e.g., 15-25 fractions) is likely needed if using CT guidance without adaptive replanning

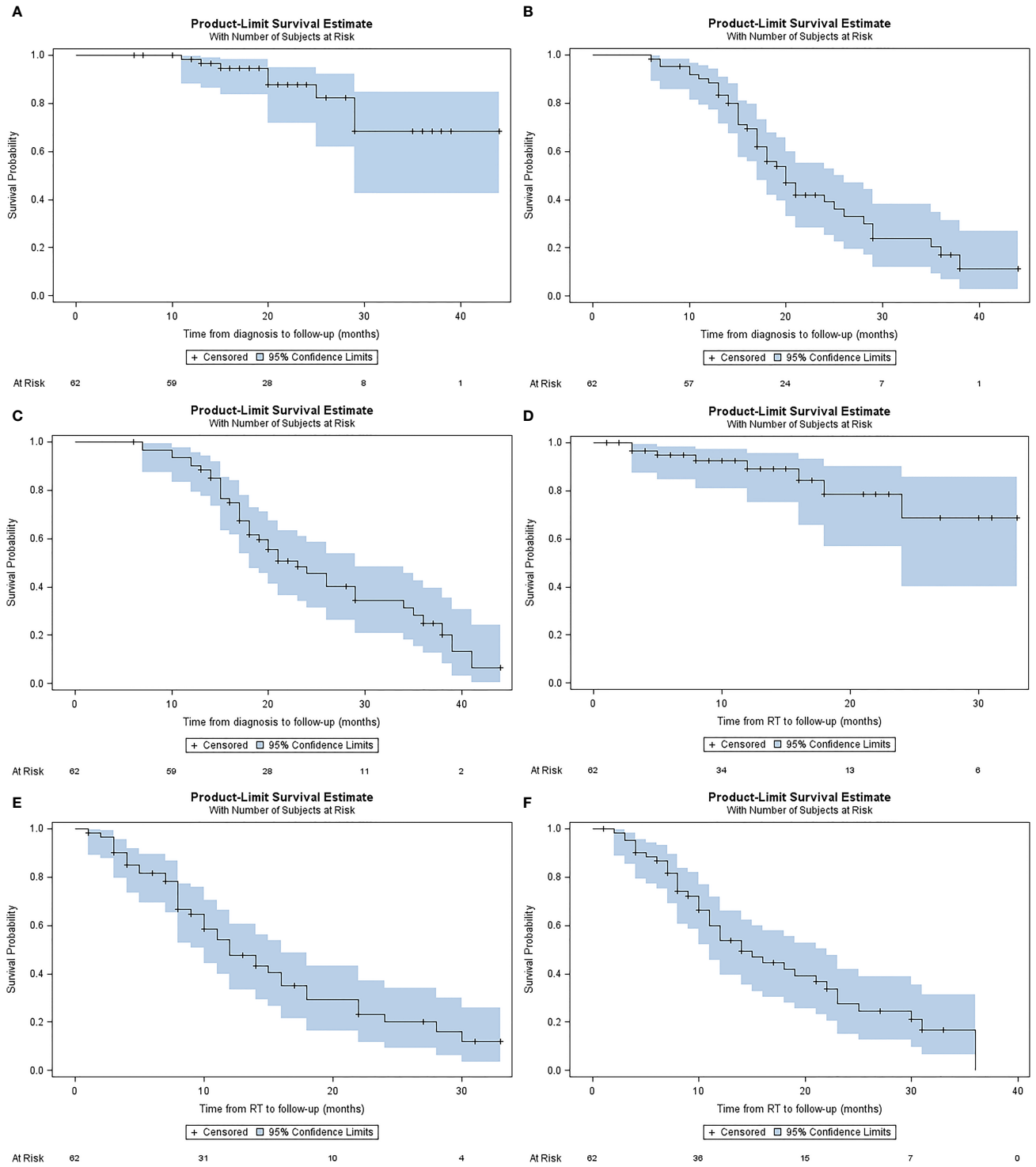

FIGURE 3 | Kaplan-Meier plots describing estimated **(A)** local control from diagnosis, **(B)** progression free survival from diagnosis **(C)** overall survival from diagnosis, **(D)** local control from A-SMART, **(E)** progression free survival from A-SMART, **(F)** overall survival from A-SMART.

(8). A limitation of the adaptive workflow is that it requires additional time and resources, although we believe this can be justified by the seemingly large gains in treatment efficacy.

Topics of interest that deserve the attention offuture studies include the development of novel prognostic biomarkers to better identify patients who should receive local therapy in addition to systemic

TABLE 3 | Multivariate analyses of factors predicting for overall survival.

Variables	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	1.009 (0.982, 1.036)	0.510		
Sex (female versus male [ref])	0.501 (0.214, 1.172)	0.110	0.546 (0.223, 1.337)	0.185
Location (body versus head [ref])	1.043 (0.313, 3.482)	0.944		
ECOG (1-2 versus 0 [ref])	1.911 (0.875, 4.174)	0.104	2.168 (0.943, 4.986)	0.068
T stage (1-3 versus 4 [ref])	1.446 (0.583, 3.586)	0.425		
N stage (1-2 versus 0 [ref])	1.214 (0.554, 2.659)	0.627		
Induction chemo drug (other versus Folfirinox [ref])	1.542 (0.754, 3.15)	0.235		
Induction chemo duration (>median versus <median [ref])	0.99 (0.492, 1.988)	0.976		
CA 19-9% change	1.004 (1.001, 1.008)	0.011	1.005 (1.001, 1.009)	0.008
Change in CA 19-9 (increase versus decrease [ref])	0.938 (0.452, 1.947)	0.864		
GTV volume (>median versus <median [ref])	2.407 (1.112, 5.21)	0.025	0.877 (0.387, 1.99)	0.753
GTV dose (>median versus <median [ref])	1.109 (0.539, 2.281)	0.779		
PTV volume (>median versus <median [ref])	2.335 (1.109, 4.916)	0.025	1.457 (0.628, 3.376)	0.380
PTV dose (>median versus <median [ref])	0.717 (0.331, 1.553)	0.398		
Elective coverage (yes versus no [ref])	0.649 (0.226, 1.863)	0.422		
Surgery (yes versus no [ref])	0.841 (0.413, 1.713)	0.633		
Post-RT chemo (yes versus no [ref])	1.009 (0.982, 1.036)	0.510		

HR, hazard ratio, CI, confidence interval; RT, radiation therapy; ECOG, Eastern Cooperative Oncology Group; GTV, gross tumor volume; PTV, planning target volume; A-SMART, ablative stereotactic magnetic resonance image guided radiation therapy.

therapy (26). It is not uncommon for some patients to experience rapid distant progression even after receiving extended chemotherapy and these patients presumably would be less likely to achieve meaningful long-term benefit from ablative RT. Assessing response after A-SMART is also currently challenging since "stable disease" can be misinterpreted on CT and MRI scans as lack of favorable response. Nearly all patients in the current analysis, who had surgery, achieved a significant histopathologic response, yet nearly all did not have any significant radiographic change, demonstrating that radiographic response is not adequate in itself to assess local treatment effect. Moreover, the discrepancy between radiographic and pathologic outcomes after preoperative therapy are well documented (27). Lastly, the cumulative dose delivered across all adapted fractions is not readily assessable on any commercially available MR Linac. Cumulative delivered dose may be associated with treatment efficacy and safety and may be useful to consider when optimizing each adapted fraction to improve the therapeutic ratio (14).

There are several limitations of this analysis including its retrospective design, single institution nature, duration of follow-up, and relatively small size. We recognize that retrospective studies may underreport toxicity but attempt to mitigate this by prospectively evaluating toxicity at each patient encounter whenever possible. We did not collect patient-reported outcomes that would have added to our understanding of patient tolerability and effects on quality of life; we plan to assess this in future patients. There was considerable heterogeneity in additional therapy delivered after A-SMART. While we report outcomes in patients who had surgery versus no surgery after A-SMART, there needs to be longer follow up to better understand the potential benefit of surgery. Follow up is also necessary regarding potential risks of operating after the delivery of such a high dose of A-SMART, which include major vascular structure by nature of patients having borderline resectable and locally advanced PDAC (28).

In conclusion, we demonstrate that induction chemotherapy and 5-fraction A-SMART appears to achieve a favorable therapeutic ratio for patients with initially inoperable PDAC, achieving durable LC for most patients and encouraging 2-year OS with minimal severe toxicity. Our findings add to the growing literature in support of significant dose escalation for inoperable PDAC and provide a strong rationale for future prospective evaluation of this novel treatment strategy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conceptualization: MC and KM; data curation: MC and RH; data analysis: MC, AK, MR, and KM; writing – original draft: MC, MR, and KM; manuscript editing and review: all authors. All authors contributed to the article and approved the submitted version.

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Caudal Approach to Laparoscopic Liver Resection—Conceptual Benefits for Repeated Multimodal Treatment for Hepatocellular Carcinoma and Extended Right Posterior Sectionectomy in the Left Lateral Position

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We had reported the novel concept of "caudal approach in laparoscopic liver resection" in 2013. In the first report, the caudal approach of laparoscopic transection–first posterior sectionectomy without prior mobilization of the liver in the left lateral position was described. Thereafter, 10 complex laparoscopic extended posterior sectionectomies with combined resection of the right hepatic vein or diaphragm were performed using the same approach. In the present study, the short-term outcomes of these cases and 42 cases of laparoscopic sectionectomies or hemi-hepatectomies (excluding left lateral sectionectomy) were compared. There was no statistically significant difference between the groups in terms of patients' backgrounds, diseases for resection, preoperative liver function, tumor number and size, as well as outcomes, operation time, intraoperative blood loss, morbidity, conversion to laparotomy, and post-operative hospital stay. Even complex laparoscopic extended posterior sectionectomy was safely performed using this procedure. This approach has the technical benefits of acquiring a well-opened transection plane between the resected liver fixed to the retroperitoneum and the residual liver sinking to the left with the force of gravity during parenchymal transection, and less bleeding from the right hepatic vein due to its higher position than the inferior vena cava. Furthermore, it has an oncological benefit similar to that of the anterior approach in open liver resection, even in posterior sectionectomy. The detailed procedure and general conceptual benefits of the caudal approach to laparoscopic liver resection for repeated multimodal treatment for hepatocellular carcinoma are described.

Keywords: laparoscopic liver resection, caudal approach, postural change, repeat hepatectomy, hepatocellular carcinoma, chronic liver disease, posterior sectionectomy

INTRODUCTION

After the introduction of laparoscopic liver resection (LLR) in the early 1990s (1–3), the procedure had been rapidly developing with technical and instrumental improvements (4) through two international consensus conferences (5, 6) and three world congresses of the International Laparoscopic Liver Society (7). Partial resections in the anterolateral segments and left lateral sectionectomy have been established as common procedures. In addition, laparoscopic hemi-hepatectomies and sectionectomies (left-medial, right-anterior, and right posterior), which have straightforward caudal–cranial transection planes suitable for the laparoscopic approach, are the next-step candidates of LLR to get established as common procedures (6). Among them, anterior and medial sectionectomies have difficulty transecting a large area of the boundary plane on both the right and left sides. On the other hand, posterior sectionectomy has a specific difficulty in acquiring a good surgical field and bleeding control because the transection plane is horizontal and deep in the subphrenic space (rib cage) beneath the large and heavy right liver in the usual supine position.

We had reported the novel concept of "caudal approach to LLR" in 2013 (8), which was followed by several researchers (9, 10), and it was defined as a main conceptual change from open liver resection (OLR) in the statement of the 2nd International Consensus Conference on LLR (6). In the first report, "caudal approach of laparoscopic transection–first posterior sectionectomy without prior mobilization of the liver in left lateral position" was described. Since the transection plane turns vertically and the plane is well opened between the retroperitoneal-fixed resected liver (posterior section) and the residual liver sunk down to the left with the force of gravity, a good surgical field is obtained in the procedure. In addition, upward standing of the right hepatic vein (RHV) from the inferior vena cava (IVC) on the transection surface decreases intravenous pressure, which leads to less bleeding. Using this approach, we performed even more complex procedures, such as laparoscopic extended posterior sectionectomy with combined resection of the RHV or diaphragm. Moreover, the caudal approach also has several conceptual benefits. Liver resection is a procedure of handling and resecting the liver protected inside the subphrenic "rib cage". In OLR, the cage is opened with a large subcostal incision followed by costal arch lifting, and the mobilized liver is picked up from the retroperitoneum. In contrast, in the laparoscopic procedure, laparoscope and forceps intrude into the cage directly from the caudal direction without destruction of the cage and with minimal mobilization of the liver (with minimal damage to the adherent structures and the liver itself, **Figure 1A**).

In this perspective, we attempted to describe the current status of the caudal approach to LLR. The short-term outcomes of our laparoscopic extended posterior sectionectomy with combined resection of the RHV or diaphragm are compared to those of the other anatomical LLRs for sections or more (excluding left lateral sectionectomy), and the detailed procedure is described. In addition, the conceptual benefits of the caudal approach for repeated multimodal treatments of hepatocellular carcinoma (HCC) are discussed.

SHORT-TERM OUTCOMES OF LAPAROSCOPIC EXTENDED POSTERIOR SECTIONECTOMY WITH COMBINED RESECTION OF RIGHT HEPATIC VEIN OR DIAPHRAGM

After the first report on the caudal approach laparoscopic posterior sectionectomy, 10 complex laparoscopic extended posterior sectionectomies with combined resection of the RHV (nine cases) or diaphragm (one case) were performed using the same approach. Herein, the short-term outcomes of these 10 cases and the other 42 anatomical LLR cases for sections or more (excluding left lateral sectionectomy) were compared.

Background-related factors, including sex, age, and body mass index; the American Society of Anesthesiologists physical status classification of the patients; diseases for resection; preoperative liver functional indicators, including plasma levels of total bilirubin and albumin, platelet counts, prothrombin time, and indocyanine green retention rate at 15 min; tumor number and size; as well as postoperative short-term outcomes, including operation time, intraoperative blood loss, conversion to laparotomy, morbidity, and post-operative hospital stay were compared between the groups (**Table 1**).

There was no statistically significant difference between the groups in terms of sex, age, body mass index, physical status class of the American Society of Anesthesiologists, diseases for resection, preoperative liver functional indicators (plasma levels of total bilirubin and albumin, platelet counts, prothrombin time, and indocyanine green retention rate at 15 min), and tumor number and size. In addition, for the short-term outcomes, there was no statistically significant difference between the groups in terms of operation time (499.00 ± 108.38 min vs. 452.12 ± 127.12 min in extended posterior sectionectomy vs. the other anatomical resections, $p = 0.253$), intraoperative blood loss (438.50 ± 425.50 ml vs. 746.43 ± 1,523.415 ml, $p = 0.261$), conversion to laparotomy (0/10 vs. 2/40 cases, $p = 0.482$), and post-operative hospital stay (16.50 ± 6.13 days vs. 23.24 ± 11.93 days, $p = 0.091$). Four cases in the anatomical LLR for section or more and zero case in the extended posterior sectionectomy groups developed major complications of grade III or above in the Clavien–Dindo classification; however, the difference was not significant ($p = 0.310$).

There are few reports on perioperative outcomes of laparoscopic major hepatectomy (hemi-hepatectomies and sectionectomies, excluding left lateral sectionectomy, and the same patient group as our anatomical LLR group). Takahara et al. analyzed the data of 929 patients in the Japanese registry and reported an intraoperative blood loss of 865.4 ml, an operation time of 441.3 min, and a complication rate of 16.4% (11). Most recently, in a study conducted by Chin et al. on 130 patients in a single high-volume center, an intraoperative blood loss of 500 ml, an operation time of 362.5 min, and a complication rate of 26.9% were reported (12). Our outcomes of anatomical LLR are comparable to those previously reported, and there are no significant differences between our outcomes in the extended posterior section LLR group and the anatomical LLR group. Although extended posterior sectionectomy with combined resection of the RHV or diaphragm is a complex procedure, the

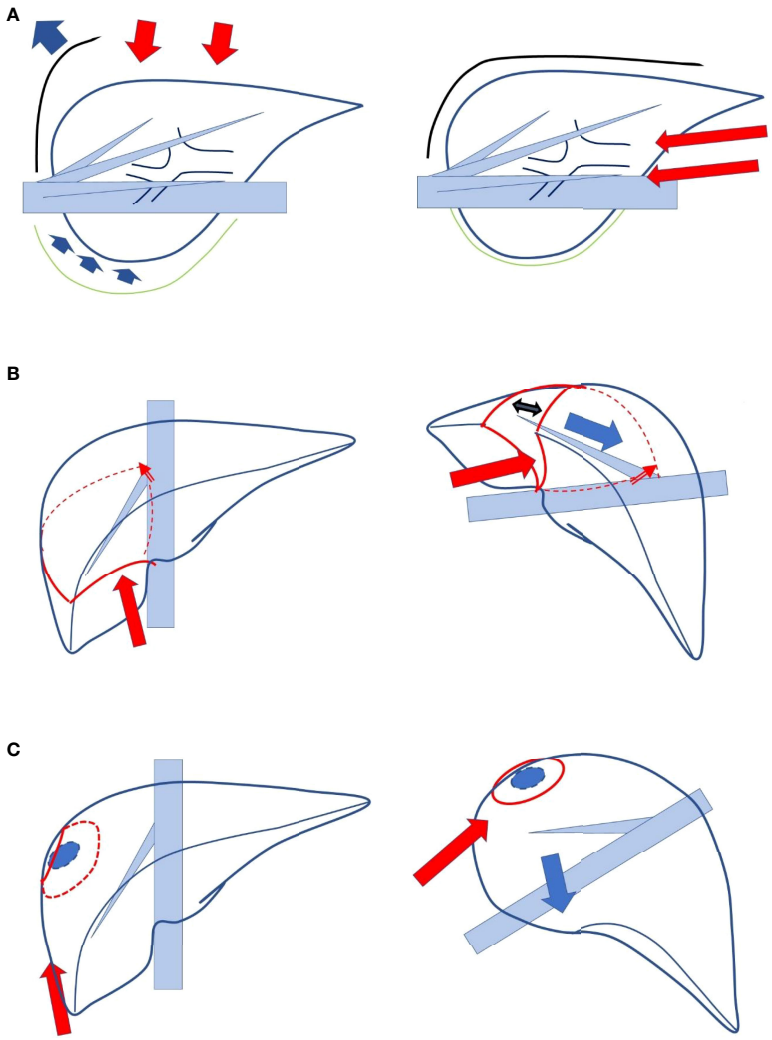

FIGURE 1 | **(A)** Liver resection is a procedure in which the liver protected inside the subphrenic "rib cage" is handled and resected. In open liver resection, the cage is opened with the big subcostal incision followed by lifting the costal arch, and the mobilized liver is picked up from the retroperitoneum (left, lateral view). In a laparoscopic procedure, laparoscope and forceps intrude into the cage directly from caudal direction without destruction of the cage and with minimum mobilization of the liver (right, lateral view). **(B)** The boundary plane between the anterior and posterior sections, the cutting plane of posterior sectionectomy, is horizontal and the large heavy liver and gravity obstruct exposure of the plane in supine position (left). In the left lateral position with transection prior to mobilization, the cutting plane between the retroperitoneal-fixed resected liver and the sunk remnant liver is well-opened (right). **(C)** The transection of segmentectomy or partial resection in segment 7 of the liver should be performed in the deep small subphrenic space with segment 6 as an obstacle in the way to the lesions. In a semi-prone position, direct access to segment 7 can be obtained with the elimination of segment 6 in the downward left direction by gravity (left).

procedure is feasible and was performed safely using the caudal approach with short-term outcomes comparable to those of other anatomical LLRs for sections or more.

DETAILED PROCEDURE AND BENEFITS OF THE CAUDAL APPROACH TO LAPAROSCOPIC POSTERIOR SECTIONECTOMY

Our LLR for posterior sectionectomy, including the extended one, is performed by placing patients in the left lateral or semi-lateral position with rotation to the left. Furthermore, liver parenchymal transection prior to mobilization is employed to obtain a well-opened transection plane.

In the first step of the procedure, dissection of the falciform and coronal ligaments is performed to increase the movability of the residual liver, which causes the liver to sink due to the force of gravity in the left direction, and results in a well-opened transection plane in the left lateral position. The root surfaces of the right and middle hepatic veins and the fissure between them are continuously exposed. The fissure is the endpoint of liver parenchymal transection in extended posterior sectionectomy combined with RHV resection. Thereafter, the hepatoduodenal

TABLE 1 | Comparison between laparoscopic extended posterior sectionectomy cases and laparoscopic liver resection cases for section or more in background factors and postoperative short-term outcomes.

	Extended Posterior Sectionectomy Cases, *n* = 10	Laparoscopic Liver Resection Cases for Section or More, *n* = 42	*p*-value
BACKGROUND FACTORS			
Age (years)	62.10 ± 12.20	68.52 ± 9.76	0.147
Sex (Male: Female)	6:4	32:10	0.300
Diseases for resection (HCC:Mets:other)	4:5:1	17:11:14	0.222
Body mass index	23.8 ± 2.1	22.8 ± 3.5	0.392
ASA-PS (1:2:3)	11:30:1	3:7:0	0.868
ICG R15 (%)	10.12 ± 4.89	9.91 ± 4.77	0.904
Total bilirubin (mg/dl)	0.60 ± 0.21	0.65 ± 0.27	0.506
Albumin (g/dl)	3.84 ± 0.48	3.88 ± 0.48	0.812
Platelet ($\times 10^4/\mu l$)	21.45 ± 6.02	21.77 ± 9.36	0.893
Prothrombin time (%)	104.40 ± 12.76	102.56 ± 13.99	0.694
Number of tumors	1.90 ± 0.99	2.06 ± 2.20	0.750
Size of tumor (mm)	42.90 ± 19.18	57.87 ± 39.45	0.252
SHORT-TERM OUTCOMES			
Operation time (min)	499.00 ± 108.38	452.12 ± 127.12	0.253
Intraoperative blood loss (ml)	438.50 ± 425.50	746.43 ± 1523.415	0.261
Conversion to laparotomy (no: yes)	10:0	40:2	0.482
Morbidity (no: yes)	10:0	38:4	0.310
Postoperative hospital stay (day)	16.50 ± 6.13	23.24 ± 11.93	0.091

HCC, hepatocellular carcinoma; Mets, liver metastasis; ASA-PS, the American Society of Anesthesiologists physical status classification. Extended posterior sectionectomy, right posterior sectionectomy with the combined resection of the right hepatic vein (n = 9) or diaphragm (n = 1). Laparoscopic liver resection cases for section or more, and sectionectomy and hemi-hepatectomy cases excluding 10 extended posterior sectionectomy and left lateral sectionectomy. ICGR15, indocyanine green retention at 15 min. Morbidity, Clavien–Dindo grade 3 or above.

ligament is encircled with vessel tape for the extracorporeal Pringle's maneuver.

Without mobilization of the liver from the retroperitoneum, the liver parenchyma (between segments 6 and 1) above the IVC is transected to expose the dorsal surface of the posterior Glissonian pedicle and IVC. In addition, the anterior surface of the Glissonian pedicle is dissected from the liver parenchyma at Rouviere's sulcus. Thereafter, the posterior Glissonian pedicle (or at least the Glissonian pedicle to segment 6) can be clamped with a bulldog clamp without the necessity of encircling and taping it.

According to the ischemic demarcation line, the liver parenchymal transection starts from the caudal edge of the liver. The demarcation line, IVC behind the liver, and root of the RHV are guides of the transection direction.

After the transection line reaches the level of Rouviere's sulcus, the posterior Glissonian pedicle is encircled and divided. The peripheral parts of the RHV are then searched and exposed on the well-opened transection plane. Exposure of the peripheral parts of the RHV eventually leads to the exposure of the main branch surface. The RHV main branch is one of the guides of the transection direction accompanied by IVC, demarcation line, and the root of the RHV as the endpoint.

The transection following the RHV and IVC eventually reaches the confluence of the veins and diaphragm, and the liver parenchymal transection is completed. After completion of liver parenchymal transection, the RHV on the transection surface of the resected liver is divided for extended posterior sectionectomy with combined resection of the RHV. Finally, retroperitoneal dissection of the liver is performed and the right posterior section, with or without the RHV, is removed.

The following are the technical benefits of this approach:

1. A well-opened transection plane between the resected posterior section fixed to the retroperitoneum and the residual liver sinking to the left with the force of gravity during parenchymal transection can be acquired.

2. Less bleeding from the RHV due to its lower intravenous pressure caused by its higher position than the IVC can be accomplished. Furthermore, the blood from the vertical transection surface runs down, and a clear view of the bleeding point can be obtained.

3. The exposed RHV on the transection surface and IVC at the bottom of the surgical field, accompanied by the liver surface ischemic line after clamping the posterior Glissonian pedicle, can be a good guide for transection direction.

Furthermore, this procedure, as a non-touch isolation procedure similar to the anterior approach in OLR, has an oncological benefit not only in hemi hepatectomy like in OLR, but also even in posterior sectionectomy.

PERSPECTIVES OF CAUDAL APPROACH TO LLR

Caudal Approach and Postural Changes in Various LLRs

Currently, all LLR procedures are performed using the caudal approach with postural changes in our institution. Caudal approach in the head-up supine to left semi-lateral position have usually been employed for hemi-hepatectomies, anterior/medial sectionectomies, and LLRs of anterolateral segments, segment 8, cranial 4 and 1. The left lateral position is applied

for posterior sectionectomy, and the semi-prone position is applied for segment 7.

As mentioned above, the boundary plane between the anterior and posterior sections, that is, the cutting plane of posterior sectionectomy, is horizontal in the supine position. Although the cutting plane should be well opened in the small subphrenic "rib cage" for a safe and stable LLR, the large and heavy right liver and gravity obstruct the exposure of the cutting plane of posterior sectionectomy in this position. In contrast, a clear view from the caudal direction (**Figure 1A**) is among the advantages of LLR. Therefore, we developed a procedure that facilitates the exposure of the cutting plane: a caudal approach laparoscopic posterior sectionectomy with parenchymal transection prior to mobilization in the left lateral position. In this procedure, the cutting plane between the retroperitoneal-fixed resected liver and the sunk remnant liver is well opened (**Figure 1B**). Moreover, transection in segmentectomy or partial resection of segment 7 should be performed in the deeper and smaller subphrenic space, with segment 6 as an obstacle in the way to lesions under the laparoscopic caudal view even in the left lateral position. Therefore, we employed the semi-prone position for the LLRs, in which direct access to segment 7 can be obtained by eliminating segment 6 in the downward and left direction due to gravity (13, 14) (**Figure 1C**). For a large tumor lodged into the diaphragm, parenchymal transection prior to mobilization is performed to acquire a good view and manipulation on the transection plane well opened by the force of gravity, similar to posterior sectionectomy in the left lateral position. Regular segmentectomy and partial resection of segment 7 are performed after the mobilization of liver from the retroperitoneum. Mobilization leads to an adequate surgical space above the liver, and stable handling of tumors and instruments is established in the area of the RHV root. Although the area is at the bottom of the abdominal cavity in the supine position, it turns to be at the top of the abdominal cavity in the semi-prone position. The movements of the instruments are relatively restricted in our semi-prone caudal approach, without intercostal ports. However, using the port, in the paravertebral area to Morrison's fossa, makes the manipulation feasible and stable with minimal risk of postoperative pleural effusion (14).

Conceptual Benefits for Repeated Multimodal Treatments in Hepatocellular Carcinoma Patients With Chronic Liver Disease

Since HCC patients mostly have underlying chronic liver diseases (CLDs), they have a higher risk of post-treatment short-term morbidity and, in the long term, have a potential need for repeat treatments for metachronous multicentric lesions from the preneoplastic background liver, and risk of liver insufficiency during the long-repeated treatment course. When considering liver resection for HCC patients with CLD, not only oncological therapeutic effects on the current tumor but also residual liver function and the degree of invasive surgical stress, especially in the diseased liver, should be considered (15, 16). Patients who undergo liver resection are exposed to three different types of surgical stress (1): general, whole-body usual surgical stress (2); decreased liver function due to reduced liver volume after resection; and (3) surgery-induced injury to structures surrounding the liver and residual liver parenchyma (such as destruction of collateral blood and lymphatic flows by laparotomy, mobilization, and parenchymal injury caused by compression during liver resection) (17). Reduction of the third stress by the laparoscopic-specific caudal approach (8–10) in LLR (**Figure 1A**), especially for patients with HCC and CLD, decreases short-term morbidity (17, 18) and may also decrease deterioration of liver function after surgery, resulting in a decreased number of deceased patients with liver insufficiency and better accessibility to repeat multimodal treatments of metachronous lesions (19).

The impact of LLR on this issue depends on the background CLD severity, operative procedures (such as extent of dissection of the peritoneal attachments and adhesions), and resection volume of the functioning liver. Our previous study evaluated the short-term outcomes of liver surface small LLR in patients with severe CLD (Child Pugh, B/C; and indocyanine green retention rate at 15 min, ≥ 40%) (20). It revealed comparable short-term outcomes in patients with severe CLD compared to those with mild-to-moderate CLD. These surgeries were performed with direct access to the surface tumors and minimum dissection of attachments and adhesions, even without inflow control, and without touching any associated structures around the tumor. Only a laparoscopic approach, not an open approach, can make this resection setting possible. Patients with small surface tumors outside the bare area, without the need for dissection of peritoneal attachments on the surface and major vessels at the bottom, could benefit from LLR. However, the survival benefits of these treatments have not been proven (21). We, with Ghent University in Belgium as a primary investigating center, also performed the international retrospective study using propensity score matching analysis of patients with Child-Pugh B cirrhosis who underwent liver resection. The study showed that LLR is beneficial for patients with Child-Pugh B cirrhosis compared to open procedures (22, 23). Furthermore, LLR is speculated to have a benefit of less deterioration of liver function after surgery due to smaller damage related to surgical manipulation mentioned before (19, 24), which can lead to long-term benefits during repeated treatment history in HCC/CLD patients.

The treatment of repeat lesions, thereafter, is another major issue for the treatment strategy of HCC/CLD patients, as they harbor the potential for multicentric metachronous lesions occurring from the preneoplastic background. Modifications of the anatomy and the formation of adhesions increase the difficulty of repeat liver resection. The laparoscopic approach makes subsequent surgeries easier because of less adhesion formation (25). Furthermore, LLR allows for better visibility and manipulation in a small operative field between adhesions under the condition of repeat resection (26), which makes total adhesiolysis unnecessary in repeat LLR. We conducted an international retrospective collective study with propensity score matching analysis for repeat liver resection, comparing

laparoscopic and open procedures (27, 28). It has been shown that laparoscopic repeat liver resection is feasible and has the short-term advantages of less intraoperative blood loss and less morbidity for selected patients. The overall survival curves after laparoscopic and open repeat liver resections were clearly separated with a better tendency in the laparoscopic group, although the disease-free survival curves were identical. The overall survival of HCC patients with CLD after liver resection is determined not only by the recurrence of the resected HCC but also by metachronous multicentric HCCs and liver insufficiency during postoperative long-term repeat treatment course (29, 30). During the long repeated treatment history of HCC/CLD patients, they should have sufficient residual liver function after each treatment, making it possible to undergo repeat treatments. We hypothesized that better overall survival after laparoscopic repeat liver resection may be caused by less deterioration of liver function (27), which made the repeat multimodal treatments more accessible, accompanied by less adhesion, and the number of deceased patients due to liver insufficiency decreased. The laparoscopic view and manipulation in the caudal approach (**Figure 1A**) facilitates better access in a small operative field between adhesions and reduces the need for adhesiolysis. This could be explained similarly to the advantage of LLR for patients with CLD noted previously. LLR, using its specific caudal approach, has conceptual benefits for HCC/CLD patients as a unique strong local treatment that makes repeated multimodal treatments more accessible. However, repeated LLRs have specific disadvantages. Disorientation can easily occur owing to the lack of tactile sensation and the lack of an overview of the entire operative abdominal field. Simulation and navigation from pre- and intra-operative imaging studies and well-planned small anatomical resection to secure tumor localization in the resected area and the tumor-free resected margin are used to overcome this disadvantage (24).

CONCLUSION

The caudal approach, which is the basic approach to LLR, can be applied to a variety of LLRs using postural changes, even in complex procedures such as extended posterior sectionectomy with combined resection of the RHV or diaphragm. Its conceptual benefits could make repeated multimodal treatments more accessible and result in longer survival in patients with HCC/CLD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Fujita health university ethics committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

TE wrote the draft. ZM supervised the whole process from data collection to writing the manuscript and revised the final draft. HK, KK, KM, YA, and AH collected the data, discussed the data during data analysis, and wrote the draft. All authors contributed to the article and approved the submitted version.

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Proton therapy in the treatment of hepatocellular carcinoma

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Liver cancer represents one of the most common causes of death from cancer worldwide. Hepatocellular carcinoma (HCC) accounts for 90% of all primary liver cancers. Among local therapies, evidence regarding the use of radiation therapy is growing. Proton therapy currently represents the most advanced radiation therapy technique with unique physical properties which fit well with liver irradiation. Here, in this review, we aim to 1) illustrate the rationale for the use of proton therapy (PT) in the treatment of HCC, 2) discuss the technical challenges of advanced PT in this disease, 3) review the major clinical studies regarding the use of PT for HCC, and 4) analyze the potential developments and future directions of PT in this setting.

KEYWORDS

proton therapy, hepatocellular carcinoma, active scanning, photon therapy, review

Introduction

Liver cancer represents the sixth most commonly diagnosed cancer and the third leading cause of cancer death worldwide in 2020 (1). Hepatocellular carcinoma (HCC) accounts for 90% of all liver cancers (2). Survival is poor with an average 5-year overall survival (OS) of around 20% (2). A certain level of cirrhosis is associated with the majority of HCCs. Locoregional recurrent disease is the main cause of death in HCC (3), while metastatic spread is limited even in advanced stages (4). Potential treatment strategies are extensive, ranging from curative approaches such as surgery or liver transplantation, which only selected patients (less than 20%) are eligible due to patients' or tumors' condition and ablation, whose efficacy is limited by tumor size or location (5), to non-curative strategies with an impact on survival for intermediate stages such as chemoembolization (6) or radioembolization (7). Advanced stages with retained liver function could benefit from systemic therapy: the recent IMbrave150 phase III trial showed the superiority of the combination of atezolizumab and bevacizumab over the standard of care treatment with sorafenib (8, 9).

In the context of localized diseases, radiotherapy (RT) represents a valid, curative, and alternative approach to surgery in various cancers (10). The case of HCC is more complex, due to several factors that historically limited the safety and efficacy of RT, such as the low radiotolerance of the liver, the need for high doses and treatment volumes of radiation for tumor control, the often impaired liver function at baseline, the impact of previous oncological treatments on liver status, and the lack of standard indications regarding the proper integration of RT with the abovementioned therapies.

However, nowadays, modern RT technologies allow safe HCC treatments with positive results in terms of local control (LC) and survival (11–13); thus, the role of RT in the treatment of HCC is slowly but steadily emerging as an effective locoregional treatment option for this disease according to several (but not all) international guidelines (14).

Proton therapy (PT) represents the most advanced radiotherapy technique currently available. In the past decade, PT registered an exponential rise in both the number of patients treated and the centers currently utilizing it worldwide, which exceeded 100 as of April 2022 (15). The unique physical properties of protons of having a finite range in tissue and a zero dose beyond the end of their path allow a dose-distribution profile which results in better sparing of healthy tissues compared with X-ray therapy at medium–low doses. These dosimetric characteristics fit well with liver irradiation and could enlarge the therapeutic window of RT in HCC treatment.

In this paper, we aim to review the rationale, the major clinical studies, the technical and economic challenges, and the potential future directions regarding the use of PT for HCC.

Protons vs. X-rays (from dosimetry to initial clinical data)

The pioneering work of the University of Michigan established the strong correlation between the mean liver dose and the risk of radiation liver toxicity (i.e., radiation-induced liver disease, RILD) (16). Baseline liver function is also a predictor of the risk of RILD (17). Several dosimetric studies established the superiority of PT dose distribution compared with photon RT in liver irradiation: in 2008, the work of Wang compared proton and photon plans for nine patients affected by primary liver tumors, showing a significant gain with PT plans in the majority of parameters with a 30% reduction in the mean liver dose resulting in a significant reduction in the risk of RILD (18). More recently, the University of Pennsylvania published a planning comparison analysis between proton and photon plans for different tumor sizes (ranging from 1 to 6 cm) and locations in the liver (four different locations: dome, caudal, left medial, and central liver), and a total of 48 plans were analyzed. Based on their analysis, the authors suggested the use of protons to preserve liver function for tumors larger than 3 cm in the dome and central locations; according to the results of their work, protons should also be considered for tumors larger than 5 cm in any location. Interestingly, 3 cm is usually the cutoff size used to consider a high risk of failure in case of ablation treatment.

Moving from *in-silico* data to initial clinical data, in recent years, an interesting research field focused on the analysis of the clinical outcomes of HCC patients treated with PT in comparison with conventional photon RT. In 2015, Qi et al. reported the results of a meta-analysis of 70 clinical studies using particle therapy (including protons and carbon ion therapy) or X-rays for HCC (stereotactic RT, SBRT, or conformal 3D RT): a comparable efficacy in terms of OS and local control (LC) was found between particle therapy and SBRT, with a significant reduction in toxicity in favor of charged particles (19). More recently, in 2019, Sanford et al. analyzed the clinical outcomes of HCC patients treated with ablative PT (n = 49) or RT (n = 84) treated at the Massachusetts General Hospital between 2008 and 2017 (20). Treatment with PT was associated with significantly improved OS in comparison with RT (median OS 31 vs. 14 months), although there was no difference in LC (93% vs. 90% at 2 years). The authors hypothesized that this survival advantage was due to a lower incidence of liver decompensation with the use of protons. However, given the retrospective nature of the study, the authors warned regarding the risk of selection bias and invited to interpret the findings only as hypothesis generating. Similarly, a very recent work by Cheng et al. analyzed the outcome of HCC patients treated at their institution with PT (n = 64) or photon (n = 349) between 2007 and 2018 (21). In order to deal with the issue of selection bias for retrospective studies, the authors used the propensity score matching (PSM) method applied to predefined patient- and tumor-related variables, thus producing more reliable and robust clinical data regarding treatment comparison. A significant advantage in OS was reported in patients treated with PT in comparison with X-rays. Moreover, although the biologically effective dose (BED) was significantly higher in the PT population, the risk of RILD was significantly lower using protons.

The evidence provided thus far confirmed that the dosimetric gain achievable with PT in comparison with X-rays translates into an effective clinical benefit for HCC patients. The next step would be to demonstrate these clinical advantages in a randomized, controlled trial, which, albeit suffering from intrinsic weaknesses such as generality, duration, and costs (22), still represents the gold standard methodology to establish evidence of new medical therapies. This is the goal of

the trial NCT03186898, a phase III randomized trial currently recruiting patients affected by unresectable HCC to determine whether OS is different between HCC patients treated with PT or X-rays.

Proton vs. other treatment options

Among the several treatment options currently available for HCC treatment, the touchstone strategies for different disease stages are surgery, radiofrequency ablation, and chemoembolization (23). In light of this, it is necessary to analyze the studies which compared PT with such strategies.

Bush et al. from the Loma Linda proton center conducted a randomized trial comparing transarterial chemoembolization (TACE) and PT for HCC patients; so far, an interim analysis has been published showing similar OS between the two treatments and a trend with better LC (88% vs. 45%, $p = .06$) and progression-free survival (48% vs. 31%, $p = .06$) favoring PT (24). Tamura et al. recently reported the results of a retrospective comparison between surgery and PT for single HCC ≤100 mm without vessel invasion (25). The authors found that the median survival time in the surgery group was significantly better than in the PT group. The performance status (PS) of the patients was confirmed to be an independent prognostic factor for survival; as a matter of fact, the difference in OS between surgery and PT disappeared after PSM. In the context of PT treatment in comparison with other key strategies in HCC, the most important data come from the very recent phase III study by Kim et al. (26). The authors from Korea conducted a single-center, non-inferiority, randomized trial to compare PT vs. standard of care radiofrequency ablation (RFA) for HCC lesions <3 cm. The primary endpoint was 2-year local progression-free survival (LPFS). To our knowledge, for the first time, PT demonstrated a similar outcome in terms of efficacy in comparison with the gold standard treatment in a phase III randomized clinical trial. As a matter of fact, the 2-year LPFS with PT vs. RFA was 92.8% vs. 83.2% in the intention-to-treat population. As expected, the tolerability profile of PT was excellent, with no change in Child–Pugh score ≥2 points after PT treatment.

Clinical studies

Table 1 illustrates the major clinical studies regarding PT for HCC patients. In general, it is important to underline that the quantity of clinical data is high: PT indeed has been used to treat HCC since the 1980s, the first experience being reported in 1983 by the University of Tsukuba, Japan. Since that time, the data from thousands of HCC patients treated with PT have been published. In terms of the quality of the studies' methodology, the majority of the reports represent institutional retrospective case series with a few prospective trials: of note, two randomized trials were published. The geographical distribution of the studies is also of note: the majority of the studies come from Eastern countries, where the incidence of HCC is the highest and where there is a high concentration of proton centers (15). The rest of the studies come from the USA, with only one retrospective series coming from the European countries (27). It is interesting to highlight that, in contrast to the European guidelines, the HCC guidelines from the USA and Asia suggest the use of PT as an effective alternative in the treatment of unresectable HCC in light of the clinical results reported in Table 1. As a matter of fact, all the studies reported positive results in terms of efficacy and safety for PT in HCC treatment. Going into detail, the comprehensive clinical experience of the University of Tsukuba assessed the effectiveness of PT for various clinical conditions of the tumor and patient. Three different treatment schedules were developed according to tumor location: lesions located adjacent to the porta hepatis (PH) and gastrointestinal (GI) tract were treated with prolonged schedules (72–77 Gy in 22–35 fractions), while lesions located ≥2 cm away from the GI tract received a more hypofractionated regimen of 66 Gy in 10 fractions. The reported 3- and 5-year OS rates were 64.7% and 44.6%, with a 5-year LC rate of 83.3%, respectively, without relevant toxicity (28). The same institution published other reports retrospectively analyzing the clinical data of a specific HCC population of patients such as patients with tumors larger than 10 cm, patients with portal vein invasion, elderly patients, and patients with poor liver function (Child–Pugh C) (29–32). Albeit retrospective, these case series illustrated the feasibility of PT in these challenging settings.

In the USA, the pivotal prospective study from the University of Loma Linda by Bush et al. established the effectiveness of PT treatment for HCC in Western countries (33). The adopted 15-fraction treatment up to 63 Gy schedule gave positive results in terms of efficacy and safety and became the backbone of the multicenter, prospective phase II study by Hong et al. (34), published in 2016, whose positive findings led to the insertion of PT as an alternative treatment option in the NCCN guideline for unresectable HCC. The study evaluated 81 patients (44 HCC and 37 intrahepatic cholangiocarcinomas); the dose regimen was 67.5 Gy in 15 fractions for peripheral tumors and 58.05 Gy in 15 fractions for lesions within 2 cm of the porta hepatis. The 2-year LC, PFS, and OS rates in the HCC population were 94.8%, 39.9%, and 63.2%, respectively, with only one patient in the HCC cohort developing G3 toxicity (thrombocytopenia). More recently, several other reports from different institutions in the USA and Eastern countries confirmed the safety and effectiveness of PT in the treatment of HCC (Table 1). Parzen et al. in 2020 evaluated the multi-institutional prospective proton registry database and identified

TABLE 1 Major clinical studies of PT and HCC.

Author, date	Center	Observation Period	Type of Study	Patient/Study Char-acteristiCs	N° Patients	Median age	Performance status	Liver function (Child-Pugh)	Proton technique	Treatment Regimen Range	Equivalent dose 2 Gy/ fr a/b = 10	tumor size (range)	median FUP (range)	LC	os	Toxicity≥g3
Su et al , 2022	Chang Gung Memorial Hospital, Taiwan	2016-2019	retrospective	PT combined with anti-PD1/PDL1	29	60	PS 0 16 pts, PS 1 13 pts	CP A5 23 pts, CP A6 6 pts	PSC	66 Gy/10 fr 3 pts 72.6 Gy /22 fr 18 pts 60 Gy/ 10 fr 2 pts 50 Gy/10 fr 2 pts 45 Gy/10 fr 1 pt 33 Gy/5 fr 2 pts 33 Gy/10 fr 1 pt	109.6 Gy_{10} 96.6 Gy_{10} 96 Gy_{10} 75 Gy_{10} 65.3 Gy_{10} 54.8 Gy_{10} 43.9 Gy_{10}	≥ 5cm 19 pts	13 mo (1/48.1)	80% at 1 yr	63% at 2 yr	1 G3 dermatitis 1 G3 thrombocytopenia 3 G3 liver enzyme increase 4 G3 GI bleeding 1 G4 bilirubine increase 1 G4 biliary stricture 2 G5 hepatic failure 1 G5 duodenal perforation
Lee et al, 2022	Chang Gung Memorial Hospital, Taiwan	11/2015-02/ 2021	retrospective	unresectable HCC with bile duct invasion	20	61.5	PS 0 7 pts, PS 1 23 pts	CP A5 9 pts, CP A6 7 pts, CP B7 2 pts, CP B8 1 pts, CP B9 1 pts	2015-2016 PSC;2017-2022 PBS	72.6 Gy/22 fr	96.6 Gy_{10}	6.3 cm (1.0–18.5)	19.9 mo (3.1-64.9)	1yr 94.7% (1yr cumulative local recurrence 5.3%)	1yr 79.4% 2yr 53.3%	3 G3 GI gastroduodenal ulcer 4 RILD
Lin et al, 2021	Chang Gung Memorial Hospital, Taiwan	2014 - 2017	retrospective	HCC patients without regional lymph node involvement or distant metastasis	43	71	PS 0 22 pts, PS 1 19 pts, PS 2 2 pts	CP A 40, CP B 3	PSC	25 pts 72.6 Gy/22 fr; 28pts 66Gy/10fr	96.6 Gy10; 109.6 Gy10	3.1 cm (1.1–17.1)	40 mo (9–62)	5yr 93.0%.	1yr 88.4%; 5yr 63.4%	1g3 skin 7 Child-Pugh score deterioration of 1 point.
Bhangoo et al, 2021	Mayo Clinic, USA	06/2015-12/ 2018	retrospective	all patients who were treated with IMPT for HCC with curative intent	37	69	PS 0 14 pts , PS 1 17 pts, PS 2 4, PS 3 2	CP A5-6 26 pts, CP B7-9 11 pts	PBS	15 pts 67.5 Gy /15 fr 13 pts 58.5 Gy /15 fr 15 pts 52.5 Gy /15 fr 6 pts 50 Gy /5 fr 37.5 Gy in 5 fx 1 (3%)	97.9 Gy10, 81.3 Gy10, 70.9 Gy10, 100 Gy10, 65.6 Gy10	5cm (3-8)	21 mo (17-30)	1yr 94%	1yr 78%	G3 pain Late 6pts increase CP by 2points
Iwata et al, 2021	Nagoya Proton Therapy Center	06/2013-12/ 2019	retrospective	elderly (≥80 years old) patients.	71	82	PS 0 44 pts, PS 1 20 pts, PS 2 4 pts, PS 3 3 pts	CP A5 49 pts, CP A6 15 pts, CP B7-9 7 pts	PSC	47 pts 66 Gy/10 Fr; 24pts 72.6 Gy/22 Fr	109.6 Gy10; 96.6 Gy10	32 mm (8–111)	33 mo (9–68)	2yr 88% (80–97%)	2yr 76% (66–87%)	1G3 dermatitis
Dionisi et al, 2020	Proton Therapy Unit, Trento	01/2018-12/ 2019	retrospective	unresectable disease	14	67	PS 0 11 pts, PS 1 5 pts, PS 2 2 pts	CP A5 10 pts, CP A6 2 pts, CP B7 1 pts	PBS	60Gy (50.31–67.5)	84 Gy10	4.5cm (1.2–13)	10mo (1-19)	100% at one year	63% at 1 y	/
Kim TH et al, 2020	National Cancer Center, Goyang, South Korea	03/2015-09/ 2018	prospective phase II	hypofractionated PBT in HCC	45	63	PS 0 45 pts	CP A 45 pts	PSC	70Gy/10fr	119 Gy10	1.6 cm (1.0–6.8)	35.1 mo (11.2–56.3)	3yr LPFS 95.2%	3yr 86.4%	Child–Pugh score showed a 1-point decrease in two patients (4.4%) and no change in 43 patients (95.6%)
Parzen et al, 2020	9 institutions in the USA	2013-2019	prospective registry	comparative efficacy of protons versus photons in patients with HCC.	30 HCC (63 total)	70.5	PS 0 13 pts, PS 1 10 pts, PS 2 5 pts, PS 3 1 pts	\	PSC or PBS	total population: 13pts 40 GyE (32.5–50)/5fr, 46pts 58.05 GyE (45–67.5)/15fr, 4pts 71.1 GyE (60.1–75)/25fr.	72 Gy10, 80.5 Gy10, 91.3 Gy10	4.3 cm (1.2–9.4)	8.2 mo	1yr 91.2%	1yr 71.5%	1G4 hyperbilirubinemia, 1G3 back pain.
Yoo et al, 2020	Samsung Medical Center, Seoul, Korea	01/2016-12/ 2017	retrospective	Evaluation of the risk of biliary complications after high-dose PBT for primary HCC	167	62	PS 0 92 pts, PS 1-2 75 pts	CP A 149 pts, CP B 15 pts, CP C 3 pts	PSC 130pts, PBS 37pts;	peripheral HCCs 66 Gy in 10 fractions hcc adjacent to the porta hepatis less than 1 cm, 72.6 Gy in 22 fractions	109.6 Gy10, 96.6 Gy10	peripheral 48pts <=2cm; 29pts >2cm; adjacent to the porta hepatis 26pts <=2cm, 54pts >2	14 months (range, 1–29 months	2ys infieldLC 86.5%	2yr OS 86.6%	2G 3 gastroduodenal ulcer 10 RILD 2 Non classic RILD
Kim TH et al, 2019	Center for Proton Therapy, Goyang, Korea	2012-2017	retrospective	PVTT	243	61	PS 0 237 pts, PS 1 6 pts	CP A 228 pts, CP B7 15 pts	NA	A=40pts PTV1 50 GyE/10 fr PTV2 30 GyE/10 fr; B=60pts PTV1 60 GyE /10 fr PTV2 30 GyE/10 fr; C=143pts PTV1=PTV2 66 GyE/10 fr	32.5 Gy10 /109.6 Gy10	all 2.2cm (1.0–17); A 6.0 cm (1.3–17), B 3.6 cm (1.0–12),	31.5 mo (2.1–68.2)	3yr LRFS 88.6%; 5yr LRFS 87.5%; 5yr LFRS a 54.60% b	3yr 61.8%, 5yr 48.1%. 5yr: a16.70%, b39.20%, c67.90%	Child–Pugh score 19 1-point decrease, 10 1-point increase, gastric or duodenal ulcers within the PBT field 1G1 3G2 1G3 GI in regimen A.

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TABLE 1 Continued

Author, date	Center	Observation Period	Type of Study	Patient/Study Char-acteristiCs	N° Patients	Median age	Performance status	Liver function (Child-Pugh)	Proton technique	Treatment Regimen Range	Equivalent dose 2 Gy/ fr a/b = 10	tumor size (range)	median FUP (range)	LC	os	Toxicity≥g3
												C 1.5 (1.0–12.7cm)		94.70% c 92.40%		
Chadha et al, 2019	MD Anderson Cancer Center	2007-2016	retrospective	HCC pts treated with PT	46	72	PS 0 (20%), PS 1 (25%), PS 2 (1%)	A 5 (26%) A6 (12%) B7 (6%) B8 (2%)	PSC	Median 67.5 GyE (24.0-91.0)/15 fr	81.6 Gy10, 67 Gy10, 73,2 Gy10	6 cm (1.5-21.0)	14.5 mo (0.4-59.8)	1yr 95%, 2yr 81%	1yr 73%, 2yr 62%	1 G3 diarrhea; 1 G3 erithema;, 4 G3 ascites 2G3 hipeblirubinema; 1G3 Upper gastrointestinal bleeding
Shibata et al, 2018	Proton Therapy Center, Fukui Prefectural Hospital, Japan	2011-2015	retrospective	effectiveness and toxicity of PT for hepatocellular carcinomas (HCC) >5 cm	29	71	PS 0: 21, PS 1: 7, PS 2: 1	A: 24; B:5	PBS	4 pts 66Gy/10fr; 13 pts 76Gy/20fr; 1 pts 80.5Gy/23fr; 1 pts 80Gy/25fr; 1 pts 67.5Gy/25fr; 5 pts 70.4Gy/ 32fr; 4 pts 76 Gy/38fr	91,3 Gy 87,4 Gy 90,6 Gy 88,5 Gy 71,4 Gy 71,6 Gy 76 Gy	6.9 Cm (50–139)	27 mo (2–72)	2yr & 4yr 95%	2yr 61%; 4yr 39%	ctcae 4.03 .. acute hyperbilirubinemia 1G3; Other patients had skin reactions Grade 2… late 6: pleural effusion 2G3, ascites 1G3, rib fracture 1G2, radiaton pneumonitis 1G2, erosions of ascending colon 1G2.
Mizuhata et al, 2018	Proton Therapy Center, Fukui Prefectural Hospital, Japan	03/2011-12/ 2015	retrospective	efficacy and toxicity of respiratory-gated PBT without fiducial markers for HCC located within 2 cm of the gastrointestinal tract.	40	72	PS 0,1: 38 \ PS 2: 2	A: 28; B: 12	PBS	1 pts 80.0 CGE/25fr 5 pts 76.0 CGE/38fr 17 pts 76.0 CGE/20fr 3 pts 74.8 CGE/ 34fr 8 pts 70.4 CGE/32fr 3 pts 70.0 CGE/35fr 1 pts 67.5 CGE/25fr 1 pts 66.0 CGE/ 30fr 1 pts 52.8 CGE/24fr	88 Gy 76 Gy 87,4 Gy 76,1 Gy 71,6 Gy 76 Gy 71,4 Gy 67,1 Gy 53,7 Gy	3.6 cm (1.1–12.4)	19.9 mo (1.2–72.3)	2yr 94%	1yr 86%; 2yr 76%	1 G3 gastric ulcer, 1 G3 ascites retention
Lee et al, 2018	Chang Gung Memorial Hospital, Taiwan	2015-2016	retrospective	HCC patients with small normal liver volume (NLV)	22	61,5	PS 0:7; PS 1: 13		PBS	median 72.6 GyE/22 fr	median 80,7 Gy_{10}	5.3cm (1.2-15.0)	15.7 mo (4.0 ±24.9)	1yr 95.5%	1yr 91.8%	1G3 esophagitis 1G3 colitis; Child±Pugh score deterioration of one point 3 pts; 1G3 liver enzyme elevations
Kim et al, 2018	National Cancer Center, Goyang, Korea	2013-2015	retrospective	inoperable or recurrent HCC	71	63	PS 0: 100%	A: 68; B: 3	PSC	66 GyE/10 fr	91,3 Gy_{10}	1.5cm (1.0–8.5)	31.3 mo (4.2–47)	3yr LPFS 89.9% (81.8–98%)	3yr 74.4% (63.1–85.7%)	Child–Pugh score 3pts 1-point increase.
Kimura et al, 2017	Radiation Oncology, Southern Tohoku Proton Beam Therapy Center	2008-2015	retrospective	HCC > 5 cm	24	73	PS 0:16; PS 1: 8	A: 100%	NA	72.6 GyE (60.8-85.8 GyE) 2.4 (2.2-6.6)Gy/fr	75 Gy_{10}	5-18 CM	17.5 mo (3-64)	2yr 87%	2yr 52.4%	2G3 radiation dermatitis;
Hong et al, 2016	Multinstitutional	2009-2015	phase II prospective	HCC and ICC	44 HCC	70,5	PS 0:14, PS 1: 26, PS 2: 3	A:32, B:9, No cirrhosis: 3	PSC	58.0 Gy (40.5-67.5) in 15 FR	42.8 Gy_{10}/ 81.5Gy_{10}	5 cm (1.9-12.0)	All pts 19.5 months (0.6 - 55.9 months)	2yr 94.8%	1yr 76.5%, 2yr 63.2%	.1 G3 thrombocytopenia.
Fukuda et al, 2016	Proton Medical Research Center, Tsukuba, Japan	2002-2009	retrospective	previously untreated HCC	129	72	PS 0:70, PS 1:50, PS 2:9	A:101 B:28	PBS	54 pts 66GyE/10fx; 45 72.6GyE/22fx; 30 77GyE/ 35fx	91,3 Gy; 80,5 Gy; 78,3 Gy	3.9 cm(1–13.5)	55 mo (43-67)	5yr 94% (82-100%)	5-year OS rates were 69% (95% CI, 49–89%) for stage 0/A patients, 66% (95% CI, 48–84%) for stage B patients, and 25% (95% CI, 11–40%) for stage C patients	NONE
Bush et al, 2016	Loma Linda University Medical Center, Usa	NA	randomizedclinical trial: PT vs TACE	interim analysis report	33	61,4	N.A.	N.A.	PSC	70.2 CGE/15 fr	86 Gy_{10}	3.2 cm(1.8-6.5)	28mo	2yr LC 88%	2yr OS 59%	2 pts requiring hospitalization for liver failure
Kim et al, 2015	National Cancer Center, Goyang, Korea	2007-2010	phase I	Dose finding	27	DL_1:70; DL_2:66; DL_3:63	PS 0:21; PS 1: 6	A: 24; B: 3	PSC	8 DL 1 60 GyE/20 fr; 7 DL 2 66 GyE/22 fr; 12 DL 3 72 GyE/24 fr	DL 1 65 Gy_{10} DL 2 71.5 Gy_{10} DL 3 80.2 Gy_{10}	DL 1 3.2cm (2-7); DL 2 2.3cm(1.5-5); DL 3 2.5cm (1.3-6.2)	31 mo (5.2-63.4)	3yr LPFS 79.9% 5yr LPFS 63.9%	3yr 56.4% 5yr 42.3%	1 patient 1-point increase in CP score.

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TABLE 1 Continued

Author, date	Center	Observation Period	Type of Study	Patient/Study Char-acteristiCs	N° Patients	Median age	Performance status	Liver function (Child-Pugh)	Proton technique	Treatment Regimen Range	Equivalent dose 2 Gy/ fr a/b = 10	tumor size (range)	median FUP (range)	LC	os	Toxicity≥g3
Lee et al, 2014	National Cancer Center, Goyang, Korea	2008-2011	retrospective	HCC with PVTT	27	55	PS 0:18; PS 1: 9	A: 18; B: 9	NA	median 55GyE (50–66 GyE)/ 20–22 fr	Max 71.5 Gy_{10}	7cm (3–16)	13.2 mo (2.4–51.7)	1yr LPFS 70.7%; 2yr LPFS 61.9%	1yr 55.6%; 2yr 33.3%	4 pts 1-point increase in CP score.
Sanford et al, 2019	Massachusetts General Hospital, USA	2008/2017	retrospective	unresectable HCC	49	65	PS 0 23 pts; PS 1 24 pts, PS 2/3 2 pts	CP A 46 38 pts, CP B/C 8 pts	PSC	Various treatmetn schedules, the most adopted being 58GyE/15fr;	67 Gy_{10}	NA	14mo (all pts)	2yr 93%	2 yr 59.1%	4 nonclassic RILD
Hojo et al, 2019	National Cancer Center Hospital East, Chiba, Japan	2008-2015	retrospective	anatomic subsegmental PT irradiation	110	74	PS 0 72 pts; PS 1-2 38 pts	CP A 95 pts, CP B 15 pts	PSC	76 Gy ()/20 fr	87.4 Gy10	74pts < 5 cm 36pts > 5 cm	36.5 mo (1-99.6 mo)	3yr 91.7%	3yr 74.2%	1 G3 radiation pneumonitis 1G3 liver dysfunction and 1 G3 bile duct stenosis. 1G5 radiation pneumonitis on day 188 after the start of PT
Tamura et al, 2019	Shizuoka Cancer Center Hospital, Japan	2003-2017	retrospective	single nodular HCC≤100 mm without vessel invasion	31	72	PS 0 20 pts; PS 1 10 pts, PS 2 1 pts	CP A 29 pts, CP B 2 pts	NA	8pts 66 GyE/10 Fr 22 pts 72.6–76 GyE/20–22 Fr 1pts 74–76 GyE/37–38 Fr	109.6 Gy10, 102.3 Gy10, 91.2 Gy10	3.5.Cm (1–9)	56.3 mo (22.2–82.3)	19.4% Local recurrence	3 yr 69.2%, 5yr 51.1%	1 G3 gastric ulcer
Sekino et al, 2019	Proton Medical Research Center, Tsukuba, Japan	2005-2014	retrospective	HCC pts with IVCTT	21	73	PS 0 12 pts; PS 1-3 9 pts	CP A 12 pts, CP B 9 pts	PBS	50–74 (median 72.6)	109.6 Gy10 median	8 cm (3.9–20)	21 mo (4–120)	100%	1yr OS 82%, 2yr OS 64%, 3 yr OS 36%	none
Yoo et al, 2020	Samsung Medical Center, Seoul, Korea	2016-2017	retrospective	compare the oncologic outcomes and toxicities between PS and PBS	172	62	PS 0 90 pts, PS 1-2 79 pts	CP A 154 pts, CP B 18 pts	133 PSC, 39 PBS	33 pts 50 Gy in 10 fractions 116 pts 60–66 Gy in 10 fractions 23 pts other schedules	75 Gy10, 109.6 Gy10, 132 Gy10	PS 3.1 (1–16); PBS 6.7 (1–19)	14 months (range, 1–31 months).	2-year 85.5%,	2-year OS 86.4%	RILD PS 2 pts PBS 1 patient 2 GI G3 (PS)
Iwata et al, 2021	Nagoya Proton Therapy Center, Japan	2013-2016	phase II	Operable or Ablation-Treatable HCC	45	68	PS 0 43 pts, PS 1 3 pts	CP A5 32, CP A6 9 pts, CP B7 4 pts	PSC	8 pts 72.6 Gy in 22 fr, 37 pts 66 Gy in 10 fr	96.6 Gy10, 109.6 Gy10	2.5 cm (1-10)	53 months (range, 9.5-75 months)	, 2yr LC 95%, 2yr PFS 62%	OS 5yr 70%	NO RILD, 1 G3 AST/ALT increase
Iizumi et al, 2021	Proton Medical Research Center, Tsukuba, Japan	2002-2014	retrospective	patients who received PBT for HCC in the caudate lobe	30	67	PS 0 12 pts, PS 1 17 pts, PS 2 1	CP A5 17 pts, CP A6 7 pts, CP B7 3 pts, CP B8 1 pts, CP C 1 pts, 1 pts N.A.	PSC	72.6 Gy in 22 fr (70%), 55 Gy in 10 fr (3.3%), 60 in 15 fr (3.3%), 74 in 37 fr (16.7%), 77 in 35 fr (6.7%)	96.6 Gy10, 85.2 Gy10, 84 Gy10, 88.8 Gy10, 93.9 Gy10	2.3 Cm (1-9)	37.5 months (3–152 months)	1yr LC 100%, 3yr LC 85.9%, 5yr LC 85.9%, 1yr PFS 65%, 3yr PFS 27.5%, 5yr PFS 22%	Os 1yr 86.6%, OS 3yr 62.8%, OS 5yr 46.1%	none
Hsieh et al, 2019	Chang Gung Memorial Hospital, Taiwan and MD Anderson Cancer Center, USA	2007-2017	retrospective	HCC who underwent definitive PT.	136	68	PS 0 68 pts, PS 1 64 pts, PS 2 4 pts	CP A5 92 pts, CP A6 25 pts, CP B7 13 pts, CP B8 6 pts	NA	72.6 Gy/22 fr ; 66 Gy/10 fr; 67,5 Gy/15 fr; 58 Gy/15 fr; 66 Gy/20 fr	80.5 Gy10, 91.3 Gy10, 81.6 Gy10, 67 Gy10, 73.2 Gy10	Median 6.8 cm in the eastern and 6.4 cm in the western pts, respectively.	10 mo (range, 5-27 months) and 23 mo (range, 3-76 mo) for the eastern and western patients, respectively	N.A.	N.A.	19 (14%) pts developed RILD
Chiba et al, 2005	Proton Medical Research Center, University of Tsukuba.	1985-1998	retrospective	pts unfit for surgery	162	62,5	PS 0 61; PS 1 79; PS 2 21;	CP A 82 CP B 62 CP C 10	PSC	72 Gy/16 fr 64 courses 78 Gy/20 fr 11 courses 84 Gy/20 fr 10 courses 50 Gy/10 fr 10 courses Miscellanous regimens 97 courses	87 Gy_{10}; 90,4 Gy_{10}; 94,5 Gy_{10}; 62,5 Gy_{10}	3 - 5 mm (108 pts)	31,7 months (3,1 to 133,2)	5 yr 89,6%	5 yr : 23,5%	Elevation of transaminase level 9,7%; Thrombocytopenia 3.2%.
Komatsu et al, 2011	Hyogo Ion Beam Medical Center, Tatsuno, Japan	2001-2009	Retrospective	All pts treated with PT for HCC	242	< 70 115 pts ≥ 70 127 pts	PS 0 172; PS 1 57; PS2 10 PS 3 3	CP A 184 CP B 55 CP C 3	PSC	76 Gy/20 Fr 70 pts 60 Gy/10 Fr 89 pts 66 Gy/10 Fr 53 pts miscellanous regimens 30 pts	104.8Gy_{10} 96 Gy_{10} 109.5 Gy_{10}	< 5 cm 196 5-10 cm 67 > 10 cm 17	31 mo	90.2% at 5 yr	38% at 5yr	1G4 dermatitis 4G3 dermatitis 1 G3 Elevation of transaminase level

(Continued)

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TABLE 1 Continued

Author, date	Center	Observation Period	Type of Study	Patient/Study Characteristics	N° Patients	Median age	Performance status	Liver function (Child-Pugh)	Proton technique	Treatment Regimen Range	Equivalent dose 2 Gy/fr $\alpha/b = 10$	tumor size (range)	median FUP (range)	LC	os	Toxicity≥g3
																1G3 GI ulcer 1 G3 biloma
Kawashima et al 2005	National Cancer Center Hospital East, Chiba, Japan	1999-2003	Propsective	Phase II study	30	70	PS 0-1 29 PS 21	CP A 20 CP B 10	PSC	76 Gy/20 Fr	$104.8Gy_{10}$	4.5 cm	31 mo	96% at 2 yr	66% at 2yr	5 G3 Elevation of transaminase level 5 G3 Leukopenia 7 G3 Thrombocytopenia 1 G3 bilirubine 8 pts developed PHI

PSC, passive scattering; PBS, pencil beam scanning; PS, performance status; CP, Child-Pugh; pts, patients. GI, gastrointestinal; PHI, proton-inducing hepatic insufficiency; mo, months; yr, years. Refer to the text for othe abbreviations.

30 HCC patients treated at nine institutions in the USA between 2013 and 2019 (35); the LC at 1 year was 91.2%, comparable with the historical series. A trend toward a statistically significant association between the BED and local control was observed.

Based on the recent reports from the Eastern countries, in addition to the already mentioned randomized trial of PT vs. ablation, the work of Kim et al. in 2019 retrospectively analyzed a large cohort (n = 243) of HCC patients treated at their institution with a risk-adapted treatment strategy according to the proximity of target to the gastrointestinal tract. Patients were treated with three different dose schedules in 10 fractions using the simultaneous boost technique to reduce the dose within 2 cm of the gastrointestinal tract; a significant association with the dose fractionation scheme, total PT dose, and OS was found (36).

Technical challenges

Protons and, more in general, charged hadrons have the unique physical property of a finite range in tissues. The range is determined by the initial energy of the proton beam and by the stopping power of the material in the beam path.

This characteristic gives the possibility to obtain very high conformal dose distributions and the possibility to lower the mean and low dose bath around the target. The majority of clinical data depended on the passive scattering (PS) delivery method. New PT installations are mainly equipped with the pencil beam scanning (PBS) delivery technique. With PBS, given the possibility to modulate the intensity of the beam, higher dose conformity can be achieved with respect to PS at the cost of being more sensitive to uncertainties.

As a matter of fact, the quality of the nominal dose distribution can be perturbed by different sources of uncertainties like setup uncertainties, daily anatomical variations, uncertainty in machine delivery parameters, tissue inhomogeneity, inaccuracies in dose calculation algorithm, inaccuracies in CT calibration curve, and perturbations induced by internal organ motion (37). Every time a moving target is treated, the combination of its motion with an active delivery technique (such as proton pencil beam scanning, intensity-modulated radiation therapy, and volumetric arc therapy) can lead to an undesired deterioration of the dose distribution. This is the interplay effect. The management of this kind of uncertainty in particle therapy was widely discussed in an AAPM report recently published (38), and a comprehensive review on the clinical necessity of adequate imaging taking into account this effect has been published (39). The evaluation of interplay effects is the main technical challenge for the commissioning of liver treatments in free breathing and/or in breath-hold (40, 41). Different methods have been proposed to mitigate the effect of the motion on the dose distribution such as repainting (42), 4D optimization taking into account the organ

TABLE 2 Clinical Trials currently open evaluating PT for HCC.

Study Title	Principal Institution	Type of trial	ClinicalTrials.gov Identifier	Estimated Enrollment	Actual Study Start Date	Estimated Study Completion Date
Feasibility of High Dose Proton Therapy On Unresectable Primary Carcinoma Of Liver: Prospective Phase II trial	Samsung Medical Center, Seoul, Korea	Monoinstitutional, Phase II	NCT02632864	66 participants	2015	2022
Proton Beam Therapy in Hepatocellular Carcinoma With Portal Vein Tumor Thrombosis (PTHP)	Samsung Medical Center, Seoul, Korea	Monoinstitutional, Phase II	NCT02571946	53 participants	2015	2022
Radiation Therapy With Protons or Photons in Treating Patients With Liver Cancer	NRG Oncology Massachusetts General Hospital Cancer Center, USA	Multicentric, Phase III	NCT03186898	186 participants	2017	2029
Stereotactic Body Proton Radiotherapy for the Treatment of Liver Cancer	Mayo Clinic, USA	Multicentric, Phase II	NCT04805788	60 participants	2021	2025
A National Phase II Study of Proton Therapy in Hepatocellular Carcinoma	Aarhus University Hospital, Denmark	Multicentric, Phase II	NCT05203120	50 participants	2022	2030

motion during the planning (43), both 4D optimization and repainting (44), motion reduction with the use of compressor (45), or forced deep expiration breath-hold (46, 47).

The setup uncertainty has another crucial role in the treatment of liver tumors (48). In particular, a comparison between vertebral body matching, diaphragm matching, and marker matching has been analyzed concluding that the last one has the best results in terms of positioning accuracy. Consideration has to be made from a radiobiological point of view if radio-opaque markers larger than 1.5 mm are used in the context of particle therapy since they can reduce the tumor control probability (TCP) and increase the dose to the surrounding critical organs (49). The diaphragm matching can be a reliable surrogate for liver tumor alignment (50). A detailed technical report of the first 17 liver patients treated with forced deep expiration breath has been reported by Fracchiolla et al. (51). The use of the Active Breathing Coordinator (ABC-ELEKTA®) reduced the residual motion of the internal organs during the delivery and increased the reproducibility of the patient anatomy. The authors also proposed a method to optimize the use of the range shifter in order to obtain a sharper lateral dose penumbra and, for facilities with more than a single treatment room, to optimize the beam time allocation.

Future directions

There is growing scientific evidence regarding the effectiveness and safety of the use of PT for HCC. In recent years, the strength of evidence increased, with several data coming from prospective studies and also from two randomized studies. Table 2 illustrates the clinical trials currently evaluating the use of protons for HCC. The superiority of PT in comparison with X-rays in terms of OS for HCC patients, which has been highlighted so far only in retrospective studies, will be evaluated in the already mentioned multicenter trial NCT03186898. The Mayo Clinic phase II trial has the goal to evaluate the safety of the use of 5-fraction stereotactic PT for the treatment of HCC. Another interesting trial is the NCT05203120 that is currently ongoing at the Danish Particle Center in Denmark, the first European prospective study for PT and HCC, whose results, if positive, could help in bridging the gap between Europe and the USA and Eastern countries in acknowledging the effectiveness of radiotherapy in the treatment of HCC. Other strategies to evaluate in the next future studies should include the combination of PT with other locoregional therapies. The positive results of the already mentioned IMbrave150 phase III trial demonstrated the effectiveness of combination therapy for advanced stage HCC. In the context of locoregional disease, the combination of local and locoregional therapy such as TACE and radiotherapy could have an impact on the oncological outcome, probably at a cost of higher toxicity, as reported by the meta-analysis by Meng et al. (52). The favorable toxicity profile of protons due to their intrinsic physical properties makes PT the option of choice in case of combined treatments, especially for complex settings such as large tumors and poor liver function. Furthermore, the combination of PT with immune checkpoint inhibitors, which has been recently retrospectively reported (53) (Table 1), should be evaluated in prospective trials for safety and effectiveness.

Author contributions

FD, AB, and GS: manuscript conception and design of the study. FD, DS, FF, LG, BS, MC, GS, IG and AB: writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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An overview of the irreversible electroporation for the treatment of liver metastases: When to use it

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Tumour ablation is an established therapy for local treatment of liver metastases and hepatocellular carcinoma. Most commonly two different kind of thermic ablation, radiofrequency ablation and microwave ablation, are used in clinical practice. The aim of both is to induce thermic damage to the malignant cells in order to obtain coagulative necrosis of the neoplastic lesions. Our main concerns about these procedures are the collateral thermic damage to adjacent structures and heat-sink effect. Irreversible electroporation (IRE) is a recently developed, non-thermal ablation procedure which works applying short pulses of direct current that generate an electric field in the lesion area. The electric field increase the transmembrane potential, changing its permeability to ions.Irreversible electroporation does not generate heat, giving the chance to avoid the heat-sink effect and opening the path to a better treatment of all the lesions located in close proximity to big vessels and bile ducts. Electric fields produced by the IRE may affect endothelial cells and cholangiocytes but they spare the collagen matrix, preserving re-epithelization process as well as the function of the damaged structures. Purpose of the authors is to identify the different scenarios where CT-guided percutaneous IRE of the liver should be preferred to other ablative techniques and why.

KEYWORDS

irreversible electroporation, IRE, CT guided, liver metastases, interventional radiology

Introduction

Liver cancer is the third cause of oncologic-related death worldwide (1), with liver metastases being the most common form of liver involving tumour. Mortality rates of this pathological condition have seen an unprecedented increment over the last few years (2). Unluckily, only 20% to 30% of patients are eligible for surgical resection at their diagnosis; therefore, numerous alternative procedures have been developed through the years (3). Today, tumour ablation is an established therapy for local treatment of liver metastases and hepatocellular carcinoma (HCC) (4), extremely efficient with metastatic lesions smaller than 3 cm, where no outcome difference between ablation and liver resection can be seen (5). The two most common kinds of thermal ablation, radiofrequency ablation (RFA) and microwave ablation (MWA), are used in clinical practice. The aim of both is to induce thermal damage to the malignant cells in order to obtain coagulative necrosis of the neoplastic lesions. Our main concern about these procedures is the collateral thermal damage to adjacent structures, in the first place, which may involve bowel, vessels, or bile ducts and, at the same time, the heat-sink effect. The heat-sink effect is the name physicians use to refer to a cooling phenomenon due to a large vessel's proximity (less than 1 cm), directly related to flowing blood that reduces the heat and, because of that, the ablation volume as well (6). Irreversible electroporation (IRE) is a recently developed, non-thermal, ablation procedure that works by applying short pulses of direct current that generates an electric field in the selected area. The electric field increases the transmembrane potential, changing its permeability to ions. Different theories have been proposed to explain this phenomenon, none of which seems to suit perfectly the physical and biological changes we have found so far; however, inducing nanopores throughout the cell membrane, allowing the interstitial ions to move according to their concentration gradient from the surrounding solution, seems to be the most appealing one. As a consequence of those changes, alteration of cell homeostasis develops, and finally, cell death occurs (7).

IRE was firstly developed to manage unresectable, highly vascularized, pancreatic neoplasm not eligible for common thermal ablation. Scientific data collected so far show discrete rates of success and high levels of safety. As years went by, this technique was applied to other organs such as the liver and prostate to obtain complete ablation of all those tumours that cannot be treated surgically or removed using heat-generating techniques. Despite a general lack of evidence, the data collected by previous studies showed encouraging results.

Irreversible electroporation does not generate heat, giving the chance to avoid the heat-sink effect and opening the path to better treatment of all the lesions located in close proximity to big vessels and bile ducts (8). Electric fields produced by the IRE may affect endothelial cells and cholangiocytes, but they spare the collagen matrix, preserving the re-epithelization process as well as the function of the damaged structures (9). The purpose of the authors was to identify the different scenarios where CT-guided percutaneous IRE of the liver should be preferred to other ablative techniques and why.

Technique

Better comprehension of tumour biology and the steady progress in radiology is allowing physicians to increase the number of cancer diagnosis while providing useful non-invasive treatment (10). Irreversible electroporation is a percutaneous or, less commonly, laparoscopic procedure that requires general anaesthesia and neuromuscular blocking agents. The underlying idea is to prevent involuntary muscle contraction that can accidentally arise from the electrical stimulation induced by the procedure on the motor neurons. Different kinds of plates and electrodes have been used through the years, but two to six parallel needle electrodes (Ø~1 mm) are mostly employed nowadays. After the insertion of the probes, their position is evaluated by CT imaging, and when all the electrodes are located correctly, 50 to 100 electric pulses are sequentially delivered (Figure 1). To mitigate the risk of arrhythmia, IRE is ECG-synchronized with the absolute refractory period of myocardial cells (11). To induce cell death, IRE needs to produce an electrical field strong enough to permanently disable the target cells homeostasis; to do so, an electric field of 300–1,000 V/cm is mandatory (12). The lethal threshold may vary according to the tissue susceptibility, but this limits decrease as more pulses are applied, eventually saturating if too many pulses are provided. Despite that, the temperature rising due to the minimal Joule effect related to the procedure increases the conductivity by 1%–3% for every Celsius degree, which may lead to a greater volume of ablation (13). Blood samples are taken before the procedure, looking for alteration in alkaline phosphatase (normal value 45–117 U/L) and bilirubin (normal value 0.2–1.0 mg/dl) levels. Due to the malignant neoplasm affecting the liver when an IRE is performed, abnormal values are not to be considered contraindications to the treatment.

Common radiofrequency thermal ablation is due to an alternating electric current, providing frictional heat directly related to the current's intensity and duration. While the main application is for unresectable tumours, RFA provides better results when applied to masses smaller than 5 cm and even better if the treated area is smaller than 3 cm, the size of the ablation is limited, and the possibility of an incomplete resection increases with larger tumours (14). Microwave ablation allows for a flexible approach to liver tumours, and it is usually performed under conscious sedation even though general anaesthesia may be required if procedural pain is problematic. Electromagnetic microwaves agitate water molecules in the surrounding area, providing higher temperature if compared with other ablation

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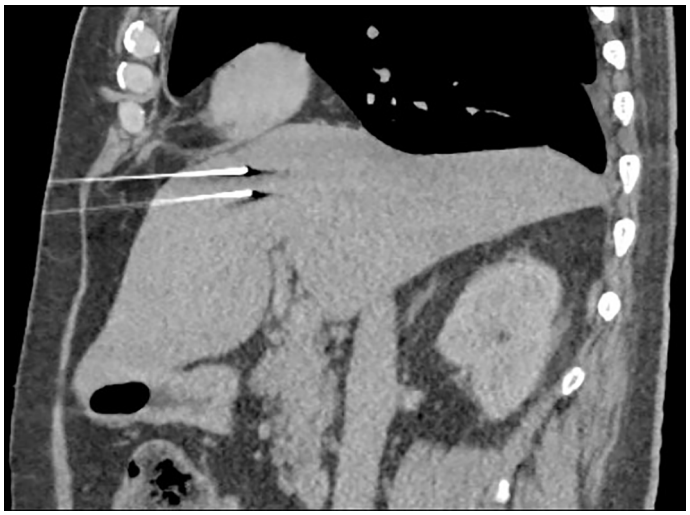

FIGURE 1
Probe position evaluation performed on CT scan. The current is directed from one probe to the other.

techniques; the result is the ability to treat larger areas of affected parenchyma with better long-term outcomes (15).

Trans-arterial chemoembolization (TACE) is an alternative treatment for patients diagnosed with a primary or secondary hepatic tumour. The best candidates are asymptomatic patients without underlying liver disease with no evidence of extrahepatic spread or vascular invasion. TACE has been performed using lipiodol chemotherapy followed by embolization with gel foam particles. The major problems are the lack of safety and the relatively low rates of success, meaning that most patients experienced an incomplete tumour embolization or a relatively fast (2 years) relapse of the disease, which is why this technique is steadily being replaced by ablation (16) (Table 1).

Target volume evaluation after IRE is conducted by ultrasound (US) or CT (17) with US sensitivity widely ranging from 20% to 84% (18), failing to establish itself as a reliable and reproducible method while, at the same time, CT is holding such a low soft tissue contrast capacity before contrast enhancement, which is not considered a feasible solution for real-time evaluation. Magnetic resonance imaging is generally more accurate in detecting liver tumours and shows an overwhelming superiority if compared to other imaging techniques when it

TABLE 1 Comparative scheme of the different types of ablation.

Ablative modality	Principles	Indications	Advantages	Limitations
RFA	Application of electrical currents *via* an electrode, resulting in resistive heating and therefore tissue hyperthermia	• BCLC O, A, B • Tumour < 3 cm • HCC	• Most extensively studied ablation technique, broad clinical experience	• Not efficient for tumour >3 cm • Not subcapsular peri-vascular or adjacent to gallbladder/diaphragm
MWA	Application of propagating microwave energy in order to induce tissue hyperthermia *via* dielectric hysteresis	• BCLC O, A, B • Similar profile to RFA • Tumour ≤5 cm	• Less heat-sink effect and shorter duration of therapy compared to RFA • Efficient in tumour volumes ≤5 cm	• Reduced efficacy in tumours >5 cm • Treatment effect varies between different vendor/device
Cryo	Gas pressures changes resulting in cooling of a cryoprobe in direct contact with tumour, resulting in fast ice crystal formation and osmotic shock	• Only limited role in HCC treatment today	• Well tolerated; less pain during ablation • Ablation processes can be monitored effectively	• High overall complication rate, such as cold shock, decreased platelet count, and bleeding • Insufficiently supported by clinical studies
IRE	Alteration of transmembrane potentials to induce irreversible disruption of cell membrane integrity	• Perivascular locations • Applicable in peribiliary locations	• No heat-sink effect • Recommended in perivascular locations • Preservation of the extracellular matrix	• Insertion of several needles sometimes necessary • Limited evidence and general lack of experience • Requires general anaesthesia

RFA, radiofrequency ablation; MWA, microwave ablation; Cryo, cryoablation; IRE, irreversible electroporation; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.

comes to smaller lesions. In the end, regardless of the method, radiological imaging may depict the morphological changes with great accuracy only when contrast agents are supplied, failing to assess the metabolic alterations immediately occurring in the treated area (19). Therapeutic response is mainly seen as a reduction of the neoplasm size but is not immediately visible (20). Due to its ability to detect viable tumours, fluorine-18 fluorodeoxyglucose (^{18}F-FDG) PET was proposed as an imaging assay to catch early clinical response (21) (Table 2). While this could predict therapeutic outcomes and allow oncologists to plan new, patient-tailored, strategies, this technique failed to come into everyday clinical practice (26). Due to its minimal heat induction and its resistance to the heat-sink effect, IRE seems to be the best option to treat metastatic lesions located near proteinaceous structures in the liver such as vessels or bile ducts, showing a pivotal advantage over classical thermal ablation strategies (27). Another common indication for IRE resection includes stage III pancreatic cancer, even though stages I, II, and IV are not eligible for this kind of treatment. Renal cell carcinoma or melanoma metastases could benefit from irreversible electroporation, as well as a local recurrent disease without any radiological sign of distant metastases. Early (<6 months) local recurrence may also be treated with IRE if the dimensional increase is below 20% and the tumour size is still within the limit for electroporation treatment (28).

Histological findings

In a recent study, Zhang et al. (27) took notes of the histological changes in rat liver after IRE was performed. Immediately after the procedure, no change was visible in the treated tissue, demonstrating that irreversible electroporation does not cause acute cell destruction. Three hours after IRE, the sinusoid experienced vascular congestion, while no changes in the larger structures, like bigger vessels and main bile ducts, could be seen. A clear histological difference between treated tissue and untreated areas may arise 6 h after the electroporation, where only pyknotic nuclei and neutrophil infiltration could be found in the treated region. Normal hepatocytes may be detected in the treated zone 24 h after the electroporation. The main hypothesis is that those new cells may be taken there by the blood supply from patent vessels. For some unknown reason, Kupffer cells, involved in both apoptosis and necrosis, were prominent at the site of electroporation; however, this procedure does not seem to induce apoptosis, while pyroptosis, karyorrhexis, and necroptosis are commonly observed instead (29).

The extracellular matrix remained undamaged after hepatocyte death, confirming that there was no extracellular protein damage linked to the procedure. The collagen scaffold helps the regrowth of normal parenchymal cells and may be used, in a close future, for experimental exogenous cell implantation (24).

Complications

Though irreversible electroporation is generally considered safe, complications arise in almost 16% of the patients. The majority of these complications are directly related to the puncture itself, but uncommon side effects such as bile duct stenosis are observed in as much as 6% of the patients (30); similar rates have been reported for portal or hepatic veins stenosis or thrombosis, which still are to be considered rare (31). On rare occasions, tumour seeding through the needle tract was speculated (32). Other severe complications include intraoperative arrhythmia and atrial fibrillation, linked to the electrical pulses, postoperative portal vein thrombosis, linked with debris clothing, and pneumothorax. The mortality rate from these side effects is as low as 3% and only if no treatment is performed. Most dangerous cardiac rhythm disturbances occurred during the ablation of a big size hepatic tumour, mostly located directly beneath the diaphragm, relatively close to the inferior cardiac border (33). Despite that, IRE still stands out as the most effective method to treat unresectable liver peripherical metastasis that is located close to the diaphragm once a proper cardiac synchronization is made.

Next to those severe and uncommon complications, there are others often encountered in clinical practice; those common complications include abdominal pain, flank pain, and extrasystole. These less serious adverse effects usually completely resolve in 30 days even if no treatment is provided (23). Since we know so little about IRE, and the literature is still moving its first steps in properly explaining and exploring this new technique, from time to time, case report studies show some extremely rare adverse effects, which may be explained, to a

TABLE 2 Irreversible electroporation in liver.

Author, year of publication, reference number	No. of lesions	Age	Type of lesions	Primary efficacy [60]
Thomson et al., 2011 (22)	63	45	HCC (17), CRLM (15), other (31)	51.6
Kingham et al., 2012 (23)	65	51	HCC (2), CRLM (21), other (5)	93.8
Narayanan et al., 2014 (24)	100	54	HCC (35), CRLM (20), other (5)	NS
Niessen et al., 2017 (25)	103	64	HCC (31), CRLM (16), other (10)	68.3

HCC, hepatocellular carcinoma; CRLM, colorectal liver metastasis.

certain extent, remembering that although IRE is a non-thermal ablation method, a little Joule effect is still theoretically possible (34).

Sporadic cases of severe portal vein stricture may be found; when this happens, the blood supply is reduced enough to require a stent placement. This device commonly leads to further complications and more interventions (24). Few studies have shown liver vessel damage after irreversible electroporation even if they all fail to assess the incidence of occurrence and underlying mechanism; therefore, the exact meaning of this rare side effect from a pathological point of view is still being debated (35). Reduced vessel patency is not immediately dangerous by itself but is strictly related to a fast deterioration in liver function, since patients undergoing IRE are usually already having chemotherapy, and a liver insufficiency could cause a sudden cessation of the treatment plan. The cause of small vessel damage after IRE is still unknown in humans, but several tests performed on laboratory animals showed oedema as being responsible for transient luminal narrowing, which usually resolves in 8 weeks (36). Some studies have supposed thermal damage due to direct contact between the plate and the vessel or post-procedural parenchymal scarring may determine sinusoid occlusion. The evidence we have today is still insufficient to determine with confidence if those conditions play a role in vascular damage or occlusion (37, 38). Incomplete or partial ablation is a typical downside in IRE, and it occurs in almost 19% of the cases mainly because of the location of the neoplasm: large bile ducts or bowel may interfere with the electrode placement, causing the procedure to be more demanding and less precise (39).

Review

When to use IRE to take advantage of its strategic role in metastases ablation is still an object of debate, mainly because the operator dependence on this procedure leads to a general lack of peer-reviewed evidence establishing a precise success rate. Moreover, patients with metastatic cancer show a wide range of co-morbidities, making it more complex to assess the exact impact of the irreversible electroporation on their general outcome (40). While tumour control was above than average for primary lesions of the liver, metastasis treated with IRE showed a poorer response, determining up to 28% of recurrence in the first 3 months after electroporation with tumours smaller than 3 cm showing a lower recurrence rate (<19%) (22). Radiofrequency ablation and microwave ablation play a pivotal role in the treatment of unresectable tumours because these techniques are safe, effective, and highly standardized, providing good outcomes with few adverse effects. IRE, however, requires the placing of more electrodes and a more accurate anatomical study. Its main role is to be performed near the big vessel and main bile ducts, and it is relatively recent when compared to other ablation techniques; therefore, among surgeons and radiologists, there is a general lack of expertise so far. Previous studies provide useful information, reporting cell death arising within the first 3 h after the treatment (23), while further investigations showed that IRE was suitable for metastases ablation even if the lesion was located near one of the main hepatic vessels, providing a fast and effective alternative to thermal ablation (40–44). Liver biomarkers and blood levels seem to be affected by this minimally invasive procedure; in fact, a modest rise can be revealed by a blood sample taken in the first 2 days after the procedure. However, since a large group of hepatocytes are being killed during the procedure, such augmentation is to be expected and showed no correlation to permanent liver damage (28). Six months of life expectancy after treatment is proven similar in both IRE and thermal ablation with the electroporation far more easily tolerated in patients with a compromised liver, which also experienced shorter hospitalization time and lower rates of re-admission (25). These findings remain valid for small liver masses up to 3 cm; bigger lesions show poorer response to non-invasive treatment and may be more efficiently approached with classical surgical techniques to the point that metastatic tumours bigger than 5 cm are hardly affected by irreversible electroporation (45). For this reason, big masses are included as current contraindications for irreversible electroporation. Metallic implants located near the procedure site aroused some hesitation regarding whether to perform the procedure, and to date, no concrete evidence can determine if they should or should not be considered absolute contraindications (Figure 2). Former studies pointed out those implants as responsible for affecting progression-free survival even though the exact mechanism was never totally understood. Today, more recent studies seem to be more indulgent about the mortality and morbidity outcomes in this category of patients; more investigations are thus mandatory (Table 3). An immediate CT scan after the procedure may detect rim enhancement in the ablated area, but said enhancement disappears 1 month later when follow-up is performed. No enhancement detected from the lesion site is the main indication of successful electroporation (46). Association with chemotherapy is an established alternative to classical IRE that significantly increases the treatment response in comparison with cytostatic agents alone such as bleomycin or cisplatin. The combined local and systemic treatment reduces the relapse risk (10.6%) and improves the life quality of our patients when chemotherapy is performed after IRE. It is postulated, incidentally, that electroporation should be performed as soon as possible after medical treatment because, due to its effect on membrane permeability, electroporation grants a higher intracellular concentration of cytostatic drugs. Unfortunately, IRE and chemotherapy association is usually reserved for palliative treatment in patients with unresectable tumours where a surgical approach is forbidden; therefore, no overall outcome impact is usually seen. This combination, however,

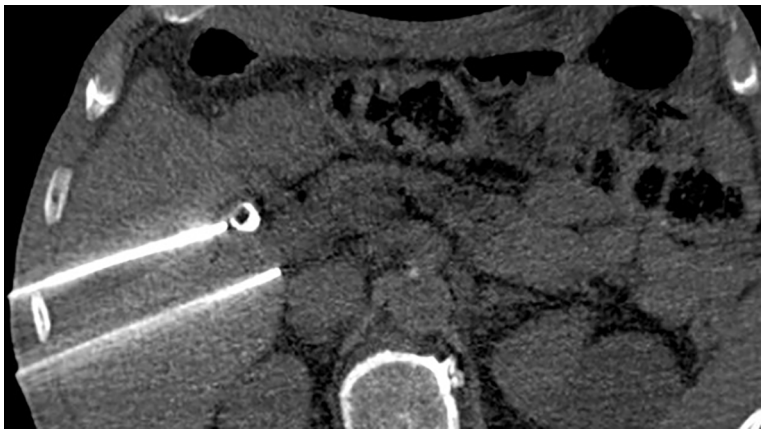

FIGURE 2
Irreversible electroporation applied near a biliary stent. Metallic devices were at first considered absolute contraindications for this kind of procedure.

may greatly affect the survival rate of patients with skin or prostate cancer. It is thought that the cell damage due to electrophoresis may induce the release of tumour-specific antigens, allowing the patients' immune system to target the tumour area and complete the task of killing neoplastic cells; if this would be proven true, it could probably explain why IRE performed in nude mice is less effective (23). An interesting experiment investigating this path provides transplantation, on mice, of two different tumours in two different sites. When one nodule was treated with IRE and chemotherapy, this induced the healing of the other, untreated, nodule (24). Is it notable that among all the studies the author encountered, only one pointed out that transient elevation of pancreatic amylase may happen. However, this rare occurrence seems to be self-limiting within 48 h (24).

Thomson et al. were able to avoid vessel or bile duct damage in the totality of their patients; at the same time they pointed out that, despite the anaesthesia, muscle contraction is still possible. Physicians should be aware that a correct electrode positioning is therefore mandatory since a correctly placed electrode simply does not change its place despite muscle contractions. However, IRE needles are less used and therefore less sophisticated than other needles such as the ones for MWA or RFA. Therefore, an accurate placing can be challenging. Repeated attempts may lead to subcapsular haematoma due to capsular puncture. This minor side effect is strictly dependent on the doctor's experience and will be less common as soon as the procedure will be performed more often (22). According to Kingham et al., IRE could potentially be game-changing when it comes to near-vessel lesions, providing a new therapeutic approach. Complication rates are the same as those of other kinds of ablation therapy such as MWA or RFA, but the outcome is promising, with an initial response rate above 98%. What is new in this paper is that the ablation procedure was performed in each liver segment, but no significant difference in success rate or complication frequency could be proven (23). Another study found that vascular side effects were involving only venous structures and portal veins in particular.

TABLE 3 IRE contraindication.

Absolute contraindication	Relative contraindication	No contraindication
Cardiac	*Cardiac*	*Cardiac*
Cardiac arrhythmias	Active coronary artery disease	History of coronary artery disease
Pacemaker	Congestive heart failure NYHA Class 2 and/or Class 3	
Congestive heart failure NYHA Class 4	· Atrial fibrillation	
Other	*Other*	*Other*
Severe ascites	Non-iatrogenic coagulation disorder	Epilepsy
	Moderate ascites	Minimal ascites

IRE, irreversible electroporation; NYHA, New York Heart Association.

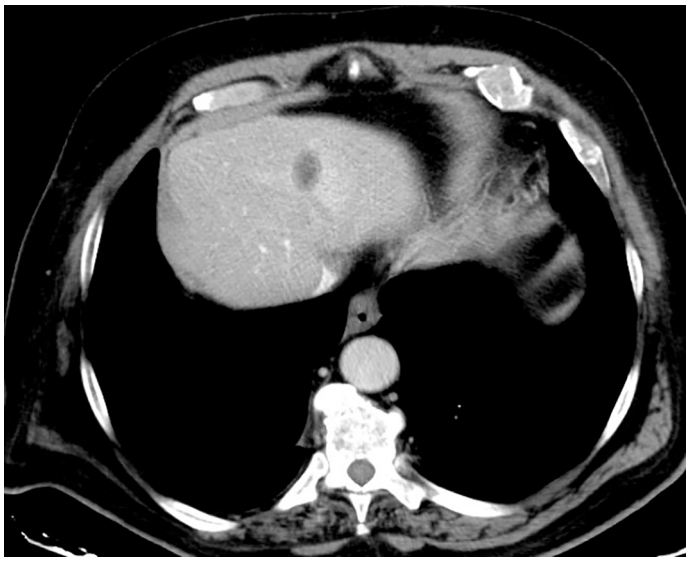

FIGURE 3
Hypodense area appears in the liver after irreversible electroporation (IRE). This low-density region represents the classical aspect of an electroporated parenchymal area.

Despite promising results on both human and murine models, long-term effects of IRE are still mostly unknown, and further investigation is mandatory (25).

Conclusions

RFA remains the most widely used thermo-ablative technique worldwide in this case scenario even though the first choice for the treatment of hepatic secondary lesions is still surgery and chemotherapy is considered a valid help to other, more effective, treatments (45) (Figure 3). The main concerns about RFA have focused on the high local recurrence rates, particularly in the treatment of masses larger than 3 cm in diameter, the potential incomplete tumour ablation near blood vessels because of the heat-sink effect, and the difficulty in US follow-up of RF lesions.

When it comes to hepatic tumours, the first indication for irreversible electroporation seems to be the treatment of metastatic lesions located in proximity to vital structures, like major bile ducts or large vessels, to spare them from thermal damage and to avoid the heat-sink effect at the same time. To date, IRE is only recommended for patients with a reasonable life expectancy or as a palliative treatment, even though preliminary studies have shown greater overall survivability if compared to chemotherapy alone (46). Metastasis size should not exceed 3.0 cm in order to avoid the chance of not obtaining a complete tumour ablation. As downsides, this procedure requires general anaesthesia and is therefore more expensive and potentially risky than the more traditional kind of ablation. An ablation technique such as IRE is needed, since its unique role in the treatment of recurrences located next to big vessels, and according to authors, more studies should be encouraged.

Author contributions

All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Efficacy and safety of transarterial chemoembolization combining sorafenib with or without immune checkpoint inhibitors in previously treated patients with advanced hepatocellular carcinoma: A propensity score matching analysis

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Purpose: To compare the efficacy and safety of transarterial chemoembolization (TACE) plus sorafenib and immune checkpoint inhibitors (T+S+ICIs) and TACE plus sorafenib (T+S) when treating patients with advanced hepatocellular carcinoma (HCC) who have previously received locoregional treatment.

Materials and methods: A retrospective analysis was performed on the patients with Barcelona Clinic Liver Cancer (BCLC) stage C HCC from May 2019 to December 2020. These patients were treated with locoregional therapy and showed radiographic progression after the treatment. Patients received either T+S+ICIs or T+S. The outcomes, including disease control rate (DCR), progression-free survival (PFS), overall survival (OS), and safety, were compared. The propensity score matching (PSM) methodology was used to reduce the influence of confounding factors on the outcomes.

Results: Forty-three patients were included in the T+S group and 33 in the T+S+ICI group. After PSM (n = 29 in each group), patients who received T+S+ICIs had a higher DCR (82.8% vs. 58.6%, p = 0.043), longer median PFS (6.9 vs. 3.8 months, p = 0.003), and longer median OS (12.3 vs. 6.3 months, p = 0.008) than those who underwent T+S. Eastern Cooperative Oncology Group performance status was an independent predictor of PFS, and age was an independent predictor of OS. The

incidence of treatment-related adverse events in T+S+ICIs was well controlled.

Conclusions: Compared with TACE combined with sorafenib, TACE combined with sorafenib plus ICIs is a potentially safe and effective treatment regimen for patients with advanced HCC who previously received locoregional treatment.

KEYWORDS

hepatocellular carcinoma, transarterial chemoembolization, sorafenib, immune checkpoint inhibitor, PD-1 inhibitor, combined therapy

Introduction

Clinical practice guidelines have recommended transarterial chemoembolization (TACE) for intermediate-stage HCC treatment (1, 2). In addition, the application scope of TACE has been expanded from Barcelona Clinic Liver Cancer (BCLC) stage A to stage C according to the Chinese guidelines for the diagnosis and treatment of HCC (3). However, TACE may increase tumor hypoxia, leading to the upregulation of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), promotion of tumor angiogenesis (4), and tumor recurrence or metastasis.

Sorafenib is a protein kinase inhibitor that hampers the activities of protein kinases, including VEGF, RAF, and PDGF, thereby exerting both antiangiogenic and direct antitumor effects. Some studies have shown that sorafenib combined with TACE treatment prolongs the progression-free survival (PFS) (5) and overall survival (OS) of patients with intermediate-advanced HCC (6). However, data from two phase II/III randomized controlled trials (RCTs), including TACE 2 trial (7) and SPACE trial (8), failed to demonstrate any clinical benefit of sorafenib combined with TACE. Thus, effective systemic therapies combined with TACE are urgently needed to improve the prognosis of patients.

Immune checkpoint inhibitors (ICIs) have shown promising clinical outcomes, and pembrolizumab and nivolumab have been approved by the US Food and Drug Administration (FDA) as a second-line systemic treatment for HCC based on phase I/II study results (9, 10). Atezolizumab combined with bevacizumab has shown the better PFS and OS than sorafenib in unresectable HCC (11).

Since TACE has local anticancer effects, it may promote antitumor immunity but inevitably induce post-TACE angiogenesis (12, 13), and sorafenib can promote "tumor vascular normalization" to alleviate hypoxia and therefore enhance the efficacy of TACE and immunotherapy. ICIs may restore the immune-supportive tumor microenvironment (TME) through inhibiting immune checkpoints. Studies have suggested the potential synergistic antitumor immunomodulatory effect when combining ICIs with other antitumor approaches to stimulate the immune system or directly kill tumor cells (14–16). In this study, we hypothesized that the comprehensive therapy of TACE plus sorafenib and ICIs might improve the treatment outcomes of patients with advanced HCC. Therefore, we compared the efficacy and safety of the TACE+sorafenib+ICI (T+S+ICI) regimen with those of the TACE+sorafenib (T+S) regimen in treating patients with BCLC stage C HCC who have previously received locoregional treatment.

Materials and methods

Study design and patient selection

This was a retrospective study that was conducted in accordance with the principles of the Declaration of Helsinki. Ethics approval was obtained from the ethical review committee of Sichuan Cancer Hospital. Informed consent was obtained from available patients and was waived in the case of deceased or otherwise unattainable patients.

Patients diagnosed with BCLC C stage HCC from 1 May 2019 to 31 December 2020, based on the HCC guidelines of the European Association for the Study of Liver, were eligible for enrollment (2). Portal vein tumor thrombus (PVTT) was categorized into four types according to the classification criteria proposed by previous authors (17). The inclusion criteria included the following: 1) patients aged between 18 and 80 years; 2) patients who had the Eastern Cooperative Oncology Group performance status (ECOG PS) of ≤2; 3) patients who had the Child–Pugh class A or B; and 4) HCC patients treated with locoregional therapy and radiographic progression seen after treatment. The exclusion criteria were as follows: 1) patients who received TACE combined with sorafenib or TACE combined with sorafenib plus ICIs as the first-line therapy; 2) patients with other malignancies; 3) patients with hepatic encephalopathy, severe ascites, esophageal or

gastric fundal variceal bleeding, or other serious medical comorbidities; 4) patients with coagulation disorders; 5) patients who received ICI treatment before TACE; and 6) patients with incomplete treatment or follow-up data.

TACE procedure

The procedure was performed with the guidance of digital subtraction angiography (DSA). Hepatic artery angiography was performed with a Yashiro-type or 5-F RH catheter (Terumo) to assess the location, number, size, and blood supply of target tumors. Subsequently, a microcatheter (Progreat; Terumo, Ann Arbor, MI, USA) was inserted into the feeding artery of tumors. Intra-arterial administration consisted of 40–60 mg of epirubicin (Pharmorubicin; Pfizer, Wuxi, China) mixed with 5–20 ml of lipiodol (Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China). Embolization was stopped following stasis of the contrast agent flow. When needed, further embolization was performed with Embosphere (100–300 μm) to achieve stasis.

Sorafenib and ICI administration

Administration of sorafenib and ICIs was initiated within 1–2 weeks after TACE therapy based on the liver condition (requiring aspartate aminotransferase (AST) level <40 U/l). Sorafenib at a dose of 400 mg was orally administered twice a day, and it was discontinued for 2 days before and after each TACE treatment session (5). Intravenous administration of 200 mg camrelizumab (Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China) or 200 mg sintilimab (Innovent Biologics, Suzhou, China) was conducted every 3 weeks. The interruption and discontinuation of drug administration depended on the presence and severity of toxic side effects according to the drug directions. Once ICI-related serious adverse events (SAEs) occurred, ICIs were discontinued, and those patients were kept in the T+S+ICI group.

Follow-up

After the first TACE, the standard-of-care clinical and radiological follow-up was scheduled at 4–6 weeks and every 3 months thereafter. The follow-up results (CT or MR images and laboratory tests) were evaluated by our multidisciplinary team (MDT) to determine the status of tumor lesions (tumor progression or not). All patients were followed up till 31 August 2021.

TACE retreatment

TACE retreatment was performed only on demand, after MDT discussion, depending upon the extension of the residual or recurrent viable tumor and patients' clinical conditions. During follow-up, the treatment of T+S+ICIs or T+S was discontinued in case of intolerable toxicity, progressive disease (PD), or change of treatment plan. The choice of the subsequent treatment, such as second-line targeted agent, ICIs (for the patients treated with T+S), radiotherapy, or best supportive care, was determined according to the results of discussion by our MDT and the patients' request.

Treatment evaluation

Tumor responses were evaluated by two diagnostic radiologists with more than 10 years of experience according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST). Objective response rate (ORR) was defined as the proportion of patients achieving complete response (CR) or partial response (PR). Disease control rate (DCR) was defined as the rate of objective response plus stable disease (SD). All objective responses were confirmed at least 4 weeks after the first observation of all patients.

PFS was defined as the time interval between the TACE procedure and the time of disease progression due to any cause. OS was defined as the period from the TACE procedure to the time of death or the last date of follow-up. Adverse events (AEs) were recorded and assessed based on the Common Terminology Criteria for Adverse Events Version 5.0.

Statistical analysis

Statistical analysis was performed using SPSS 25.0 (IBM). The propensity score model enrolled the following variables: age, sex, ECOG PS, hepatitis B surface antigen level, AFP, Child–Pugh class, and intrahepatic major tumor size. The 1:1 nearest-neighbor method was used to deduce the matched pairs between the two groups, with a caliper width of 0.03 of the standard deviation of the logit of the propensity score. Before and after propensity score matching (PSM), the quantitative data were expressed as frequency, mean ± standard deviation (SD), or median with a 95% confidence interval (CI). To determine the significant differences between the two groups, continuity correction and independent-samples t-test, chi-square test, or Fisher's exact test were used. Survival curves of PFS and OS were analyzed by the Kaplan–Meier method using the log-rank test. The Cox proportional hazard model was used for univariate and multivariate analyses to determine the prognostic factors. All statistically significant ($p < 0.15$) factors identified by the univariate analysis were entered into a Cox proportion hazards regression model to identify the independent predictors. All statistical analyses were based on the two-tailed hypothesis tests with a significance level of $p < 0.05$.

Results

Patient characteristics

Seventy-six patients with BCLC C stage HCC were included in this study. The average tumor size was 9.6 ± 4.8 cm. There were 43 patients in the T+S group and 33 patients in the T+S+ICI group (Figure 1). Nineteen patients received camrelizumab, and 14 patients received sintilimab in the T+S+ICI group.

Following PSM, 58 patients were analyzed (29 patients in the T+S group and 29 patients in the T+S+ICI group) (Figure 1). The baseline characteristics before and after PSM of the two groups were similar (p > 0.05) (Table 1).

Treatment outcomes

Tumor response evaluation

The DCR was maintained higher for patients in the T+S+ICI group than for those in the T+S group before (84.8% vs. 55.8%, p = 0.007) and after (82.8% vs. 58.6%, p = 0.043) PSM (Table 2). The ORR was higher for patients in the T+S+ICI group than for those in the T+S group before (60.6% vs. 27.9%, p = 0.004) PSM. However, there was no difference in ORR (58.6%% vs. 34.5%, p = 0.065) after PSM (Table 2).

Survival analysis

Before PSM, the median PFS was 7.1 months (95% CI 5.773–8.427) in the T+S+ICI group and 3.5 months (95% CI 2.087–4.913) in the T+S group (p = 0.001) (Figure 2A), and the median OS was 12.3 months (95% CI 9.719–14.881) in the T+S+ICI group and 6.3 months (95% CI 4.559–8.041) in the T+S group (p = 0.004) (Figure 2B).

After PSM, the median PFS was 6.9 months (95% CI 4.805–8.995) in the T+S+ICI group and 3.8 months (95% CI 2.218–5.383) in the T+S group (p = 0.003) (Figure 2C), and the median OS was 12.3 months (95% CI 10.36–14.24) in the T+S+ICI group and 6.3 months (95% CI 4.647–7.953) (p = 0.008) (Figure 2D) in the T+S group.

Subgroup analysis

Subgroup analyses of patients in the two groups before PSM

In patients with AFP of <400 ng/ml, the median PFS was 7.2 months (95% CI: 5.374–8.826) in the T+S+ICI group and 3.5 months (95% CI 1.926–4.274) in the T+S group (p = 0.008) (Figure 3A); the median OS was 15 months (95% CI: 8.433–21.567) in the T+S+ICI group and 6.3 months (95% CI 2.979–8.821) in the T+S group (p = 0.006) (Figure 3B). In patients with AFP of ≥400 ng/ml, the median PFS was 7.1 months (95% CI 2.331–12.069) in the T+S+ICI group and 3.1 months (95% CI 1.528–5.472) in the T+S group (p = 0.049) (Figure 3C); the median OS was 12 months (95% CI: 8.634–15.366) in the T+S+ICI group and 5.9 months (95% CI 4.985–7.615) in the T+S group (p = 0.202) (Figure 3D).

In patients with Child–Pugh class A, the median PFS was 7.1 months (95% CI: 5.970–8.230) in the T+S+ICI group and 4.1 months (95% CI 3.121–5.079) in the T+S group (p = 0.048) (Figure 4A); the median OS was 15 months (95% CI 0.979–

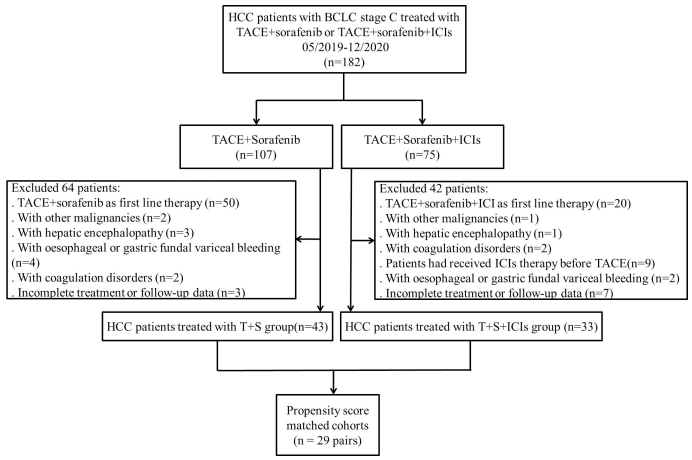

FIGURE 1
Flowchart shows the patients' selection process. BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization; ICIs, immune checkpoint inhibitors; T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors.

TABLE 1 Patient demographics and baseline characteristics before and after propensity score matching.

Characteristics	Before PSM			After PSM		
	T+S (n = 43)	T+S+ICIs (n = 33)	p value	T+S (n = 29)	T+S+ICIs (n = 29)	p value
Age (years)	50.9 ± 11.5	54.6 ± 9.7	0.324	51.3 ± 11.2	53.7 ± 10.2	0.792
<50	16 (37.2)	16 (48.5)		13 (44.8)	14 (48.3)	
≥50	27 (62.8)	17 (51.5)		16 (55.2)	15 (51.7)	
Sex			0.434			>0.999
Men	39 (90.7)	28 (84.8)		26 (89.7)	26 (89.7)	
Women	4 (9.3)	5 (15.2)		3 (10.3)	3 (10.3)	
ECOG PS			0.987			0.945
0	9 (20.9)	7 (21.2)		6 (20.7)	5 (17.2)	
1	31 (72.1)	24 (72.7)		21 (72.4)	22 (75.9)	
2	3 (7.0)	2 (6.1)		2 (6.9)	2 (6.9)	
HBV			0.827			>0.999
Positive	36 (83.7)	27 (81.8)		24 (82.8)	24 (82.8)	
Negative	7 (16.3)	6 (18.2)		5 (17.2)	5 (17.2)	
Cirrhosis			0.610			0.773
Yes	31 (72.1)	22 (66.7)		21 (72.4)	20 (69.0)	
No	12 (27.9)	11 (33.3)		8 (27.6)	9 (31.0)	
Child–Pugh			0.109			0.773
A	25 (58.1)	25 (75.8)		20 (69.0)	21 (72.4)	
B	18 (41.9)	8 (24.2)		9 (31.0)	8 (27.6)	
AFP (ng/mL)			0.339			>0.999
<400	23 (53.5)	14 (42.4)		12 (41.4)	12 (41.4)	
≥400	20 (46.5)	19 (57.6)		17 (58.6)	17 (58.6)	
AST (U/L)	75.3 ± 62.3	69.9 ± 60.9	0.706	83.2 ± 70.6	74 ± 63.9	0.854
ALT (U/L)	37.1 ± 24.3	44.3 ± 35.7	0.299	39.1 ± 26.9	45.4 ± 37.8	0.616
Albumin (g/L)	34.0 ± 5.0	35.8 ± 5.4	0.130	34.3 ± 5.2	35.2 ± 4.6	0.625
Tumor size (cm)			0.878			0.599
<10	24 (55.8)	19 (57.6)		13 (44.8)	15 (51.7)	
≥10	19 (44.2)	14 (42.4)		16 (55.2)	14 (48.3)	
Vascular invasion	31 (72.1)	27 (81.8)	0.323	21 (72.4)	24 (82.8)	0.345
EHS	27 (62.8)	17 (51.5)	0.324	17 (58.6)	16 (55.2)	0.791
Type of PVTT			0.269			0.197
I+II	16 (37.2)	17 (51.5)		10 (34.5)	16 (55.2)	
III	14 (32.6)	8 (24.2)		11 (37.9)	8 (27.6)	
Number of TACE			0.054			0.146
1	25 (58.1)	10 (30.3)		15 (51.7)	8 (27.6)	
2	11 (25.5)	14 (42.4)		10 (34.5)	13 (44.8)	
3	7 (16.3)	9 (27.3)		4 (13.8)	8 (27.6)	
Prior therapy			0.687			0.803
DEB-TACE/cTACE	29 (67.4)	20 (60.6)		20 (69.0)	18 (62.1)	
DEB-TACE/cTACE+RFA	7 (16.3)	8 (24.2.)		5 (17.2)	7 (24.1)	
Surgery+cTACE/RFA	7 (16.3)	5 (15.2)		4 (13.8)	4 (13.8)	

Data were presented as n (%) or mean ± standard deviation. PSM, propensity score matching; T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors; ECOG PS, Eastern Cooperative Oncology Group performance status; HBV, hepatitis B virus; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine transaminase; EHS, extrahepatic spread; PVTT, portal vein tumor thrombus; type I, tumor thrombi involving segmental branches of portal vein or above; type II, tumor thrombi involving right/left portal vein; type III, tumor thrombi involving the main portal vein; DEB-TACE, drug-eluting bead transarterial chemoembolization; cTACE, conventional transarterial chemoembolization; RFA, radiofrequency ablation.

29.021) in the T+S+ICI group and 6.8 months (95% CI 3.474–10.126) in the T+S group (p = 0.05) (Figure 4B). In patients with Child–Pugh class B, the median PFS was 5.1 months (95% CI 0.000–10.921) in the T+S+ICI group and 3 months (95% CI 2.584–3.416) in the T+S group (p = 0.011) (Figure 4C); the median OS was 12.0 months (95% CI 9.649–14.351) in the T+S+ICI group and 6.3 months (95% CI 2.618–9.982) in the T+S group (p = 0.075) (Figure 4D).

In patients with tumor size of <10 cm, the median PFS was 10.1 months (95% CI 6.894–13.306) in the T+S+ICI group and

TABLE 2 Summary of response rates before and after propensity score matching.

Best overall response, n (%)	Before PSM			After PSM		
	T+S (n = 43)	T+S+ICIs (n = 33)	p value	T+S (n = 29)	T+S+ICIs (n = 29)	p value
Complete response	0 (0)	0 (0)	> 0.999	0 (0)	0 (0)	> 0.999
Partial response	12 (27.9)	20 (60.6)	0.004	10 (34.5)	17 (58.6)	0.065
Stable disease	12 (27.9)	8 (24.2)	0.719	7 (24.1)	7 (24.1)	> 0.999
Progressive disease	19 (44.2)	5 (15.2)	0.007	12 (41.4)	5 (17.2)	0.043
Objective response rate	12 (27.9)	20 (60.6)	0.004	10 (34.5)	17 (58.6)	0.065
Disease control rate	24 (55.8)	28 (84.8)	0.007	17 (58.6)	24 (82.8)	0.043

Data are numbers of patients, with percentages in parentheses. PSM, propensity score matching; T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors.

3.5 months (95% CI 2.300–4.7) in the T+S group (p = 0.004) (Figure 5A); the median OS was 12.3 months (95% CI 8.449–16.151) in the T+S+ICI group and 6.8 months (95% CI 4.097–9.503) in the T+S group (p = 0.029) (Figure 5B). In patients with tumor size of ≥10 cm, the median PFS was 4 months (95% CI 1.678–6.322) in the T+S+ICI group and 3 months (95% CI 0.441–5.559) in the T+S group (p=0.128) (Figure 5C); the median OS was 10.2 months (95% CI 3.093–17.307) in the T+S+ICI group and 5.9 months (95% CI 3.023–8.777) in the T+S group (p = 0.06) (Figure 5D).

In patients with type I or II (type I+II) PVTT, the median PFS was 7.2 months (95% CI 2.568–11.832) in the T+S+ICI group and 3.1 months (95% CI 2.708–3.492) in the T+S group (p = 0.031) (Figure 6A); the median OS was 12.3 months (95% CI: 10.457–14.143) in the T+S+ICI group and 6.2 months (95% CI 5.416–6.984) in the T+S group (p = 0.076)

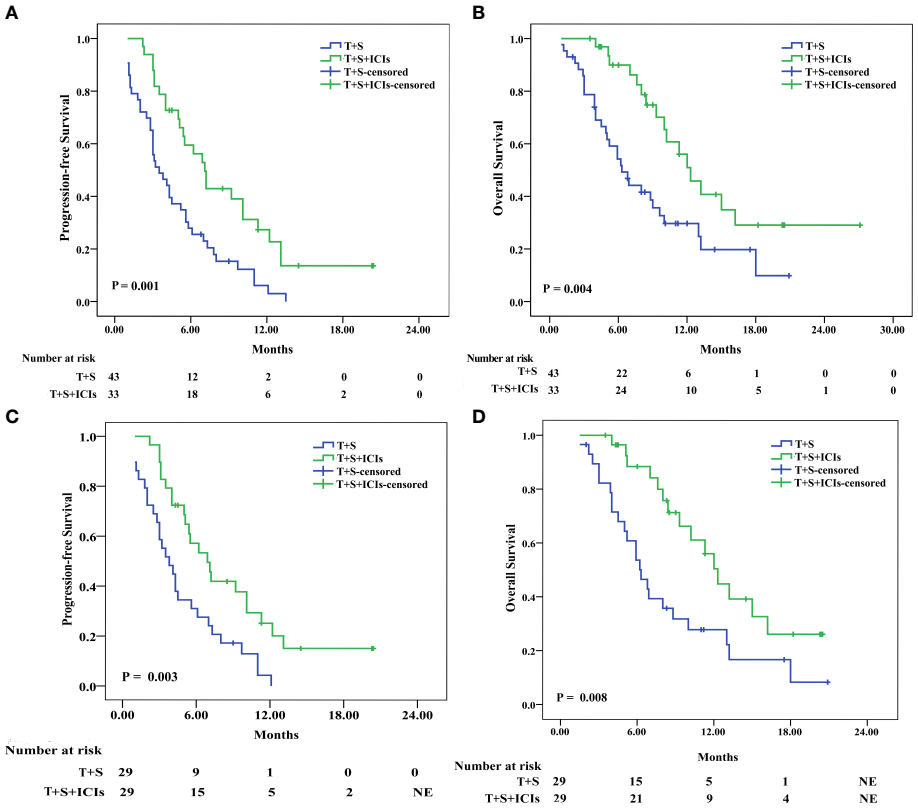

FIGURE 2
Kaplan–Meier analyses of progression-free survival and overall survival before **(A, B)** and after **(C, D)** propensity score matching in patients treated with T+S or T+S+ICIs. T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors.

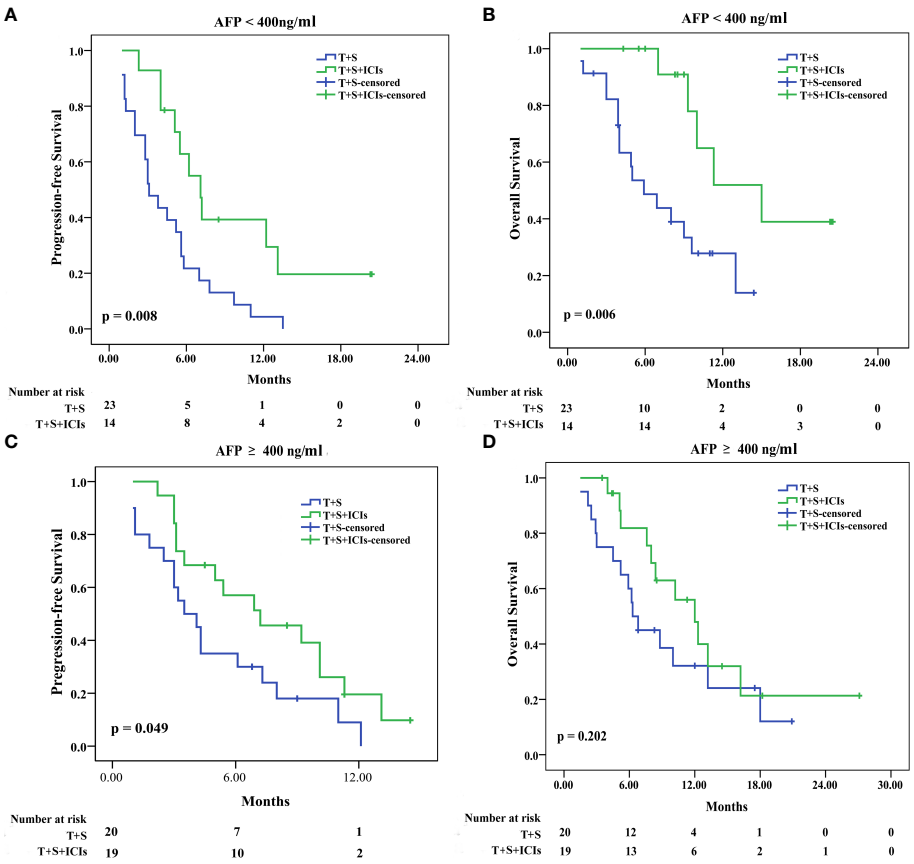

FIGURE 3
Subgroup analysis for progression-free survival and overall survival stratified by AFP level <400 ng/ml **(A, B)** and ≥400 ng/ml **(C, D)**. T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors.

(Figure 6B). In patients with type III PVTT, the median PFS was 6.9 months (95% CI 000–14.028) in the T+S+ICI group and 2.8 months (95% CI 0.967–4.633) in the T+S group (p = 0.001) (Figure 6C); the median OS was 10.2 months (95% CI 7.248–13.152) in the T+S+ICI group and 5 months (95% CI 1.835–8.165) in the T+S group (p = 0.004) (Figure 6D).

Univariate and multivariate analyses

In the matched cohort, after screening, ECOG PS and treatments influencing the PFS were selected for multivariate analysis (Table 3). The Cox proportional hazard model showed that the ECOG PS (0 + 1 vs. 2) [hazard ratio (HR) = 0.276; 95% CI 0.095–0.800; p = 0.018] and treatment (T+S+ICIs vs. T+S) (HR = 0.376; 95% CI 0.207–0.682; p = 0.001) were independent predictive factors for PFS (Table 3). Multivariate analysis indicated that age (<50 vs. ≥50 years) (HR = 2.052; 95% CI 1.040–4.048; p = 0.038) and treatment (T+S+ICIs vs. T+S) (HR = 0.386; 95% CI 0.195–0.764; p = 0.006) were independent predictive factors for OS (Table 4).

Safety

To assess the safety of the two groups in real clinical practice, the incidence of AEs was reported in cohorts matched previously (Table 5); SAEs (more than grade 4) did not occur in this study. Ten (30.3%) patients experienced reactive cutaneous capillary endothelial proliferation (RCCEP) (grade 1/2) on the skin and three (9.2%) patients experienced hypothyroidism (grade 1/2) in the T+S+ICI group; no patient experienced that symptom in the T+S group (respectively, p < 0.05) (Table 5). Also, no treatment-related deaths occurred in this study.

Discussion

This study revealed that T+S+ICIs conferred a significant survival benefit compared with T+S in patients with BCLC stage C HCC who previously received locoregional treatment. This finding was associated with an increase in median OS from 6.3 to 12.3 months, which might be attributed to the higher ORR and

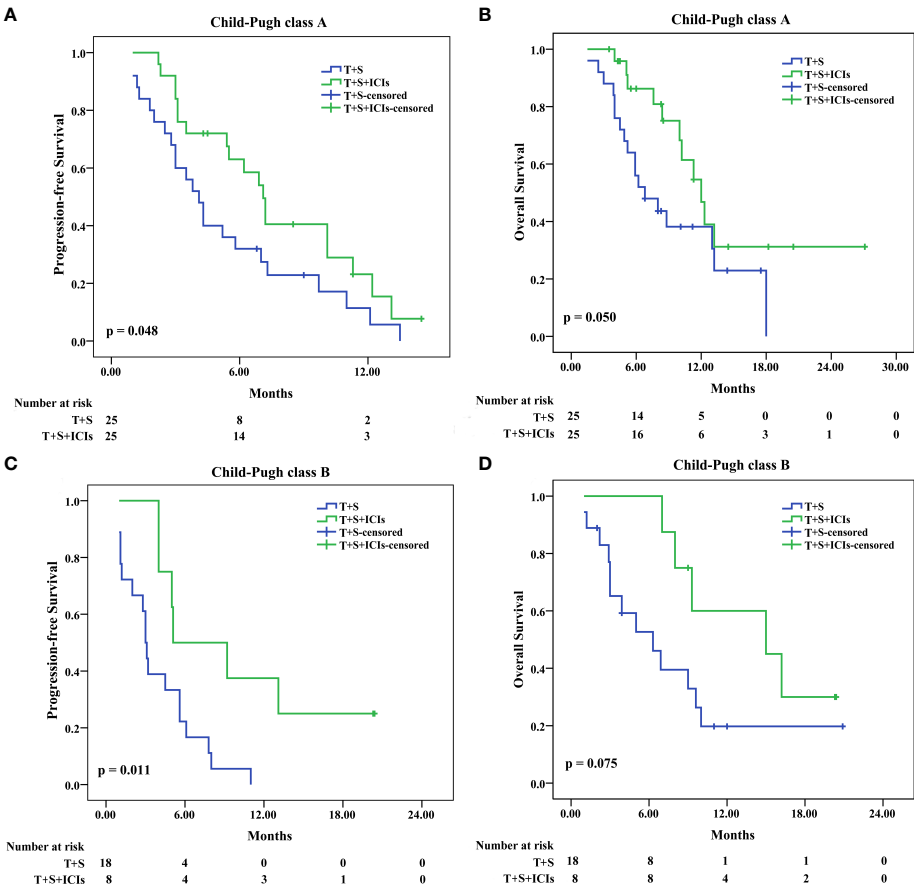

FIGURE 4
Subgroup analysis for progression-free survival and overall survival stratified by Child–Pugh class A **(A, B)** and B **(C, D)**. T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors.

DCR and longer PFS in patients receiving T+S+ICIs rather than those treated with T+S. Multivariate analyses also showed that combining ICIs on the basis of TACE plus sorafenib was an independent predictor for prolonged PFS and OS. These results indicated that the TACE combined with sorafenib and ICI regimen might be a superior treatment option in patients with BCLC C stage HCC who previously received locoregional treatment, which might be due to the following reasons: 1) TACE can lead to tumor necrosis after occlusion of feeding arteries and release of tumor antigens, which can be captured by antigen-presenting cells. This can activate tumor-specific immune responses (18), change the cytokine spectrum and the activity level of T cells and immune cell subsets (18), and transfer TME into Th1 dominance to improve the regulatory T-cell level and obtain favorable survival prognosis (19). 2) Sorafenib may counteract the hypoxia-induced angiogenesis after TACE (12, 20), regulate VEGF-mediated immunosuppression within tumors and TME (21, 22), and enhance the immunomodulatory effect by reversing VEGF-mediated immunosuppression and promoting T-cell infiltration into tumors (23, 24). Therefore, the combination of TACE, sorafenib, and ICIs has a synergistic antitumor effect, contributing to improved survival outcomes in patients with advanced HCC.

Patients with advanced HCC who were administered with nivolumab or pembrolizumab as systemic first-/second-/third-/fourth-line treatment had an ORR of 12% and a median OS of 11 months (25). However, patients with unresectable HCC who received first-line lenvatinib plus pembrolizumab treatment had an ORR of 46% and a median OS of 12.6 months (26). Thus, combination therapy significantly improved the ORR and OS. A previous study suggested that the median PFS and OS in patients with BCLC C stage TACE-refractory HCC who received TACE+sorafenib+ICI treatments were 10.8 and 13.5 months, respectively, which were higher than the results of this study. The reason may be that the patients' average liver tumor diameter in the previous study was smaller compared to this study (6.1 ± 2.5 vs. 9.6 ± 4.8 cm) (27). In the TRIPLET study (28), HCC patients in BCLC stage C who received hepatic artery

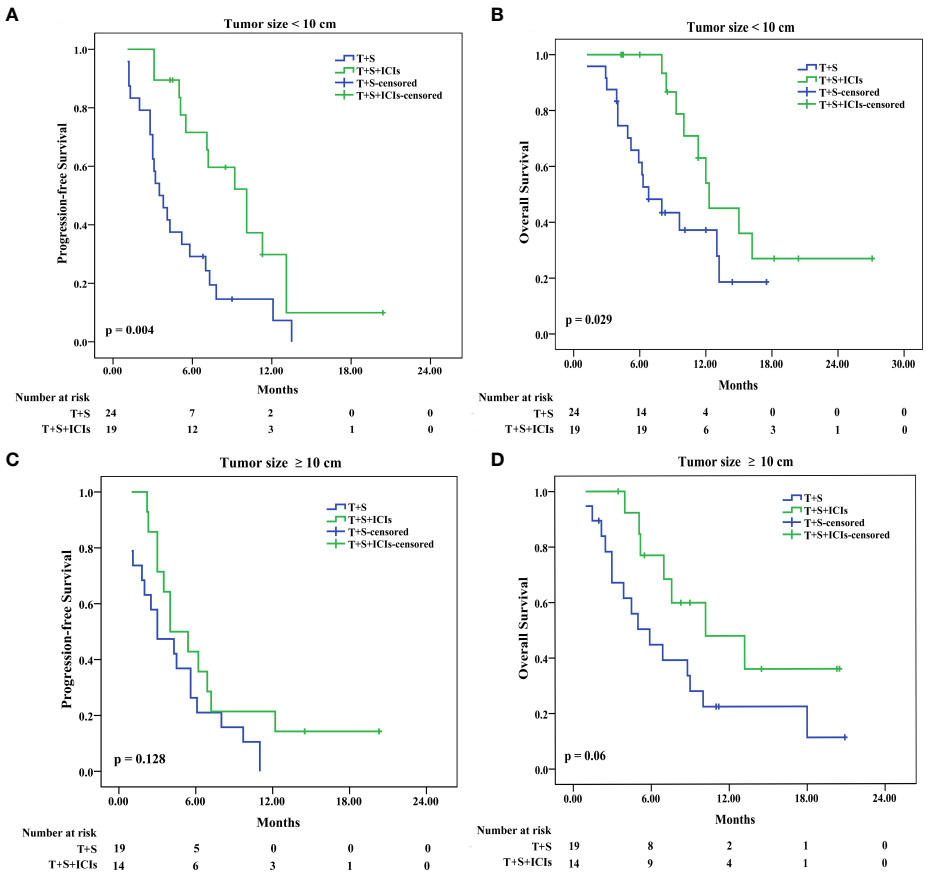

FIGURE 5
Subgroup analysis for progression-free survival and overall survival stratified by tumor size <10 cm **(A, B)** and ≥10 cm **(C, D)**. T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors.

infusion chemotherapy (HAIC) combined with apatinib and camrelizumab had an ORR and DCR of 61.54% and 92.3%, respectively. These results were better than the data obtained in this study, and the reason may be that all patients in the TRIPLET study received no previous treatment (in this study, patients with BCLC C stage HCC were previously treated with locoregional therapy). Cai et al. (29) assessed the TACE+lenvatinib+PD-1 inhibitor for patients with advanced HCC and reported an ORR of 56.1%, a DCR of 85.4%, and a PFS of 7.3 months; these results were consistent with this study.

The main PVTT is the independent risk factor for the survival of HCC (30, 31). In this study, subgroup analyses showed that T+S+ICIs provided a better PFS and OS than T +S in the patients with type III PVTT but not in those with type I+II PVTT. The reason might be that TACE exerted its antitumor property mainly by controlling intrahepatic PVTT rather than extrahepatic PVTT (20). Thus, a treatment strategy combining TACE with a more potent systemic therapy was urgently needed for patients with extrahepatic PVTT. Our results revealed the necessity of the additional treatment with ICIs to TACE plus sorafenib for such patients.

In this study, AEs were mild to moderate and could be managed easily. Chemoembolization- and sorafenib-related AEs were similar to those reported in previous studies (5, 8, 32). The incidence rate of RCCEP (30.3%) was lower in the T+S+ICI group than the result in a previous study (67%) (33). After receiving thyroxine, glucocorticoid, and ICI interruption treatments, patients with hypothyroidism recovered within 2 weeks.

There were several limitations in the present study. Firstly, this study was a retrospective analysis, which carries limitations in terms of selection bias and the control of other confounding factors. We implemented the PSM methodology to account for the effect caused by confounding factors. A randomized clinical trial is required to validate the findings from this study. Secondly, this study has a small sample size. Lastly, only patients with BCLC stage C HCC were included in this study. Thus, the findings from this study may not be generalized to other unresectable HCC populations.

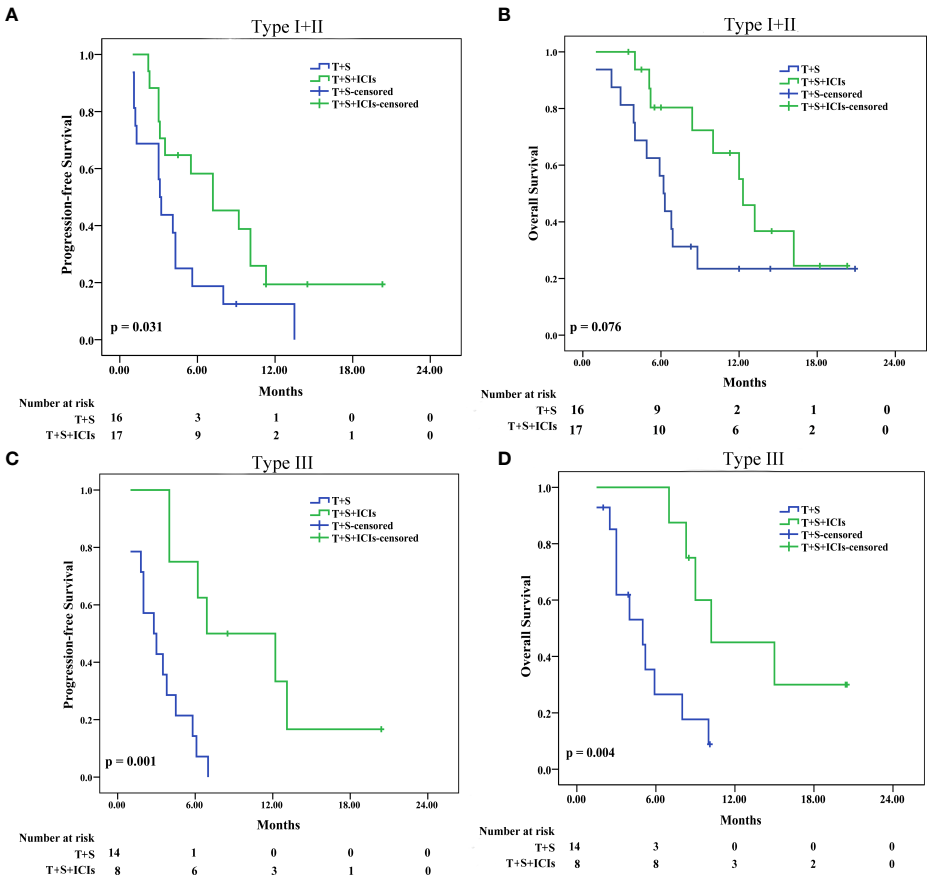

FIGURE 6
Subgroup analysis for progression-free survival and overall survival stratified by type I+II PVTT **(A, B)** and type III PVTT **(C, D)**. T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors; PVTT, portal vein tumor thrombus; type I, tumor thrombi involving segmental branches of portal vein or above; type II, tumor thrombi involving right/left portal vein; type III, tumor thrombi involving the main portal vein.

TABLE 3 Univariate and multivariate predictors of progression-free survival.

Variables	Univariate Cox analysis			Multivariate Cox analysis		
	HR	95% CI	p value	HR	95% CI	p value
Sex (men/women)	1.718	0.795-3.714	0.169			
Age (years) (<50/≥50)	0.851	0.482-1.499	0.576			
ECOG PS (0 + 1/2)	2.819	0.993-8.003	0.052	0.276	0.095-0.800	0.018
HBV infection (positive/negative)	1.312	0.588-2.927	0.507			
Cirrhosis (yes/no)	1.113	0.669-1.723	0.518			
Child–Pugh class (A/B)	0.849	0.459-1.569	0.601			
AFP (ng/mL)(<400/≥400)	0.812	0.457-1.441	0.476			
Tumor size (cm) (<10/≥10)	0.797	0.456-1.394	0.427			
Extrahepatic metastasis (yes/no)	1.176	0.623-1.876	0.685			
PVTT (I+II/III)	0.741	0.517-1.564	0.522			
Albumin level (g/L) (<35/≥35)	0.880	0.501-1.547	0.657			
TBIL (μmol/L) (<20/≥20)	1.290	0.726-2.291	0.385			

(Continued)

TABLE 3 Continued

Variables	Univariate Cox analysis			Multivariate Cox analysis		
	HR	95% CI	p value	HR	95% CI	p value
ALT (U/L) (<35/≥35)	0.805	0.460-1.408	0.447			
AST (U/L) (<40/≥40)	1.350	0.608-2.801	0.495			
Number of TACE (1/2+3)	0.829	0.467-1.470	0.521			
Treatment (T+S+ICIs/T+S)	2.483	1.378-4.473	0.002	0.376	0.207-0.682	0.001

ECOG PS, Eastern Cooperative Oncology Group performance status; AFP, alpha-fetoprotein; PVTT, portal vein tumor thrombus; type I, tumor thrombi involving segmental branches of portal vein or above; type II, tumor thrombi involving right/left portal vein; type III, tumor thrombi involving the main portal vein; TBIL, total bilirubin; ALT, alanine transaminase; AST, aspartate transaminase; T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors.

TABLE 4 Univariate and multivariate predictors of overall survival.

Variables	Univariate Cox analysis			Multivariate Cox analysis		
	HR	95% CI	p value	HR	95% CI	p value
Sex (men/women)	1.638	0.712-3.768	0.246			
Age (years) (<50/≥50)	0.620	0.325-1.183	0.147	2.052	1.040-4.048	0.038
ECOG PS (0 + 1/2)	2.328	0.811-6.683	0.116	0.473	0.159-1.413	0.180
HBV infection (negative/positive)	1.111	0.433-2.852	0.827			
Cirrhosis (yes/no)	1.211	0.687-1.821	0.649			
Child–Pugh class (A/B)	0.778	0.383-1.577	0.486			
AFP (ng/mL) (<400/≥400)	1.344	0.687-2.631	0.388			
Tumor size (cm) (<10/≥10)	0.786	0.415-1.489	0.461			
Extrahepatic metastasis (yes/no)	1.298	0.795-2.157	0.298			
PVTT (type I+II/III)	0.456	0.452-1.461	0.736			
Albumin level (g/L) (<35/≥35)	1.135	0.594-2.167	0.702			
TBIL (μmol/L) (<20/≥20)	1.083	0.554-2.116	0.816			
ALT (U/L) (<35/≥35)	0.636	0.333-1.217	0.172			
AST (U/L) (<40/≥40)	1.271	0.530-3.050	0.592			
Number of TACE (1/2+3)	0.590	0.307-1.133	0.113	1.609	0.807-3.208	0.176
Treatment (T+S+ICIs/T+S)	0.426	0.222-0.820	0.011	0.386	0.195-0.764	0.006

ECOG PS, Eastern Cooperative Oncology Group performance status; AFP, alpha-fetoprotein; PVTT, portal vein tumor thrombus; type I, tumor thrombi involving segmental branches of portal vein or above; type II, tumor thrombi involving right/left portal vein; type III, tumor thrombi involving the main portal vein; TBIL, total bilirubin; ALT, alanine transaminase; AST, aspartate transaminase; T+S, transarterial chemoembolization+sorafenib; T+S+ICIs, transarterial chemoembolization+sorafenib+immune checkpoint inhibitors.

TABLE 5 Treatment-related adverse events (TRAE).

Event, n (%)	T+S (n=43)			T+S+ICIs (n=33)			p value		
	Any grade	Grade 1/2	Grade 3/4	Any grade	Grade 1/2	Grade 3/4	Any grade	Grade 1/2	Grade 3/4
Any TRAE	40(93.0)	37(86.0)	6(14.0)	33(100.0)	29(87.9)	7(21.2)	0.122	0.815	0.405
Fatigue	14(32.6)	10(23.3)	4(9.3)	15(45.5)	10(30.3)	5(15.2)	0.251	0.489	0.434
Decreased appetite	12(27.9)	9(20.9)	3(7.0)	15(45.5)	11(33.3)	4(12.1)	0.113	0.224	0.442
Vomiting or nausea	14(32.6)	12(27.9)	2(4.7)	11(33.3)	9(27.3)	2(6.1)	0.943	0.951	0.785
Abdominal pain	12(27.9)	11(25.6)	1(2.3)	10(30.3)	9(27.3)	1(3.0)	0.819	0.868	0.849
Fever	13(30.2)	10(23.3)	3(7.0)	13(39.4)	11(33.3)	2(6.1)	0.404	0.330	0.873
Dose reduce or interruptions	5(11.6)	4(9.3)	1(2.3)	7(21.2)	4(21.1)	3(9.1)	0.256	0.691	0.190
Hypertension	3(7.0)	2(4.7)	1(2.3)	5(15.2)	3(9.1)	2(6.1)	0.250	0.439	0.407
Hand and foot syndrome	8(18.6)	6(14.0)	2(4.7)	8(24.2)	5(15.2)	3(9.1)	0.550	0.883	0.439
Diarrhea	2(4.7)	2(4.7)	0(0.0)	4(12.1)	4(12.1)	0(0.0)	0.231	0.231	_
Alopecia	3(7.0)	3(7.0)	0(0.0)	2(6.1)	2(6.1)	0(0.0)	0.873	0.873	_

(Continued)

TABLE 5 Continued

Event, n (%)	T+S (n=43)			T+S+ICIs (n=33)			p value		
	Any grade	Grade 1/2	Grade 3/4	Any grade	Grade 1/2	Grade 3/4	Any grade	Grade 1/2	Grade 3/4
Pruritus	5(11.6)	5(11.6)	0(0.0)	6(18.2)	5(15.2)	1(3.0)	0.421	0.652	0.251
Rash	1(2.3)	1(2.3)	0(0.0)	4(12.1)	3(9.1)	1(3.0)	0.088	0.190	0.251
Proteinuria	8(18.6)	6(14.0)	2(4.7)	12(36.4)	9(27.3)	3(9.1)	0.081	0.148	0.439
Increased AST	7(16.3)	6(14.0)	1(2.3)	8(24.2)	6(18.2)	2(6.1)	0.387	0.616	0.407
Increased ALT	6(14.0)	4(9.3)	2(4.7)	8(24.2)	6(18.2)	2(6.1)	0.251	0.256	0.785
Decreased neutrophil count	6(14.0)	6(14.0)	0(0.0)	6(18.2)	6(18.2)	0(0.0)	0.616	0.616	–
Increased blood bilirubin	6(14.0)	6(14.0)	0(0.0)	8(24.2)	7(21.2)	1(3.0)	0.251	0.405	0.251
Gastrointestinal hemorrhage	3(7.0)	3(7.0)	0(0.0)	2(6.1)	2(6.1)	0(0.0)	0.873	0.873	–
Hypothyroidism	0(0.0)	0(0.0)	0(0.0)	3(9.1)	3(9.1)	0(0.0)	0.044	0.044	–
RCCEP	0(0.0)	0(0.0)	0(0.0)	10(30.3)	10(30.3)	0(0.0)	<0.001	<0.001	–

Data are numbers of patients, with percentages in parentheses. TACE, transarterial chemoembolization; ICIs, immune checkpoint inhibitors; AST, aspartate aminotransferase; ALT, alanine transaminase; RCCEP, reactive cutaneous capillary endothelial proliferation.

In conclusion, compared with TACE combined with sorafenib, TACE combined with sorafenib plus ICIs is a potentially safe and effective treatment regimen for patients with advanced HCC who previously received locoregional treatment.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was reviewed and approved by Sichuan Cancer Hospital. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Conception and design: G-HX and X-QH. Collection and assembly of data: X-GY, Y-YS, H-QW, and D-SL. Manuscript writing: all authors. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Urolithin A targets the AKT/WNK1 axis to induce autophagy and exert anti-tumor effects in cholangiocarcinoma

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Urolithin A (UA; 3,8-dihydroxybenzo[c]chromen-6-one), a metabolite generated by intestinal bacteria during the biotransformation of ellagitannins, has gained considerable attention in treating several cancers. Cholangiocarcinoma (CCA) remains one of the most lethal cancers; it grows in a special environment constantly exposed to both blood and bile. Since UA is known to undergo enterohepatic recirculation, we hypothesized that UA might have significant antitumor effects in CCA. Here, we investigated the therapeutic potential of UA in CCA and aimed to elucidate its mechanisms, including autophagy. UA treatment inhibited cell proliferation and induced G2/M phase cell cycle arrest in CCA cells. UA also suppressed cell migration and invasion, but did not cause apoptosis. Furthermore, Western blotting and immunocytochemistry demonstrated increased LC3-II accumulation, while electron microscopy demonstrated induced autophagosomes after UA treatment, suggesting that UA upregulated autophagy in CCA cells. In xenograft mice treated with UA, tumor growth was inhibited with increased LC3-II levels. On the other hand, phospho-kinase array demonstrated downregulation of the AKT/WNK1 pathway. LC3-II expression was elevated in WNK1 knocked down cells, indicating that WNK1 is the key signal for regulating autophagy. Thus, UA exerted antitumor effects by suppressing the AKT/WNK1 signaling pathway and inducing autophagy. In conclusion, UA, a natural, well-tolerated compound, may be a promising therapeutic candidate for advanced CCA.

KEYWORDS

Urolithin A, UA, cholangiocarcinoma, autophagy, WNK1

Introduction

Natural compounds have been extensively researched over the past several decades for their potential in cancer prevention and treatment (1). Ellagitannins (ETs) are naturally occurring polyphenolic compounds with a wide range of pharmacological effects, including antioxidant, anti-inflammatory, and antitumor effects (2, 3). ETs are hydrolyzed in the gut to release ellagic acid (EA), mainly present in pomegranates, strawberries, blueberries, nuts, and dried fruits (4). However, the absorption of EA is limited due to its hydrophobic nature (5).

Urolithins are metabolites of EA produced by the intestinal bacteria (6). Urolithins are much better absorbed than ETs and EA, and may provide various health benefits such as anti-obesity, antimicrobial, anti-inflammatory, anti-tumor effects (7–9). Various types of urolithins have been identified, including urolithin A (UA; 3,8-dihydroxybenzo[c]chromen-6-one), B (UB), C, and D (10, 11). Urolithins are produced in the gut from tetrahydroxy-urolithin by removal of one of the lactone rings of ellagic acid, and the subsequent removal of a hydroxyl group, resulting in the formation of UA and UB (11). Of these, UA is the major microbial metabolite observed in human, which possess anti-inflammatory and antioxidant properties (12, 13). UA has been found to induce mitophagy efficiently and improve mitochondrial function in the model organism, Caenorhabditis elegans (14). In addition, antitumor effects of UA on lung, prostate, colon, bladder, pancreatic, and neuroblastoma cancers have also been demonstrated (15–21). Several reports indicate that UA induces autophagy, but not mitophagy, *in vitro* and *in vivo* (18, 22, 23). Espín et al. reported the pharmacokinetics and tissue distribution of urolithins in Iberian pigs, which feed on oak acorns rich in ellagitannins (24). An analysis of urolithins in plasma, urine, bile, jejunum, colon, and feces revealed that UA undergoes enterohepatic recirculation and, therefore, persists in the body for long periods (24).

Cholangiocarcinoma (CCA) is the second most common primary hepatic malignancy, accounting for 10–20% of newly-diagnosed liver cancers with features of biliary tract differentiation (25, 26). Unfortunately, most CCAs are diagnosed at an advanced stage and have to be treated with systemic chemotherapy instead of surgery. However, effective chemotherapy for CCA is still limited, and the development of new therapies is required. Since CCA grows in a special environment that is constantly exposed to both blood and bile, we hypothesized that UA would have significant antitumor effects in CCA because of enterohepatic recirculation. Despite promising effects in other cancers, the antitumor effects of UA in CCA are currently unknown. We aimed to investigate the antitumor effects of UA in CCA and elucidate its mechanism, including autophagy.

Materials and methods

Cell cultures

Human intrahepatic cholangiocarcinoma cell lines, HuCCT-1 and SSP-25, were obtained from the RIKEN cell bank. All cell lines were cultured in RPMI-1640 medium (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan), supplemented with 10% fetal bovine serum (FBS), in an incubator with 5% CO2 at 37°C.

Cell viability assays

Cell viability was measured using a Cell Counting Kit-8 assay (Dojindo, Kumamoto, Japan), and evaluated by the absorption of WST-1. The cells were seeded at a density of 4.0×10^3 cells/well on 96-well plates. After overnight incubation, the cells were treated with or without different concentrations of UA (Cayman Chemical Co., Ann Arbor, MI, USA) for 48 h.

Wound-healing assay (scratch assay)

The cells were grown to confluence in 12-well plates, and then a straight wound was made using a sterile 200-µL pipette tip. UA (10 or 40 µmol/L) was then added to the cells. The straight wound was photographed and measured under a microscope at 0 and 12 h. These investigations were independently performed three times.

Transwell invasion assay

Transwell assay was performed using Corning®Matrigel™ Invasion Chamber with 8.0-µm pore membranes (top chamber) for the 24-well culture plate (Corning, NY, USA). The cells were seeded at a density of 2×10^5 (HuCCT-1) cells or 1×10^5 (SSP-25) cells with serum-free FBS in the top chamber of the 24-well plate, and treated with or without UA (10 or 40 µmol/L). Complete medium was added to the lower chamber. After incubation for 24 h, the invading cells were fixed with 10% formalin, stained with crystal violet, and microscopically counted.

Western blot analysis

The cells were lysed in lysis buffer, and 20 µL of protein lysate sample was fractionated on polyacrylamide gels (TGX™ FastCast™ Acrylamide Kit; Bio-Rad Laboratories, Hercules, CA, USA) and then electroblotted to nitrocellulose membranes. The membranes were blocked with 5% skim milk in phosphate

buffered saline-Tween 20 (PBS-T). The membranes were incubated with primary and then secondary antibodies. They were then treated with enhanced chemiluminescence detection reagents (Amersham™; Cytiva, Marlborough, MA, USA), and chemiluminescent signals were visualized as bands using a LAS 4000 mini analyzer (Cytiva).

Antibodies against phospho-cdc2 (Try15), cyclin D1, cyclin B1, cleaved caspase-3, caspase-3, phospho-AKT (Ser473), AKT, phospho-WNK1 (Thr60), WNK1, phospho-GSK-3β (Ser9), GSK-3β, phospho-mTOR (Ser2448), and mTOR were purchased from Cell Signaling Technology (Beverly, MA, USA). Monoclonal beta-actin antibody (FUJIFILM Wako Pure Chemical Corp.) was used to probe an internal control.

Flow cytometry analysis

The cells were seeded in 60-mm dishes and cultured overnight, and then treated with or without UA (40 μmol/L) for 24, 48 and 72 h. After treatment, floating and attached cells were collected and stained, and flow cytometric analysis was performed using a flow cytometer (FACSCanto II, BD Biosciences; San Jose, CA, USA). Cell cycles were evaluated by PI staining (PI solution, Dojindo) and apoptosis was detected using the Annexin V Cell Apoptosis Detection Kit 1 (BD Biosciences) according to the manufacturer's instructions. Camptothecin (Merck, Darmstadt, Germany) was used as a positive control for the apoptosis assay.

Detection of autophagy

Autophagic cells were detected with LC3 using autophagy watch (Medical & Biological Laboratories, Aichi, Japan), according to the manufacturer's instructions. For Western blot analysis, HuCCT-1 and SSP-25 cells were treated with UA (40 μmol/L) and/or Chloroquine (CQ; 20 μmol/L) for 24 h, and the analysis was performed with 20 μL of protein lysate sample using anti-LC3 monoclonal antibody-HRP-DirecT (Autophagy watch). For immunocytochemistry, the cells were evaluated using anti-LC3 monoclonal antibody (Autophagy watch), with Alexa Fluor 488-conjugated goat anti-rabbit IgG (H + L; Thermo Fisher Scientific, Waltham, MA, USA) as the secondary antibody. All sections were counterstained using 4′,6-diamidino-2-phenylindole (DAPI; Fluoromount-G; Southern Biotech, Birmingham, AL, USA). HuCCT-1 cells were seeded in 4-well glass slides (Lab-Tek® Chamber Slide™ system; Thermo Fisher Scientific) and incubated for 24 h, and then treated under the respective conditions for 24 h. Images were obtained using a confocal laser scanning fluorescence microscope (FV3000; Olympus, Tokyo, Japan).

Transmission electron microscopy

HuCCT-1 cells were seeded at a density of 1.5×10^5 cells/well on 6-well plates. After overnight incubation, the cells were treated with UA (40 μmol/L) and/or CQ (20 μmol/L) for 24 h, and the samples were pre-fixed with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C. After fixation, the specimens were post-fixed with 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 45 min. They were subsequently dehydrated in a graded series of ethanol and embedded in epoxy resin. Ultra-thin sections were cut using an Ultracut-UCT (LEICA, Wetzlar, Germany) with a diamond knife, and stained with 2% uranyl acetate in distilled water for 15 min followed by a lead staining solution for 5 min. Sections were examined with a JEM-1400 plus (JEOL, Tokyo, Japan) electron microscope.

Human Phospho-kinase array

Phosphorylated proteins were analyzed using the Human Phospho-Kinase Array Kit (ARY003C; R&D Systems, Minneapolis, MN, USA). HuCCT-1 and SSP-25 cells were treated with or without UA (40 μmol/L) for 3 h and according to the manufacturer's instructions. Signals were detected using chemiluminescence detection reagents (Amersham™, Cytiva), and array images were analyzed using the ImageJ software.

Transfection

Small interfering RNA (siRNA) transfection was performed using Lipofectamine RNAi-MAX (Thermo Fisher Scientific) according to the manufacturer's instructions. HuCCT-1 cells were transfected with the desired siRNA using siGENOME non-targeting siRNA (siNT) control pool and siGENOME human WNK1 siRNA SMART pool (Dharmacon, Lafayette, CO, USA). Two days after transfection, the cells were treated with each condition for 3 or 24 h.

In vivo experiments

The protocols for all animal studies were approved by Nagoya City University Center for Experimental Animal Science, and the mice were housed according to the guidelines of Nagoya City University for Animal Experiments. Female nude mice (BALB/c Slc-nu/nu), aged 7 weeks, were obtained from Japan SLC Inc. The mice were acclimatized for 2 weeks before the experiments, and were kept in individual cages with unrestricted access to food and water. All mice were maintained under specific pathogen-free conditions with a 12-h light/dark cycle. To prepare the xenograft models, HuCCT-1

cells were injected into the mouse flanks with 5×10^6 cells in 100 µL of media. One day after implantation, the mice were randomly allocated into two groups. Two weeks after subcutaneous tumor transplantation, UA (20 mg/kg, 3 times a week) or dimethyl sulfoxide (DMSO; control) was administered by oral gavage, as in a previous study (20). The maximum tumor diameter (L) and the diameter at right angles to that axis (W) were measured using calipers twice a week, and the tumor volume was calculated according to the formula: (L×W2)/2. The weights of the mice were also recorded twice a week. The mice were sacrificed 35 days after the start of medication, and the transplanted tumors were excised and fixed in formalin or frozen in liquid nitrogen for protein lysate.

In vivo immunohistochemistry

The tumors were excised, and immediately fixed in formalin and embedded in paraffin blocks. Then, the block specimens were sectioned (4 µm) and stained using Ki-67 antibodies (Cell Signaling Technology). High spot areas were captured under a microscope, and the positive areas were counted visually. Data were expressed as means ± SD (Standard Deviation) of five independent experiments.

Statistical analysis

The data were analyzed using Student's *t* test and Mann-Whitney *U* test. Differences were considered statistically significant at $P < .05$. Data were expressed as means ± SD.

Results

UA treatment inhibited cell proliferation and induced G2/M phase cell cycle arrest in cholangiocarcinoma cell lines

The chemical structure of UA is shown in Figure 1A. To assess sensitivity for UA, a cell viability assay was performed with HuCCT-1 and SSP-25 cells. We found that the viabilities of the two cell lines treated with UA for 48 h were reduced in a dose-dependent manner (Figure 1B). We further explored the effect of UA on the cell cycle using flow cytometry (FACS). HuCCT-1 and SSP-25 cells treated with 40 µmol/L UA for 48 h showed accumulation of cells in the G2/M phase compared to the controls (control vs. 40 µmol/L UA in HuCCT-1 cells: 21.3± 1.9% vs. 31.5 ± 3.7%; and in SSP-25 cells: 40.9 ± 3.9% vs. 48.5 ± 1.3%, respectively, $P < 0.05$) (Figure 1C). As shown in Supplementary Figure 1, the G2/M phase cell accumulation was also observed under the conditions treated with UA for 24 or 72 h. Then, to confirm major cell cycle regulators of the G2/M phase, we examined the changes in phospho-cdc2 (Try15), cyclin B1, and cyclin D1 using Western blot analysis. HuCCT-1 cells treated with UA for 48 h upregulated the expression of phospho-CDC2 (Tyr15) and cyclin B1 without influencing cyclin D1 levels, consistent with the observed G2/M cell cycle arrest (Figure 1D).

Effects of UA on cell migration, invasion, and apoptosis progression in cholangiocarcinoma cell lines

To evaluate the effects of UA on cell migration, we conducted a wound-healing assay. UA treatment (40 µmol/L) significantly suppressed cell migration in both HuCCT-1 (0, 10, and 40 µmol/L UA: 81.2 ± 9.0%, 74.6 ± 15.5%, and 38.1 ± 9.3%, respectively, $P < 0.01$) and SSP-25 (0, 10, and 40 µmol/L UA: 74.1 ± 7.1%, 64.8 ± 1.9%, and 36.6 ± 3.0%, respectively, $P < 0.01$) cells (Figure 2A). We also performed the transwell assay to evaluate the effects of UA on cell invasion. UA significantly inhibited cell invasion at 40 µmol/L in both HuCCT-1 (0, 10 and 40 µmol/L UA: 1.0 ± 0.097, 0.91 ± 0.094, and 0.43 ± 0.106, respectively, $P < 0.01$) and SSP-25 (0, 10, and 40 µmol/L UA: 1.0 ± 0.119, 0.90 ± 0.091, and 0.63 ± 0.143, respectively, $P < 0.01$) cells (Figure 2B).

To investigate the effects of UA on apoptosis, we used the AnnexinV-FITC/PI staining method with flow cytometry. As shown in Supplementary Figure 2, 30 µmol/L Camptothecin for 24 h was used as a positive control for the apoptosis assay. Interestingly, there was no difference between control and UA treatment in the percentage of apoptotic cells in HuCCT-1 cells, treated with or without UA for 24h (0, 10, and 40 µmol/L UA: 10.6%, 10.3% and 8.4%) (Figure 2C). And, as shown in Supplementary Figure 3, there was also no difference between them under the conditions treated with UA for 48 or 72 h. We also examined the effects of UA on apoptosis-related factors, total and cleaved caspase-3, using Western blot analysis. There were no apparent changes in the total and cleaved caspase-3 in HuCCT-1 cells treated with 40 µmol/L UA for 0, 1, 3, 6, or 24 h (Figure 2D).

UA-mediated upregulation of autophagy in cholangiocarcinoma cells

Increased LC3-II levels and the formation of LC3 puncta were used to determine whether UA treatment induced autophagy in cholangiocarcinoma cells. To confirm the contribution of UA treatment to autophagy, we performed autophagy flux assay with CQ, which blocks the fusion of autophagosomes with lysosomes and inhibits late-stage

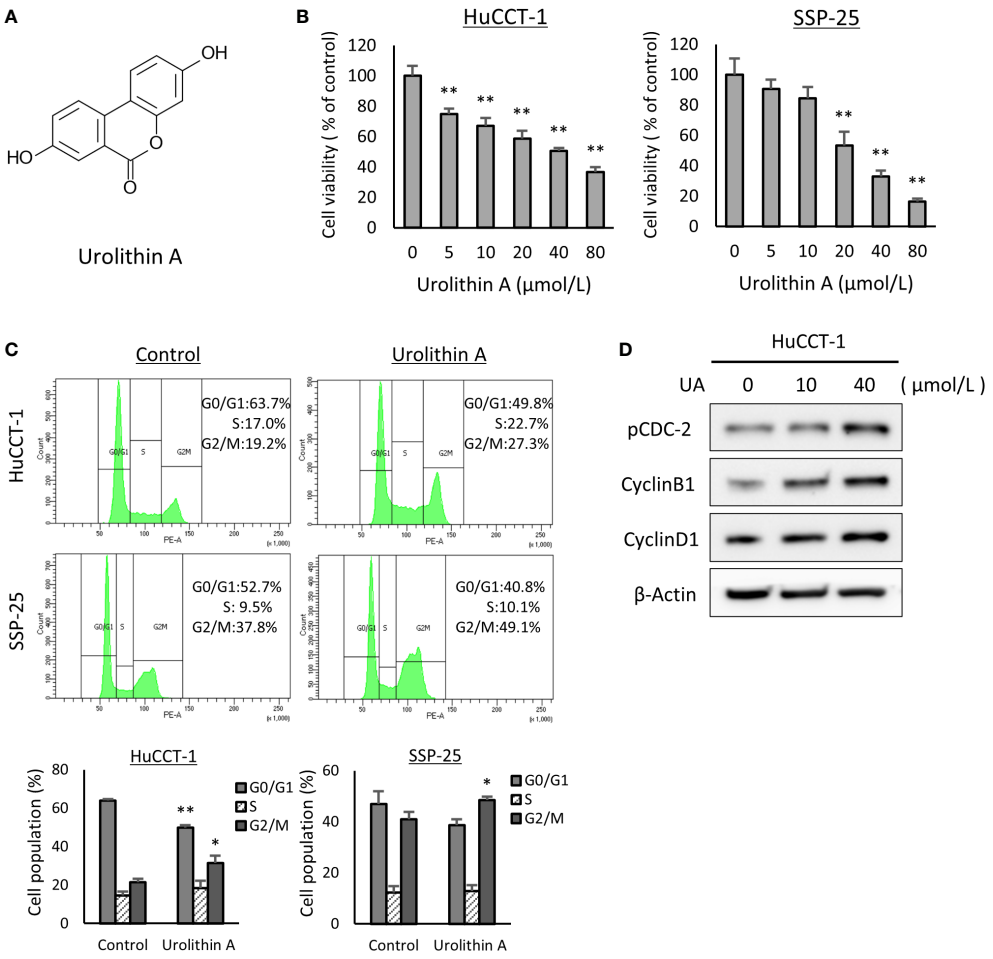

FIGURE 1
UA treatment inhibits cell proliferation and induces G2/M phase cell cycle arrest in cholangiocarcinoma cell lines. **(A)** Chemical structures of UA. **(B)** HuCCT-1 and SSP-25 cells were treated with UA at 0–80 μmol/L for 48 h Cell viability was measured using the Cell Counting Kit-8 assay. Data represent the means of three independent experiments. Bars, standard deviation; **P < 0.01. **(C)** HuCCT-1 and SSP-25 cells were treated with 0 or 40 μmol/L UA for 48 h Cell cycles were determined using flow cytometry. Data represent the means of three independent experiments. Bars, standard deviation; *P < 0.05; **P < 0.01. **(D)** HuCCT-1 were treated with 0, 10, or 40 μmol/L UA for 48 h Expression of cell cycle regulators was analyzed by Western blotting for phospho (p)-cdc2 (Try15), cyclin B1, and Cyclin D1. β-actin was used as internal loading control.

autophagy. We first examined the effects of UA on autophagy using Western blot analysis. It was found that UA treatment for 24 h caused an increase in LC3-II levels in HuCCT-1 and SSP-25 cells. CQ induced LC3-II expression, and addition of UA led to further accumulation of LC3-II in HuCCT-1 cells (Figure 3A). In addition, immunofluorescent staining revealed that UA, CQ, and their combination significantly increased LC3 puncta accumulation in the cytoplasm of cells compared to control (Figure 3B). Furthermore, transmission electron microscopy (TEM) demonstrated that there were more autophagosomes and autolysosomes in HuCCT-1 cells treated with UA for 24 h. After combined treatment with UA and CQ, autophagosomes that had stopped prematurely were clearly observed in the cytoplasm (Figure 3C).

UA inhibited xenograft tumor growth *in vivo*

The above-mentioned results demonstrated the efficacy of UA in cholangiocarcinoma cells. To verify these effects *in vivo*, we subcutaneously injected HuCCT-1 cells into the flank of nude mice as xenograft models. UA (20 mg/kg, 3 times a week) or DMSO (control) was administered by oral gavage for 35 days (Figure 4A). There was no body weight loss in the treatment group compared to the control group during the treatment (data not shown), which suggested that the volume of UA used was not harmful to the mice. Tumor volume and weight significantly reduced in the UA-treated mice compared to controls (Figures 4B, C). The proliferative potential of mice tumor

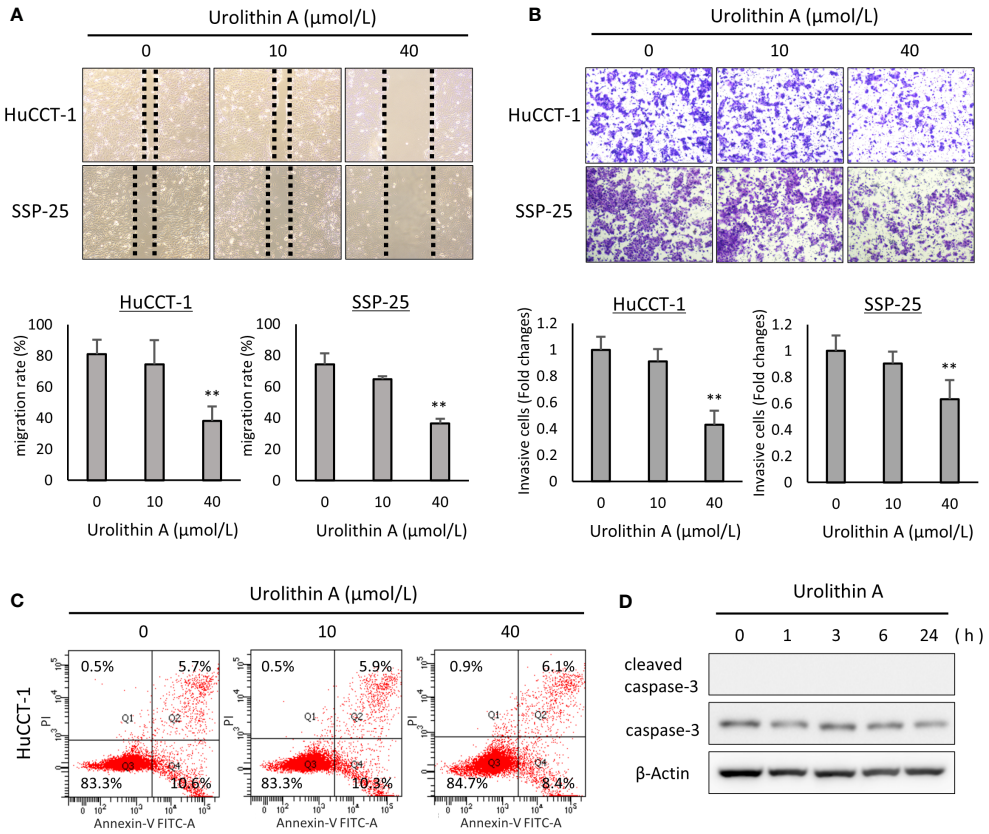

FIGURE 2
Effects of UA on cell migration, invasion, and apoptosis progression in cholangiocarcinoma cell lines. **(A)** Representative images obtained at 12 h after a scratch wound was made in confluent monolayers of HuCCT-1 and SSP-25 cells. After the scratch, 0, 10, or 40 μmol/L of UA were added. The migration rates were quantified by measuring the area of the injured region. Data represent the means of three independent experiments. Bars, standard deviation; **P < 0.01. **(B)** Representative transwell-membrane images stained with crystal violet show invasion cells after 12 h of treatment with 0, 10, or 40 μmol/L UA in HuCCT-1 and SSP-25 cells. Quantitative analysis of the invasion cells was expressed as fold change relative to untreated controls. Data represent the means of three independent experiments. Bars, standard deviation; **P < 0.01. **(C)** HuCCT-1 cells were treated with 0, 10, or 40 μmol/L UA for 24 h, and then stained with annexin-V FITC and PI. Apoptosis cells were evaluated using flow cytometry. **(D)** HuCCT-1 cells were treated with 40 μmol/L UA for 0, 1, 3, 6, or 24 h Expression of apoptosis-related factors was analyzed by Western blotting for cleaved caspase-3 and caspase-3. β-actin was used as an internal loading control.

samples were analyzed by Ki-67 immunostaining. The number of Ki-67 positive cells in the high spot area was significantly suppressed in the UA treatment group compared to the control group (Figure 4D). Western blot analysis revealed that the UA treatment group had significantly higher LC3-II levels than the control group (Figure 4E). These results suggested that UA could suppress tumor growth and might induce autophagy in cholangiocarcinoma.

UA treatment downregulated AKT and WNK1 pathways, and induced autophagy in cholangiocarcinoma cells

To clarify the key regulatory pathways of UA treatment, we utilized the human Phospho-kinase array. UA treatment downregulated the expressions of phospho-WNK1, phospho-AKT, and phospho-GSK-3β in HuCCT-1 and SSP-25 cells (Figure 5A). The significant changes of phosphorylation for WNK1 and AKT were also confirmed in the two cell lines using Western blot analysis, but were not seen in GSK-3β (Figure 5B). Therefore, we hypothesized that UA treatment might induce autophagy *via* the AKT/WNK1 pathway. To verify our hypothesis, we analyzed LC3-II expression in WNK1 knocked down HuCCT-1 cells. Western blot analysis for LC3-II revealed that the targeted knockdown of WNK1 elevated LC3-II protein without UA treatment, suggesting the importance of WNK1 in the activation of autophagy. Furthermore, UA treatment indicated similar up-regulation of LC3-II, regardless of the knockdown of WNK1. These results suggested that UA induced autophagy mainly *via* the AKT/WNK1 pathway (Figure 5C). In addition, Western blotting with

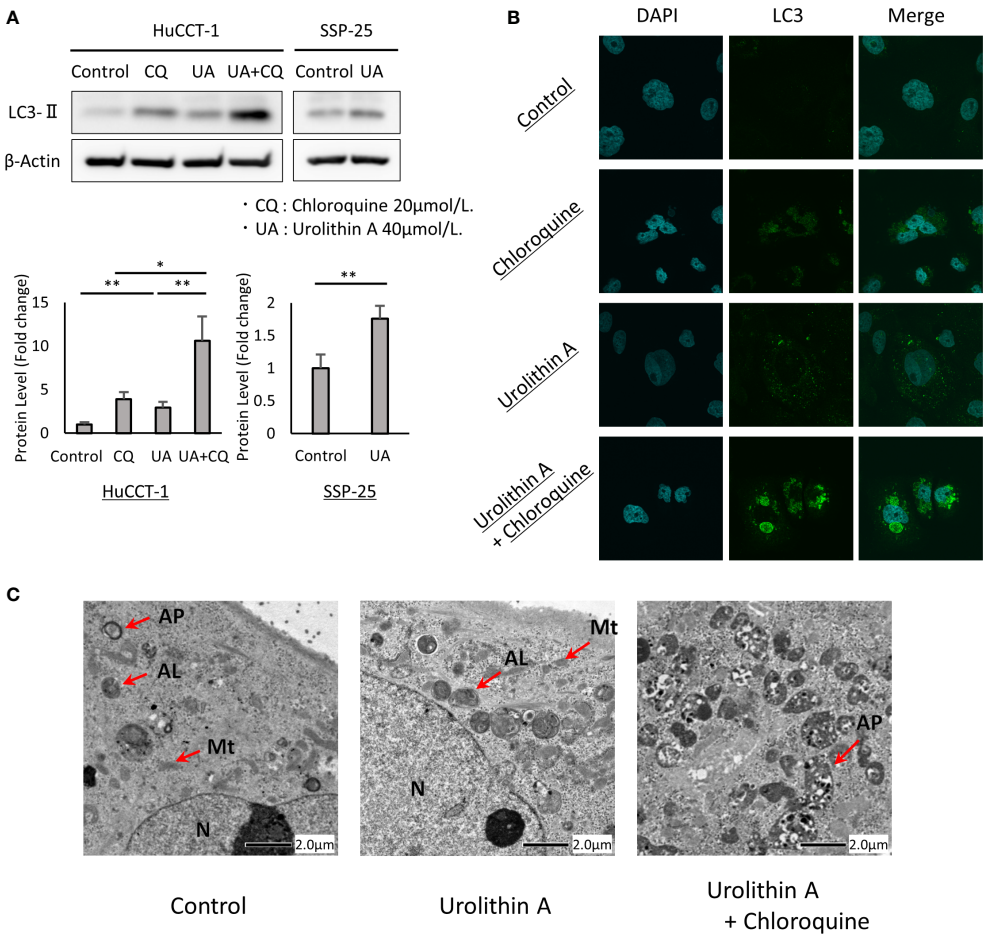

FIGURE 3
UA-mediated upregulation of autophagy in cholangiocarcinoma cells. **(A)** HuCCT-1 and SSP-25 cells were treated with 20 μmol/L CQ and 40 μmol/L UA for 24 h Autophagy was detected by Western blotting for LC3-II. β-actin was used as an internal loading control. LC3-II levels were normalized against β-Actin and represented the means of three independent experiments. Bars, standard deviation; *P < 0.05; **P < 0.01. **(B)** Immunofluorescence for LC3 (green) was performed after the same treatment as shown in **(A)**. Blue staining denotes DAPI-labeled nuclei. **(C)** Electron microscopy after the same treatment as shown in **(A)**. N, nucleus; Mt, mitochondrion; AP, autophagosome; AL, autolysosome.

IGF-1, known as the AKT-WNK1 signal activator, demonstrated that UA downregulated phospho-WNK1 levels even in HuCCT-1 cells treated with IGF-1. We further assessed the mTOR activity, which is another pathway for induction of autophagy, by measuring phosphorylation of mTOR, and found that the mTOR pathway was not affected by UA treatment and WNK1 knockdown (Figure 5D).

Discussion

Systemic chemotherapy with a combination of gemcitabine and is globally considered the standard first-line therapy for advanced CCA (27). However, effective chemotherapy for CCA is still limited, and the development of new therapies has not proressed sufficiently. Many targeted therapies for CCA, targeting FGFR2 fusions (28), IDH mutations (29, 30), major downstream pathways (31), and growth factor receptors (32), have been reported. However, clinical trials on therapies that appeared promising on basic research have not led to clinical breakthroughs due to various challenges (33).

UA is a metabolite generated by intestinal bacteria after ingestion of EA- and ET-rich foods and health supplements (34). UA is reported to have antitumor effects in many cancers, such as lung, prostate, colon, bladder, pancreatic, and neuroblastoma cancers (15–21). Given that UA undergoes enterohepatic recirculation (24), we speculated that UA might have significant antitumor effects in CCA, which grows in a special environment that is constantly exposed to both blood and bile. In this study, we demonstrated that UA showed antitumor

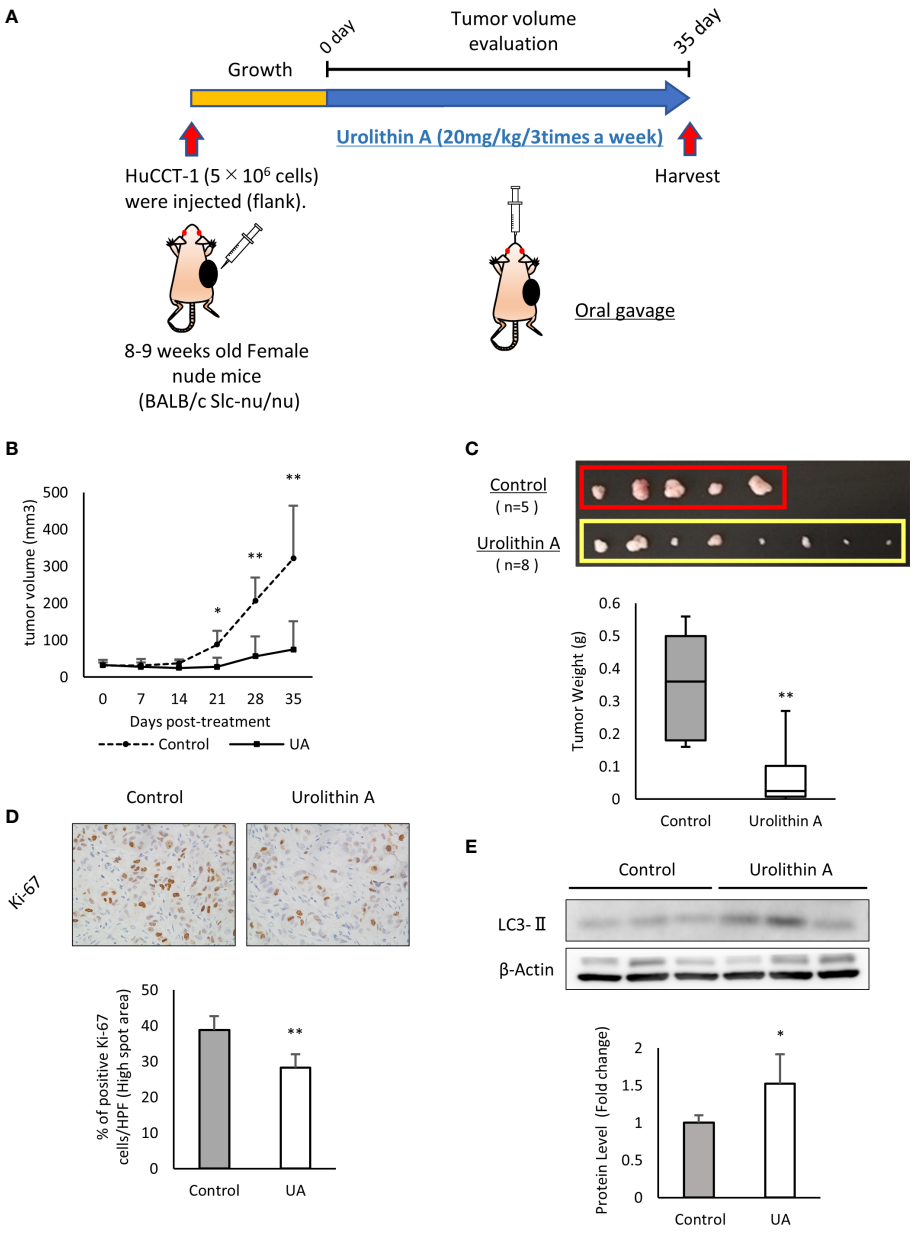

FIGURE 4
UA inhibits xenograft tumor growth *in vivo*. **(A)** Experimental design for UA treatment in the xenograft model. HuCCT-1 cells were injected into the flank of nude mice. UA (20 mg/kg, 3 times a week) or DMSO (control) were administered by oral gavage for 35 days. **(B)** The volume of subcutaneous tumors in the xenograft model was measured twice a week. Data represent the means of the control group (n = 5) or the UA group (n = 8). Bars, standard deviation; *P < 0.05; **P < 0.01. **(C)** Representative macroscopic images of tumors in nude mice obtained at day 35 after the start of the treatment. Data represent the means of the control group (n = 5) or the UA group (n = 8). Bars, standard deviation; **P < 0.01. **(D)** Representative Ki67-stained immunohistochemical images of the two groups. The positive rates of Ki67 staining were quantified by measuring five high spot areas from each tumor. Data represent the means of the control group (n = 4) or the UA group (n = 4). Bars, standard deviation; **P < 0.01. **(E)** Autophagy was detected by Western blotting for LC3-II in the two groups. β-actin was used as an internal loading control. LC3-II levels were normalized against β-Actin and represented the means of three independent experiments. Bars, standard deviation; *P < 0.05.

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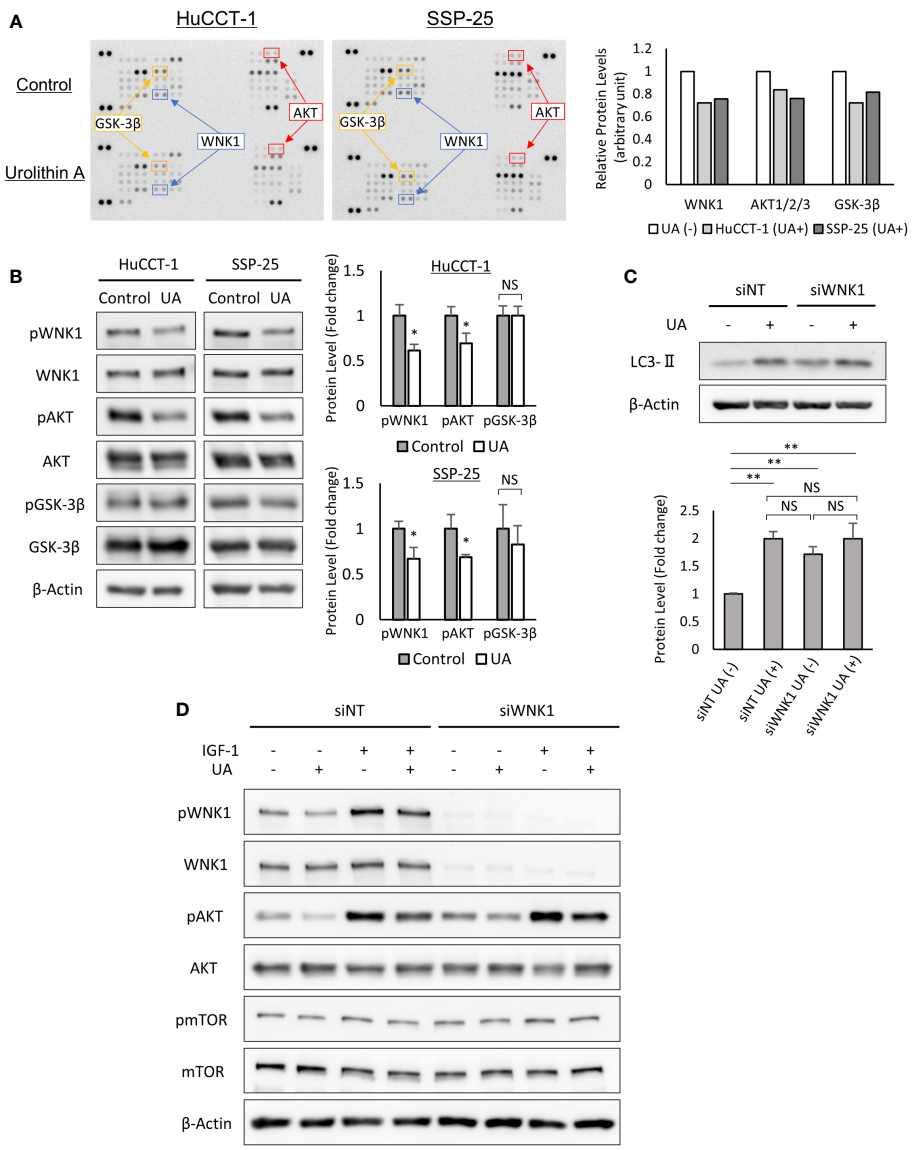

FIGURE 5
UA treatment downregulated AKT and WNK1 pathways, and induced autophagy in cholangiocarcinoma cells. **(A)** HuCCT-1 and SSP-25 cells were treated with 40 μmol/L UA for 3 h and analyzed using the human Phospho-Kinase array. Relative levels of protein phosphorylation (normalized intensity for each antibody) were quantified as a ratio of the UA-treated sample to the untreated one. **(B)** Results of the human Phospho-Kinase array were verified by Western blotting. β-actin was used as an internal loading control. Protein phosphorylation levels were normalized against β-Actin and represented the means of three independent experiments. Bars, standard deviation; NS, not significant; *P < 0.05. **(C)** Western blotting for LC3-II in WNK1 knocked down HuCCT-1 cells. Cells were treated with 40 μmol/L UA for 24 h β-actin was used as an internal loading control. LC3-II levels were normalized against β-Actin and represented the means of three independent experiments. Bars, standard deviation; NS, not significant; **P < 0.01. **(D)** Western blotting for WNK1 (Thr60 and total), mTOR (Ser2448 and total), and AKT (Ser473 and total) in HuCCT-1 cells transfected with control (siNT) or WNK1-specific (siWNK1) small interfering RNAs. Cells were treated with 40 μmol/L UA and 50 ng/mL IGF-1 for 3 h.

effects by inhibiting cell viability, migration, and invasion in HuCCT-1 and SSP-25 cells (Figure 1). In addition, UA administration dramatically reduced tumor growth in a xenograft mice model (Figure 4).

The mechanism of the antitumor effects of UA is characterized by various factors that regulate intracellular molecule targets, ultimately influencing cancer cell survival. Our data suggested that UA showed antitumor effects mainly *via* autophagy in cholangiocarcinoma cells (Figure 3). Autophagy is a self-degradative process required to maintain cellular homeostasis, development, differentiation, survival, and death (35). In cancer, suppression or induction of autophagy can

exert antitumor effects through promotion of cell death or survival, which are two main therapeutic targets (36). Thus, it is essential to identify key autophagy targets for new therapeutic agents. Previous studies have reported a cross-talk relationship between autophagy and apoptosis in anti-tumor therapy (37), but in our study, UA treatment did not lead to apoptosis (Figure 2). We suggest that UA significantly affects cancer cell survival by inducing autophagy in cholangiocarcinoma.

Autophagy is mainly mediated through the PI3K/Akt/mTOR and AMPK/mTOR signaling pathways, the molecular mechanisms by which mTOR kinase induces autophagy (36). We examined the change in mTOR phosphorylation after UA treatment and found that UA did not cause any change in mTOR phosphorylation (Figure 5D). Kankanamalage et al. reported that reduced WNK1 expression accelerates autophagy independently of the mTOR signaling pathway (38). In concordance with that report, we found that WNK1 knockdown induced autophagy regardless of the mTOR signaling pathway in HuCCT-1 cells (Figures 5C, D).

WNKs (With-no-lysine kinases) are a family of four serine-threonine protein kinases, WNK1–4, with an atypical placement of the catalytic lysine (39). Initial attention was focused on these enzymes as regulators of blood pressure because mutations of two family members, WNK1 and WNK4, caused pseudohypoaldosteronism type II, a heritable form of hypertension (39). WNK1 was also reported to be involved in PI3K-AKT pathway activation in several cancers (40). Likewise, our study indicated that IGF-1 stimulation upregulated AKT phosphorylation in WNK1 knockdown cells, indicating that WNK1 was downstream of AKT (Figure 5D). From these results, we proposed a schematic representation of the signaling pathway involved in the inhibition of cancer growth by UA-modulation of the AKT/WNK1 axis (Figure 6).

According to recent pharmacokinetic studies, UA is reported to undergo phase-II metabolism, to be mainly glucuronides, after absorption (41). Several *in vitro* studies showed that UA phase-II metabolites have lower bioactivity than deconjugated UA, including anti-tumor effects and inflammation (42–44). However, in the present study, UA oral administration exerts significant anti-tumor effects in xenograft model. Some reports indicated UA glucuronides are susceptible to β-Glucuronidase, which is known to present at high concentration in the microenvironment of most solid cancers (45, 46). On the basis of these findings, we speculated that β-Glucuronidase might be related to deconjugation of UA in *in vivo* study. Further investigation is needed.

In terms of safety of UA supplementation, a human clinical study revealed that UA was biologically safe and improved mitochondrial function in older adults (47). A recent randomized, double-blind, placebo-controlled clinical study demonstrated that daily 1000-mg UA supplementation in healthy older adults for 4 months was biologically safe, and improved muscle endurance and mitochondrial health (48). In our study, the UA dose used in mice (20 mg/kg) was convertible to a human equivalent dose (HED) of approximately 1.62 mg/kg for adults (49), and is expected to be safe. The potential clinical application of UA appears promising on the basis of its safety and benefits.

Collectively, our *in vitro* and *in vivo* data revealed that UA exerted antitumor effects by suppressing the AKT/WNK1 signaling pathway and inducing autophagy. Thus, UA, a natural, well-tolerated compound, may be a promising therapeutic candidate for advanced CCA.

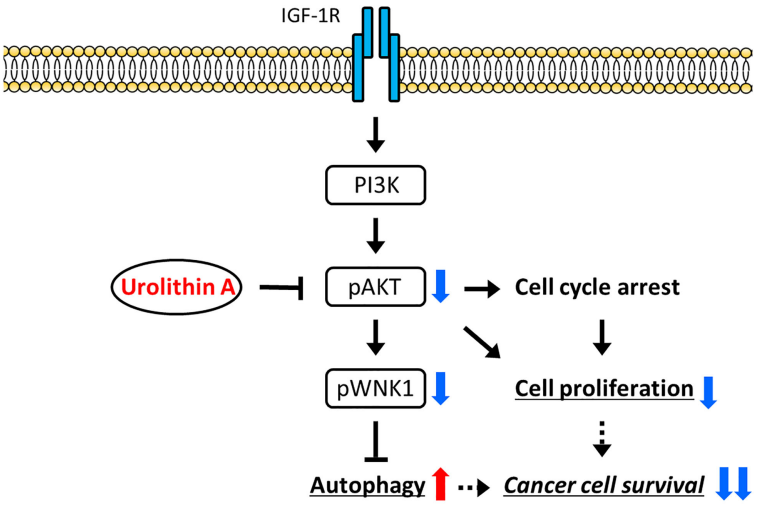

FIGURE 6
A proposed model of the mechanism. UA treatment reduces cell proliferation by inhibiting the activation of AKT, and inducing autophagy *via* the WNK1 pathway. As a result, cancer cell survival is suppressed by UA in cholangiocarcinoma cells.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Nagoya City University Center for Experimental Animal Science.

Author contributions

AK and HS, MY designed the study, and drafted the paper. HS mainly contributed *in vitro* and *in vivo* data on cholangiocarcinoma cells. MN and NJ, KK, GA, TT performed *in vivo* experiments. MY and IN, YH, YK analyzed the data. SA and KH, HK finalized and revised the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.963314/full#supplementary-material

SUPPLEMENTARY FIGURE 1
UA treatment inhibits cell proliferation and induces G2/M phase cell cycle arrest in cholangiocarcinoma cell lines. HuCCT-1 and SSP-25 cells were treated with 0 or 40 µmol/L UA for 24 and 72 h. Cell cycles were determined using flow cytometry. Data represent the means of three independent experiments. Bars, standard deviation; **P < 0.01.

SUPPLEMENTARY FIGURE 2
Positive control for the apoptosis assay. HuCCT-1 cells were treated with 30 µmol/L Camptothecin for 24 h, and then stained with annexin-V FITC and PI. Apoptosis cells were evaluated using flow cytometry.

SUPPLEMENTARY FIGURE 3
Effects of UA on apoptosis progression. HuCCT-1 cells were treated with 0 or 40 µmol/L UA for 48 or 72 h, and then stained with annexin-V FITC and PI. Apoptosis cells were evaluated using flow cytometry.

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The role of robotic surgery for the treatment of hilar cholangiocarcinoma: A systematic review

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Background: The role of robotic surgery (RS) for hilar cholangiocarcinoma (HC) is under investigation. Surgical resection is the only curative modality of treatment but extremely complex and high risk of morbidity and mortality may occur. The aim of this study is to perform a systematic review of perioperative and oncological outcomes of RS for HC, across a comprehensive range of outcomes reported in recent literature.

Materials and Methods: PRISMA checklist was used as a basis for writing the systematic review and studies' selection. Literature documenting RS for HC was analyzed by searching PubMed and Cochrane Library from 2009 to May 2022. The search terms, either independently or in combination, were used according to PICOT framework. The target population are patients treated with robotic surgical approach for HC.

Results: 12 studies with 109 patients were included after screening process. The Bismuth classification in all series except one was: 21 type I, 7 type II, 12 type IIIa, 26 type IIIb and 4 type IV. Mean operative time for a total of 21 patients was 644 minutes. Other two case series reported a median operative time of 375 with a console time of 276 minutes. Mean blood loss for case reports and two case series was 662 milliliters. Blood transfusion rate for all operation was 33.3%. Overall Conversion rate was 2.8%. Pooled post operative morbidity and mortality was 39.8% and 1.8% respectively. Mean LOS for case reports and one case series for a total of 17 patients was 16 days. R0 resection rate for the 11 papers was 74.3%. Seven out of 12 studies reported on the oncological follow up: median observation time ranged from 5 to 60 months, recurrence rate was 52.6% (range 0-90%) reported only in 19 patients (10/19).

Conclusions: RS for HC was feasible and safe. However, although this systematic review could not be conclusive in most of the analyzed items, RS for the treatment of HC could represent the best tool for a future meticulous

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and precision surgery. The review's results certainly indicate that further research in urgently is required on this field.

KEYWORDS

hilar cholangiocarcinoma, Klatskin tumor, robotic liver resection, liver resection, biliary tumor

Introduction

Biliary malignancies are the second most prevalent primary liver neoplasia, following hepatocellular carcinoma, and their incidence is on the rise, with an estimated 1.8% annual increase (1). Hilar cholangiocarcinoma (HC) is a rare hepatobiliary malignancy, with an incidence of 1.2 cases/100,000 people in Western countries (2). HC, also referred to as Klatskin tumor (KT), arises from the epithelial cells of the bile ducts and presents a highly aggressive behavior with a high propensity for vascular, perineural, and liver invasion. Due to the late onset of symptoms and lack of effective non-surgical treatments, the mean disease-specific survival is still less than 1 year after diagnosis (2).

Surgical resection with curative intent has been recognized as the primary therapy and sole procedure for curing patients with resectable local disease (3). Oncological biliary tract resection is one of the most challenging abdominal procedures, with high rates of major morbidity and recurrence. Less bleeding, minimal liver damage, and a good oncological outcome are the cornerstones for the treatment of HC. The optimal surgical treatment for an oncological resection of HC is radical extrahepatic bile duct resection in conjunction with major hepatectomy, radical lymphadenectomy, and Roux-en-Y hepaticojejunostomy reconstruction (4, 5).

Robotic surgery (RS) has recently emerged as an alternative for minimally invasive liver surgery; however, its role in biliary tract cancer remains unclear. RS will find its place in hepatobiliary surgery since it can facilitate the most technically challenging procedures such as biliary anastomoses. A robotic approach has been introduced to overcome some of the typical limitations of laparoscopy, including a wider range of movements and enhanced instrument dexterity, a three-dimensional view of the surgical field, a reduction in surgeon tremors, and a shorter learning curve. RS has demonstrated promising results in terms of morbidity, mortality, length of hospital stay, and postoperative recovery in a subset of patients (6). Furthermore, an improvement in perioperative outcomes could have an impact on oncological results, thereby improving long-term survival and recurrence rates. The role of RS in HC remains a subject of discussion, as it is still debatable whether it can produce optimal and appropriate results. In the past 12 years, only a few case reports or small single-center case series have examined the efficacy of RS in the surgical treatment of HC.

The aim of this study is to conduct a comprehensive systematic review of the perioperative and oncological outcomes of RS for HC, as reported in recent literature. The ultimate objective is to demonstrate RS' state of the art, while taking into account safety, feasibility, and efficacy in this new frontier of treatment for KT.

Materials and methods

Literature search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist formed the basis for writing the systematic review, and the PRISMA flow diagram was used to report the selection of studies (7). All steps were performed independently by two authors (FAC and MR). Final decisions on eligibility were resolved by consensus.

The literature documenting RS for HC was analyzed by searching PubMed and The Cochrane Library from 2009 to May 2022. Independent or combination search terms were used according to the PICOT framework (Figure 1). The keywords or combinations used were as follows: (hilar cholangiocarcinoma OR perihilar cholangiocarcinoma OR Klatskin tumor) AND (minimally invasive surgery OR robot OR robotic-assisted surgery) AND (liver resection). A methodical search was conducted for relevant systematic reviews, randomized controlled trials, observational studies (prospective or retrospective cohort and case–control or matched case–control studies), case series, and reports using a search strategy guided by oncological or surgical information, abstract, and keywords related to our research question. Only published articles in the English language were screened. With the exception of multicenter studies, articles with the largest series or the most recent publication date were selected when more than one article was reported by the same institution or author.

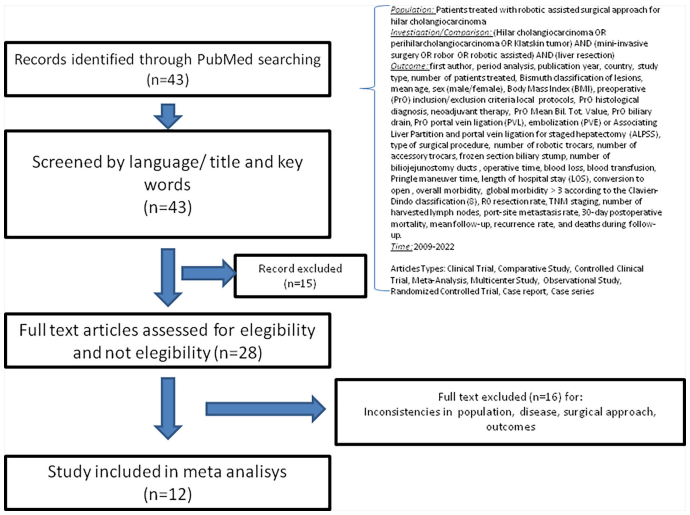

FIGURE 1
Flow diagram depicting the systematic review's article selection process based on the PRISMA diagram (7).

Study inclusion criteria

The titles and abstracts of all the studies were screened and a full text assessment was then conducted. Patients treated for HC with robotic liver resection surgery with or without biliary tract resection, cholecystectomy, and lymphadenectomy were the target population. Studies were eligible if they included an evaluation and report on one of the perioperative or oncological outcomes of the robotic resection performed for HC. All types of resections for KT were included. The PICOT (Population, Intervention, Comparison, Outcome, and Time) framework (Figure 1) was used to define the study selection criteria. The following studies or data were excluded: robotic surgical procedures for staging or palliative care, those that lacked HC cases in the results, and the lack of a robotic approach, abstracts, editorials, or reviews. The quality of the primary studies was not a criterion for exclusion.

Outcomes

The following data, clinical, and oncological outcomes were collected: first author, period analysis, publication year, country, study type, number of patients treated, Bismuth classification of lesions, mean age, sex (male/female), body mass index (BMI), preoperative (PrO) inclusion/exclusion criteria local protocols, PrO histological diagnosis, neoadjuvant therapy, PrO Mean Bil. Tot. Value, PrO biliary drain, PrO portal vein ligation (PVL), embolization (PVE) or Associating Liver Partition and portal vein ligation for staged hepatectomy (ALPSS), type of surgical procedure, number of robotic trocars, number of accessory trocars, frozen section biliary stump, number of biliojejunostomy ducts, operative time, blood loss, blood transfusion, Pringle maneuver time, length of hospital stay (LOS), conversion to open, overall morbidity, global morbidity > 3 according to the Clavien–Dindo classification (8), R0 resection rate, TNM staging, number of harvested lymph nodes, port-site metastasis rate, 30-day postoperative mortality, mean follow-up, recurrence rate, and deaths during follow-up.

Data extraction and quality assessment

Two reviewers (FAC and MR) independently screened the titles, abstracts, and full texts of the selected studies and extracted demographic and clinical outcome data. In the case of disagreement, they reviewed the papers together to reach consensus. The methodological quality of the studies was evaluated using the Oxford Centre for Evidence Medicine's critical appraisal tool, checklists of the Dutch Cochrane Centre, BMJ editor's checklists, and the checklists of the EPPI Centre (9). The overall quality of the primary studies was rated as either very low, low, moderate, or high.

Statistical analyses

All of the analyses were conducted using data from the included studies. When available, patient characteristics and outcomes were reported as numbers or percentages, mean ±

standard deviation, or median (interquartile range or range), as reported in the primary studies.

To provide a pooled estimate of the outcomes, we calculated the total percentages of dichotomous outcomes by adding the numbers of events and patients from the original primary studies. Some of the included studies reported continuous outcomes with means and no standard deviations, while others reported medians and ranges. These heterogeneities in the effect measure prevented us from combining certain outcomes globally (operative time, blood loss in milliliters, Pringle time in minutes, number of lymph nodes, and length of stay). However, for such outcomes, we calculated the mean of case reports and case series that reported outcomes for individual patients. Moreover, due to the lack of data across studies, we were unable to provide a pooled estimate of the port site. Analyses were carried out using Stata and Excel software.

Results

Study characteristics and population

The flowchart in Figure 1, which depicts the selection of articles for the systematic review, is based on the PRISMA diagram. The initial search returned 43 English-language results. After examining the titles and keywords, 15 citations were eliminated as they were deemed irrelevant. Twenty-eight studies were assessed for eligibility through full-text evaluation. Due to inconsistencies in population, disease, surgical approach, or outcomes, 14 records were removed following full-text screening. The remaining 12 studies (10–21) were included in the quantitative synthesis of this systematic review. Eight case reports (10, 12, 14, 16, 17, and 19-21) and four case series (11, 13, 15, and 18) describing only single-center RS experiences for HC were selected. Four were from China, three from Italy, three from the US, one from Brazil, and one from Spain. No randomized controlled trials were retrieved. All the results are summarized in Tables 1 and 2. Table 3 details the quality assessment of each included study. All studies were deemed to be of low or very low quality.

The analysis included a total of 109 patients, with the largest study reporting a series of 48 cases (15). Except for one case report (12), all the subjects in this review were over 54 years old. Only one study reported preoperative exclusion criteria for robotic surgical resection, along with preoperative neoadjuvant therapy, major underlying disease, Bismuth type IV, and stage beyond T4 (18). Information regarding preoperative diagnosis and preoperative biliary drainage was present in six (10, 16, and 18-21) and seven studies (10, 12, 13, 16, 18, 19, and 21), respectively. With one exception, extension of local disease was expressed according to the Bismuth classification in all series and reports (11). Except for one series (12), the Bismuth classification was as follows: 21 type I, 7 type II, 12 type IIIa, 26 type IIIb, and 4 type IV. Only four studies (10, 12, 18, and 20) were linked to PrO future liver remnant (FLR) evaluation and applied portal vein embolization or ligation or ALPSS to prevent postoperative liver failure. Robotic major liver resection enlarged to segment 1, biliary carrefour resection, and lymphadenectomy were performed in 72/109 cases (66%). With one exception (11), all studies disclosed information regarding the frozen section of the biliary stump. Only two authors (18, 20) reported more than one bile duct anastomosis.

Operative time was provided by all series except one (12). Case reports (10, 14, 16, 17, and 19-21) and two case series (13, 18) for a total of 21 patients had a mean operative time of 644 min. Another two case series reported median operative times of 375 (11) min and a console time of 276 min (15). Data on blood loss and blood transfusion rate were reported in all series except two (11, 19). The mean blood loss for case reports (10, 12, 14, 16, 17, 20, and 21) and two case series (13, 18) for a total of 21 patients was 662 milliliters. The case series by Li et al. reported a median of 150 ml for 48 patients (15). The blood transfusion rate for all operations was 33.3% (22/66). All studies reported the conversion rate, and its global ratio was 3/109, resulting in a total percentage of 2.8%. Only one article discussed the reasons for conversion (18).

One paper failed to provide complete data on postoperative morbidity (19). The pooled postoperative morbidity rate was 43/108 (39.8%). The stratified incidence of combined morbidity for severe complications (Clavien–Dindo classification grade ≥ 3) was 8/69 (11.6%). All studies reported the postoperative mortality rate, with a pooled total of 2/109 (1.8%). Postoperative deaths were caused by abdominal infection (11) and liver failure (13).

Two studies (11, 16) did not provide data on LOS. The mean LOS for case reports (10, 12, 14, 17, and 19-21) and one case series (13) for a total of 17 patients was 16 days; the case series by Li et al. (15) reported a median of 9 days (range, 4–52) and the case series by Cillo et al. (18) also reported a median of 9 days.

Pathological TNM staging was reported in all but six studies. According to data from four studies (14, 17, 20, and 21), the mean number of harvested lymph nodes was 14. Only one study (11) failed to report data on the status of margins at the final pathological examination. The rate of R0 resection for the 11 papers analyzed was 74.3% (52/70). Seven out of 12 studies reported on the oncological follow-up: the median observation time ranged between 5 and 60 months, and the recurrence rate was 10/19 (52.6%; range, 0–90%) (10, 13, 16, and 18-21). Inadequate data and the heterogeneity of the study population or metrics employed prevented a cumulative analysis of disease and overall survival. It should be highlighted that the patients included in these case reports and series may have been clinically selected. This may limit the generalizability of results for a larger population of patients with the same disease.

TABLE 1 Studies on robotic surgery for HC: study, patients, and procedural characteristics.

Study	Year	Study period	Study type	No. of patients	Bismuth type	Age (years)	Male/ Female	BMI	Inclusion/ exclusion criteria	Pre op biopsy positive	Neadj therapy	Bil Tot value	Pre op biliary drain (*n*, %)	Pre op PVE/PVL/ ALPSS (*n*, %)
Giulianotti et al. (10)	2010	2009	Case Report	1	3a	66	1M	23	na	1/1 (100%)	no	na	1/1 (100%)	1 PVE
Liu et al. (11)	2012	2009–2011	Case series	39	na	na	na	na	na	na	na	na	na	na
Zhu et al. (12)	2014	2011	Case Report	1	IIIa	43	M	na	na	na	na	23 mmol	1/1 (100%)	PVE
Xu et al. (13)	2016	2009–2012	Case series	10	1 type II/1 type IIIb/4 type IIIa/4 type IV	57.6*;54**	8M/2F	na	na	na	na	145.6 mmol/L *	6/10 (60%)	na
Quijano et al. (14)	2016	2011–2014	Case Report	1	IIIb	na	na	na	na	na	na	na	na	na
Li et al. (15)	2020	2017–2019	Case series	48	20 type I/6 type II/5 type IIIa/17 type IIIb	62.9*	28M/20F	2.7*	na	na	0/48 (0%)	30 pts (62.5%)	na	na
Machado et al. (16)	2020	2019	Case Report	1	IIIb	76	F	30	na	1/1 (100%)	1/1 (100%)	na	1/1 (100%)	na
Marino et al. (17)	2020	2019	Case Report	1	IIIb	57	1M	na	na	na	na	5.2 mg/dl	na	na
Cillo et al. (18)	2021	2019–2020	Case series	4	4-3b	60.5*	1M/3F	na	Y	3/4 (75%)	1/4 (25%)	4.67 *	4/4 (100%)	0/4 (0%)
Sucandy et al. (19)	2021	2020	Case Report	1	IIIb	77	1F	na	na	1/1 (100%)	na	na	1/1 (100%)	na
Di Benedetto et al. (20)	2022	2021	Case Report	1	3a	74	1F	na	na	1/1 (100%)	no	na	na	1 ALPSS + PVE
Tee et al. (21)	2022	2021	Case Report	1	I	58	M	49		1/1 (100%)	0/1 (0%)	na	1/1 (100%)	na

ALPSS, associating liver partition and portal vein ligation for staged hepatectomy; BMI , body mass index; F , female; HC , hilar cholangiocarcinoma; M , male; na, not available; PVE , portal vein embolization; PVL, portal vein ligation; * , mean; ** , median. na, not applicable.

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TABLE 2 Studies on robotic surgery for HC: study, patients, and procedural characteristics.

Study	Surgery	Nr Rob trocars	Nr Acc Trocar	Frozen section bil stump	Nr ducts of biliary anasto	Operative time (min)	Blood loss (ml)	Blood trasfusion	Pringle time (min)	LOS (days)	Conversion to open (*n*, %)	Global morbidity (*n*, %)	Morbidity >3 Clavien–Dindo	R0 res rate (*n*, %)	TNM	Number of lymph nodes	Port site metastasis (*n*, %)	Post-operative death (*n*, %)	Follow-up (months)	Recurrence rate (*n*, %)	Deaths during follow-up
Giulianotti et al. (10)	Right ext Hep+S1 +limphadenectomy+biliary carrefour res+hepatico-jejunostomy	4	1	1/1 (100%)	1	540	800	1/1 (100%) with 1 EC	0/1 (0%)	11	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	T2N0	na	na	0/1 (0%)	8	0/1 (0%)	0/1 (0%)
Liu et al. (11)	L Hep 3/biliary res and hepjejunostomy 15/others 21	4	2	na	na	355*; 375**	na	na	na	na	1/39 (2.6%)	1/39 (2.6%)	na	na	na	na	1/39 (2.6%)	1/39 (2.6%)	na	na	na
Zhu et al. (12)	Right Hep+S1+lymphadenectomy +biliary carrefour res+hepatico-jejunostomy	na	0/1 (0%)	1/1 (100%)	na	na	700	na	na	14	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	na	na	na	0/1 (0%)	na	na	na
Xu et al. (13)	R (5)/L (4)/trisegm Right(1)Hep enl Sg1, biliary tract res and lymphadenectomy	4	2	8/10 (80%)	na	703*	1360*	6/10 (60%)	na	16 **	0/10 (0%)	9/10 (90%)	3/10 (30%)	7/10 (70%)	na	na	na	1/10 (10%)	60	9/10 (90%)	na
Quijano et al. (14)	Left Hep+S1+lymphadenectomy +biliary carrefour res+hepatico-jejunostomy	4	2	1/1 (100%)	na	510	1000	na	na	16	1/1 (100%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	T2N0	13	na	0/1 (0%)	na	na	na
Li et al. (15)	R/L Hep enl Sg1, biliary tract res and lymphadenectomy	4	1	48/48 (100%)	na	276** (only console time)	150*	13/48 (27.1%)	14/48 (29,2%)	9**	0/48 (0%)	28/48 (58.3%)	5/48 (10.4%)	35/48 (72.9%)	na	na	na	0/48 (0%)	na	na	na
Machado et al. (16)	Left Hep+S1+lymphadenectomy +biliary carrefour res+hepatico-jejunostomy	4	1	1/1 (100%)	1	480	740	2 ec	na	na	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	T1aN0	na	na	0/1 (0%)	5	0/1 (0%)	0/1 (0%)
Marino et al. (17)	Left Hep+S1+lymphadenectomy +biliary carrefour res+hepatico-jejunostomy	4	2	1/1 (100%)	1/1 (100%)	295	280	na	0/1 (0%)	6	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	na	9	na	0/1 (0%)	na	Na	na
Cillo et al. (18)	Left Hep+S1+lymphadenectomy +biliary carrefour res+hepatico-jejunostomy	4	4	4/4 (100%)	2,75*	850*	840*	0/4 (0%)	19,25*	9**	1/4 (25%)	3/4 (75%) bil first grade A, ileous, asympt segm ileous ischemia	0/4 (0%)	3/4 (75%)	T3Nx/ T2aN1/ T4N1/ T4N0	>14 on 3/ 4 and 0 on 1 pt	na	0/4	7,5**	1/4 (25%)	0/4 (0%)
Sucandy et al. (19)	Left Hep+S1+lymphadenectomy +biliary carrefour res+hepatico-jejunostomy	na	na	1/1 (100%)	1	360	na	na	na	6	0/1 (0%)	na	na	1/1 (100%)	na	na	na	0/1 (0%)	12	0/1 (0%)	0/1 (0%)
Di Benedetto et al. (20)	Right ext Hep+S1 +lymphadenectomy+biliary carrefour res+hepatico-jejunostomy	4	2	1/1 (100%)	2	370 (previous ALPSS)	450	0/1 (0%)	0/1 (0%)	19	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	T4N1	21	na	0/1 (0%)	13	0/1 (0%)	0/1 (0%)
Tee et al. (21)	bile duct res, cholecystectomy, hilar lymph and roux hep jejunostomy	5	3	1/1 (100%)	1	540	100	0/1 (0%)	na	5	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	T2N1M0	12	na	0/1 (0%)	12	0/1 (0%)	0/1 (0%)

ALPSS, associating liver partition and portal vein ligation for staged hepatectomy; LOS, length of stay; *, mean; **, median. na, not available.

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TABLE 3 Critical appraisal of the included studies based on the Critical Appraisal tool of the Center for Evidence-Based Management-CENMa (9).

Study	Year	Did the study address a clearly focused question/ issue?	Is the research method (study design) appropriate for answering the research question?	Are both the setting and the subjects representative with regard to the population to which the findings will be referred?	Is the researcher's perspective clearly described and taken into account?	Are the methods for collecting data clearly described?	Are the methods for analyzing the data likely to be valid and reliable? Are quality control measures used?	Was the analysis repeated by more than one researcher to ensure reliability?	Are the results credible, and if so, are they relevant for practice?	Are the conclusions drawn justified by the results?	Are the findings of the study transferable to other settings?	Overall level, and quality of evidence
Giulianotti et al. (10)	2010	Yes	Cannot tell	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Level IV, very low quality
Liu et al. (11)	2012	Yes	No	Cannot tell	Yes	No	No	Cannot tell	Yes	Yes	Yes	Level IV, very low quality
Zhu et al. (12)	2014	Yes	Cannot tell	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Level IV, very low quality
Xu et al. (13)	2016	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Level IV, low quality
Quijano et al. (14)	2016	Yes	Yes	Cannot tell	Yes	Yes	No	Yes	Yes	Yes	Yes	Level IV, very low quality
Li et al. (15)	2020	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Level IV, low quality
Machado et al. (16)	2020	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Level IV, low quality
Marino et al. (17)	2020	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Level IV, very low quality
Cillo et al. (18)	2021	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Level IV, low quality
Sucandy et al. (19)	2021	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Level IV, very low quality
Di Benedetto et al. (20)	2022	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Level IV, low quality
Tee et al. (21)	2022	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Level IV, low quality

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Discussion

HC is a malignant disease with a poor prognosis and survival rate (22). Even among patients with localized disease, few tumors are amenable to anatomical radical resection due to a high incidence of local invasion and proximity to hilar hepatic vessels. Thus, very aggressive surgical treatment is generally required to increase the rate of curative resection and long-term survival (23). The results of this systematic review are based on the analysis of a small number of studies with a high probability of bias. In addition, the total number of enrolled subjects is very small, and the overall experience is much lower than that of the published counterpart treated with conventional open surgery.

Both open and minimally invasive surgical approaches for HC are acknowledged to be technically challenging due to the anatomical characteristics of the hepatic hilum and the biological characteristics of cholangiocarcinoma, which requires clean radial margins for curative resection. The complexity of these procedures stems from the need for precise hilar dissection and lymphadenectomy, as well as biliary reconstruction, which has primarily acted as a barrier to the propagation of the laparoscopic technique. Compared to minimally invasive surgery for hepatocellular carcinoma, minimally invasive resection for HC is a relatively new field in HPB surgery. However, the rapid development of surgical expertise and equipment has increased the use of laparoscopic and robotic techniques to treat all Bismuth classification grades of KT (24). In 2010, Giulianotti et al. (10) used the Da Vinci Robotic Surgical System (Intuitive Surgical, Sunnyvale, CA, USA) to perform an extended right hepatectomy, biliary carrefour resection, and left hepaticojejunostomy for HC. Their experience demonstrated the technical feasibility of a robotic approach to HC. Among the advantages cited by the authors, the robotic platform provides surgeons with three-dimensional stereoscopic visualization, and EndoWrist enables surgeons to perform various complicated and challenging maneuvers, including biliary anastomoses, with greater stability than traditional laparoscopic instruments (25). In recent years, interest in and reliance on robotic approaches for treating lesions classified as Bismuth grades I–III have increased as a result of these initial experiences. In this review, we reported 71 major enlarged caudate lobe liver resections on 109 patients (65.1%). Robotic surgery is best suited for procedures requiring high level precision and dexterity, and the reconstructive phase is where the majority of technical benefits are reported. Our data demonstrated that robotic-assisted treatment of HC is feasible and reproducible. In particular, the curative intent of KT treatment has been maintained. However, only one of the case series included in this review specified inclusion and exclusion criteria as well as restrictive allocation criteria for the robotic approach (18). These results are indicative of a clear selection bias among the patients enrolled in all the studies under consideration. Xu et al. reported robotic liver resection for enlarged caudate lobe, lymphadenectomy, resection of the extrahepatic bile duct, and Roux-en-Y hepaticojejunostomy to treat Bismuth type-IV HC in four patients (13). It was concluded that resection indications for Bismuth type-IV HC should be determined with caution because trisectionectomy was associated with more uncontrollable accidents due to the extreme complexity of the procedures (13). The paper did not provide a way to split the outcome of KT type IV from the other types. In the context of the curative intention-to-treat program, it could not be ruled out that many KT type IV patients received suboptimal oncological surgical treatment due to restrictive allocation criteria for minimally invasive procedures. Due to the biological nature of cancer, restrictive selection criteria are necessary irrespective of the surgical approach (robotic or laparoscopic). Complex Roux-en-Y hepaticojejunostomy with single or multiple bile ducts were rarely described in a minimally invasive setting. The robotic approach, with its degree of freedom and stability, could be the best way to circumvent all laparoscopic limitations. One author performed biliary reconstruction of multiple bile stumps with a robotic technique in 75% of cases, and their paper confirmed that the robotic approach was the absolute novel opportunity to also perform minimally invasive hepaticojejunostomies even when multiple ducts are present (18).

No intraoperative accidents were reported, and the overall conversion rate in this review was 2.8%. These data are more favorable than the 10% reported by the best high-volume center series regarding minimally invasive liver resection (26). The shorter learning curve for robotic-assisted surgery compared to conventional laparoscopic surgery may likely account for these results (27). A recent meta-analysis focusing on distal pancreatic resections also confirmed this effect (28).

In this review, the mean operative time and mean blood loss were highly variable. The duration of robotic surgery is typically longer than that of an open or laparoscopic approach. However, the longer operative time is still under investigation. These findings are probably due to the fact that the learning curve for HC is still in its infancy. Chen et al. observed improvements in operative time after 52 cases of robotic major hepatectomy (29). Li et al. reported 48 cases of radical robotic resection for HC, with a median operative time that only takes into account the console time (276 min; range, 170–500 min) and a mean blood loss of 150 ml (range, 20–1,500 ml) (15). In 2020, Ratti et al. compared 16 cases of laparoscopic resection versus 32 cases of open approach for HC (30). In that study, the operative time, blood loss, and transfusion rate in the laparoscopic group vs. the open group were 360 ± 290 min vs. 275 ± 200 ml and 380 ± 250 ml vs. 470 ± 390 ml, respectively. In this review, the total

blood transfusion rate for all operations was 33.3%. In comparative studies by Zhang et al., the estimated blood loss and incidence rate of blood transfusion were 620.0 ± 681.2 ml and 57%, respectively (31). These data suggest that the robotic approach could further facilitate a precise and effective control of intraoperative bleeding.

This review reported good results in terms of morbidity and mortality. Pooled postoperative global morbidity was 39.8%, whereas morbidity stratified by severity according to the Clavien–Dindo classification ≥3 was 11.6%. The average pooled morbidity and mortality rates reported by open series were 14%–75% and 0%–17%, respectively (32). These data may indicate that the HC robotic approach is the final challenge in the learning curve, leading to improved outcomes.

Better survival rates depend on oncological outcomes. The overall analysis reveals a pooled R0 rate of 74.3%, which may be a satisfactory outcome when compared to large series of open surgery for HC (33). There was a paucity of data on survival and recurrence, and these data were unfortunately based on short follow-up and observation periods. Currently, information on postoperative HC recurrence is limited. Seven out of 12 studies reported on the oncological follow-up leading to a total recurrence percentage of 52.6%, ranging from 0%–90% (10, 13, 16, and 18-21). Lu et al. reported a 66.5% tumor recurrence rate after open resection with a median follow-up of 22.7 months (34).

The first report concerning the robotic approach for the treatment of HC was published in 2010 (10), 8 years after the first paper on robotic liver resection (35). In their review, Cipriani et al. reported fewer than 200 minimally invasive procedures (laparoscopic and robotic) for HC (32). The latter approach and its slow propagation are coincident with the technical challenges involved and the low resectable rate of KT. None of the centers that have dealt with RS in KT have identified any absolute contraindications to the robotic approach. The benefits of suturing in biliojejunostomy anastomosis and the advantageous possibility of performing liver resection in a restricted space have been identified with the use of the robotic approach. Different centers, different skills in HPB surgery, and substantial bias in patient selection and surgical procedures influence the final black-and-white results. Presently, the potential benefits in terms of short-term and oncological outcomes are only theoretical and must be investigated through a comparative study of laparoscopic and open approaches. This review supports the feasibility and efficacy of RS for HC after assessing surgical and oncological outcomes. The next step could be a multicentric comparative study to validate and strengthen the results. Randomized controlled trials will be necessary to further confirm this hypothesis.

Study limitations

This systematic review has several limitations. First, the literature search was carried out by only consulting the two most relevant scientific databases for medical practice (PubMed and The Cochrane Library). Second, the review was limited by the lack of randomized controlled trials, large observational cohort studies, and comparative studies in general. In fact, the totality of studies we found were case reports and case series. As a result, the quality of the included studies was rated as low or very low, limiting the strength and reliability of our results; however, a recent study has demonstrated that it is possible to write rigorous clinical practice guidelines and recommendations for rare diseases or areas where there is little or low- or very-low-quality evidence (36). Due to the absence of a control group, we were unable to conduct a comparative meta-analysis of outcomes and could only perform a descriptive pooled estimation on a subset of outcomes. Moreover, we combined results from the case reports (i.e., involving 1 patient) with a case series that involved a larger number of patients (4, 10, 39, and 48) without weighing the data. Finally, we were unable to statistically investigate the heterogeneity of studies. Different patient demographic characteristics (13, 14) and surgical intervention characteristics (11, 14) were the clinical heterogeneity sources in this review. It must be stated that, as robotic surgery is still in its infancy, the patients included in this review (i.e., in the primary studies, case reports, and series) may have been clinically selected patients. This may limit the generalizability of results and necessitate the application of this technique to a wider audience of patients with the same disease.

Implications for future research

Our systematic review provides preliminary evidence on oncological RS for HC. The review's results certainly indicate that this topic urgently requires additional research. Particularly, it would be of utmost importance to increase the number of patients (and the number of studies) on this topic, as well as to generate evidence of higher methodological quality in terms of study design, execution, and the reporting of findings.

Conclusion

Despite the fact that this systematic review was inconclusive, RS for the treatment of HC could certainly represent the best tool for future meticulous and precise surgery that is currently only possible with expert hands and extensive skill with liver RS.

To treat a disease as particular as KT, it is necessary to consider a number of specific aspects, including patient and center characteristics, organizational factors, and team acceptance. The main criticism in the majority of series is the very long operative time. However, if surgery must become increasingly precise today, RS for the treatment of HC may become one of the best indications and potentially the most suitable tool for quality surgery.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

AB, MR and FAC contributed equally to this work and share first authorship. AB, MR and FAC contributed to conception and design of the study. FAC organized the database. MR performed the statistical analysis. AB and FAC wrote the first draft of the manuscript. AB, MR, AP, GV, MB and FAC wrote sections of the manuscript. AP, GV, and MB contributed equally to this work about data analysis and revision. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case report: Conversion therapy to permit resection of initially unresectable hepatocellular carcinoma

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Most patients with hepatocellular carcinoma (HCC) are diagnosed when the disease is already at an advanced stage, so they are not eligible for resection and their prognosis is poor. The combination of transarterial chemoembolization (TACE) with immune checkpoint inhibitors or tyrosine kinase inhibitors can improve unresectable HCC to the point that patients can be treated with surgery. Here we describe two cases of such "conversion therapy". One patient was a 52-year-old man in Child-Pugh class A with treatment-naive HCC whose 11.3-cm tumor had invaded the middle hepatic vein and right branch of the portal vein. He was treated with TACE plus camrelizumab, and radical resection was performed 3 months later. No evidence of recurrence was observed during 5-month follow-up. The other patient was a 42-year-old man in Child-Pugh class A with HCC involving a 11.4-cm tumor and severe liver cirrhosis. The patient was treated with TACE and lenvatinib, but the embolic effect after one month was unsatisfactory, so the regional treatment was changed to hepatic artery infusion chemotherapy and transcatheter arterial embolization. Radical resection was performed 2 months later, and no recurrence was evident at 1-month follow-up. These cases demonstrate two conversion therapies that may allow patients with initially unresectable HCC to benefit from resection.

KEYWORDS

hepatocellular carcinoma, transarterial chemoembolization, immune checkpoint inhibitor, conversion therapy, tyrosine kinase inhibitor

Introduction

Globally, hepatocellular carcinoma (HCC) is the sixth most common malignancy and the third leading cause of cancer-related death (1). Surgery and liver transplantation are still the best radical treatments for HCC patients, which can provide good long-term survival. Unfortunately, about 70% of HCC patients are diagnosed at an advanced stage of the disease and are therefore ineligible for surgery (2). Recently, so-called "conversion therapies" have been described that can improve unresectable HCC enough that the patient can undergo resection, leading to much better prognosis (3, 4).

Several types of conversion therapy have been described, most often involving transarterial chemoembolization (TACE) (3). Immune checkpoint inhibitor (ICIs) and tyrosine kinase inhibitors (TKIs) have also proven promising for treating advanced HCC, alone and together (5, 6). Conversion therapies remain in the exploratory stage and there are no consensus standards.

Here we describe two patients with unresectable HCC in whom different types of conversion therapy proved effective at downgrading the cancer enough that the patients could be treated with resection, leading to recurrence-free survival.

Case reports

Case 1

A 52-year-old male patient was admitted to our hospital on May 22, 2021 due to pain in the right upper abdomen. He had been diagnosed with chronic infection with hepatitis B virus more than 20 years before, and he had a 10-year history of hypertension. Laboratory analysis revealed that the alpha-fetoprotein (AFP) level was 3.72 ng/ml, and the albumin level was 30.4 g/L (Supplemental Table 1). Dynamic enhanced computed tomography revealed multiple low-density shadows in the right lobe of the liver that were fused with one another (11.3 x 12.0 x 11.9 cm). Tumor invasion of the middle hepatic vein and right branch of the portal vein were observed, together with retroperitoneal lymph node metastasis (Figures 1A, B). The patient was assigned an Eastern Cooperative Oncology Group Performance Status (ECOG-PS) of 0, BCLC-C stage, Child-Pugh class of A and modified albumin-bilirubin (mALBI) stage of 2b.

The patient was initially given transarterial chemoembolization (TACE) involving 6 g eluting beads of pirarubicin (50 mg) as well as the PD-1 inhibitor camrelizumab (200 mg) once every 3 weeks for a total of nine weeks. The patient did not experience obvious adverse reactions, except mild fever during the night following TACE. On August 21, 2021, dynamic computed tomography showed no significant change in the size of multiple lesions in the liver, but extensive necrosis of lesions was observed, without obvious enhancement (Figures 1C, D). AFP at this time was 2.06 ng/ml, still within the normal range. Given the apparent success of the conversion therapy, the patient was treated by open right hemihepatectomy and cholecystectomy on August 27, 2021. Postoperative pathology showed coagulative necrosis of all hepatic tumors, hyperplasia of surrounding fibrous tissue, lymphocyte infiltration, and no residual cancer cells (Figures 1E, F).

After surgery, the patient continued to receive camrelizumab once every 3 weeks for a total of 15 weeks. During follow-up until July 10, 2022, no tumor recurrence was detected based on computed tomography or AFP.

Case 2

A 42-year-old male patient was admitted to our hospital on August 27, 2021 after dynamic computed tomography revealed a lump in the right lobe of the liver and AFP was found to be elevated. For more than 30 years, the patient had had cirrhosis associated with hepatitis B virus infection. AFP on admission was 992.8 ng/ml, and albumin was 31.5 g/L (Supplementary Table). Dynamic enhanced computed tomography revealed a lesion (11.4 x 8.9 x 10.0 cm) on the inferior segment of the right anterior lobe without macrovascular invasion or extrahepatic metastases, but with liver cirrhosis and splenomegaly with collateral circulation (Figures 2A, B). The patient was assigned an ECOG-PS score of 0, BCLC-A stage, Child-Pugh class of A and mALBI stage of 2b.

Given the patient's large tumor, cirrhosis and < 45% residual liver volume, he was not considered eligible for surgery. After the absence of contraindications was confirmed, the patient was given superselective TACE involving raltitrexed (4 mg) and oxaliplatin (100 mg) as an emulsion in 40% iodized poppy oil (10 ml), as well as the tyrosine kinase inhibitor lenvatinib (8 mg, once daily). The patient experienced no obvious adverse reactions during treatment, except mild fever. One month later, dynamic computed tomography revealed that the lesion had shrunk slightly (9.9 x 7.4 cm), about half the lipiodol in the lesion had washed away, and the area without lipiodol deposition still showed partial enhancement. AFP at this time was 140.33 ng/ml. This suggested inadequate embolization by TACE, so the patient was switched to hepatic arterial infusion chemotherapy (HAIC) plus transcatheter arterial embolization. After 3 days of HAIC involving calcium leucovorin (600 mg), fluorouracil (4.0 mg) and oxaliplatin (200 mg), the patient underwent transcatheter arterial embolization. During treatment, the patient experienced no serious adverse events, except for mild fever. The patient was discharged and given lenvatinib therapy for 2 months.

On December 1, 2021, dynamic enhanced magnetic resonance imaging showed that the tumor had shrunk substantially (9.5 x 7.9 x 8.7 cm), the original lesion showed extensive necrosis, and some active lesions were situated around

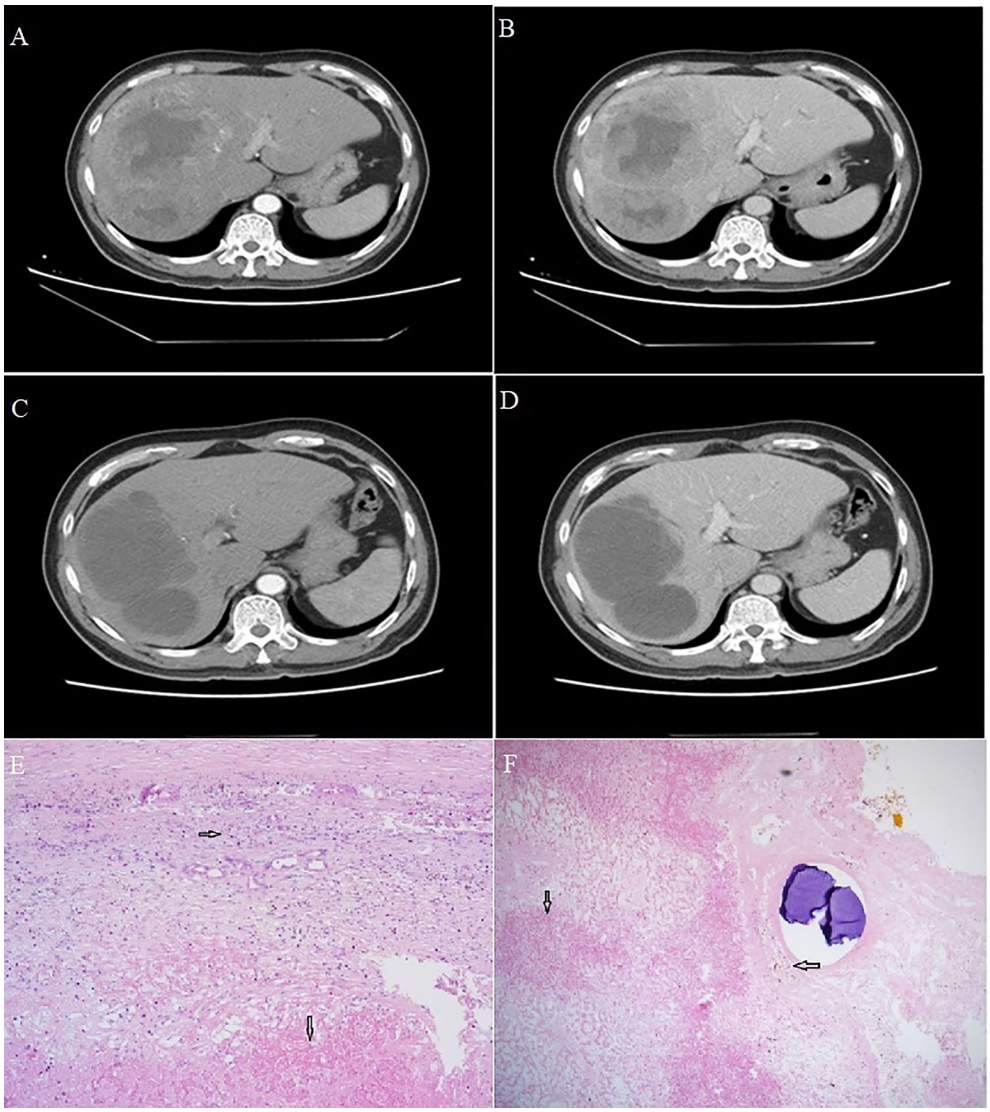

FIGURE 1
Computed tomography of Case 1. **(A, B)** Scans at admission showed a large, space-occupying lesion in the right liver, obvious inhomogeneous enhancement in the arterial phase, relatively low density in the portal phase, and a large area of non-enhancement in the tumor. **(C, D)** Scans after conversion therapy and before resection showed extensive necrosis in the primary lesion, with no residual activity in the arteriovenous phase. Sections of hepatocellular carcinoma tumor from Case 1 after conversion therapy and resection. **(E, F)** Visible are necrotic lesions (downward arrows), hyperplasia of surrounding fibrous tissue and lymphocyte infiltration (rightward arrow) and hemosiderin deposition (leftward arrow). Magnification, 40x.

the original one (Figures 2C-F). AFP was 70.06 ng/ml at this time. The apparent success of the conversion therapy and the patient's strong desire for surgery led to open right liver tumor radical resection and cholecystectomy on December 10, 2021. Histopathology showed coagulative necrosis, a few surviving cancer cells around the tumor, and some degenerated cancer cells. The lesion area also showed substantial fibrous hyperplasia with lymphocyte infiltration (Figure 3). No tumor recurrence was detected during follow-up through July 5, 2022, based on computed tomography and AFP (Supplemental Figure S1).

Discussion

The two cases in this report demonstrate that local regional therapy plus TKIs or ICIs can improve initially unresectable HCC enough that patients can undergo resection and have better prognosis (Figure 4).

First-line treatment of unresectable HCC is usually TACE and atezolizumab-bevacizumab or durvalumab-tremelimumab. If the above treatment fails, other options include regorafenib, cabozantinib, and remolumab (7). On their own, TKIs or ICIs

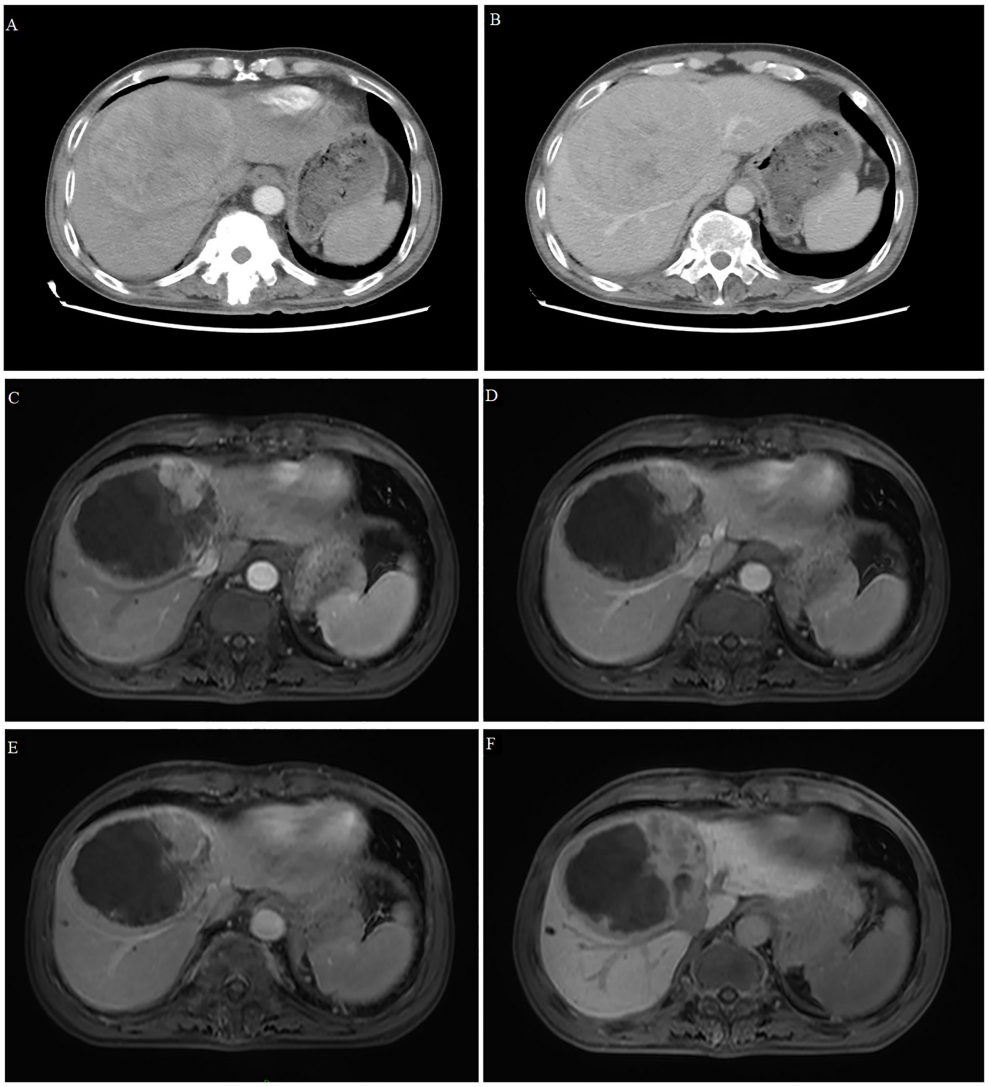

FIGURE 2
Computed tomography and magnetic resonance imaging of Case 2. **(A, B)** Tomography scans at admission showed a large, space-occupying lesion in the right liver, obvious inhomogeneous enhancement in the arterial phase **(A)** and relatively low density in the portal phase **(B)**. **(C–F)** T1-weighted magnetic resonance imaging after conversion therapy and before resection showed extensive tumor necrosis, but there was still a small active area around the tumor showing high signal intensity in the early arterial phase **(C)** and low signal intensity in the late arterial phase **(D)**, portal vein phase **(E)** and hepatobiliary phase **(F)**.

are associated with objective response rates only around 20% (5), while combining immune drugs with targeted drugs can improve objective response (4, 6, 8). For example, lenvatinib combined with pembrolizumab in one trial led to median progression-free survival (mPFS) of 9.3 months and median overall survival (mOS) of 22 months in patients with unresectable HCC (9). In addition, ORIENT-32 study found that sintilimab plus bevacizumab showed a significant mPFS and mOS benefit versus sorafenib for patients with unresectable, HBV-associated HCC (10). Similarly, this study chose the combined treatment of local regional therapy plus immunotherapy, all the patients achieved tumor downstaging and the opportunity of surgical resection, and theoretically they could get longer OS. This combination regimen may provide a novel treatment option for unresectable HCC patients.

Pembrolizumab showed a high objective response rate in clinical trials and therefore was the first PD-1 inhibitor to be approved for clinical use (11). Its high cost in mainland China led our Case 1 to opt for the locally produced PD-1 inhibitor camrelizumab, which has shown similar efficacy to

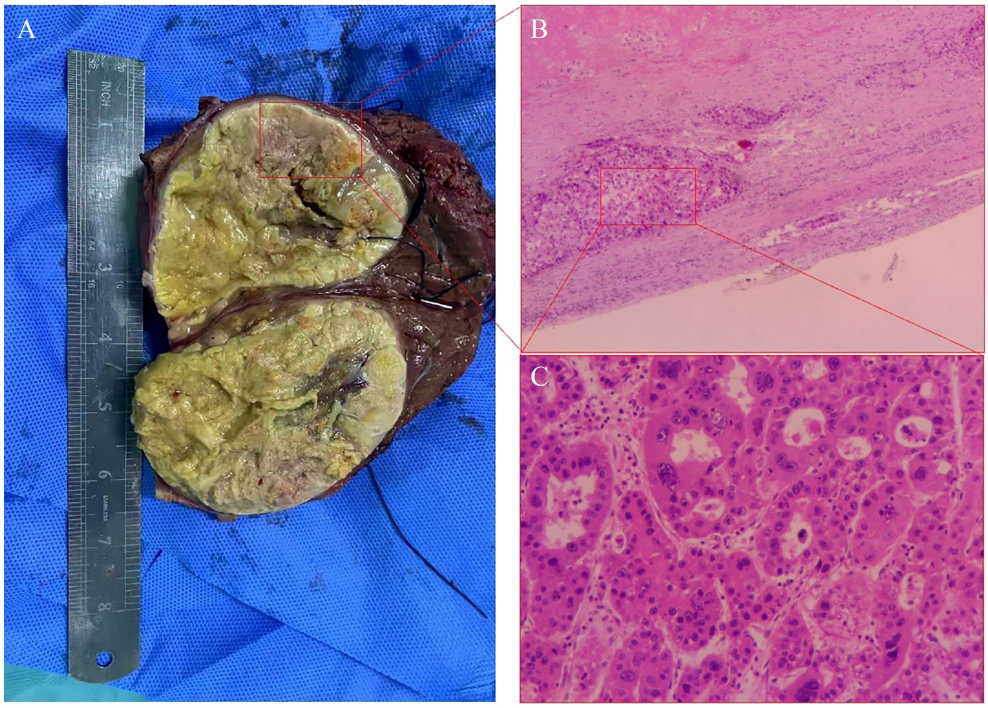

FIGURE 3
Histopathology of tumor tissue in Case 2 after conversion therapy and resection. **(A)** Viable cancer cells were observed (inside the red box), as well as necrotic cells (outside the red box). **(B)** The primary lesion showed massive necrosis with lymphocyte infiltration, and scattered tumor cell nests surviving around the lesion (red box). Magnification, 40x. **(C)** Higher-magnification image of the red box in panel **(B)** shows some degenerated tumor cells and some giant tumor cells. Magnification, 100x.

pembrolizumab (12). In that patient's conversion therapy, TACE presumably killed tumor cells by embolizing the tumor and causing cytotoxicity because of the chemotherapeutics, while camrelizumab restored endogenous anti-tumor T cell responses and induced tumor cell apoptosis (13). In this way, TACE and camrelizumab exerted synergistic anti-tumor effects.

HAIC can continuously infuse high concentrations of cytotoxic drugs into tumor-associated arteries, leading to

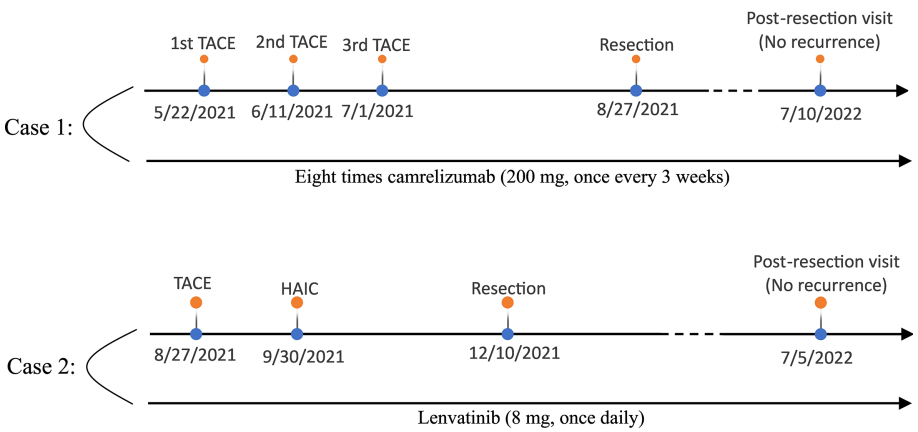

FIGURE 4
The treatment timeline of the two patients (top: case 1; below: case 2).

strong anti-tumor effects without significantly damaging the liver. Trials have reported that HAIC involving fluorouracil, calcium folinate and oxaliplatin led to median progression-free survival of 7.8 months and median overall survival of 13.9 months, much better than the corresponding survival times of 4.3 and 8.2 months for sorafinib (14, 15). In fact, the Chinese Society of Clinical Oncology recommends this HAIC regime for advanced HCC. We selected a conversion regime for Case 2 involving TACE, lenvatinib and HAIC. We attribute the efficacy of this approach to several effects: (1) TACE and HAIC induce tumor ischemia and necrosis, leading to direct anti-tumor effects; (2) the small-molecule kinase inhibitor lenvatinib prevents this ischemia and hypoxia from upregulating vascular endothelial growth factor, fibroblast growth factor and platelet-derived growth factor, in turn inhibiting angiogenesis and thereby leading to direct as well as indirect anti-tumor effects; and (3) lenvatinib may normalize tumor vessels, facilitating the distribution and delivery of anticancer drugs such as pirarubicin. However, at present, both TACE and HAIC are local regional therapy schemes for HCC, and there are no official guidelines to determine which treatment is the best local treatment. Therefore, we are carrying out relevant clinical research to explore which treatment is the best treatment.

Some studies have shown that for cirrhotic patients with HCC, laparoscopic liver resection (LLR) is superior to open liver resection in perioperative safety and postoperative recovery time, and there is no significant difference in OS (16, 17). In addition, LLR can reduce postoperative abdominal adhesion and provide opportunities for reoperation or salvage liver transplantation after tumor recurrence, further prolonging OS (18). However, the tumor of the two patients in this study was large (>10cm) and located in an unfavorable resection position (right anterior segment), the LLR was difficult and had no advantage in reducing the incidence of postoperative complications, so the two patients finally chose open liver resection.

According to the XXL trial, if the HCC patients beyond the Milan criteria achieve partial or complete response after tumor downstaging, the prognosis of liver transplantation is better than that of continuous systemic therapy (19). In addition, for cirrhotic patients with HCC, liver transplantation can completely cure liver cirrhosis, so the prognosis of liver transplantation is better than that of liver resection. Considering the advantages of liver transplantation over liver resection or systemic treatment, it may be better for patients in this study to choose liver transplantation after liver tumor recurrence, especially for cirrhotic patients with HCC.

How best to evaluate the efficacy of conversion therapy is unclear. The imaging-based evaluation criteria RECIST and mRECIST are commonly used to examine treatment response in HCC. Unlike RECIST, mRECIST focuses on blood supply to the tumor rather than tumor size. We applied mRECIST criteria to both patients in our study, and we found the postoperative assessment to be partial or complete response, consistent with other laboratory and histopathological indicators that we examined. Our results suggest that mRECIST may be accurate and effective for evaluating the efficacy of conversion therapy, which should be explored in further clinical studies.

In Case 2, AFP decreased substantially during treatment, suggesting that the level may be useful for evaluating the efficacy of conversion therapy. If AFP does not change during conversion therapy, applying other targeted therapy or immunotherapy may be beneficial. Resected surgical specimens from both our patients showed lymphocyte infiltration. It would be interesting to examine whether this observation is associated with the good prognosis that we observed for both patients after conversion therapy.

Conclusions

These two cases indicate that for patients with normal liver function, combining local regional therapy of TACE or/and HAIC with ICIs or TKIs may be a tolerable and effective way to render initially unresectable HCC amenable to surgery – even radical surgery – that may improve long-term survival. Our clinical experience should be explored in cohort studies.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

J-HZ performed the research. KC and J-HZ designed the study and wrote the paper. All authors approved the final version of the manuscript.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.946693/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Alpha-fetoprotein levels in Case 2 at different stages of treatment. HAIC, hepatic arterial infusion chemotherapy.

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Neoadjuvant chemotherapy for borderline resectable and upfront resectable pancreatic cancer increasing overall survival and disease-free survival?

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Background: Pancreatic ductal adenocarcinoma (PDAC) is the most common pancreatic neoplasm. Surgery is the factual curative option, but most patients present with advanced disease. In order to increase resectability, results of neoadjuvant chemotherapy (NAC) on metastatic disease were extrapolated to the neoadjuvant setting by many centers. The aim of our study was to retrospectively evaluate the outcome of patients who underwent upfront surgery (US)-PDAC and borderline (BR)-PDAC, and those resected after NAC to determine prognostic factors that might affect the outcome in these resected patients.

Methods: One hundred fifty-one patients between January 2012 and March 2021 in our department were reviewed. Epidemiological characteristics and pre-operative induction treatment were assessed. Pathological reports were analyzed to evaluate the quality of oncological resection (R0/R1). Post-operative mortality and morbidity and survival data were reviewed.

Results: One hundred thirteen patients were addressed for US, and 38 were considered BR and referred for surgery after induction chemotherapy. The pancreatic resection R0 was 71.5% and R1 28.5%. pT3 rate was significantly higher in the US than BR (58,4% vs 34,2%, p= 0.005). The mean OS and DFS rates were 29.4 months 15.9 months respectively. There was no difference between OS and DFS of US vs BR patients. N0 patients had significantly longer

OS and DFS (p=<0.001). R0 patients had significantly longer OS (p=0.03) and longer DFS (P=0.08). In the multivariate analysis, the presence of postoperative pancreatic fistula, R1 resection, N+ and not access to adjuvant chemotherapy were bad prognostic factors of OS.

Conclusions: Our study suggests the benefits of NAC for BR patients in downstaging tumors and rendering them amenable to resection, with same oncological result compared to US.

KEYWORDS

borderline pancreatic cancer, neoadjuvant chemiotherapy, downstaging treatment, pancreatic surgery outcomes, FOLFIRINOX regimen

Introduction

Pancreatic cancer (PC) is one of the most aggressive solid tumor entities and the fourth leading cause of cancer-related mortality in western countries. It is projected to become the second leading cause of cancer-related death in 2030 (1). Pancreatic ductal adenocarcinoma (PDAC) is the most common histological subtype (>85%) of pancreatic neoplasms. Surgery is the only potential curative treatment of PDAC but, unfortunately, only 20% of patients are eligible for such treatment (2). Indeed, after staging, PDAC is classified into resectable, borderline resectable (BR), locally advanced (LA), or metastatic diseases. Resectable disease is anatomically defined as having the following criteria (i) absence of extra pancreatic disease; (ii) no involvement of the superior mesenteric artery (SMA), hepatic artery, and coeliac axis; and (iii) patency of the superior mesenteric vein (SMV)/portal vein (PV) confluence. Beyond resectable criteria, tumors might remain technically resectable, but surgery carries higher risk of positive margins (R1) with also a higher risk of post-operative complications. Surgery is more challenging and requires frequently associated vascular resection. This might compromise adjuvant treatments and, thus, put patients at a higher risk of recurrence (3). In fact, survival in such patients remains very low, even for those who achieve R0 resection. It is estimated that the 5-year survival rates can hardly reach 20% with more than 80% distant metastatic disease risk (2). Complementary adjuvant treatments are often associated to achieve better OS and DFS (4). Unfortunately, up to 25% of patients with resectable tumors are unable to receive post-operative therapy due to frequent morbidity of pancreaticoduodenectomy and prolonged recovery (5).

These clinical observations suggest that upfront surgery (US) may not be the optimal strategy for BR PDAC. Neoadjuvant chemotherapy (NAC) for BR is becoming the trend in most specialized centers. This strategy has several objectives, (i) the possibility of downstaging the tumoral load to achieve higher rates of R0 resection and (ii) improving the selection of surgical candidates as patients with progressive disease refractory to chemotherapy will not be suitable for pancreatectomy.

Since multiple studies showed encouraging results in metastatic disease with regimens such as FOLFIRINOX (6) and gemcitabine/Nab-paclitaxel (6), many centers extrapolated these results and incorporated these regimens in the pre-operative setting for advanced tumors (7, 8). One study even managed to prove the effectiveness and cost-effectiveness of induction FOLFIRINOX regimen in patients with resectable PDAC (2).

The aim of our study was to retrospectively evaluate the outcome of patients with US-PDAC and BR-PDAC, resected after NAC, and to determine prognostic factors that might affect the outcome in these resected patients.

Methods

Study design

After the institutional review board approval, all US- and BR-patients at Robert Debre University Hospital between January 2012 and March 2021 were retrospectively identified from institutional databases. Among them, 151 patients were finally selected, 113 US, and 38 patients BR-PDAC who underwent surgery after induction chemotherapy (Flow chart—Figure 1).

Inclusion patients

All patients with PDAC were discussed during our institutional multidisciplinary oncological meeting (MOM). The MD Anderson Cancer Centre (MDACC) classification was used for staging (9). BR patients were referred to pre-

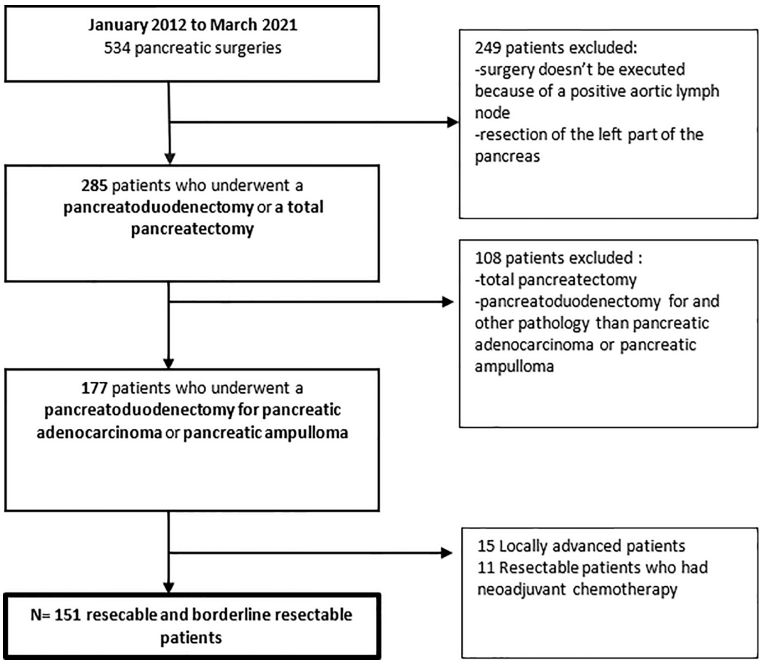

FIGURE 1
Patients selection and Flowchart.

operative chemotherapy. Post-chemotherapy reassessment was performed using triple-phase computed tomography (CT) scan and magnetic resonance imaging (MRI) with diffusion-weighted phase. Pancreatectomy was considered in the patients with no newly developed metastases on less than 4 weeks imaging before surgery and who did not experience obvious tumoral locoregional growth.

Induction chemotherapy

For BR-PDAC, different protocols upon comorbidity were used: (i) modified FOLFIRINOX regimen consisted of oxaliplatin (85 mg per square meter of body-surface area), irinotecan (180 mg per square meter, reduced to 150 mg per square meter after a protocol-specified safety analysis), leucovorin (400 mg per square meter), and fluorouracil (2400 mg per square meter) every 2 weeks; (ii) FOLFOX regimen consisted of oxaliplatin (85 mg per square meter of body-surface area), leucovorin (400 mg per square meter), and fluorouracil (2400 mg per square meter) every 2 weeks; (iii) GEMOX regimen consisted of gemcitabine (850 mg square meter for dose on day 1 and day 8) and oxaliplatin (100 mg square meter for dose on day 2) every 21 days. Patients were monitored for adverse effects and managed mainly in the outpatient clinic.

Surgery and histopathological evaluation

During laparotomy, once the presence of hepatic metastases and peritoneal carcinomatosis were excluded, harvesting of aorto-caval lymph nodes (LN station 16) was performed. Then, pancreaticoduodenectomy was performed either with the posterior approach or the artery first technique as described by Pessaux et al. (10). Pancreatico-jejunal anastomosis was performed in duct-to-mucosa technique with a stent left in place. Lymphadenectomy was done based on recommendations of the ISGPS (11). All operative sites were drained mostly by unique right-side drains. Pancreatic and bile duct margins were sent for frozen section and re-resection was performed in case invaded margins. On the specimen, the retro-portal lamina was inked to identify the posterior margins. All surgeries were performed by an expert pancreatic surgeon (TP or RK). In our study, we do not separate open pancreatectomy to mini-invasive pancreatectomy, because in the mini-invasive approach, we follow all the steps of open approach, as previously reported (12). Histopathological analysis was performed according to current international TNM classification at the time of resection. We stratified margins into R0 or absence of tumoral contact (margin > 1 mm) and R1 or microscopical tumoral contact (margin ≤ 1 mm) (8). As post-operative complications, we evaluated only the post-operative

pancreatic fistula (POPF, grade B or C) (13), delayed gastric emptying (DGE, grade B or C) (14), and post-pancreatectomy hemorrhage (PPH, grade B or C), according to International Study Group of Pancreatic Surgery (ISGPS).

Follow up

In line with institutional guidelines, all patients are followed after surgery with biological tumoral markers (CA 19.9) and radiological examination (CT scan) every 3 months for the first 2 years and every 6 months thereafter.

Statistical analysis

For the descriptive analysis, the quantitative variables were expressed as mean and standard deviation; the qualitative variables as numbers and percentages. The Student's t test was used to compare the quantitative characteristics and the chi-square test for categorical characteristics. The variables were dichotomized, when possible, to facilitate the comparisons. When the Student's t test could not be used because the variances were not homogeneous, the Mann–Whitney test was applied. When the chi-square test was not valid because the number was lower than 5, the Fisher's exact test was used. Survival analysis was performed using the Kaplan–Meier and log-rank test method for the endpoints. The variables entered in the Cox model and regression model were those with a univariate p value < 0.20 or clinical significance. The results were expressed as hazard ratio with 95% confidence intervals for the Cox model, and odds ratio with 95% confidence intervals for the logistic regression model. A p value less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS20.0 (SPSS, Inc., Chicago, IL, USA).

Ethical considerations

The study design (from retrospective observation) was based on a medical database that did not require patient consent, according to French legislation (15). This study was performed in compliance with national legislation regarding epidemiological studies (Declaration N°2206749 v 0). Moreover, in accordance with national ethical directives, the requirement for written informed consent was waived because the study was strictly observational and all data were blinded (16). According to the French Public Health Code, this research also did not require an ethical committee. Patients were informed that the study was being carried out *via* the hospital's registry of ongoing studies.

Results

Between January 2012 and March 2021, 177 patients underwent duodenopancreatectomy for PDAC. Of those 113 patients who had US for resectable disease, 38 patients had surgery for BR disease after induction chemotherapy (see flowchart—Figure 1).

Patients' data at diagnosis

Patients' data at diagnosis are summarized in Table 1. Baseline characteristics [age, sex, body mass index (BMI), comorbidity, and American society of anesthesiologists (ASA) score] were statistically similar and they are shown in Table 1. Pre-operative biliary drainage and CA 19.9 value were statistically similar and they are shown in Table 2. All patients had a PDAC localized in the head of the pancreas or in the uncus

TABLE 1 Baseline population characteristics.

	All patients (n = 151)	Upfront surgery (n = 113)	Borderline (n = 38)	p-value*
Women	85	61 (54%)	24 (63.2%)	0.351
Men	66	52 (46%)	14 (36.8%)	0.351
Age at surgery (mean)	67	67	66	0.607
Mean BMI (kg/m2)	24.8	24.9	23.8	0.089
Diabetes	39 (25.8%)	33 (29.2%)	6 (15.8%)	0.134
BMI > 35 kg/m2	7 (4.6%)	6 (5.3%)	1 (2.6%)	0.680
High blood pressure	64 (42.4%)	49 (43.4%)	15 (39.5%)	0.708
Weaned or active smoking	57 (37.7%)	42 (37.2%)	15 (39.5%)	0.431
ASA I	19 (12.6%)	16 (14.2%)	3 (7.9%)	0.4
ASA II	70 (46.4%)	51 (45.1%)	19 (50%)	1
ASA III	46 (30.5%)	32 (28.3%)	14 (36.8%)	0.538
ASA IV	1 (0.7%)	1 (0.9%)	0	1

*Comparison between the Upfront Surgery and the Borderline.

TABLE 2 Neoadjuvant data.

	All patients (n = 151)	Upfront surgery (n = 113)	Borderline (n = 38)	p-value*
Tumor size (mean) (mm)	25.2	23.7	29.3	0
Biliary drainage	97 (64.2%)	71 (62.8%)	26 (68.4%)	0.564
Endoscopic drainage	89 (58.9%)	67 (59.3%)	22 (57.9%)	1
Biliary prosthesis	85 (56.3 %)	61 (54%)	24 (63.2%)	0.351
CA19.9 <37 U/ml	32 (21.2%)	24 (21.2%)	3 (21.1%)	0.308
CA19-9 (median and extremes)	103 (0.8-19648)	103 (0.8-19648)	83 (2.1-12000)	
Neoadjuvant chemotherapy	38 (25.2%)	0	38 (100%)	0
FOLFIRINOX**	35 (23.2%)	0	35 (92.1%)	
GEMOX	3 (2%)	0	3 (7.9%)	

*Comparison between the Upfront Surgery and the Borderline group.
**Three patients changed for FOLFOX because of a bad tolerance.

or in the peri-ampullary tissue. Mean tumor diameter was 29.3 mm for BR-PDAC patients vs. 23.7 mm for US patients (Table 2).

Chemotherapy data

BR-PDAC patients' data following chemotherapy are shown in Table 2. Induction chemotherapy alone with no radiotherapy. All BR-PDAC patients received pre-operative chemotherapy, 92.1% (n=35) FOLFIRINOX regimen, and 7.9% (n=3) GEMOX regimen (Table 2). In addition, 3 patients (8.5%) experienced severe side effects of irinotecan with FOLFIRINOX and were switched to FOLFOX. Moreover, 32 patients (91.5%) who completed neoadjuvant therapy tolerated their treatment without hospital admission or emergency department care. The median duration of neoadjuvant treatment was 2.1 months, with an average of 5.2 cures per patient. The mean reduction in tumor size was 28.7%, from a median size of 30 mm to 20 mm (Table 3). Surgical exploration was performed 5–6 weeks following chemotherapy completion. The 78.8% of patients (119) received an adjuvant chemotherapy regimen. Table 5 summarizes the data of the different protocols used. Often, a different protocol was used due to toxicity problems or a compromised performance status. The median duration of adjuvant chemotherapy was 5.1 months, with an average of 6.8 cures per patient.

Post-operative data

The rate of venous resections was significant higher in the BR patients than in the US patients (52.6% vs. 23%, p=0.001) (Table 4).

Histopathological and post-operative data are summarized in Table 4. Resection quality rate showed 71.5% R0 and 28.5% R1. Concerning tumor size, T3 rate was significantly higher in the US than BR (58.4% vs. 34.2%, p= 0.005). Lymphadenectomy resulted in mean of 19.6 lymph node per procedure with a positive rate of 70.8% and an average of two involved LN per patient in the BR-PDAC, vs. 63.2% and one involved LN in US patients. Comparisons between POPF, PPH, and DGE rates were not significative in two groups (Table 5). As expected, POPF rate was 11.5% in the US patients vs. 5.3% in the chemo-inducted patients. Moreover, 15 patients had POPF during the post-operative period. All these patients received post-operative Somatostatin analogues for at least 7 days. Among the four

TABLE 3 After neoadjuvant chemotherapy data.

Borderline n = 38	Before neoadjuvant chemotherapy	After neoadjuvant chemotherapy
Tumor size (mean) (mm)	29.2***	19.9****
Tumor size (median and extremes) (mm)	30 (15-50)	21 (0-40)
Regression (mean) (mm)		8.5
Regression (median and extremes) (mm)		6 (0-25)
Percentage of regression (mean)		29.9%
CA19.9 < 37 U/ml	7 (18,4%) *	11 (28.9%) **
CA19.9 (mean)	1072.2*	241.7**
CA19-9 (median and extremes)	83 (2.1-12000) *	36 (2.1-2101.6)

*5 missing data, ** 5 missing data, *** 2 missing data, **** 3 missing data.

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TABLE 4 Pathological data on post-resection specimens.

	All resected patients (n = 151)	Upfront surgery resected (n = 113)	Borderline resected after NAC (n = 38)	p-value*
Venous resection	46 (30.5%)	26 (23%)	20 (52.6%)	0.001
Tumor size (mean) (mm)	26.3	27.2	23.5	0.143
R0 >1 mm	108 (71.5%)	86 (76.1%)	22 (57.9%)	0.039
R1	43 (28.5%)	27 (23.9%)	16 (42.1%)	0.039
T0	1 (0.7%)	0	1 (2.6%)	0.441
T1	19 (12.6%)	11 (9.7%)	8 (21.1%)	0.089
T2	40 (26.5%)	25 (22.1%)	15 (39.5%)	0.054
T3	79 (52.3%)	66 (58.4%)	13 (34.2%)	0.005
T4	11 (7.3%)	9 (8%)	2 (5.3%)	0.731
N+	104 (68.9%)	80 (70.8%)	24 (63.2%)	0.687
Number of N+ (mean)	2.9	3	2.6	0.552
Collected lymph nodes (mean)	19.6	20.4	17.5	0.104
LN ratio (mean)	0.14	0.15	0.13	0.552
Venous emboli	79 (52.3%)	61 (54%)	18 (47.4%)	0.574
Perineural sheathing	108 (75.5%)	82 (72.6%)	26 (68.4%)	0.679
Lymph emboli	85 (56.3%)	67 (59.3%)	18 (47.4%)	0.257

*Comparison between the Upfront Surgery and the Borderline group.

TABLE 5 Post-operative data.

	All patients (n = 151)	Upfront surgery (n = 113)	Borderline (n = 38)	p-value*
Grade B-C pancreatic fistula	15 (9.9%)	13 (11.5%)	2 (5.3%)	0.131
Grade B-C gastroparesis	19 (12.6%)	12 (10.6%)	7 (18.4%)	0.258
Grade B-C hemorrhage	14 (9.3%)	10 (8.8%)	4 (10.5%)	0.752
D30 mortality	6 (4%)	4 (3.5%)	2 (5.3%)	0.642
D90 mortality	11 (7.3%)	7 (6.2%)	4 (10.5%)	0.272
1-year survival	113 (88.4%)	89 (78.8%)	24 (63.2%)	
3-year survival	38 (25.1%)	37 (32.7%)	8 (21.1%)	
Mean survival (month)	29.4	30,3	26.6	
Mean survival (day)	894	921	809	
6 months tumor recurrence	10 (6.6%)	7 (6.2%)	3 (8.3%)	
1-year recurrence	44 (29.1%)	35 (31%)	9 (236%)	
3-year recurrence	77 (60%)	57 (50.4%)	20 (52.6%)	0.851
Mean DFS (day)	495	471.5	456	
Mean DFS (month)	15.9	15.5	13.5	
Adjuvant chemotherapy	119 (78.8%)	88 (77.9%)	31 (81.6%)	0.819
FOLFIRINOX	32 (26.9%)	16 (18,1%)	16 (51,6%)	<0.001
GEMZAR + XELODA	74 (62,1%)	68 (77,2%)	6 (19,4%)	<0.001
LV5 FU2	6 (5%)	1 (1,1%)	5 (16,1%)	<0.001
FOLFOX **, ***	7 (5,9%)	3 (3,4%)	4 (12,9%)	0.053

*Comparison between the Upfront Surgery and the Borderline group.
**Patients who had neoadjuvant FOLFOX went on an adjuvant therapy with FOLFOX.
***Bad tolerance for FOLFIRINOX because of post-operative complication or bad general condition.

patients with a grade C pancreatic fistula, two died during the first 90 post-operative days due to PPH, and two needed a redo-surgery during the same hospitalization. One of the patients with a grade B pancreatic fistula died during the first 30 post-operative days due to a mesenteric ischemia.

Among the patients, 19 had DGE during the post-operative period. A medical treatment was managed for all these patients in first place, with the administration of prokinetic drugs sometimes associated with a nasogastric tube. A nasojejunal tubes were necessary for three patients. One of them was reoperated at the 21st post-operative day due to an early stenosis of gastrojejunal anastomosis.

In addition, 14 patients had PPH. Two patients had a parietal bleeding that was controlled by surgical hemostasis. Two patients had a bleeding from a hemorrhagic ulcer of the gastrojejunal anastomosis. One of them received a surgical hemostasis, and in the other the bleeding was spontaneously interrupted. A portal vein bleeding was the cause of the death for two patients, despite redo-surgery for hemostasis. Two patients had a bleeding from the superior mesenteric artery, treated by radiological embolization followed by surgical hemostasis, in one of them. Bleeding came from fissure of proper hepatic artery pseudoaneurysm in two patients, and radiological embolization and stenting were performed in both patients, the post-procedure outcome for one of them was fatal. Three patients with sentinel bleeding, without cause, detected to arteriography. The last patient had a bleeding from a branch of superior mesenteric artery treated by radiological embolization. In the cohort patients, six patients died in the first 30 post-operative days and a total 11 patients died during the first 90 post-operative days. Mortality was for the first 30 post-operative days, four (3.5%) in the US patients vs. two (5.3%) in the BR groups (p=0.64). For the first 90 post-operative days, seven (6,2%) in the US patients *vs.* four (10.5%) in the BR groups (p=0.27). Among the six patients who died within the first 30 post-operative days, one died due to a pulmonary embolism, two patients died because of a hemorrhagic shock, one of multiple organ failure after a PPH, one had a several cardiac arrest, and one died of a mesenteric ischemia. Among the five other patients who died within the first 90 post-operative days, two died because of a mesenteric ischemia, two died because of multiple organ failure after a PPH. For one of these patients, the reason of the death is unknown.

Survival and recurrence

In the cohort population, 78.8% of patients received adjuvant CHT, 77.9% in the US vs. 81.6% in the BR patients (p=0.82). As shown in Table 5, the global mean post-operative OS was 29.4 months, whereas median post-operative DFS was 15.9 months. Figure 2 shows no statistically significant difference in OS between US and BR patients. BR patients vs. US patients 1 and 3 years OS were not statistically significant, 73.5% and 23% vs. 85.8% and 23.6%, respectively. This was also not significant for 1 and 3 years DFS, 73.5% and 38.2% vs. 68.2% and 42.1% for BR patients vs. US patients (p=0.89), respectively. No statistically significant difference OS and DFS was evidenced in the US group based on the value of Ca19.9 considering a cutoff of 120 U/ml (p=0.76 in OS and P=0.26 in DFS) or 500 U/ml (p=0.62 in OS and p=0.96 in DFS). Figure 3 shows the results according to the nodal invasion (N0 vs N+). A statistically significant difference in OS was observed after 1 and 3 years in OS for N0 versus N+ patients, 88.5% and 58.4% vs. 80.3% and 32.4% (p=0<001). For the 1 and 3 years DFS, a statistically significant difference was observed, 86.4% and 61.4% vs. 61.9% and 32% between N0 vs. N+

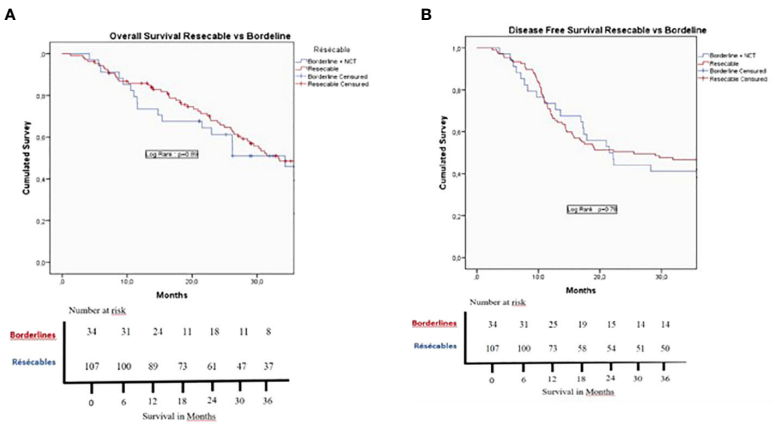

FIGURE 2
Kaplan–Meier survival curves for survival rates. **(A)** Overall survival and **(B)**. Disease- free survival for the Upfront surgery group (red curve) and the Borderline group (blue curve). There is no significant difference for OS (p=0.,89) and DFS (p=0.,78) between the two groups. Patients diedead at the 90th post-operative day were excluded of the survival analysis.

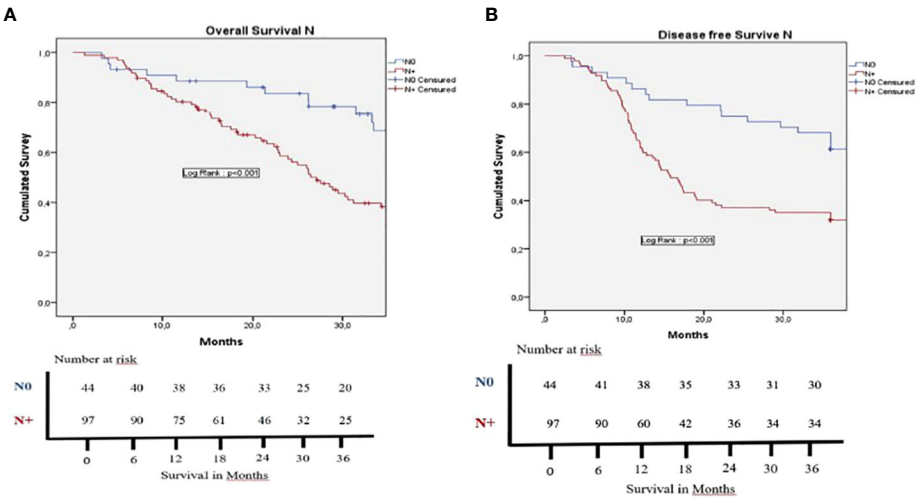

FIGURE 3
Kaplan-Meier survival curves for survival rates. **(A)** Overall survival and **(B)** Disease free survival for the N+ (positive collected lymphe nodes at the pathology analysis) group (red curve) and the N0 (no positive collected lymphe node at the pathology analysis) group (blue curve). There is a significant difference for OS (p < 0,001) and DFS (p < 0,001) between the two groups. Patients dead at the 90th post-operative day were excluded of the survival analysis.

patients (p<0.001). In Figure 4, the outcome according to the status of margin invasion (R0 vs. R1) is shown. A statistically significant difference in OS at 1 and 3 years was observed between R0 and R1 patients, 86.8% and 46.4% vs 73.2% and 29% (p=0.03). However, no statistically significant difference was observed in DFS at 1 and 3 years between R0 and R1 patients, 70% and 46% vs 68.3% and 29.3% (p=0.08). When we stratify all the variable that can influenced the OS and DFS in the cohort population, we found that in the multivariate analysis that POPF, R1, N+ and not access to adjuvant chemotherapy were bad prognostic factors of

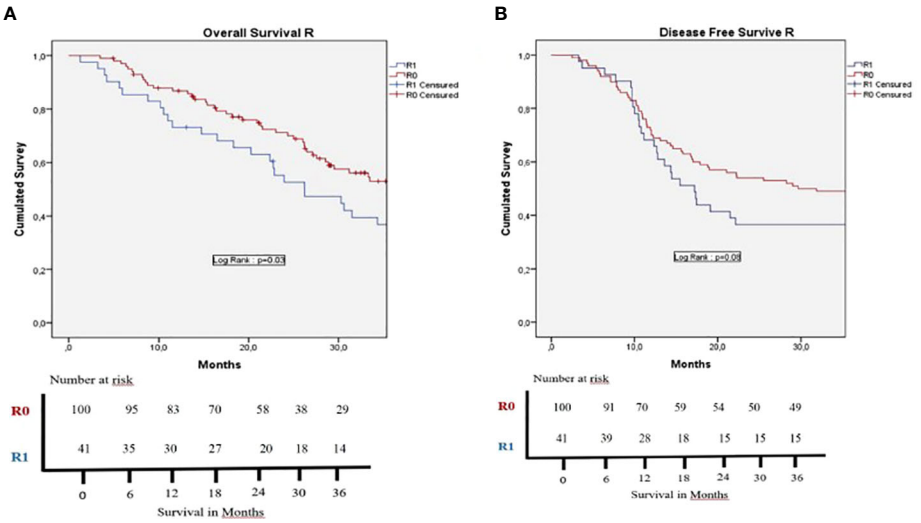

FIGURE 4
Kaplan-Meier survival curves for survival rates. **(A)** Overall survival and **(B)** Disease free survival for the R0 (resection margin > 1mm at the pathology analysis) group (red curve) and the R1 (resection margin < 1mm at the pathology analysis) group (blue curve). There is a significant difference for OS (p =0,03) and no significant difference for DFS (p =0,08) between the two groups. Patients dead at the 90th post-operative day were excluded of the survival analysis.

OS (Table 6); and we found that PPH, N+ were bad prognostic factors of DFS (Table 7).

Discussion

Our study showed that mean OS and DFS in BR patients after NAC and in the US patients were 26.6 and 13.5 months vs. 30.3 and 15.5 months, respectively. In the BR patients, the tumor diameter dropped after pre-operative chemotherapy significantly, with a mean percentage of regression of 29.9%. No evidence of tumor was seen on the control CT scan for five patients. In the two groups, the rate of post-operative pT3 tumors was significantly higher in the US patients (p= 0.005), while after NAC, BR patients, who were initially in more advanced tumor status, had a similar OS and DFS of US patients at 1–3 years (p=0.89 and p=0.78). In our cohort, according to nodal status, the patients had a significant better OS and DFS when they did not have a nodal infiltration by the tumor (N+) (p<0.001). Equally, according to margin status, the patients had a significant better OS when there was a microscopic tumoral invasion of the margin (p =0.03) and a non-significant better DFS when there was a microscopic tumoral invasion of the margin (p =0.08).

Most of the patients with PDAC present with locally advanced or metastatic disease, in fact only 15%–20% present with upfront resectable disease. To date, the only potentially curative therapy for PDAC remains surgical resection. NAC is increasingly used to target occult disease if present, select patients, and possibly downstage tumors.

Induction chemotherapy for borderline tumors is acquired but it is place for resectable borderline. PDAC is not standardized and its role is not well definite for the different results reported by the literature (17–19).

Most of the patients in our study were highly selected, most had good performance status (OMS ≤ 2), with no contraindications to NAC, especially vascular anatomical abnormalities for subsequent major pancreatic surgery, and BR patients received pre-operative induction chemotherapy mainly FOLFIRINOX regimen (84.2% of

TABLE 6 Univariate and multivariate Cox-regression analysis of the overall survival with Borderline group and Upfront surgerygroup.

Variable	Cohort	Univariate *p* value	Multivariate HR	95% CI	*P* value
Women	85	0.562			
Biliary drainage	97				
Endoscopic drainage	89				
Biliary prosthesis	85				
ASA1	19				
ASA 2	70				
ASA3	46				
High Blood Pressure	64				
Diabetes	39				
Weaned or active smoking	51				
BMI >35kg/m2	7				
Ca 19.9 < 37 U/ml	32				
Neoadjuvant chemotherapy	35				
B-C pancreatic fistula	25	0.048	3.746	1.073-13.086	0.038
B-C hemorrhage	14	0.269	3.170	0.318-31.565	0.325
B-C Gastroparesis	19				
R1	43	0.036	2.716	1.268-5.818	0.010
Positive collected lymph nodes	104	0.036	2.695	1.068-6.797	0.036
Venous emboli	79	0.238			
Perineural sheathing	108	0.466			
Lymph emboli	85	0.300	0.712	0.363-1.4	0.325
T1	19	0.005			
T2	40	0.016			
T3	79	0.011			
T4	11	0.114			
Venous resection	46	0.121	0.478	0.201-1.136	0.095
Adjuvant chemotherapy	119	0.045	3.485	1.226-0.904	0.019

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TABLE 7 Univariate and multivariate Cox-regression analysis of the recurrence with Borderline group and Upfront surgery group.

Variable	Cohort	Univariate *p* value	Multivariate HR	95% CI	*P* value
Men	66	0.508			
Women	85	0.508			
Biliary drainage	97	0.393			
Endoscopic drainage	89	0.317			
Biliary prosthesis	85	0.139			
ASA1	19	0.323			
ASA 2	70	0.299			
ASA3	46	0.071			
High Blood Pressure	64	1			
Diabetes	39	0.455			
Weaned or active smoking	51	0.862			
BMI >35 kg/m2	7	0.699			
Ca 19.9 < 37 U/ml	32				
Neoadjuvant chemotherapy	35	0.846			
B-C pancreatic fistula	25	0.787			
B-C hemorrhage	14	0.009	3.29	1.014-10.67	0.047
B-C Gastroparesis	19	0.329			
R1	43	0.106			
Positive collected lymph nodes	104	0.002	0.395	0.223-0.7	0.001
Venous emboli	79	0.194			
Perineural sheathing	108	0.003	0.613	0.346-1.086	0.094
Lymph emboli	85	0.250			
T1	19	0.006	1.604	0.593-4.341	0.352
T2	40	0,353			
T3	79	0.002	0.783	0.482-1.273	0.324
T4	11	0.532			
Venous resection	46	0.477			
Adjuvant chemotherapy	119	0.001	1.226	0.612-2.458	0.565

patients). Despite its high toxicity profile often necessitating dose re-adjustments or change of regimen in frail patients; FOLFIRINOX has proven superiority over other regimens in many studies, mainly the ACCORD trial that showed prolonged survival with minimal impairment in quality of life in well-selected patients (6). In the borderline group, only the 52.6% of patients received vascular resection. This point was marked during the latest international consensus, given that the major determinants of resectability in PDAC remain anatomical findings on imaging (mainly size and vessel involvement), biologic behavior of the tumor (Ca 19-9), and the patient's characteristics (OMS and co-morbidities) (20). By carefully selecting patients, our study showed how NAC succeeded in downstaging tumors and affecting biological behavior, while preserving a good performance status allowing patients to undergo a highly morbid surgical procedure like a pancreaticoduodenectomy.

In order to study the effect of NAC on survival, follow up was continued post-operatively, from the histopathologic study of surgical specimens to the surgical morbidity and mortality. Patients were then followed with markers and imaging every 3 months for 2 years post-operatively and every 6 months thereafter.

In our series, even if in the borderline group, the tendency is to have fewer pancreatic fistulas; there is no significant difference compared to the US (5.3% vs. 11.5%). This is mainly due to a lack of statistical power, but the tendency is clearly towards fewer POPF after neoadjuvant treatment. In the literature, the results are conflicting. Cools et al.'s data using the ACS-NSQIP–targeted pancreatectomy from 2014–2015 showed a statistically significant

difference in terms of Type C pancreatic fistula between patients that received NAT and US patients (21). Denbo et al. considering all types of pancreatectomy (Whipple et. DP), no difference was found between patients that received NAT and US patients (P = 0.96) (22). Extremely interesting are the results of the study by Marchegiani et al. that reports the experience of the Verona team. In fact, NAT significantly reduces the incidence of pancreatic fistula (P = 0.05), but based on the Modified Accordion Severity Grading System and average complication burden (ACB) used to compare the patients treated with NAT with the patients who underwent US, the results show that the patients who develop a fistula post-NAT are associated with an increase in clinical burden (23–27). These results introduce, in our opinion, an aspect that is often overlooked, the toxicity of chemotherapy. The toxicity of FOLFIRINOX grade 3/4/5 can reach up to 50% (ASCO 2022). This often results in surgical management of fragile patients who may have a more complicated post-operative course. Although NAT allows us to operate on patients with a "hard" pancreas and better selected (exclude patients who develop metastases during chemotherapy), on the other hand, the pre-operative management requires multidisciplinary management.

This aspect of patient fragility also results in difficult access to adjuvant chemotherapy. As we have well shown in our results, among the OS risk factors, pancreatic fistula and lack of access to adjuvant chemotherapy are themselves negative risk factors.

Since multiple series showed that radical surgical resection with negative margins is the key to achieve better survival, margins were noted in all specimens, especially the retroperitoneal margin. A minimum of 1-mm margin has been adopted by the current Royal College of Pathologists' guidelines for pancreatoduodenectomy specimens (28). In fact, many studies showed that the survival benefit of negative margin was lost when the tumor was within 1 mm of the resection margin (R1 < 1 mm). Our study showed an R0 resection in 76.1% of US patients vs. 63.2% of BR patients, R1 (margin inferior to 1 mm) in 11.5% of US patients vs. 15.7% of BR patients and R1 (microscopical contact with the tumor, margin 0 mm) in 12.4% of US vs. 21.1% of BR patients. In pancreatic surgery, R0 resection is generally reported to be achieved in 70%–80% of cases, but, unfortunately, the definition of R0 resection is not yet worldwide standardized. When 1-mm margin was used, R0 resection rate dropped to 5%–26% (29–33). A meta-analysis of 19 studies by Chandrasegaram et al. found that the rate of R0 resection with a 0-mm margin was 72%, while that with a 1-mm margin was 41% (34). Yamamoto et al. noted a drop in R0 resections after the revised classification from 84% to 43% (35). Chang et al. reported on 365 patients, 46% of whom were resected with a margin wider than 1.5 mm. Patients with a margin wider than 1.5 mm were actual long-term survivors, as compared to a margin of less than 1.5 mm (36). In our series, in the 76.1% and 63.1% of resected patients (US and BR patients), the resection margin was ≥ 1 mm. One of the reasons that can explain high rate of R0 resections was likely achieved due to the artery first approach, common in our technique. As described by Pessaux et al., resection starts by isolating the mesenteric artery at the origin and along its upper/right border in contact with the adventitia allowing us to dissect the artery up to the last fat cell and thus gaining margins (10).

The aim of our study was to assess the effect of NAC on BR pancreatic tumors compared to the patients that received US and how these changes might affect OS and DFS. As expected, a significant effect on tumor size were observed in histopathological post-operative analysis: higher T3 rate was found in the US patients, despite a mean lower size of the tumor shown on pre-operative CT scan. Our study did not show any significant difference, concerning OS and DFS between two groups. In our series, patients with R1 resection had worse OS than patients with R0 resection (p=0.03). At the same time, N+ patients had a worse OS and DFS at 1–3 years when compared to N0 patients (p<0.001 respectively), the points that are largely admitted in the literature and recently by Netherlands studied showing the effect of margin and lymph node status in all pancreatectomies for cancer (37).

In our study, we analyze prognostic factor that can have an impact on OS such as presence of POPF, R1 margin, presence of nodal tumoral invasion (N+), and absence of adjuvant chemotherapy as being bad prognostic factors at multivariate analysis. Bilici et al. showed that median survival time was better in R0-resected patients when compared with R1-resected patients (22 months *vs.* 15 months) (38). Li et al. analyzed retrospectively prognostic factor that impacted OS and showed that R1 and N+ were important prognostic factors for OS after pancreatic resection. Moreover, the authors found a statistical difference in OS for the patients that have POPF (p<0.05) (39). In the study of Girgis et al., multivariate analysis predicting overall survival, the absence of adjuvant chemotherapy negatively impacted the OS (P < 0.001) (40). Recently, Strobel et al. reviewed all patients undergoing upfront resection for resectable and borderline-resectable PDAC between 2001 and 2011. The extent of lymph node involvement was the strongest predictor of 5 years OS. Patients with pN0R0 had a 5-year OS rate of 38.2% (41); in our experience, patient with pN0R0 had a 3-year OS rate of 70%.

Our study had several limits. The retrospective design from one center, and limited number of included patients especially for BR-disease. No intention-to-treat analysis was performed. The previous results might suggest the benefit from such strategy in highly selected patients. We also admit the presence of few missing data that we were not able to retrieve and that may alter the interpretation of the result.

In conclusion, the present study confirms the favorable outcomes of radical pancreatectomy for BR-patients after NAC. This seems to allow significant downstaging of BR-patients both in tumors size and LN with similar 1 and 3 years OS and DFS when compared to US patients. In the lack of prospective randomized trials, our policy is to propose US for resectable and routine NAC for BR tumors. The artery-first technique seems to help achieving better R0 margin rates.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

VF and AM are 2 first co-authors because they have contributed in same manner to the development of work. (I). Conception and design: TP, AM, RR, VF, RK (II). Administrative support: AM, VF, LR, MBa (III). Provision of study materials or patients: TP, OB, RK (IV). Collection and assembly of data: AM, RR, VF, LR, MBa (V). Data analysis and interpretation: AM, RR, TP, SS, OB, RK (VI). Manuscript writing: All authors (VII). All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Laparoscopic versus open liver resection for hepatocellular carcinoma in elderly patients: A systematic review and meta-analysis of propensity score-matched studies

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Purpose: Laparoscopic liver resection (LLR) is a widely practiced therapeutic method and holds several advantages over open liver resection (OLR) including less postoperative pain, lower morbidity, and faster recovery. However, the effect of LLR for the treatment of hepatocellular carcinoma (HCC) in elderly patients remains controversial. Therefore, we aimed to perform the first meta-analysis of propensity score-matched (PSM) studies to compare the short- and long-term outcomes of LLR versus OLR for elderly patients with HCC.

Methods: Databases including PubMed, Embase, Scopus, and Cochrane Library were systematically searched until April 2022 for eligible studies that compared LLR and OLR for the treatment of HCC in elderly patients. Short-term outcomes include postoperative complications, blood loss, surgical time, and length of hospital stay. Long-term outcomes include overall survival (OS) rate and disease-free survival (DFS) rate at 1, 3, and 5 years.

Results: A total of 12 trials involving 1,861 patients (907 in the LLR group, 954 in the OLR group) were included. Compared with OLR, LLR was associated with lower postoperative complications (OR 0.49, 95% CI 0.39 to 0.62, $P < 0.00001$, $I^2 = 0\%$), less blood loss (MD −285.69, 95% CI −481.72 to −89.65, $P = 0.004$, $I^2 = 96\%$), and shorter hospital stay (MD −7.88, 95% CI −11.38 to −4.37, $P < 0.0001$, $I^2 = 96\%$), whereas operation time (MD 17.33, 95% CI −6.17 to 40.83, $P = 0.15$, $I^2 = 92\%$) was insignificantly different. Furthermore, there were no significant differences for the OS and DFS rates at 1, 3, and 5 years.

Conclusions: For elderly patients with HCC, LLR offers better short-term outcomes including a lower incidence of postoperative complications and shorter hospital stays, with comparable long-term outcomes when compared

with the open approach. Our results support the implementation of LLR for the treatment of HCC in elderly patients.

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KEYWORDS

hepatocellular carcinoma (HCC), laparoscopic liver resection (LLR), open liver resection (OLR), meta-analysis, elderly

Introduction

Liver cancer is one of the most common cancers and a major global health challenge (1). According to GLOBOCAN 2020, liver cancer is the fourth leading cause of cancer death, causing an estimated 830,180 deaths in 2020 globally (2). Hepatocellular carcinoma (HCC) represents about 90% of primary liver cancers and constitutes a major health problem worldwide (3). Furthermore, modern advances in healthcare systems have greatly extended life expectancy (4), and the increased incidence of HCC is closely related to the aging of the population.

Surgical resection is one of the most effective treatments of choice for early HCC. Since Reich et al. reported the first laparoscopic liver resection (LLR) in 1991 (5), this minimally invasive technique has advanced continuously. Nowadays, this minimally invasive technique has gained increasing acceptance for some major well-known benefits, including a lower incidence of postoperative complications, shorter hospital stay, faster recovery, and better quality of life (6–8).

However, several factors such as the presence of comorbidities and the age of the patients may have a significant effect on the efficacy and safety of this minimally invasive technique. Age is a challenging feature given the significant heterogeneity of general conditions among individuals of the same age range and the growing number of elderly patients in good clinical condition presenting with HCC (9). Also, elderly patients are infrequently included in the range of randomized clinical trials, resulting in a lack of understanding of the benefits and risks of treatment strategies (10). Due to the factors that are mentioned above, clinicians are required to reconsider the treatment indications of this minimally invasive technique. Moreover, to surmount the existing selection and confounding biases inherent in non-randomized studies, we elected to limit to studies that performed propensity score matching (PSM), because a great number of research (11–14) have shown that PSM studies are comparable to RCTs empirically in terms of their capability of deriving unbiased estimates.

Accordingly, in order to summarize the present high-quality evidence, we performed a meta-analysis of PSM studies to compare the short- and long-term outcomes of LLR versus OLR for the treatment of HCC in elderly patients.

Methods

We conducted our study on the basis of the updated PRISMA statement (15) (Supplementary Material 1), and the protocol was registered in the International Platform of Registered Systematic Review and Meta-analysis Protocols (INPLASY 202240156). We systematically searched the PubMed, Embase, Scopus, and Cochrane Library databases for PSM studies up to April 2022. The search used broad search terms containing "HCC", "liver cancer", "hepatoma", "laparoscopic", "open liver resection", "hepatectomy", "elderly", and "propensity score" (the comprehensive search strategies are listed in Supplementary Material 2).

Eligibility criteria

The inclusion criteria were as follows: 1) population: elderly patients (≥65 years old) with pathology-confirmed HCC; 2) intervention: LLR; 3) comparison: OLR; 4) outcomes: short-term outcomes including postoperative complications, blood loss, surgical time, and length of hospital stay and long-term outcomes including 1-, 3-, and 5-year overall survival (OS) rates and 1-, 3-, and 5-year disease-free survival (DFS) rates; and 5) design: PSM.

Data extraction and quality assessment

Two authors (SW and HY) independently searched relevant studies and extracted data. The characteristics of the included studies (e.g., author, years of publication, study design,

population, number of patients, patient characteristics, outcomes, and covariates included in the PSM model) are recorded in Table 1.

Two authors (GY and SW) independently evaluated the methodological quality of the included studies by using the Newcastle–Ottawa Scale for cohort studies. The Newcastle–Ottawa Scale contains three categories (including eight subcategories), and each study is able to acquire a maximum of 9 stars. The detailed grading standards are as follows: a score of 7 to 9 stars is graded as a high-quality study, a score of 4 to 6 stars is considered an average-quality study, whereas a score of 0 to 3 stars is classified as a low-quality study.

Statistical synthesis and analysis

We computed the pooled odds ratio (OR) with 95% confidence interval (CI) for dichotomous outcomes and the mean difference (MD) with 95% CI for continuous outcomes. For survival data, we used the hazard ratio (HR) with 95% CI reported in the included studies. If the HR data were not reported in the original study, we imputed the HR by digitizing the Kaplan–Meier survival curves (16). The heterogeneity between studies was assessed by the Higgins inconsistency (I^2) statistics (17). Substantial heterogeneity was identified when the I^2 value >30%, and a random-effects model was employed to perform the analysis; otherwise, a fixed-effects model would be used. Funnel plots were generated to assess the possibility of publication bias, and the Egger regression test was used to measure funnel plot asymmetry (18). We considered P <0.05 to be statistically significant and P <0.10 as an indicator of trends.

Subgroup analysis stratified by types of hepatectomy [minor versus major hepatectomy, based on the Second International Consensus Conference on Laparoscopic Liver Resections (19)] and age groups (≥65, ≥70, or ≥75) was performed to investigate the potential source of heterogeneity. Finally, a sensitivity analysis was conducted to explore the effect of an individual study by the consecutive exclusion of each study at one time.

Results

Study identification and characteristics

The initial search identified 608 articles (114 from PubMed, 174 from Embase, 274 from Scopus, and 46 from Cochrane Library). Among them, 376 were duplicated articles, and 147 studies were excluded by screening the abstracts. During the evaluation of the full text, 73 studies were further removed for various reasons. Eventually, a total of 12 trials (20–31) involving 1,861 patients (LLR versus OLR: 907 versus 954) were included in our study (flowchart in Figure 1).

Table 1 presents the characteristics of the included studies. The number of patients in each study ranged from a minimum of 51 to 438. Among the 12 included studies, four were performed in China (22, 23, 29, 30), three in Japan (20, 27, 28), two in Korea (25, 31), one in Singapore (26), one in France (21), and one study in Italy, France, and Spain (24), respectively. Different studies define "elder patients" individually. Three studies (21, 30, 31) had an inclusion criterion of ≥65 years, eight studies (20, 22–27, 29) comprised patients aged ≥70 years, and one study (28) included patients who were 75 years old and above. The LLR and the OLR groups were comparable in terms of age, gender, characteristics of the tumor, and the American Society of Anesthesiologists score. The types of hepatectomy were diverse among each study: eight studies (20, 22, 23, 26–30) performed minor hepatectomy and four studies (21, 24, 25, 31) included minor and major hepatectomy. The postoperative complications were graded according to the Clavien–Dindo classification, and a postoperative complication of Clavien–Dindo grade ≥III was defined as a major complication (32).

In addition, the length of hospital stay, surgical time, and blood loss were expressed as median with range or interquartile range. Thus, we converted the above data into mean and standard deviation by utilizing the methodology that was developed by Wan et al. (33).

Quality assessment

Table 2 presents the quality assessment by the Newcastle–Ottawa Scale. All included studies had high quality with a quality score ≥7. Six studies (20, 22, 24–26, 28) did not adjust for some important confounders (such as age, sex) or the covariates included in the PSM model were not reported, and the duration of follow-up in seven studies (21–23, 26–29) was limited.

Funnel plots and Egger regression test for all short-term outcome measures were used to further test for potential publication bias (Supplementary Material 3). No significant differences were found with respect to the endpoints of postoperative complications (P = 0.92), blood loss (P = 0.4164), length of hospital stay (P = 0.8368), or surgical time (P = 0.5373). Furthermore, since the number of trials in the analysis of long-term outcomes was limited, we could not reliably assess the publication bias.

Short-term outcomes

A total of 11 studies presented the postoperative complications (Monden et al. only reported the major postoperative complications). Overall, the incidence of postoperative complications in the LLR group was lower than that in the OLR group, 31.8% (236/741) versus 45.2% (356/788), respectively. Our

TABLE 1 Characteristics of the included studies.

Study	Design	Population	Number of patients	Patient characteristics	Outcome	Covariates included in the PSM model
Monden 2022, in Japan	Single center, PSM	Patients aged ≥70 years with HCC who underwent LLR and OLR between January 2010 and June 2021	150 (LLR: 75, OLR: 75)	LLR: age 75 (70–83)[a]; male rate 71%; size of the largest tumor 24 mm (10–82)[a]; Child–Pugh A 96% OLR: age 75 (70–90)[a]; male rate 68%; size of the largest tumor 21 mm (2.7–80)[a]; Child–Pugh A 95%	Short-term outcomes: major postoperative complications, surgical time, blood loss, hospital stay, R0 resection	Age, sex, BMI, history of abdominal surgery, comorbid diseases, history of aspirin prescription, ASA classification, hepatitis status, Child–Pugh classification, tumor size, preoperative blood test, and surgical procedures
Wen 2021, in China	Single center, PSM	Patients aged over 65 with HCC who underwent liver resection between January 2015 and September 2018	142 (LLR: 71, OLR: 71)	LLR: age 68 (66, 72)[b]; male rate 76%; tumor size 5.5 cm (4.0, 7.5)[b]; liver cirrhosis 38 OLR: age: 69 (66, 72)[b]; male rate: 80%; tumor size: 6.0 cm (4.0, 8.0)[b]; liver cirrhosis 35	Short-term outcomes: postoperative complications, surgical time, blood loss, hospital stay Long-term outcomes: OS and DFS rates at 1 and 3 years	Age, sex, BMI, ASA grade, preoperative blood test, previous abdominal surgical history, comorbidities, tumor characteristics, and intraoperative records
Delvecchio 2021, in Italy, France, and Spain	Multicenter, PSM	Consecutive hepatocellular carcinoma liver resection cases in patients with ≥70 years of age	438 (LLR: 219, OLR: 219)	LLR: age 75 (70–93)[a]; male rate 72%; size of the largest tumor 35 mm (9–160)[a]; Child–Pugh A 98% OLR: age 75 (70–89)[a]; male rate 76%; size of the largest tumor 40 mm (7–150)[a]; Child–Pugh A 97%	Short-term outcomes: postoperative complications, surgical time, hospital stay Long-term outcomes: OS and DFS rates at 1, 3, and 5 years	Gender, comorbidity, ASA score, Child–Pugh score, Milan stage, number of tumors, tumor size, tumor locations, and type of hepatic resection
Nomi 2020, in Japan	Multicenter, PSM	Patients (age ≥ 75 years) who underwent liver resection for HCC between April 2010 and December 2017	310 (LLR: 155, OLR: 155)	LLR: age 78 (75–93)[a]; male rate 58%; size of the largest tumor 28 mm (2–120)[a] OLR: age 78 (75–87)[a]; male rate 67%; size of the largest tumor 28 mm (2–150)[a]	Short-term outcomes: postoperative complications, blood loss, hospital stay, R0 resection	Sex, smoking, alcohol consumption, platelet count, underlying hepatic disease, tumor size, and type of resection
Dumronggittigule 2020, in Korea	Single center, PSM	HCC patients aged ≥70 years after hepatectomy between 2003 and 2018	82 (LLR: 41, OLR: 41)	LLR: age 73 (71, 79)[b]; male rate 68%; tumor size 3.8 cm (2.5, 6.4)[b]; Child–Pugh A 95% OLR: age 73 (71, 75)[b]; male rate 85%; tumor size 4.0 cm (2.9, 6.9)[b]; Child–Pugh A 90%	Short-term outcomes: postoperative complications, surgical time, blood loss, hospital stay, R0 resection Long-term outcomes: OS and DFS rates at 1, 3, and 5 years	Child–Turcotte–Pugh classification, tumor number, maximum size, location, extent and difficulty of liver resection
Chen 2020, in China	Single center, PSM	Patients aged 70 or over who underwent hepatectomy for HCC between January 2013 and December 2018	128 (LLR: 64, OLR: 64)	LLR: age 71 (70–77)[a]; male rate 64%; size of the largest tumor NR OLR: age 72 (70–76)[a]; male rate 59%; size of the largest tumor NR	Short-term outcomes: postoperative complications, surgical time, blood loss, hospital stay, R0 resection	Age, gender, BMI, ASA score, Charlson comorbidity index, underlying liver disease, tumor location, and type of hepatectomy

(Continued)

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TABLE 1 Continued

Study	Design	Population	Number of patients	Patient characteristics	Outcome	Covariates included in the PSM model
Kim 2020, in Korea	Single center, PSM	Patients older than 65 years with solitary treatment-naive HCC who underwent liver resection	182 (LLR: 91, OLR: 91)	LLR: age 70 (65–82)[a]; male rate 75%; tumor size 2.6 cm (0.9, 14.0)[a]; liver cirrhosis 44 OLR: age 69 (65–84)[a]; male rate 77%; tumor size 2.9 cm (0.3, 13.2)[a]; liver cirrhosis 47	Short-term outcomes: surgical time, blood loss, hospital stay	Tumor size, sex, protein induced by vitamin K absence or antagonist II, and cirrhosis
Badawy 2019, in Japan	Single center, PSM	Elderly patients (≥70 years) who underwent liver resection for malignant liver tumors between March 2009 and July 2016	80 (LLR: 40, OLR: 40)	LLR: age 75 (72, 79)[b]; male rate 68%; tumor size 32 mm (4–45)[a]; Child–Pugh A 98% OLR: age 76 (73, 79)[b]; male rate 58%; tumor size 24 mm (5–48)[a]; Child–Pugh A 95%	Short-term outcomes: postoperative complications, surgical time, blood loss, hospital stay Long-term outcomes: OS and DFS rates at 1, 3, and 5 years	NR
Goh 2018, in Singapore	Single center, PSM	Elderly patients (≥70 years) who underwent liver resection for HCC	64 (LLR: 32, OLR: 32)	LLR: age 73 (70–88)[a]; male rate 72%; size of the largest tumor 30 mm (14–80)[a] OLR: age 75 (70–83)[a]; male rate 72%; size of the largest tumor 35 mm (5–90)[a]	Short-term outcomes: postoperative complications, surgical time, blood loss, hospital stay	NR
Cauchy 2016, in France	Multicenter, PSM	Elderly patients aged 65 years and older who underwent major liver resection for HCC	144 (LLR: 72, OLR: 72)	NR	Short-term outcomes: postoperative complications	Sex, age, ASA score, BMI, comorbidities, presence of severe underlying fibrosis, indication for hepatectomy tumor characteristics, type of resection, and extent of resection
Wang 2015, in China	Single center, PSM	Elderly patients (≥70 years) who underwent LLR or OLR for malignant liver carcinoma	90 (LLR: 30, OLR: 60)	LLR: age 71 (70–81)[a]; male rate 83%; size of the largest tumor 4 cm (1.5–10)[a]; Child–Pugh A 100% OLR: age 73 (71–84)[a]; male rate 75%; size of the largest tumor 5 cm (2–10)[a]; Child–Pugh A 98%	Short-term outcomes: postoperative complications, surgical time, blood loss, hospital stay	Age, sex, comorbid illness, Child–Pugh class, ASA grade, tumor size, tumor location, and extent of hepatectomy
Chan 2014, in China	Single center, PSM	Patients aged ≥70 years old who received liver resections for malignant liver tumors between January 2002 and December 2012	51 (LLR: 17, OLR: 34)	LLR: age 73 (70–94)[a]; male rate 59%; size of the largest tumor 3 cm (0.8–9.5)[a]; Child–Pugh A 100% OLR: age 74 (70–83)[a]; male rate 59%; size of the largest tumor 3 cm (1–10)[a]; Child–Pugh A 97%	Short-term outcomes: postoperative complications; surgical time, blood loss, hospital stay	Age, tumor size, and tumor location

HCC, hepatocellular carcinoma; LLR, laparoscopic liver resection; OLR, open liver resection; ASA, American Society of Anesthesiologists; OS, overall survival; DFS, disease-free survival; N, number of studies.
[a]Data presented as median and range.
[b]Data presented as median and interquartile range.

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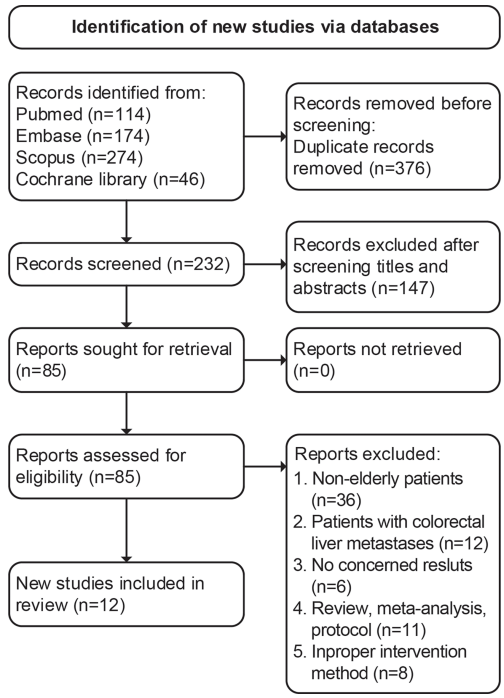

FIGURE 1
PRISMA 2020 flow diagram for the meta-analysis.

meta-analysis demonstrated that LLR was associated with a lower incidence of postoperative complications (OR 0.49, 95% CI 0.39 to 0.62, $P < 0.00001$, $I^2 = 0\%$; Table 3, Supplementary Material 3). In addition to overall postoperative complications, the incidence of pulmonary complications was significantly lower in the LLR group (OR 0.24, 95% CI 0.14 to 0.40, $P < 0.00001$, $I^2 = 0\%$; Table 3, Supplementary Material 3). Moreover, six studies reported the rate of R0 resection, and there was no difference in the rate of R0 resection between the OLR and LLR groups (OR 1.06, 95% CI 0.30 to 3.74, $P = 0.92$, $I^2 = 69\%$; Table 3, Supplementary Material 3).

A total of seven studies reported blood loss during the operation. The meta-analysis demonstrated that LLR was associated with a significant less blood loss than OLR (MD −285.69, 95% CI −481.72 to −89.65, $P = 0.004$, $I^2 = 96\%$; Table 3, Supplementary Material 3). Also, LLR was related to a shorter length of hospital stay (MD −7.88, 95% CI −11.38 to −4.37, $P < 0.0001$, $I^2 = 96\%$; Table 3, Supplementary Material 3). Moreover, there was no significant difference in surgical time (MD 17.33, 95% CI −6.17 to 40.83, $P = 0.15$, $I^2 = 92\%$; Table 3, Supplementary Material 3). However, considering the significant heterogeneity in the pooled results, the results should be interpreted with caution.

Long-term outcomes

Four studies (20, 24, 25, 30) reported the long-term outcomes including the 1-, 3-, and 5-year OS and DFS rates, and the meta-analysis indicated that there was no significant difference in the 1-, 3-, and 5-year OS rates between the LLR and the OLR groups (1-year OS: HR 0.60, 95% CI 0.36 to 1.00, $P = 0.05$, $I^2 = 7\%$; 3-year OS: HR 0.82, 95% CI 0.59 to 1.14, $P = 0.24$, $I^2 = 0\%$; 5-year OS: HR 0.77, 95% CI 0.55 to 1.09, $P = 0.15$, $I^2 = 20\%$; Supplementary Material 3). Similarly, the pooled results showed no significant difference in the DFS rates at 1, 3, and 5 years between the LLR and the OLR groups (1-year DFS: HR 0.65, 95% CI 0.43 to 1.00, $P = 0.05$, $I^2 = 43\%$; 3-year DFS: HR 0.82, 95% CI 0.64to 1.04, $P = 0.10$, $I^2 = 28\%$; 5-year

TABLE 2 Quality assessment of the included studies by the Newcastle–Ottawa Scale.

Study	Newcastle–Ottawa Scale components								Quality score
	1	2	3	4	5	6	7	8	
Monden 2022	*	*	*	*	**	*		*	8
Wen 2021	*	*	*	*	**	*	*	*	9
Delvecchio 2021	*	*	*	*	*	*	*	*	8
Nomi 2020	*	*	*	*	*	*		*	7
Dumronggittigule 2020	*	*	*	*	*	*	*	*	8
Chen 2020	*	*	*	*	**	*		*	8
Kim 2020	*	*	*	*	*	*	*	*	8
Badawy 2019	*	*	*	*	*	*	*	*	8
Goh 2018	*	*	*	*	*	*		*	7
Cauchy 2016	*	*	*	*	**	*		*	8
Wang 2015	*	*	*	*	**	*		*	8
Chan 2014	*	*	*	*	*	*		*	7

1, Representativeness of the exposed cohort; 2, selection of the non-exposed cohort; 3, ascertainment of exposure; 4, demonstration that the outcome of interest was not present at the start of the study; 5, comparability of cohorts on the basis of the design or analysis; 6, assessment of outcome; 7, was follow-up long enough for outcomes to occur; 8, adequacy of follow-up of cohorts. *: get one point; **: get two points.

DFS: HR 0.79, 95% CI 0.54 to 1.16, P = 0.24, I^2 = 60%; Supplementary Material 3).

Subgroup and sensitivity analyses

Prespecified subgroup analyses stratified by types of hepatectomy were performed to investigate the potential discrepant treatment effect and potential sources of heterogeneity (Table 3, Supplementary Material 3). A total of eight studies (20, 22, 23, 26–30) reported patients with minor hepatectomy, and the remaining three studies (21, 24, 25) included both minor and major hepatectomy defined as the combined hepatectomy group.

The pooled ORs for postoperative complications in the two subgroups were 0.44 (95% CI 0.31 to 0.61, P < 0.00001, I^2 = 0%) for minor hepatectomy and 0.55 (95% CI 0.40 to 0.75, P = 0.0002, I^2 = 0%) for combined hepatectomy. The results indicated that LLR was associated with a lower incidence of postoperative complications for patients with minor or major hepatectomy. Moreover, for patients with minor hepatectomy, LLR was associated with less blood loss (MD −402.09, 95% CI −616.68 to −187.50, P = 0.0002, I^2 = 96%), shorter length of hospital stay (MD −8.17, 95% CI −12.24 to −4.10, P < 0.0001, I^2 = 95%), and comparable surgical time (MD 10.56, 95% CI −19.79 to 40.90, P = 0.50, I^2 = 94%). However, for patients with combined hepatectomy, there was no significant difference in blood loss (MD 22.66, 95% CI −502.36 to 547.68, P = 0.93, I^2 = 91%) and length of hospital stay (MD −7.12, 95% CI −14.75 to 0.52, P = 0.07, I^2 = 97%), but a longer surgical time (MD 39.26, 95% CI 18.97 to 59.54, P = 0.0001, I^2 = 35%) was observed. However, the significant heterogeneity and limited number of studies in this subgroup weakened the credibility of this conclusion.

Furthermore, since there were three different definitions of elderly patients (at least 60, 70, or 75 years old), we performed a subgroup analysis based on the age groups. The subgroup analysis showed that the incidence of postoperative complications was similar in three different subgroups (≥65: OR 0.36, 95% CI 0.21 to 0.61, P = 0.0001, I^2 = 0%; ≥70: OR 0.59, 95% CI 0.45 to 0.79, P = 0.0003, I^2 = 0%; ≥75: OR 0.36, 95% CI 0.21 to 0.61, P = 0.0002), and the subgroup of ≥70 years old showed similar results with the overall analysis. However, the subgroup of ≥65 years old showed no difference in the length of

TABLE 3 Results of this meta-analysis.

Outcome	*N*	Result (laparoscopic versus open liver resection)
Postoperative complications	10	OR 0.49, 95% CI 0.39 to 0.62, P < 0.00001, I^2 = 0%
Subgroup analysis		
Minor hepatectomy	7	OR 0.44, 95% CI 0.31 to 0.61, P < 0.00001, I^2 = 0%
Combined hepatectomy	3	OR 0.55, 95% CI 0.40 to 0.75, P = 0.0002, I^2 = 0%
		Test for subgroup difference: I^2 = 4%
Minor complications	10	OR 0.63, 95% CI 0.49 to 0.81, P = 0.0004, I^2 = 0%
Major complications	11	OR 0.50, 95% CI 0.36 to 0.69, P < 0.0001, I^2 = 0%
		Test for subgroup difference: I^2 = 23%
Pulmonary complications	6	OR 0.24, 95% CI 0.14 to 0.40, P < 0.00001, I^2 = 0%
R0 resection	6	OR 1.06, 95% CI 0.30 to 3.74, P = 0.92, I^2 = 69%
Blood loss	7	MD −285.69, 95% CI −481.72 to −89.65, P = 0.004, I^2 = 96%
Subgroup analysis		
Minor hepatectomy	5	MD −402.09, 95% CI −616.68 to −187.50, P = 0.0002, I^2 = 96%
Combined hepatectomy	2	MD 22.66, 95% CI −502.36 to 547.68, P = 0.93, I^2 = 91%
		Test for subgroup difference: I^2 = 54%
Length of hospital stay	9	MD −7.88, 95% CI −11.38 to −4.37, P < 0.0001, I^2 = 96%
Subgroup analysis		
Minor hepatectomy	6	MD −8.17, 95% CI −12.24 to −4.10, P < 0.0001, I^2 = 95%
Combined hepatectomy	3	MD −7.12, 95% CI −14.75 to 0.52, P = 0.07, I^2 = 97%
		Test for subgroup difference: I^2 = 0%
Surgical time	10	MD 17.33, 95% CI −6.17 to 40.83, P = 0.15, I^2 = 92%
Subgroup analysis		
Minor hepatectomy	7	MD 10.56, 95% CI −19.79 to 40.90, P = 0.50, I^2 = 94%
Combined hepatectomy	3	MD 39.26, 95% CI 18.97 to 59.54, P = 0.0001, I^2 = 35%
		Test for subgroup difference: I^2 = 58%

N, number of included studies; OR, odds ratio; CI, confidence interval; MD, mean difference; OS, overall survival; DFS, disease-free survival.

hospital stay (MD −2.26, 95% CI −4.56 to 0.03, $P = 0.05$, $I^2 = 69\%$) but had longer surgical time (MD 40.82, 95% CI 15.29 to 66.36, $P = 0.002$, $I^2 = 65\%$).

In addition, based on the Clavien–Dindo classification (grades I to II as minor complications, grades III to V as major complications), we divided the data of postoperative complications into minor and major complications. The results indicated that both major and minor postoperative complications were in favor of LLR (major: OR 0.50, 95% CI 0.36 to 0.69, $P < 0.0001$, $I^2 = 0\%$; minor: OR 0.63, 95% CI 0.49 to 0.81, $P = 0.0004$, $I^2 = 0\%$; Table 2, Supplementary Material 3).

Furthermore, the sensitivity analysis by excluding each study showed no significant difference in the short-term outcomes (Supplementary Material 3).

Discussion

Considering the increase in overall life expectancy and the rising incidence of HCC, more elderly patients are considered for liver resection. Despite the advancement of laparoscopic techniques, only a few studies have focused on the potential benefits of LLR in the elderly population. In view of the scarcity of high-quality evidence, we performed this meta-analysis of PSM studies to compare the short- and long-term outcomes of LLR versus OLR for elderly patients with HCC. The results demonstrated that LLR significantly reduces postoperative complications, blood loss, and length of hospital stay, whereas the operation time was insignificantly different. Additionally, in terms of long-term survival rate, there were no significant differences between the LLR and the OLR groups. However, it should be noted that these benefits might only apply to a selected group of patients, undergoing less technically demanding minor laparoscopic hepatectomies.

Generally, the elderly are considered a vulnerable group because of the aging process, with numerous comorbidities and lower reserve capacity (34). In general, elderly patients with underlying functional status can influence the surgeons' decision-making on surgical procedure selection. OLR for the treatment of HCC is a major abdominal surgery with high risks and difficulties, especially for elderly patients (35, 36). When choosing the clinical outcomes of our study, we compared LLR with OLR on different levels in terms of safety (postoperative complications), difficulty (operative time, blood loss), efficiency (length of hospital stays), and long-term results (OS and DFS rates). The results of our meta-analysis were broadly consistent with previous meta-analyses (35–37), indicating that LLR is a favorable approach for elderly patients that delivers improved short-term outcomes in terms of postoperative complications, blood loss, and length of hospital stay. Moreover, we further analyzed the pulmonary complications and survival rates between LLR and OLR. Our meta-analysis revealed that LLR was associated with an obviously lower incidence of pulmonary complications and no significant difference in OS or DFS rates between the LLR and the OLR groups, thereby dispelling the concerns that the laparoscopic approach may be inferior to the standard open approach in oncological efficiency.

Significantly lower rates of postoperative complications for the LLR group including a lower risk for both minor and major complications were proven in our meta-analysis. Furthermore, pulmonary complications are one of the potentially life-threatening complications after hepatectomy, especially for elderly patients. Our meta-analysis discovered a significantly lower incidence of pulmonary complications in the LLR group, and there might be several reasons for the difference. First, in open hepatectomy, the large abdominal incisions may increase the risk of wound infection and severe pain, which in turn would increase the risk of postoperative pulmonary complications. This might also be associated with delayed postoperative rehabilitation and longer hospital stay. Second, some studies have demonstrated that intraoperative fluid overload is a strong risk factor for pulmonary complications after hepatic surgery (38–40). Therefore, the lower intraoperative blood loss in the LLR group might be helpful in decreasing intraoperative fluid administration.

Another advantage of LLR is less intraoperative blood loss. The decreased blood loss in the LLR group could be attributed to the fact that the length of the incision was relatively small in laparoscopic surgery. Secondly, the hemostatic effect of the artificial pneumoperitoneum and a better view of the surgical field could also diminish blood loss (41, 42). Furthermore, the prevalence of liver cirrhosis differs among studies, but the majority is classified as Child–Pugh A, which might explain the reduced blood loss as well. Nevertheless, considering the significant heterogeneity and potential mistakes in calculating intraoperative blood loss (43), the results need to be interpreted with caution.

Concerning long-term outcomes, we observed that the laparoscopic approach had a potential long-term survival advantage, but it was not statistically significant. Moreover, it is interesting to note that the individual participant data meta-analysis of PSM studies by Syn et al. (44) demonstrated a long-term survival benefit in favor of LLR over OLR for patients with colorectal liver metastases. Although the survival benefit was not definitively confirmed in our meta-analysis, the potential clinical and biological mechanisms underlying the survival benefit associated with LLR should be attracted. First of all, many studies demonstrated that postoperative morbidity was an independent risk factor for long-term survival (45–47). The laparoscopic approach might provide a survival advantage by decreasing postoperative morbidity. Furthermore, by reducing the adverse effects of postoperative morbidity on the timing of postoperative chemotherapy, patients who had LLR have a quicker recovery and earlier resumption of chemotherapy regimens than patients with OLR (48, 49). Secondly, since laparoscopic surgery is a relatively newer surgical technique, it

requires skilled surgeons with extensive experience. Thus, surgeons who routinely perform LLR may be more experienced, and the accumulated experience is associated with improved outcomes after hepatectomy for HCC (50). Moreover, the laparoscopic approach could preserve the liver parenchyma and portal pedicles or reduce the rates of dense adhesions, which may also reduce the incidence of postoperative complications and increase the feasibility of salvage surgical resection in the future (51).

However, the current study had several limitations. First and foremost, our study was limited by the retrospective and non-randomized design of the included studies. Although all included studies employed the PSM method to reduce the impact of the measured potential confounders, some unmeasured but important potential confounding factors might be overlooked. Moreover, most of the included studies had a limited sample size. Of those, nine studies were typically defined as small studies (<100 patients per arm), which may lead to a small study effect bias (52).

Secondly, there was a significant between-study heterogeneity in several outcomes, which might be derived from the differences in age ranges, liver function, number and location of lesions, general condition of the individual patient, surgeons' experience, perioperative care protocols, pre- and postoperative chemotherapy, and other factors. Some studies included patients at wide study intervals, which may introduce biases due to advances in the mastery of surgical skills and improvements in surgical instruments (53). Noteworthy, the covariates for matching were different between the included studies, and some studies did not adjust for some important confounders such as age, sex, and liver function classification. Future research should further dissect the matching covariates to draw more accurate results.

Last but not the least, our meta-analysis only evaluated the overall and pulmonary complications. Some specific and important complications including bile leak, abscesses, and intra-abdominal infection between the two therapies could not be adequately compared, which should be further evaluated in the future.

Conclusion

In conclusion, our meta-analysis of PSM studies suggests that LLR has improved short-term outcomes including a lower incidence of postoperative complications, less blood loss, and shorter length of hospital stay, with comparable long-term outcomes for elderly patients with HCC when compared with the open approach. However, most of the existing data are about the results of minor hepatectomy, and laparoscopic major hepatectomy in elderly patients should be carefully evaluated and preferably performed in expert centers. Furthermore, considering the limited number of included studies with small sample sizes, significant heterogeneity and potential bias were found among the included studies. Well-designed, multicenter RCTs with a large sample size are needed to further evaluate the short- and long-term outcomes of LLR versus OLR for elderly patients with HCC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

SW and HY conceived the idea, performed the analysis, and wrote the initial draft of this paper. GY, JW and SX contributed to the collection and interpretation of data. QY helped to frame the idea of the study and provided technical support. HY contributed to the revision of this paper and the final approval of the version to be published. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.939877/full#supplementary-material

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Thermal ablation in pancreatic cancer: A scoping review of clinical studies

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Background: Pancreatic cancer is a deadly cancer with a 5-year survival rate less than 10%. Only 20% of patients are eligible to receive surgery at diagnosis. Hence, new therapies are needed to improve outcomes for non-surgical candidates. Thermal ablation techniques can offer a non-invasive alternative to surgery.

Aim: The aim of this review is to map the literature for the use of thermal ablative techniques: Radiofrequency ablation (RFA), High-intensity focused ultrasound (HIFU), Microwave ablation (MWA), and Laser ablation (LA) in the management of patients with PC.

Methods: A search strategy was applied to PUBMED and EMBASE using keywords concerning pancreatic cancer, radiofrequency ablation, ultrasound ablation, laser ablation, and microwave ablation. The studies that fit this inclusion criteria were summarized in table format and results reviewed for interpretation.

Results: 72 clinical studies were included. Most of the included studies related to RFA (n=35) and HIFU (n=27). The most common study design was retrospective (n=33). Only 3 randomized control trials (RCT) were included, all of which related to RFA. Safety outcomes were reported in 53 of the 72 studies, and survival outcomes were reported in 39. Statistically significant survival benefits were demonstrated in 11 studies.

Conclusion: The evidence for the benefit of MWA and LA in PC patients is limited. RFA and HIFU are safe and feasible therapies to be used in PC patients. Further RCTs where thermal techniques are standardized and reported are necessary in the future to elucidate thermal ablation's clinical utility, and before an evidence-based decision on its routine use in PC management can be considered.

KEYWORDS

thermal ablation, pancreatic cancer, radiofrequency ablation, high-intensity focused ultrasound, microwave ablation, laser ablation

1 Introduction

Pancreatic cancer (PC) is a deadly disease, which according to GLOBOCAN cancer statistics accounted for 2.6% of new cancer cases, and 4.7% of cancer deaths globally in 2020 (1). This makes it the 7th leading cause of cancer death worldwide (1). Surgery is the main curative treatment option for PC, but according to the American Cancer Society, fewer than 20% of patients are candidates for surgery (2).

For those with locally advanced and metastatic disease, chemotherapy (CHT) regimens like FOLFIRINOX have been shown to be effective in prolonging survival (3, 4). However, survival is dismal at this stage irrespective of CHT regimen, with the use of FOLFIRINOX (OS: 11.1 months) (4) and Nab-Paclitaxel+Gemcitabine (OS: 8.5 months) (5) giving a modest survival advantage over Gemcitabine (OS: 6.8 months (P<0.001)) (4) (OS: 6.7 months(P<0.001)) (5).

Radiotherapy (RT) can be used as chemosensitization in many cancers, but it's chemosensitization in PC remains controversial. In the phase III LAP07 trial, after 4 cycles of induction gemcitabine +/- erlotinib, patients with locally advanced pancreatic cancer (LAPC) whose tumors were controlled were randomized to receive either CHT or chemoradiotherapy (CRT) for a further 2 months. There was no significant difference in OS in the CHT group (16.5 months) compared to the CRT group (15.2 months) (6). If the evidence for the combination of RT and CHT is inconclusive, perhaps thermal techniques could provide an opportunity to improve patient outcomes.

Thermal ablation has been defined as the use of temperatures >50°C for >4 min, or >512 CEM43°C (7) and has already demonstrated efficacy for managing other solid malignancies such as colorectal cancer and prostate cancer (8, 9). Ablative temperatures can be generated by several modalities including: RFA, LA, MWA, and HIFU. The preclinical evidence for the use of these techniques in PC models has been the subject of a systematic review from 2018 by Saccomandi et al. (10). The question that remains is whether these techniques can be effective in clinical studies of patients with PC.

Abbreviations: PC, Pancreatic cancer; RFA, Radiofrequency Ablation; HIFU, High-Intensity Focused Ultrasound; MWA, Microwave ablation; LA, Laser ablation; RCT, Randomized control trial; CHT, Chemotherapy; FOLFIRINOX, Folinic acid, fluorouracil, irinotecan hydrochloride, and oxaliplatin; MPC, Metastatic pancreatic cancer; OS, Overall survival; RT, Radiotherapy; LAPC, Locally advanced pancreatic cancer; CRT, Chemoradiotherapy; CEM43°C, Cumulative equivalent minutes at 43°C; PDAC, Pancreatic ductal adenocarcinoma; NET, Neuroendocrine tumor; US, Ultrasound; RECIST 1.1, Response evaluation criteria in solid tumors 1.1; CT, Computed tomography; BR, Borderline resectable.

The objective of this scoping review is to map the literature that has been published from clinical studies in this space. This review will focus on RFA, HIFU, MWA and LA, and will aim to address issues surrounding:

1. The safety and efficacy of these methods.
2. Standardization of these methods.
3. The potential future directions of this field.

2 Methods

This literature review was undertaken according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews guidelines (11).

2.1 Eligibility criteria

Texts were included in this review if they described an ablative intervention in PC patients, were in English, and if the full text was available *via* open access. Papers were excluded if they described *in vitro* studies, *in vivo* studies, review papers, abstracts, posters, or letters. Journal pre-proofs were included where available.

2.2 Information sources

The search was conducted on EMBASE and PUBMED. This was supplemented by relevant studies that were cited in the studies from EMBASE and PUBMED.

2.3 Search

Search strategies were undertaken for each modality on both databases. The strategies are described in detail in Figure 1.

2.4 Selection of sources of evidence

The abstracts of the results of each search were screened by the authors on the PUBMED and EMBASE databases. When the full text could not be retrieved automatically, the authors searched the internet using the article title. When abstracts, letters, and posters were retrieved by the automated feature, the authors searched the internet for corresponding full articles. The retrieved full texts were inspected, and if they fit the inclusion criteria, included in the review.

RFA

PUBMED	EMBASE
Radiofrequency ablation [T/A] OR radio frequency ablation [T/A] OR radio-frequency ablation [T/A] OR RFA [T/A] OR radiofrequency hyperthermia [T/A] AND Pancreatic cancer [T/A] OR Pancreas [T/A] OR Pancreatic Neoplasm [T/A] OR PDAC [T/A] OR Pancreatic Ductal Adenocarcinoma	'Radiofrequency ablation' [T/A] OR 'Radiofrequency hyperthermia' [T/A] AND 'Pancreas Cancer' [T/A] OR 'Pancreas' [T/A] OR 'Pancreas Tumour' [T/A] OR 'Pancreatic ductal carcinoma cell line' [T/A]

HIFU

PUBMED	EMBASE
High-intensity focused ultrasound [T/A] OR HIFU [T/A] OR ultrasound ablation [T/A] OR focused ultrasound [T/A] OR high-intensity focused ultrasound AND Pancreatic cancer [T/A] OR Pancreas [T/A] OR Pancreatic Neoplasm [T/A] OR PDAC [T/A] OR Pancreatic Ductal Adenocarcinoma	'High intensity ultrasound' [T/A] OR HIFU [T/A] OR 'ultrasound ablation' [T/A] OR 'focused ultrasound' [T/A] OR 'high intensity focused ultrasound' [T/A] OR 'high intensity focused ultrasound ablation' [T/A] AND 'Pancreas Cancer' [T/A] OR 'Pancreas' [T/A] OR 'Pancreas Tumour' [T/A] OR 'Pancreatic ductal carcinoma cell line' [T/A]

MWA

PUBMED	EMBASE
Microwave ablation [T/A] OR MWA [T/A] OR micro wave ablation [T/A] OR micro-wave ablation [T/A] OR microwave hyperthermia [T/A] OR microwave [T/A] OR micro-wave [T/A] OR micro wave [T/A] AND Pancreatic cancer [T/A] OR Pancreas [T/A] OR Pancreatic Neoplasm [T/A] OR PDAC [T/A] OR Pancreatic Ductal Adenocarcinoma	'Microwave thermotherapy' [T/A] OR 'Microwave ablation' [T/A] OR MWA [T/A] OR 'micro wave ablation' [T/A] OR 'micro-wave ablation' [T/A] OR 'microwave hyperthermia' [T/A] OR 'microwave radiation' [T/A] AND 'Pancreas Cancer' [T/A] OR 'Pancreas' [T/A] OR 'Pancreas Tumour' [T/A] OR 'Pancreatic ductal carcinoma cell line' [T/A]

LA

PUBMED	EMBASE
Laser ablation [T/A] OR Laser hyperthermia [T/A] OR thermal ablation [T/A] AND Pancreatic cancer [T/A] OR Pancreas [T/A] OR Pancreatic Neoplasm [T/A] OR PDAC [T/A] OR Pancreatic Ductal Adenocarcinoma	'Laser ablation system' [T/A] OR laser hyperthermia [T/A] OR laser ablation [T/A] OR thermal ablation [T/A] AND 'Pancreas Cancer' [T/A] OR 'Pancreas' [T/A] OR 'Pancreas Tumour' [T/A] OR 'Pancreatic ductal carcinoma cell line' [T/A]

FIGURE 1
Search Strategies. These terms were applied to the title/abstract of texts in each database for each thermal technique.

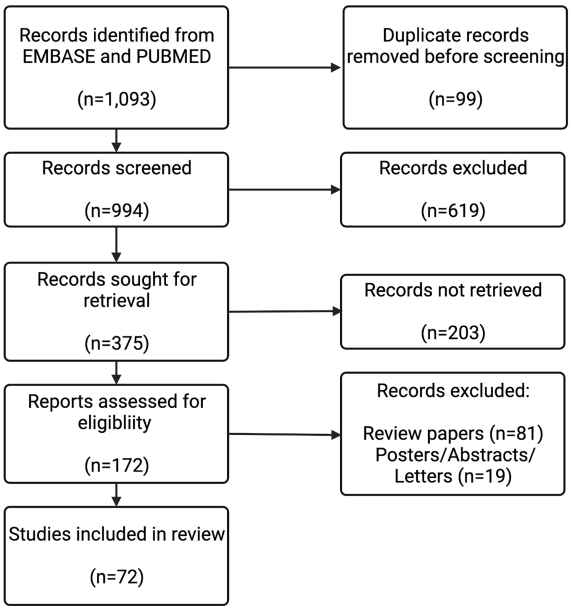

FIGURE 2
Study Selection Flow Diagram. The results from the 8 search strings described (n=1,093) were screened for duplicates. These results from each string were combined, and then screened by their abstracts (n=994). Full texts for the results that were deemed to be relevant from the abstract were sought (n=375). Full texts were assessed (n=172). Review papers (n=81), and posters/abstracts/letters (n=19) were excluded. 72 full-text records were included in this review.

2.5 Data recorded

Data was sought for: year of publication, country of origin, aim of study, stage of disease, patient number, concurrent treatment course, size and type of lesion, parameters of the ablation procedure (frequency utilized/power transferred/energy generated/thermal dose), outcomes measured, results of the study, side effects reported, and the timing of the delivery of the ablation.

3 Results

The search strings resulted in 994 papers for screening. After screening and retrieval, 172 papers were assessed for eligibility. 100 of these were review papers, posters, abstracts, or letters, which left 72 papers to be included in this review. The flowchart summarizing the exclusion process is depicted in Figure 2.

Figure 3 represents the population included in this review, both in terms of studies for each modality, and numbers of patients receiving each modality. There were considerably more studies and patients detailing RFA and HIFU than MWA and LA.

3.1 Radiofrequency ablation

3.1.1 Summary of results

The search strategy for RFA resulted in 35 clinical studies being included in this review, making it the most described ablation technique for PC. The studies detail the use of RFA in the treatment of pancreatic ductal adenocarcinoma (PDAC) (n=637), neuroendocrine tumors (NET) (n=10), and cystic lesions (n=8).

The frequent outcomes measured in the studies detailing RFA treatment were survival measures (n=34), radiological responses (n=18), and pain responses (n=6), as represented in Figure 4. Statistically significant survival benefits were reported in 5 of the studies, while a significantly improved pain response was noted in 3 studies. Side effects were reported in 31 of the studies. The side effects of note reported for RFA were peri-pancreatic fluid collections (n=13; 1.54%), pancreatic fistula (n=11; 1.3%), venous thrombosis (n=10; 1.19%), pancreatitis (n=7; 0.83%), and gastrointestinal hemorrhage (n=5; 0.59%).

The target ablation temperature was reported in 14 studies, and it ranged from 30°C to 105°C. The power settings were reported in 15 studies. The power settings ranged from 5-10W

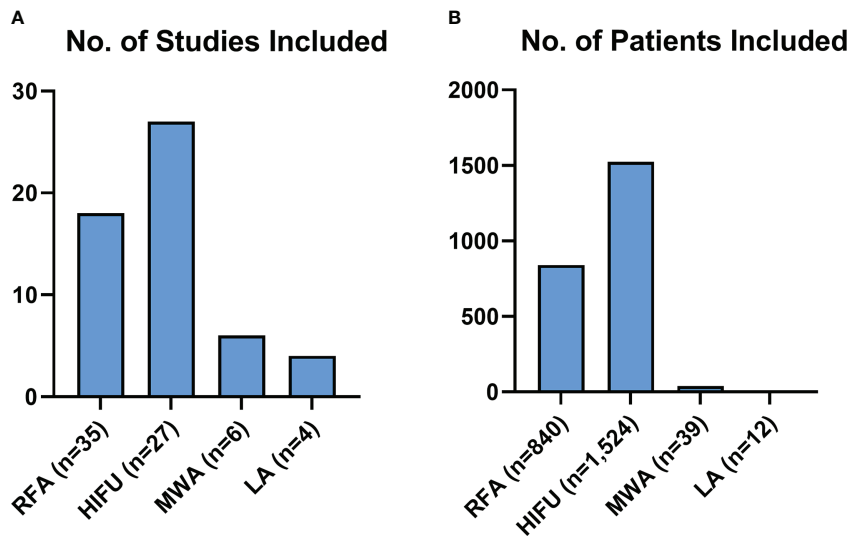

FIGURE 3
Review Population Graphs. Figure 3 describes the makeup of all the subjects of this review for each modality, both in terms of studies **(A)** and patients included **(B)**.

(12) to 200W (13). The duration of the delivery of RFA was described in 26 of the studies, with the longest duration of ablation being 60 minutes (14), and the shortest being 50 seconds (15).

3.1.2 Notable studies

Two RFA studies included in this review were concerned with immunomodulation/immunostimulation (16, 17). In 1986, Falk et al. added immunostimulatory compounds (Copovithane/

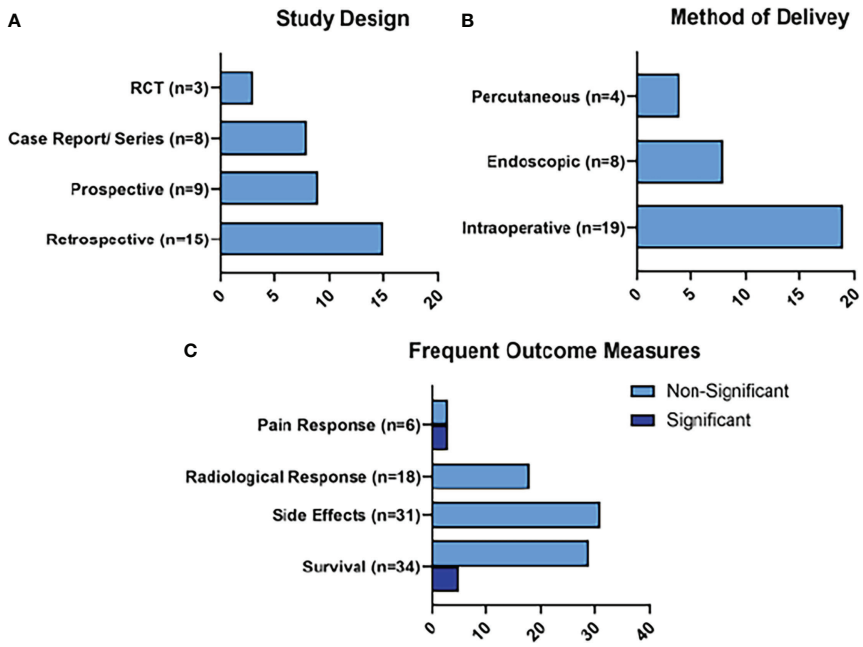

FIGURE 4
Summary of RFA Results. Figure 4 represents the main characteristics of the RFA studies included in this review in terms of study design **(A)**, method of delivery of RFA **(B)**, and frequent outcome measures **(C)**. Frequent outcome measures are subdivided into those that reached statistical significance, and those that did not.

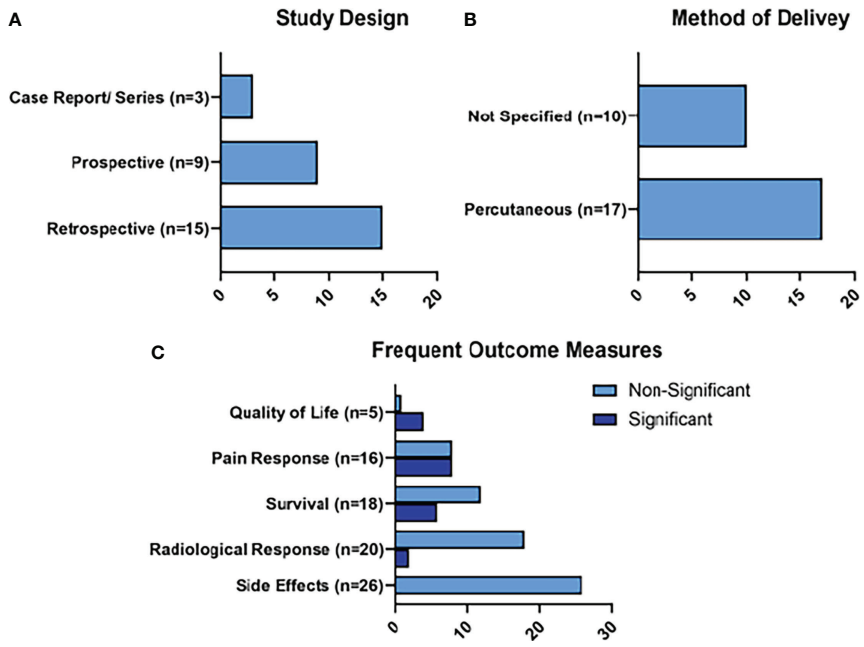

FIGURE 5
Summary of HIFU Results. Figure 5 represents the main characteristics of the HIFU studies included in this review in terms of study design **(A)**, method of delivery of HIFU **(B)**, and frequent outcome measures **(C)**. Frequent outcome measures are subdivided into those that reached statistical significance, and those that did not.

PZ-73C/NED-137) to the treatment of a cohort of 77 PC patients receiving CHT and RFA treatment. They discovered significant percentage survival benefits at 6 months (60.1% vs. 29.8% P<0.008), and 12 months (35% vs. 6% P<0.001) in the patients receiving immunostimulation over those who were not (17).

More recently, Giardino et al. performed a prospective study of the immunomodulatory properties of RFA in the treatment of LAPC (16). Patients were excluded if they had any previous medical oncology treatment. RFA was applied intraoperatively with ultrasound (US)-guided RFA at 90°C using the Uniblate single cool-tip probe™, which has a built-in thermocouple for thermal monitoring. The mean application time was 6 minutes. 30% of patients experienced complications. These were 1 hemorrhage (managed conservatively), 1 ulcer, and 1 pancreatic pseudocyst (16). They analyzed two immunological parameters: serum cell populations (CD8 and CD4 T cells, Treg cells, NK cells, dendritic cells, and monocytes), and serum cytokines (IL-6, CCL-5, SDF1, VEGF, TGF-B, TNF-a). Both CD4 and CD8 T cells demonstrated a significant increase in number from day 3 to day 30 (16). There was a particular increase in effector memory T cells, while no expansion of Treg cells were observed. There was significant enhancement in dendritic cells at day 30 which are fundamental in presenting tumor-associated antigen (16).

The highest quality study assessing the utility of RFA as an up-front therapy comes from Frigerio et al. who published results from a RCT in 2021 (18). They compared the use of RFA with subsequent CHT or CRT (group A), against standard CHT or CRT only (group B). The only requirement for the CHT/CRT regimens used for both cohorts was that they had to have a documented efficacy for treating PDAC that was at least as good as gemcitabine-based therapy. The lack of restriction on the choice of CHT/CRT regimen was chosen because the authors did not want to preclude the participants from receiving novel therapies.

100 patients with LAPC were recruited for this study. 16 of the 48 patients randomized to group A did not receive RFA due to findings of metastases (n=10), or safety concerns (n=6). US-guided RFA was performed during a laparotomy using a Uniblate™ device with the temperature never exceeding 90°C. One month after RFA, group A received CHT or CRT (18).

The OS in group A was 14.2 months, while the median OS in group B was 18.1 months (p=0.639), demonstrating a non-statistically significant reduction in OS. The PFS in group A was increased to 8 months compared to 6 months in group B however this did not reach statistical significance (p=0.570). Three grade B pancreatic fistulas, one delayed gastric emptying, and one abdominal collection requiring treatment were observed as RFA-associated complications (18). Currently, this is the only published RCT on the effect of RFA on OS in LAPC. While the results are disappointing, when they are taken in the greater context of advancements made in LAPC treatment (novel CHT

combination therapies) the results may not be very relevant to the current LAPC treatment landscape. This will be explained further in the discussion section.

3.2 High-intensity frequency ultrasound

3.2.1 Summary of results

The search strategy yielded 27 studies that referred to the use of HIFU in patients with PC. 928 of the tumors treated were PDAC, there were 2 NET treated, and the remainder were referred to as PC or unspecified.

The most common outcomes were radiological responses (n=20), survival outcomes (n=18), pain responses (n=18), and quality of life responses (n=5), as represented in Figure 5. Two of the radiological responses, 6 of the survival responses, 8 of the survival responses, and 4 of the quality of life outcomes were statistically significant. Some of the more commonly reported side effects of HIFU treatment included: pancreatitis (n=9; 0.6%), pseudocyst formation (n=5; 0.32%), and skin burns (n=58; 3.8%). These skin burns were mild in 49 of the cases, and more severe in 9, sometimes requiring plastic surgery (n=3).

The power settings and/or energy administered was reported in 25 of the studies, and the timing of HIFU was reported in 23. The range of power settings was from 100 W (19) to 1,350W (20). The sonification times ranged from 725 seconds (21) to 6,000 seconds (19).

3.2.2 Notable study

HIFU has been described as a surgical tool to facilitate resection. Wang et al. published a retrospective analysis of feasibility and safety of HIFU in 30 patients with BR disease (22). These patients had an *in-situ* gastric tube (which was removed during the subsequent operation) filled with degassed water to improve the acoustic path. The median power of the HIFU was 274 W (± 87 W) for an average sonification time of 1452 (± 370s). 7-9 days after HIFU, 27/30 patients underwent surgical resection. 18 of the patients who underwent surgery also had 21 days of gemcitabine regimen. From the original group of 30 patients, the total resectable rate was 90%. 25 of the cases were R0 resections, while 2 were R1 resections (22). This resection rate of patients with BR disease has been shown to vary across studies. A prospective study in 2019 including 249 BR patients determined the resectable rate to be 24.1% after neoadjuvant treatment (23), while a 2019 retrospective study of 151 reported their resectable rate as 63.6% (24). It appears HIFU can offer a potential effective alternative to neoadjuvant CHT for patients with BR PC.

HIFU has further been described as a therapy in combination with CHT. Li et al. retrospectively compared the use of HIFU in combination with the S-1 (Tefagur/Gimeracil/Oteracil) CHT regimen (n=61), versus S-1 regimen alone (n=59) in metastatic gemcitabine-refractory PDAC (25). S-1 was administered twice daily for one week. This was repeated every 3 weeks until disease progression or toxicity. 2-6 cycles (median= 4) were applied to each patient. Further details of the HIFU treatment were not reported other than the fact that it was delivered percutaneously under US guidance (25). Overall survival was 10.3 months for the HIFU and S-1 group compared to 6.6 months for S-1 monotherapy. PFS was 5.1 months compared to 2.3 months, respectively. In the combination group, 1 patient had a complete response, and 15 had partial response (RECIST 1.1). In the monotherapy group, 5 patients showed a partial response. There was a significant benefit in the proportions of responders in the HIFU group. No grade 3 or 4 adverse events were noted, while patients in the combination group experienced transient nausea, vomiting, anorexia, and diarrhea. Some slight skin burns also occurred in the HIFU group (25).

3.3 Microwave ablation

3.3.1 Summary of results

There were less clinical studies available concerning the use of MWA than for RFA and HIFU. From the search strategy, 6 clinical studies of MWA fitted the inclusion criteria. 4 studies treated PDAC, 1 study treated an intraductal papillary mucinous neoplasm (IPMN), and 1 study treated an insulinoma.

Radiological responses were reported as an outcome measure in all 6 of the studies. Survival was reported in 3, and technical success was reported in 3 (Figure 6). Side effects were reported in all 6 of the studies. 3 liver or pancreatic abscesses were observed (7.7%), 2 pseudocysts were reported (5.12%), and 2 cases of severe local pain were reported (5.12%).

Temperature was not reported in any of the studies, while power was reported in two studies (20W and 100W) and mean cumulative energy output was reported in 1 (9,627W). Duration of the MWA was also only described in 3 studies, and averaged 148 seconds.

3.3.2 Notable studies

MWA was notably utilized as a method of treating insulinomas. Egorov et al. detailed MWA performed on 7 patients (26). These patients had insulinomas and were symptomatic with hyperinsulinism at presentation. They were deemed unfit for surgery, or at high risk of postoperative complications. MWA was performed percutaneously, during a laparotomy, and laparoscopically. The treatment was effective in all patients to render them normoglycemic at 3 days, without any recurrence at the end of follow-up which was 31 months long (26). There were 2 pancreatic fistulas observed. 1 patient developed a pancreatic fistula 1 month after MWA which was drained (26).

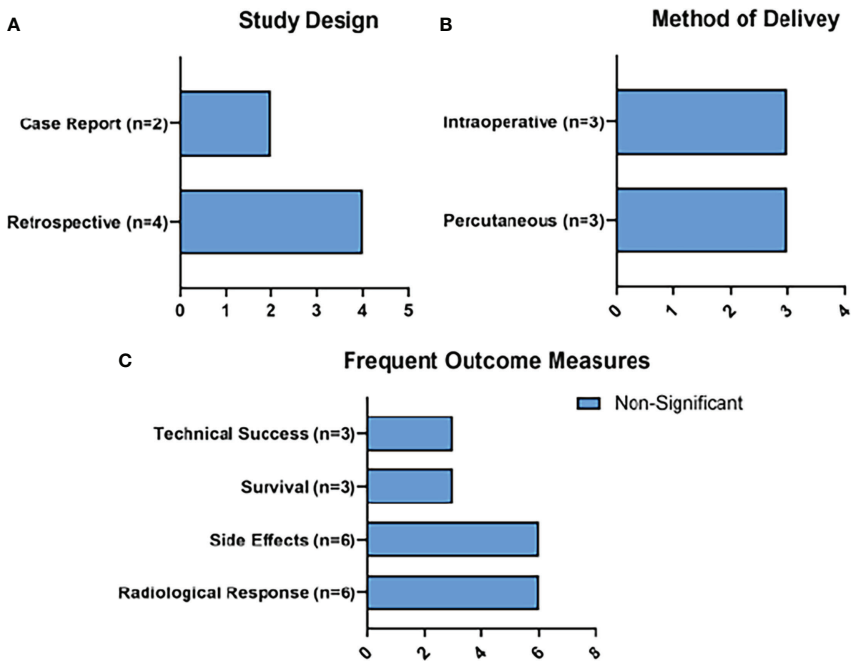

FIGURE 6
Summary of MWA Results. Figure 6 represents the main characteristics of the MWA studies included in this review in terms of study design **(A)**, method of delivery of MWA **(B)**, and frequent outcome measures **(C)**. None of the frequent outcome measures reached statistical significance.

3.4 Laser ablation

3.4.1 Summary of results

From the search strategy, 4 clinical studies examining the use of LA fit the inclusion criteria. 11 patients were treated for PDAC, and 1 had an IPMN. Power settings in these studies ranged from 2W-5W, energy delivered ranged from 800J-14,000J, and the study that detailed ablation time ranged from 200 seconds -600 seconds.

Radiological response (n=3) and symptom response (n=3) were the most frequently reported outcomes. Survival outcomes were only reported in 1 study (Figure 7). Side effects were reported in 2 of the studies, with the only reported side effect of LA being 3 cases of peripancreatic fluid collections.

3.4.2 Notable studies

In 2018, Di Matteo et al. conducted a small prospective cohort study of the feasibility and safety of endoscopic ultrasound-guided (EUS) LA in the treatment of locally advanced PDAC which was unresponsive to previous CRT (27). Feasibility was measured by CT imaging as evidence of coagulative necrosis post-ablation. Safety was measured by the occurrence of adverse events. They applied the LA at a different power (2-4W), energy (800-1,200J) and duration (200-600s) in each of the 9 participants to demonstrate safety and feasibility at a range of operating settings (27). CT scans at 24 hours, 7 days and 30 days after ablation demonstrated well-defined coagulative necrotic areas. The ablated areas decreased in all cases at 30 days. No major adverse events were recorded, but 3 patients showed peri-pancreatic fluid that spontaneously disappeared. Median overall survival was 7.4 months. It was determined that a power of 4 W and 1,000 J achieved the largest ablation volume without adverse effects, and concluded that EUS- guided LA was safe and feasible.

4 Discussion

The aim of this scoping review was to characterize the evidence on the use of RFA, HIFU, MWA and LA. The sources of evidence were heterogeneous, with the most common study design being retrospective review (n=33), followed by case studies (n=16). There were only 3 RCTs reviewed, and they were all for RFA (18, 28, 29). Based on the numerous studies detailing the use of RFA and HIFU compared to MWA and LA, it seems the interest of the scientific community is currently focused on RFA and HIFU. The interest in RFA in particular as a cancer therapy is further reflected in the report by Research Nester (a market research firm) that predicts the global gastrointestinal RFA systems market to grow at a compound annual growth rate of 6% from 2022-2030 (30). While many of the studies in this review

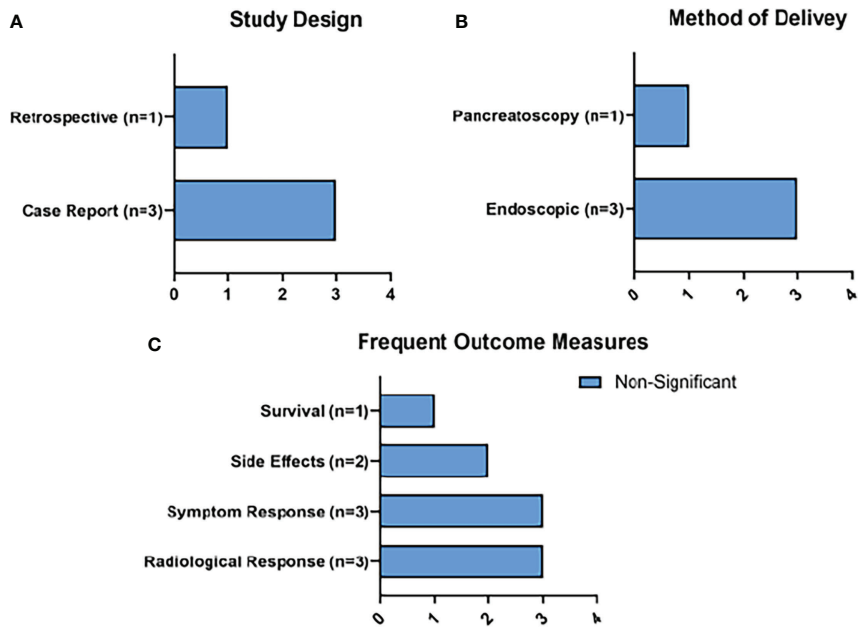

FIGURE 7
Summary of RFA Results. Figure 7 represents the main characteristics of the RFA studies included in this review in terms of study design **(A)**, method of delivery of MWA **(B)**, and frequent outcome measures **(C)**. None of the frequent outcome measures reached statistical significance.

demonstrated benefits to patients with the use of these techniques, the predominance of retrospective study design over RCTs is a serious limitation of the evidence for these techniques. Furthermore, there is a lack of standardization in the application of ablation in terms of temperature recorded, exposure time, and energy applied, which makes it difficult to compare results.

The evidence for the efficacy of these techniques depends on the outcome measured. Radiological tumor responses were commonly seen in these studies, and pain reduction was frequently reported in patients following treatment. Survival was reported in 56 of the studies, and 11 of these demonstrated statistically significant improvements in OS (12, 25, 31–33), median survival time (34, 35), and disease specific survival (33). However, the RCTs for RFA did not show this survival benefit (18, 29).

The occurrence of side effects and adverse events was reported in 53 of the 72 studies, and the majority were mild or moderate. The complications of note were pancreatitis (n=17), fistula formation (n=16), and pseudocyst formation (n=7). Skin burns occurred exclusively in HIFU studies (n=42). Grade I to Grade III burns accounted for 90% of the burns. In one of the HIFU studies (36), two of the burns were of grade III severity and required plastic surgery. The pancreatitis was generally classified as mild or moderate, except for in one HIFU study (36), and two RFA studies (37, 38) where it was severe.

4.1 Evidence for effect on overall survival

The goal of any cancer therapy is to prolong survival, and many of the trials address the effect that ablation has on OS. Most of the trials that have shown statistically significant improvements in OS take the form of retrospective studies, and while some of them have large patient numbers included, there has been a limited number of RCTs published that address the effect of ablation on OS.

A particularly notable retrospective study that showed a statistically significant improvement in OS is a study by Ning et al. (2019) that examined the outcomes of 523 cases of unresectable PDAC. 347 patients received HIFU treatment and gemcitabine, while 176 patients received gemcitabine monotherapy. OS was 7.4 months in the combination group compared to 6 months in the monotherapy group (P=0.004) (32). One of the main limitations of this study is a lack of randomization and potential selective bias. However, the improvement in OS is encouraging, and suggests that further studies could provide stronger evidence for the use of HIFU.

In a study that built on the retrospective evidence, Sofuni et al. (2021) performed a prospective clinical safety trial to evaluate the effects of HIFU for unresectable PC (34). 176 patients received HIFU and CHT, and 89 patients received CHT only. The CHT regimens in this trial included gemcitabine monotherapy, S-1 monotherapy, Gemcitabine plus S-1 therapy, Gemcitabine plus Nab-paclitaxel, and

FOLFIRINOX. The median survival time after diagnosis was 21.3 months in the HIFU and CHT group compared to 9.5 months in the CHT only group (P<0.001) (34). Although this was a prospective study, there was a possible selection bias based on the timing of HIFU, prior therapy, and CHT regimen of the patients. A strength of this study, however, is that the included CHT regimens are more representative of current options for LAPC. Despite its lack of randomization, this study still adds to the evidence for ablation to prolong OS in LAPC and highlights the need for RCTs in the future.

The highest-quality RCT available on thermal ablation in PC to date was published by Frigerio et al. (2021) in which they randomized 100 patients with LAPC to receive either CHT/CRT from an oncologist, or up-front US-guided RFA followed by CHT/CRT (18). The only requirement for the CHT/CRT regimens used for both cohorts was that they had to have a documented efficacy for treating PDAC that was at least as good as gemcitabine-based therapy. The lack of restriction on the choice of CHT/CRT regimen was chosen in the trial design stage because the authors did not want to preclude the participants from receiving novel therapies. As we will see, this is part of the reason why the data from this RCT may have been less relevant at the date of publication.

This trial was conceived in 2013 following previous retrospective studies (37, 38) hoping to treat LAPC and achieve an OS that exceeded 14 months. The authors point towards two factors that they believe impacted their results, and that would have led them to consider another approach to this trial, in retrospect.

The first is that they did not recruit the desired number of patients to this trial. They estimated the required sample size to be 126 patients based on the primary endpoint of OS at 1 year. Only 100 patients were enrollled, and then there was a high dropout rate in the RFA arm of 33.3% due to findings of metastases or safety concerns (18). Ultimately, the study lacked power to detect any significant differences between groups.

The second issue was that in the prolonged period that it took to enrolll, treat, and follow-up on the patients, significant advancements in the treatment of LAPC were made. Namely, the acceptance and success of the combination therapies FOLFIRINOX and Nab-Paclitaxel and Gemcitabine. These advancements made the results of this trial less relevant to LAPC treatment at the time of publication, because many patients received 'outdated' CHT regimens. What the authors ultimately concluded from their work was that upfront RFA does not provide a benefit to LAPC patients and therefore shouldn't be offered (18).

This is not to say, however, that RFA has no place in the management of LAPC. The PELICAN trial is currently in progress. This study is a multicenter superiority RCT examining the effect of second line RFA and CHT versus CHT only on OS in LAPC patients who have stable disease or partial response following at least 2 months of CHT therapy (39). Wherever possible, patients will receive FOLFIRINOX or nab-paclitaxel and gemcitabine. The primary endpoint will be OS, and secondary endpoints will include PFS, radiological tumor response, quality of life, pain, and immunomodulatory effects. The study is aiming to enroll 228 patients (40).

The PELICAN trial is designed to be more relevant to the current landscape of LAPC treatment than the RCT performed by Frigerio et al. When the first RCT was conceived, gemcitabine monotherapy was the dominant treatment for patients with LAPC, and so RFA was added as an upfront treatment. However, in the current era of FOLFIRINOX, improved OS and the possibility of downstaging and resection are known to be possible, meaning that LAPC patients should avail of these treatment regimens before trying less-established therapies. Therefore, this clinical trial of RFA as a second-line treatment should yield more representative evidence for the future application of RFA in the clinic.

4.2 Further potential applications of ablation

For patients with BR PC, RFA could be used as a surgical adjunct to improve resectability. Surgical resection is the only curative option for PC, but only 20% of patients are eligible at diagnosis (2). Furthermore, the rate of R0 resection (tumor-free margin of 1mm) can be low. In a cohort study conducted by Hank et al. in 455 patients who underwent upfront resection for PC, the R0 rate was 23.5%, the R1 (tumor free margin less than 1mm) resection rate was 22.9%, and the R2 (direct invasion of the margin) resection rate was 53.6% (36). They also showed that R0 resection rate was a significant prognostic factor for overall survival. The median OS was 62.4, 24.6, and 17.2 months for R0, R1 (>1mm), and R2 (direct) respectively (36).

In one notable study included in this review, Kumar et al. described the use of RFA as an adjunct to pancreaticoduodenectomy in 6 patients with locally advanced disease where blood vessel involvement prevented resection and vessel reconstruction (41). 4 of these 6 patients achieved R1 margin status after use of RFA, and there were no intraoperative complications. There could be potential for surgeons to use RFA in combination with resection to improve chances of R0 and R1 resections, and better patient outcomes.

The immunomodulatory effects of ablation could be a promising avenue of future clinical trials. The concept of combining RFA and immunostimulatory agents was actually first reported by Falk et al. in 1986 in patients with PDAC receiving RFA and CHT. Survival was significantly increased in patients with immunostimulation (17). More recently, Giardino et al. examined the effect that RFA has on the immune system. Following RFA performed after laparotomy, serum cytokines were not greatly modulated but a number of populations of immune cells were elevated (16). These findings should be treated with caution however, due to the small sample size and the possible

confounding factor of post-surgery inflammation. If future larger clinical studies could replicate these findings however, there is rationale to combine thermal therapy with immunotherapies to ameliorate this largely immunosuppressive cancer.

4.3 Standardization

There are notable limitations to the studies included in this review. The standardization of how heat is delivered is not consistent across studies, which makes comparison difficult. Only 16 of the studies in this review included temperature readings (15 RFA and 1 HIFU). All of the RFA studies relied on thermal sensors incorporated into their RFA probes for this reading: Starburst XL, RITA Systems (n=5); Cool-tip, Radionics (n=3); Uniblate™, AngioDynamics (n=3); Celon POWER, Olympus (n=2); Habib™ 4X, AngioDynamics (n=1). The only HIFU study to report temperature readings was by Vidal-Jove et al. They reported that 'the median intensity of treatment was 350W, which corresponded to a median temperature of 70°C (42). However, no detail is given about where and when this temperature reading was recorded.

The international working group on image-guided tumor ablation have published a standardization of terminology and reporting criteria (43). They say that temperature measurements should include precise specification of where the temperature was measured. Most of the studies that report temperature in this review provide this information, but two RFA studies do not include this detail, and the only HIFU study to report temperature doesn't report this either (41, 42, 44). The standard reporting criteria also say that it should be specified when during the ablation the temperature measurements were acquired. This information is not clear in any of the studies included in this review. When the reporting of, and standardization of the ablation is inconsistent, it makes it very difficult to draw conclusions about their effects. For example, it could be that the statistically significant improvements in OS seen in the retrospective trials did not transfer to the RCTs due to inconsistent treatment deliveries.

To be able to report this information in clinical trial papers, accurate thermal monitoring must also be in place. Thermal probes can be positioned within the tumor volume and in healthy adjacent tissue to provide information about the temperature in the treatment volume, and to provide safety warnings at the desired ablation boundary. These probes can be used to monitor RFA treatment especially, however they are less suitable to monitor HIFU treatment because their placement negates HIFU's main clinical advantage, in that it is non-invasive. For HIFU, MRI based thermometry can non-invasively monitor temperature in real time, but it is far more expensive. In the future, all studies should employ a recognized method of thermal monitoring, be consistent with consensus guidelines for tumor ablation (45), and the standardization of terminology and reporting criteria (43).

4.4 Future directions

The most probable future application of ablation will take the form of adjuvant second-line treatment in combination with CHT. The future progression of this therapeutic field clearly will rely on quality RCTs which detail standardized thermal dosages and delivery methods of thermal ablation according to international expert consensus. The last update was posted on the 1st of August 2017 which said that the trial was recruiting. As previously mentioned in the discussion section, the results of the PELICAN trial will also inform what direction this field goes in (40).

Another possibly interesting direction to pursue with ablation could come in the form of combination therapies with immunotherapies in order to turn an immunologically 'cold tumor' hot. In this vein, there is a phase II trial (NCT04156087) of patients with non-resectable PC to undergo minimally invasive MWA in combination with a CTLA-4 mAb, a PD-L1 mAb, and adjuvant gemcitabine. The study will examine PFS and is estimated to be completed in 2023. Should the data from this trial prove to be significant, the previously underwhelming response to immunotherapy seen in PC could be overcome, providing a new treatment option to the PC patients who require it most.

4.5 Conclusion

In conclusion, ablative techniques like RFA and HIFU still require more standardization and investigation before they can be applied confidently to PC in the clinical setting. The positive effects on OS that these techniques have demonstrated in numerous retrospective studies provides encouragement for the utility of these therapies. Ultimately however, progress will not be made until these benefits are translated to adequately powered RCTs that compare ablation techniques to current gold-standard treatment regimens for unresectable PCs. In order to achieve this and provide reproducible results across treatment centers and research groups, thermal monitoring and reporting of achieved temperatures in the tumor volumes must be standardized according to current consensus from international working groups. Beyond its effects on OS, these ablative techniques could have applications in combination with immunotherapies, as a surgical adjunct, or for palliation of PC-associated pain. However, it must be acknowledged that in some clinical scenarios, neoadjuvant thermal therapy is in competition with more established techniques like surgery and other tumor reducing strategies. All in all, thermal ablation remains an promising area of cancer research which merits further investigations.

Author contributions

WF and GH contributed to conception and design of the review. WF performed the initial database search and wrote the original draft. SG and AP-M revised and contributed to the draft. All authors contributed to manuscript revision, read, and approved the final submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Multidisciplinary team diagnosis and treatment of pancreatic cancer: Current landscape and future prospects

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The pathogenesis of pancreatic cancer has not been completely clear, there is no highly sensitive and specific detection method, so early diagnosis is very difficult. Despite the rapid development of tumor diagnosis and treatment, it is difficult to break through in the short term and the overall 5-year survival rate of pancreatic cancer is less than 8%. In the face of the increasing incidence of pancreatic cancer, in addition to strengthening basic research, exploring its etiology and pathogenesis, it is urgent to optimize the existing diagnosis and treatment methods through standard multidisciplinary team (MDT), and formulate personalized treatment plan to achieve the purpose of improving the curative effect. However, there are some problems in MDT, such as insufficient understanding and enthusiasm of some doctors, failure to operate MDT according to the system, lack of good communication between domestic and foreign peers, and lack of attention in personnel training and talent echelon construction. It is expected to protect the rights and interests of doctors in the future and ensure the continuous operation of MDT. To strengthen the research on the diagnosis and treatment of pancreatic cancer, MDT can try the Internet +MDT mode to improve the efficiency of MDT.

KEYWORDS

multidisciplinary team (MDT), pancreatic cancer, current landscape, future prospects, diagnosis and treatment

1 Introduction

Pancreatic cancer is one of the most common malignant digestive system tumors, about 227,000 patients die of pancreatic cancer every year around the world (1–4). According to the latest data from the American Cancer Society, its incidence and mortality are almost equal, and the incidence is tenth in malignant tumors, the

incidence is fifth in female malignant tumors, and fourth in male malignant tumors (5). In the UK, pancreatic cancer accounts for 5.6% and 5.3% of cancer-related deaths in men and women, respectively, ranking fifth (6). China is the largest developing country in the world, with the acceleration of urbanization, the changes of lifestyle and diet, and the aging and environment, the incidence of pancreatic cancer is faster than that of in developed countries, but the growth rate of pancreatic cancer is the fastest in the whole sphere. Although pancreatic cancer did not rank in the top five of cancer-related deaths in China, the proportion of pancreatic cancer-related deaths increased by 9% in the past decade, and this proportion also increased sharply (7). Therefore, pancreatic cancer has become a rigorous public health problem threatening human life and health, and has attracted more and more attention.

The rapid progress of pancreatic cancer leads to a very high mortality rate. In the past few decades, the level of diagnosis and treatment of pancreatic cancer has been significantly improved in China. Although the prognosis has improved slightly in recent years, the survival time of most patients with pancreatic cancer is less than one year, and the 5-year survival rate is still less than 8%. Pancreatic cancer has proved to be a major diagnosis and treatment problem faced by medical circles at home and abroad.

2 Current status of diagnosis of pancreatic cancer

With the rapid application of modern high-tech, the advancing diagnostic methods has been developed quickly, and a variety of new drugs are widely used clinically, tumor diagnosis and treatment has undergone unprecedented improvement, but the early diagnosis rate of pancreatic cancer is still disappointing (8–11). Early detection and diagnosis of pancreatic cancer is vital t for the survival and prognosis of patients (9).

2.1 Imaging examination

At present, a sort of imaging examinations has been used in the diagnosis of pancreatic masses. The methods featured by different advantages and limits, which could provide complementary evidence and confirmation of each other. A proper selection of imaging methods not only improve the diagnostic efficiency and accuracy, but also reduce the unnecessary cost.

B-ultrasound shows the size and scope of the tumor, lymph node metastasis, pancreaticobiliary dilatation, etc. It is known with the advantages of simple, noninvasive and low cost. Also, it is a common screening method for abdominal tumors. At the same time its performance easily affected by fat, intestinal gas, ascites and other factors. It is hardly to show the whole picture of pancreas and not suitable for the early diagnosis of pancreatic cancer (12).

CT scan not only identify the tumor, but also provide effective preoperative evaluation for the invasion of pancreatic surrounding tissue, lymph node and distant metastasis. It is a common imaging examination method in the diagnosis of pancreatic cancer. However, the sensitivity of CT may decline with the decrease of tumor diameter (13).

The spatial resolution of MRI is lower than CT in the diagnosis of pancreatic cancer, and the evaluation of tumor resectability is similar to that of CT. Diffusion weighted imaging (DWI) could identify small lesions of pancreatic cancer, but it is not able to distinguish tumor lesions from inflammatory lesions. Magnetic resonance cholangiopancreatography (MRCP) could obtain the image of pancreaticobiliary duct with contrast agent, which is mainly used to detect the dilatation or stenosis of pancreaticobiliary duct, but its application in the diagnosis of pancreatic cancer is limited (14).

Positron emission computed tomography (PET-CT), which combines functional imaging with anatomical imaging, plays an important role in the diagnosis, staging and recurrence detection of tumors. In addition, it is powerful to analyze the metabolism of the lesions, especially in the differential diagnosis of pancreatic cancer and benign lesions (especially pancreatic head cancer and mass type chronic pancreatitis) out. However, the cost of PET-CT is relatively high and limits its utility in pancreatic cancer early screening (15).

Ultrasound endoscopy (EUS), especially the fine needle biopsy technique (EUS FNA), has a unique diagnostic value compared with other imaging examinations. Because of the invasive nature of EUS FNA, it is not suitable for the first choice of detection of pancreatic cancer. In addition, ERCP is often used to drain bile by self-expanding stent. It is not supposed to be a valuable mean in early diagnosis of pancreatic cancer (16).

2.2 Tumor biomarkers

Carbohydrate antigen 19-9 (CA19-9) is a marker of pancreatic cancer. The sensitivity and specificity of CA19-9 in the diagnosis of pancreatic cancer are 79% ~ 81% and 82% ~ 90% (17), respectively. False positive results were found in patients with liver cirrhosis and gastrointestinal cancer.CA19-9 is not used in the early diagnosis of pancreatic cancer, but often applied to evaluate curative effect and detect postoperative recurrence. Carcinoembryonic antigen (CEA) is highly expressed in patients with pancreatic cancer, gastric cancer and colorectal cancer, but its diagnostic specificity for pancreatic cancer is poor (18). In addition, other tumor markers (such as CA242, CA50, CA72-4, etc.) are not commonly used in the diagnosis of pancreatic cancer because of their low sensitivity and specificity.

2.3 Liquid biopsy

2.3.1 Circulating tumor cells

Circulating tumor cells (CTCs) fall off from primary or metastasis tumor cells of peripheral blood. CTC may have experienced epithelial mesenchymal transition (EMT), with stronger mobility and invasiveness, and it is easier to adhere to the vascular wall and penetrate into the blood circulation, which is an important reason for tumor metastasis. CTC has integrity of the

tumor data, including not only DNA information, but also genome and proteome which is consistent with the source of tumor tissue

The value of CTC in the early diagnosis of tumor has been confirmed in many kinds of tumor research. In the mouse model of pancreatic cancer, Rhim et al. (19) found that EMT occurred in some pancreatic cells at the early stage of tumor development, and these cells were considered as early tumor cells. Before malignant transformation, pancreatic epithelial cells can be detected in blood samples of patients with pancreatic cystic lesions. These results suggest that appearance of CTC is earlier than tumor formation *in situ* and may be a tumor marker for early diagnosis of pancreatic cancer.

CTC specific gene expression could be considered as an alternative marker for early diagnosis of tumor. This kind of research mainly detects the expression of epithelial protein to validate the presence of CTC. For example, Soeth et al. (20) detected cytokeratin 20 (CK20) in bone marrow and venous blood of patients with pancreatic cancer, and found that high level of CK20 was associated with tumor staging of UICC. Zhang et al. (21) combined immunostaining of CK, CD45, DAPI and fluorescence *in situ* hybridization (FISH) with chromosome 8 centromere probe (CEP8) method to improve the identification efficiency of CK-/diploid CTC in pancreatic cancer.

CTC also be taken as a marker for the diagnosis of early pancreatic cancer, asymptomatic patients and patients with normal CA19-9. Xu et al. (22) used a similar method in 40 patients. When the cut-off value set at CTC ≥ 2/7.5ml and CA19-9 > 37 μmol/L, the diagnostic rate of pancreatic cancer reached 97%. In addition, DCLK1, another marker of CTC, may also be used in the early diagnosis of pancreatic cancer. Qu et al. (23) found that the level of DCLK1 increased in patients with TNM stage I and II, but decreased in patients with TNM stage III and IV. Although CTC has great potential value in the early diagnosis of pancreatic cancer, it is difficult to capture CTCs from the blood due to the scarcity of CTCs, which limits its clinical application.

2.3.2 Circulating tumor DNA

In 1977, Leon et al. found circulating tumor DNA (ctDNA) in the serum of tumor patients. In 1983, Shapiro et al. (24) first detected ctDNA in the blood of patients with pancreatic cancer. Studies have shown that ctDNA mainly comes from necrotic tumor cells, apoptotic tumor cells, CTC and exosomes secreted by tumor cells.

The length of ctDNA is about 134-144bp and the half-life is about 2 hours. It can be detected in blood, saliva, urine and other body fluids. ctDNA contains gene information of tumor cell with specific mutations. By capturing and sequencing these important DNA fragments, we could obtain tumor specific mutations information, which is helpful in tumor diagnosis and individual medication guidance.

Studies have shown that more than 90% of patients with pancreatic intraepithelial neoplasia have KRAS gene mutation, and the mutation rate of KRAS gene is directly related to the grade of pancreatic intraepithelial neoplasia (25). Detection of KRAS mutation in ctDNA is expected to be applied to the early diagnosis of pancreatic cancer. Bettegowda et al. (26) detected ctDNA in serum of 640 patients with different types and stages of tumor by using dPCR, including 155 patients with pancreatic cancer. The results revealed that the detection rate of ctDNA in patients with localized pancreatic cancer was 48%. The ratio increased with the increase of tumor clinical stage. Similarly, Sausen et al. (27) found that 43% patients being identified of ctDNA in total resectable pancreatic cancer cases. However, other studies have reported that patients with chronic pancreatitis (10% - 15%) will also have KRAS mutations, combined detection of KRAS mutations and serum creatinine levels

CA19-9 can improve the sensitivity (98%) and specificity in the diagnosis of pancreatic cancer degree (77%) (28). In addition, the study found that the methylation analysis of ctDNA can works as a potential marker of pancreatic cancer to distinguish chronic pancreatitis from pancreatic cancer (29). Although ctDNA provides a possibility for the early diagnosis of pancreatic cancer, the sensitivity of existing technologies is not satisfying, and the standardization of detection methods still needs to be settled.

2.3.3 Exosomes

Exosomes are largely secreted in the process of carcinogenesis, which is different from ctDNA that released by tumor necrosis cells, exosomes are secreted by living cells, so exosomes could be distinguished earlier in the blood, which is more suitable for the early diagnosis of pancreatic cancer. Serum exosome derived proteins or miRNAs may be proper candidate markers, such as protein markers (CD44v6, TSPAN8, EpCAM, CD104) and miRNAs (miR-1246, miR-4644, miR-3976, miR-4306). The expression of these proteins and miRNAs in serum exosomes of patients with pancreatic cancer was significantly up-regulated. Combined detection of these proteins and miRNAs would effectively improve the sensitivity of diagnosis of pancreatic cancer (30). In addition, studies have shown that exosome derived DNA mutations (such as KRAS and TP53) can also be selected in the diagnosis of pancreatic cancer, and the diagnostic efficiency is better than CTC, but exosome KRAS mutations can also occur in healthy people (29). Studies have shown that GPC-1, an exosome membrane protein, can be chooses to differentiate pancreatic cancer patients from chronic pancreatitis patients and healthy people with specificity and sensitivity up to 100% (31).All the above results indicate that exosomes are expected to become a new type of biomarker. The ideal marker for early diagnosis of pancreatic cancer still supposed to be validated by a large number of studies.

Although pathological diagnosis is the gold standard for the diagnosis of pancreatic cancer, imaging diagnosis plays an important role in screening, differential diagnosis and staging of pancreatic cancer. Decisions about diagnostic management and resectablity should involve multidisciplinary consultation at a high-volume center with application of appropriate imaging studies. At present, ultrasound, Computed Tomography (CT), Magnetic Resonance Cholangiopancreatography (MRCP) and Endoscopic Ultrasonography (EUS) are the main early screening methods for pancreatic cancer. Ultrasound examination is the most

economical and noninvasive examination method, and it is the first-line screening method for patients with suspected pancreatic cancer (10). However, ultrasound examination highly depends on the experience and physical condition of ultrasound doctors (32). Enhanced CT is the first choice of pancreatic imaging in the world, and it is also the first choice of postoperative evaluation of pancreatic cancer recurrence. However, enhanced CT has some radiation, which limits it as a routine screening for asymptomatic high-risk population. Endoscopic ultrasonography and cholangiopancreatography are better than CT in the early screening of pancreatic cancer (33, 34). Therefore, most scholars suggest that MRCP, Magnetic resonance imaging (MRI) and EUS should be included in the initial screening of pancreatic cancer, while CT and ERCP are excluded (9, 35). However, combined with the actual economic situation of our country, MRI examination is still carried out after ultrasound and CT examination. In addition, EUS still cannot be popularized in domestic hospitals while only installed in some large medical institutions. Although positron emission tomography/computed tomography (PET/CT) has been widely used in the diagnosis of tumors, its conventional tracer 18F-fluorodeoxyglucose (18F-FDG) has little effect in the detection of early pancreatic ductal adenocarcinoma (36, 37).

3 Current status of treatment of pancreatic cancer

3.1 Surgery

Surgical treatment is the basic treatment for pancreatic cancer, and it is also the only way to achieve the curative effect of pancreatic cancer (10, 38, 39). Recent studies have shown that less than 20.0% of pancreatic cancer patients have access to surgical treatment (40). Even after R0 resection, some patients still have postoperative tumor recurrence and distant metastasis, which affect the postoperative survival rate. For patients with unresectable pancreatic cancer, preoperative neoadjuvant therapy can be managed to transform them into resectable patients. Systemic therapy is accepted in all stages of pancreatic cancer. This includes neoadjuvant therapy (resectable or borderline resectable), adjuvant therapy, and first-line or subsequent therapy for locally advanced, metastatic, and recurrent disease (41).

3.1.1 Pancreaticoduodenectomy

Pancreaticoduodenectomy (PD) was put forward by Whipple in 1935, which was also the classic surgical method for pancreatic cancer. It is mainly used for the head and neck of the pancreas (head, neck, and hook). Foreign statistics show that the most common complications of this operation include delayed gastric emptying, pancreatic fistula and wound infection incidence rate is 42%~47% (42). Bassi and other (43)studies that compared PD among different conditions, PD has no statistical significance in the proportion of complications, mortality and length of hospital stay, but the incidence of bile leakage and ascites in PD group is higher than that in pancreaticogastrostomy group, which may be due to the fact that PD group will not be invaded by pancreatic fistula, whether PD or pancreaticogastrostomy is still controversial.

3.1.2 Pylorus preserving duodenectomy

Pylorus preserving pancreaticoduodenectomy (PPPD) was first proposed by Watson in 1944. It is believed that PPPD can reduce the incidence of dumping syndrome, reduce intraoperative bleeding and shorten the operation time. However, some scholars doubt that PPPD will increase the proportion of delayed gastric emptying, compared with PD, surgery does not significantly change the mortality or survival rate of patients, and does not conform to the relevant procedures of tumor resection. Therefore, the choice of surgery on PD or PPPD is still controversial.

There are many other surgical conduction, such as distal pancreatectomy, extended resection, portal vein resection, arterial resection and reconstruction, and extended lymphadenectomy (44), which have also been accepted in clinical utility.

3.1.3 Minimally invasive treatment of pancreatic tumors

Due to the deep anatomic location and complex surrounding tissue structure of the pancreas, the development of minimally invasive surgery of the pancreas is more obvious than that of other digestive system tumors. With the in-depth study of minimally invasive treatment of pancreatic tumors, certain progress has been made recently. Pryor et al. (45)have studied that laparotomy and laparoscopy are the most effective methods for the treatment of pancreatic tumors. Compared with patients on different surgical treatment, the incidence of complications was 43% vs 7%, and the mortality was 29% vs 0%, which showed the obvious advantages of laparoscopic surgery compared with traditional open surgery.

With the development of medical technology, surgical robots have gradually entered people's field of vision. Robotic surgery improves the efficiency and accuracy of surgery. Of course, there are also some disadvantages, such as the robot does not have the touch of traditional surgery, there are errors in tactile judgment. At present, the development direction of surgery is gradually toward precision and minimally invasive, which requires us to better use endoscopic technology and surgical robot, as well as the combination of the both. Regarding some experts worried that minimally invasive treatment cannot reach the R0 margin affect the OS, disease-free survival (DFS), etc., Halit et al (46) reported a study of 396 patients with borderline resectable and resectable pancreatic adenocarcinoma, minimally invasive pancreatic surgery (MIPS) was associated with better OS and DFS than open pancreatic surgery (OPS). Centralization of MIPS should be stimulated, and pancreatic surgeons should be encouraged to pass the learning curve before implementing MIPS for pancreatic adenocarcinoma in daily clinical practice.

3.2 Chemotherapy

Advanced patients or patients pre- and post-operative should be treated with chemotherapy (47). Pancreatic cancer is not

sensitive to chemotherapy. Gemcitabine, albumin paclitaxel, fluorouracil (including capecitabine, S1) and other single drug regimens can be exerted for 6 months. Patients in good condition could be considered the combination with chemotherapy (48).

Almost all pancreatic cancer patients need chemotherapy. Early patients need postoperative chemotherapy to prevent recurrence. In late stage, chemotherapy is needed to relieve symptoms and prolong survival. Therefore, chemotherapy has always been a hot topic in the treatment of pancreatic cancer.

3.2.1 Fluorouracil single therapy

Since 1950s, 5-fluorouracil (5-fluorouracil, 5-Fu) based chemotherapy has been a major chemotherapy regimen for pancreatic cancer. Although the combination of adriamycin, mitomycin C, cyclophosphamide, methotrexate vincristine and cisplatin can improve the effect of 5-FU, none of them extend the OS of patients.

3.2.2 Gemcitabine single therapy

Gemcitabine (GEM) is the first chemotherapy drug that can prolong the survival period of patients with pancreatic cancer. In a randomized controlled trial (49), 126 patients with advanced pancreatic cancer were divided into two groups. One group received GEM treatment and the other group received 5-Fu treatment. The clinical benefits of the two groups were evaluated by pain index, Karnofsky (KPS) and body mass. The results showed that GEMC group had better clinical benefits (23.8% vs 4.2%, P = 0.0022); At the same time, the mOS of GEM group was longer than that of 5-FU group (5.65mo vs 4.41mo, p=0.0025), and the one-year survival rate was higher than that of 5-FU group (18% vs 2%, P = 0.0025). Therefore, GEM is classified as a first-line chemotherapeutic agent for advanced pancreatic cancer.

3.2.3 GEM based combination chemotherapy

After the single efficacy of GEM was verified, a series of GEM based combination chemotherapy developed rapidly from the 1990s to the early 21st century. The efficacy of GEM combined with capecitabine was verified in two clinical phase III trials. Cunningham et al. (50) selected 533 patients with advanced pancreatic cancer were randomly divided into two groups, one group received chemotherapy combined with GEM plus capecitabine (GEMCAP group), and the other group received a single chemotherapy regimen of GEM (GEM group). The results showed that the OS of GEMCAP group was slightly prolonged, but the difference was not statistically significant. The 1-year overall progression free survival (PFS) in GEMCAP group was significantly higher than that in GEM group (13.9% vs 8.4%, P = 0.004). Herrmann et al. (51) showed that there was no significant difference in mOS and 1-year survival between GEMCAP group and gem group, but efficacy analysis showed that patients with higher KPS had longer mOS, and GEMCAP regimen could significantly improve PFS (P = 0.022). The National Comprehensive Cancer Network (NCCN) has classified the GEMCAP protocol as an alternative for advanced pancreatic cancer treatment, and shows that the premise of choosing this protocol bring better physical fitness and behavioral status (KPS:90-100 score).

Japan proposed GEM plus S-1 as a chemotherapy regimen for advanced pancreatic cancer. Okabayashi (52) and other studies suggested that S-1 and GEM alone had no significant difference in OS. However, Meta-analysis of Li (53) in patients with pancreatic cancer after S-1 combined with GEM adjuvant therapy showed that GEM and S-1 in patients with non resectable pancreatic cancer significantly improved the patient's OS and PFS. Wada et al. (54) Proposed GEM combined with S-1 chemotherapy twice a week, which can reduce adverse reactions and economic burden without weaken therapeutic efficacy.

Heinemann and Colucci (55) and other phase III clinical trials confirmed that GEM combined with platinum chemotherapy drugs did not improve the survival time of patients with Heinemann compared with GEM chemotherapy alone. A total of 400 patients with advanced pancreatic cancer were randomized to receive GEM plus cisplatin or GEM monotherapy. The results showed that there was no significant difference in mOS and PFS between the two groups. However, the results of a large meta-analysis showed that GEM combined with cisplatin could effectively improve the quality of life of patients compared with GEM monotherapy group (P = 0.010). Therefore, NCCN lists GEM combined platinum chemotherapy drugs as one of the treatment options for advanced pancreatic cancer, but limited to patients with familial pancreatic cancer.

A series of phase I clinical trials confirmed that GEM combined with oxaliplatin, irinotecan or pemetrexed cannot significantly prolong OS in patients with pancreatic cancer (47). GERCOR and GISCAD tests showed that GEM combined with oxaliplatin can improve PFS, but it has no significance on OS (56).

3.2.4 Chemotherapy for pancreatic cancer patients with BRCA gene mutation

Although GEMCAP combined with cisplatin is not widely recommended in the clinical treatment of early pancreatic cancer, studies have confirmed that familial pancreatic cancer or pancreatic cancer with BRCA mutation is more sensitive to platinum-based chemotherapy (57).

BRCA1 and BRCA2 mutations can lead to ineffective repair of damaged DNA in homologous recombination and increase the risk of malignant tumor. Cisplatin, as an alkylating drug, can combine with DNA to form intrastrain crosslinks, change the structure of DNA and affect DNA replication. Under normal circumstances, these crosslinks can be repaired by homologous recombination, but patients with BRCA gene mutation cannot complete effective repair, BRCA deficient cells are more sensitive to platinum-based chemotherapy. In a retrospective study conducted by Johns Hopkins University in 2010, 468 patients with metastatic pancreatic cancer who were treated with cisplatin-based chemotherapy were evaluated. It was found that patients with family history of breast cancer, ovarian cancer or pancreatic cancer had significantly longer mOS than those without such family history (22.9mo vs 6.3 mo). P<0.01). At the same time, Lowery (58) and other research results also showed that BRCA1 or

BRCA2 mutant pancreatic cancer patients can use PARP inhibitor or platinum chemotherapy drug to achieve 27.6 months on mOS. PARP family protein binding with DNA and participate in the repair of DNA damage. Therefore, inhibition of PARP can hinder the damage and repair of DNA and ultimately induce cell apoptosis (59). These two studies all suggest that platinum-based chemotherapy drugs may be effective in improving mOS in familial pancreatic cancer or BRCA gene mutation patients.

3.2.5 Oxaliplatin + folic acid + fluorouracil regimen

CONKO-003 trial of second-line chemotherapy for pancreatic cancer showed that compared with folate + fluorouracil (FF) regimen, the OFF regimen increased relative to GEMCAP resistant patients (2.9 mo vs 2.0 mo, P=0.019), OS was also significantly prolonged (5.9 mo vs3.3mo, P=0.01), but the neurotoxicity of the regimen was apparently higher than that of the regimen (60). The NCCN guidelines recommend OFF regimen as one of second-line chemotherapy regimens for GEMCAP resistance in advanced pancreatic cancer.

3.2.6 5-Fu + folic acid + irinotecan + oxaliplatin regimen

In the ACCORD II/III trial, 342 patients with metastatic pancreatic cancer who had not received any treatment were randomized to receive FOLFIRINOX chemotherapy or GEMCAP monotherapy. The former mOS (11.1 mo vs 6.8 mo, P<0.001) or PFS (6.4 mo vs 3.3 mo, P<0.001) are significantly higher than the latter, and the tumor is more sensitive to the former regimen (31.6% vs 9.4%, P<0.001), which suggests that combined chemotherapy can improve the survival rate of metastatic pancreatic cancer patients compared with single dose of chemotherapy (61). Compared with GEMCAP monotherapy, FOLFIRINOX regimen had a higher incidence of grade 3 and 4 adverse reactions, but the 6 months health status and quality of life scores showed that the overall quality of life of FOLFIRINOX group was higher than that of GEMCAP group, which may be related to the significantly improved survival rate of FOLFIRINOX regimen (62). Currently, the FOLFIRINOX regimen is considered to be a first-line chemotherapy regimen of advanced pancreatic cancer in general condition. The combination of 27 GEMCAP and paclitaxel regimen is rich in stroma, which can block chemotherapeutic drugs from entering cancer cells and increase chemotherapy resistance. In recent years, a new scheme of paclitaxel combined with GEMCAP for metastatic pancreatic cancer has been proposed abroad. Nano paclitaxel is a combination of human albumin and paclitaxel by using nanotechnology to import drugs into cancer cells in the form of nanoparticles and increase the bioavailability of drugs. The uptake of paclitaxel nanoparticles by pancreatic stromal cells requires specific albumin binding proteins, such as cysteine rich secreted protein (SPARC). In a phase I/II clinical trial, the expression level of SPARC in 36 patients was detected by immunohistochemistry and used as a biomarker, the patients were divided into high expression SPARC group and low expression SPARC group. The results showed that the mOS of high expression SPARC group was significantly higher than that of low expression SPARC group, which suggested that GEMCAP combined with Nano-paclitaxel showed important antitumor activity. However, another phase II trial using paclitaxel as a second-line treatment for metastatic pancreatic cancer has found no significant correlation between the expression of SPARC and prognosis. In phase III clinical trials such as Von Hoff, a total of 861 patients with untreated advanced pancreatic cancer were randomly divided into GEMCAP combined with paclitaxel chemotherapy or GEMCAP single chemotherapy. The results showed that GEMCAP, combined with paclitaxel group had significant improvement in mOS, PFS and tumor sensitivity, but the incidence of myelosuppression and peripheral neuritis in this group was equally higher. MPACT detailed analysis of SPARC expression and patient survival at the 2014 European Society of Clinical Oncology Conference also showed that SPARC was not associated with patient survival.

Currently, GEMCAP combined with paclitaxel or FOLFIRINOX is a first-line treatment for pancreatic cancer. However, pancreatic cancer is a highly malignant tumor, and nearly half of the patients are ineffective for first-line treatment. At this time, chemotherapy drugs such as fluorouracil, capecitabine, pemetrexed and oxaliplatin can play an essential role. However, there is no standardized treatment plan for patients with advanced pancreatic cancer who are tolerant of first-line and second-line chemotherapy.

3.2.7 Neoadjuvant chemotherapy for the operation of pancreatic cancer

For the resectable or borderline resectable pancreatic cancer patients, they can receive the neoadjuvant chemotherapy or adjuvant therapy (63, 64). There were many clinical trials suggested that the FOLFIRINOX add radiotherapy is the preferred new adjuvant therapy (41, 65, 66). Janssen QP, et al. reported that 351 patients (68.6%) were treated with FOLFIRINOX alone (8 studies) and 161 patients (31.4%) were treated with FOLFIRINOX and radiotherapy (7 studies). The pooled estimated median OS was 21.6 months (range 18.4–34.0 mo) for FOLFIRINOX alone and 22.4 months (range 11.0–37.7 mo) for FOLFIRINOX with radiotherapy. The pooled resection rate was similar (71.9% vs. 63.1%, p = 0.43) and the pooled R0 resection rate was higher for FOLFIRINOX with radiotherapy (88.0% vs. 97.6%, p = 0.045). Other pathological outcomes (ypN0, pathologic complete response, perineural invasion) were comparable (67). Giovinazzo F, et al. (68)found that gemcitabine based neo-adjuvant therapies (GEM-NAT) in borderline resectable pancreatic ductal adenocarcinoma (BR-PDAC). A meta-analysis of individual participant data (IPD) was conducted on 271 patients who received GEM-NAT. Pooled median patient-level OS was 22.2 months (95%CI 19.1–25.2). R0 rates ranged between 81 and 95% (I2 = 0%, p = 0.64), respectively. Median OS was 27.8 months (95% CI 23.9–31.6) in the patients who received NAT-GEM followed by resection compared to 15.4 months (95%CI 12.3–18.4) for NAT-GEM without resection and 13.0 months (95%CI 7.4–18.5) in the group of patients who received upfront surgery (p < 0.0001). R0

rates ranged between 81 and 95% (I2 = 0%, p = 0.64), respectively. Overall survival in the R0 group was 29.3 months (95% CI 24.3–34.2) vs. 16.2 months (95% CI 7·9–24.5) in the R1 group (p = 0·001). GEM-NAT may result in a good palliative option in non-resected patients because of progressive disease after neoadjuvant treatment (68).

The standard treatment of resectable pancreatic cancer is surgery followed by adjuvant chemotherapy. Neoadjuvant chemotherapy appears to be equally efficient in converting irresectable in resectable disease and more efficient with regard to systemic tumor progression and overall survival compared to neoadjuvant chemoradiation therapy. Despite these convincing findings from mostly small phase II trials, neoadjuvant therapy has not yet proven superiority over upfront surgery in randomized trials (63, 66, 69–72). Vivarelli et al (64) suggested that the choice of the best multimodal treatment of resectable pancreatic cancer should probably be based on the biological behavior of the tumor rather than on the loco-regional staging of the tumor, which currently represents the cornerstone of the decision-making process with regard to first-line treatment. More effective and individualized systemic therapeutic regimens will probably stem from a better knowledge of clinic-pathological prognostic factors such as molecular profiling and novel biomarkers.

3.3 Radiotherapy

Radiotherapy is an important treatment for pancreatic cancer, which is the first choice for locally advanced pancreatic cancer (73). Generally speaking, the sensitivity of pancreatic cancer to radiotherapy alone is rather poor. The current view is that radiotherapy can be combined on the basis of chemotherapy for patients with advanced stage, but there are still differences in the effectiveness. A study has shown that chemoradiotherapy improves overall survival compared with chemotherapy alone, but the adverse reactions are also significantly enhanced. Another study suggested that the overall survival rate after chemoradiotherapy was slightly lower than that after chemotherapy alone (15.3 mo vs 16.5 mo). In last years, the radiotherapy technology has also been improved significantly, such as three-dimensional conformal radiotherapy, which focuses on raising the radiation dose and gradually improving the stereotactic radiotherapy technology of primary tumor. Although there are many problems with these technologies, the latest radiotherapy combined with chemotherapy is very promising for the treatment of patients with advanced pancreatic cancer.

3.4 Targeted therapy

Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor that plays an important role in cell cycle regulation. 90% of all pancreatic cancer samples are highly expressed in EGFR. Therefore, targeting small molecule inhibitors of EGFR tyrosine kinase domain is a promising drug for cancer therapy. In a large clinical phase II trial, 569 patients with advanced pancreatic cancer were randomly divided into GEM combined with erlotinib or GEM monotherapy. The results showed that mOS and PFS in the combination group were obviously higher than those in the single drug group. Subsequently, the trial also analyzed the number of KRAS and EGFR in 117 patients, and found that neither of them could predict the longer survival of patients with combination regimen. In addition, EGFR monoclonal antibody (cetuximab) combined with GEM was also used. Immunohistochemistry showed that 92% of the tumor tissues were EGFR positive, but it did not improve the mOS, PFS or tumor sensitivity. Türeci Ö found that zolbetuximab-induced antibody-dependent cell-mediated cytotoxicity (ADCC), and in mouse xenograft tumors derived from human pancreatic cancer cell lines, including GEM-refractory ones, zolbetuximab slowed tumor growth, benefited survival, and attenuated metastases development (74).

With the research of pancreatic cancer related genes and signaling pathways, targeted therapy has become a new method for the treatment of pancreatic cancer, including directly targeting tumor antigen, growth factor receptor, changing gene or biochemical channels, directly responding to host immune response (75). Olaparib can be used for targeted therapy in pancreatic cancer patients with BRCA1/2 mutation (76).

Activation of the tyrosine kinase domain of EGFR to activate the downstream RAS/RAF/MEKPI3K/AKT and JAK/STAT signaling pathways is essential for cell proliferation and survival. This makes the research and development of EGFR small molecule inhibitors become a hot spot in the field of tumor therapy. Currently, EGFR inhibitors such as Nimotuzumab and Afatinib are currently undergoing phase I clinical trials. In addition, insulin-like growth factor receptor (IGFR) can also regulate cell proliferation by activating signal pathways such as PI3K/AKT, but IGFR monoclonal antibodies and MK-0646 have not been effective for pancreatic cancer.

On the other hand, 90% of pancreatic cancer has a mutation in the KRAS gene, which activates RAF/MEK/ERK and PI3K/AKT channels, leading to uncontrollable cell growth. This makes KRAS a potential target for pancreatic cancer treatment. However, its inhibitors, either alone or in combination, are not effective in the treatment of pancreatic cancer. Therefore, the inhibitors of its downstream signaling pathway are tried to treat pancreatic cancer, such as the use of MEK1/2, an inhibitor of the oral administration of the drug. But compared with GEM, the drug does not prolong the mOS of patients with pancreatic cancer. Trametinib is a reversible MEK1/2 inhibitor. Although it has not significantly improved the mOS of patients, it has been used in the treatment of advanced pancreatic cancer. At present, more drugs blocking KRAS signaling pathway are being developed, among which PI3K inhibitors and AKT inhibitors have entered the clinical trial stage.

3.5 Immunotherapy

Programmed death 1(PD-1)/programmed cell death-Ligand 1 (PD-L1) immunotherapy can be considered for pancreatic cancer

patients with disease progression after surgery or first-line chemotherapy (47, 77, 78). MSI or MMR genes closely related to pancreatic cancer should be detected before immunotherapy (7, 79, 80). Immunotherapy with antibodies targeting PD-1, PD-L1, cytotoxic T lymphocyte associated antigen 4 (CTLA-4) has not shown clinical activity in unselected pancreatic cancer, emphasizing the need for combination of immunotherapy approaches or other therapeutic strategies (81).

Pancreatic cancer cells are able to escape human immune system monitoring by various mechanisms, such as negative regulation of T cell response (82), secretion of cytokines inhibiting the immune system, and down regulation of major histocompatibility complex-I (MHC-I) expression. This provides a basis for the discovery of tumor specific antigen, the development of tumor vaccine and antibody (83).

Ipilimumab is a specific monoclonal antibody against CTLA-4 (84). Its combination with CTLA-4 can enhance the activity and function of T cells. It has been confirmed by FDA for the treatment of melanoma. Currently, clinical trials have combined it with the FOLFIRINOX scheme and allogeneic tumor vaccine in the treatment of pancreatic cancer. Tumor vaccine is promising in the field of tumor immunotherapy. Allogeneic pancreatic cancer vaccine is injected into another patient from a cancer cell vaccine. It hopes to express specific tumor antigens and be recognized by the host immune system, thereby stimulating the immune response to the host's own tumor. The only tumor vaccine approved by FDA is the Sipuleucel-T cancer vaccine, which is used to treat steroid resistant prostate cancer. CRS-207 is still undergoing the studying. It is an attenuated vaccine of Lester, which can express mesothelin (mesothelin is a glycoprotein overexpressed on pancreatic cancer cell surface), and its mechanism is bacteria invading macrophages to produce mesothelin. Subsequently, activation of mesothelin cytotoxic T cells eventually induces apoptosis of tumor cells expressing mesothelin. Currently, phase CRS-207 clinical trials of CRS-207 and GVAX, a master cell vaccine expressing human granulocyte macrophage colony-stimulating factor, are being carried out. Jung and his colleges found that the combination of Navoximod and atezolizumab demonstrated acceptable safety, tolerability, and pharmacokinetics for patients with advanced cancer (NCT02471846) (85).

Other immunotherapy (80, 86–90), such as tumor antibody development and transformation of lymphocytes, are promising new technologies for the treatment of pancreatic cancer. However, more clinical data are needed to confirm the clinical value. CDK1/2/5 inhibition by dinaciclib provides a novel strategy to overcome IFNG-triggered acquired resistance in pancreatic tumor immunity (91).

4 Necessity of MDT

The condition of patients with pancreatic cancer is complex. At present, the treatment of pancreatic cancer in large hospitals in China involves pancreatic surgery, gastroenterology, oncology, radiotherapy, pathology, medical imaging, nuclear medicine and other clinical fields. Each department has certain limitations. Therefore, MDT should go through the whole process of pancreatic cancer treatment, including the choice of treatment decision, surgery and chemoradiotherapy, and targeting (39, 92, 93). It is of great significance for the treatment of patients with pancreatic cancer to combine various departments to achieve the best therapeutic effect.

In recent years, MDT model has become one of the important models of international medicine (94–96). Its purpose is to transform the traditional individual and empirical medical model into a modern group cooperative decision-making model. The National Comprehensive Cancer Network (NCCN) guidelines bring MDT discussion into the necessary procedures, and the Chinese Medical Association has also brought MDT into the treatment of each patient with pancreatic cancer (97, 98), including medicine, technology, nursing and other disciplines, the use of multidisciplinary linkage can improve the survival of patients and ensure the quality of life of patients. And the path map of MDT model in pancreatic cancer as show Figure 1 (97, 98). MDT treatment mode brings together the advantages of various departments, and plays an irreplaceable role in improving the treatment level, formulating the corresponding treatment plan, reducing over treatment, and diagnosis and treatment of pancreatic cancer in China (99).

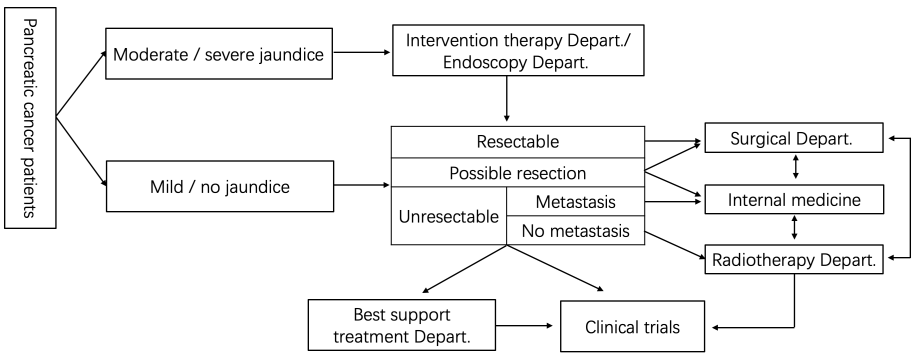

FIGURE 1
The path map of MDT model in pancreatic cancer.

5 Current landscape of MDT

At present, there are still a few doctors in MDT who lack the awareness of multidisciplinary diagnosis and treatment. Due to the limitations of the existing medical system and the different treatment methods of pancreatic cancer belong to different disciplines. It is easy for some patients with pancreatic cancer not to get the most reasonable treatment or to receive a single treatment repeatedly in a single specialty for a long time.

The MDT of pancreatic cancer regularly holds MDT forums to discuss difficult cases, improve the level of diagnosis and treatment, and formulate personalized and optimal treatment plan for patients in strict accordance with the corresponding clinical treatment guidelines. The operation and treatment process of MDT team for pancreatic cancer follow NCCN treatment guidelines and Chinese pancreatic cancer treatment guidelines. Although the working process of MDT is perfect, some doctors can't participate in it for some reasons, which leads to the interruption of MDT and can't implement it well. The most challenge when conduct MDT model maybe how to make the best choice in the face of multiple treatment decisions. Usually, the surgery department should act as the leader in MDT model, and when disagreement happens, the pancreatic surgeon makes the decision.

At present, there are some limitations in the implementation of MDT, such as nutritionists and psychiatrists cannot play a role in the whole treatment of patients, so the benefits of MDT for patients will be impaired.

Although MDT of pancreatic cancer is mostly difficult cases, it would promote the communication between domestic and foreign counterparts, but in the actual process, there is not enough communication at home and abroad (100, 101). MDT discuss the diagnosis and treatment of a case in various disciplines, which is a good opportunity for young doctors to learn and improve, and is conducive to the cultivation of young doctors' diagnosis and treatment thinking. But in fact, young doctors rarely participate in MDT due to busy work and other reasons, which is not conducive to talent cultivation and talent echelon construction.

6 Future prospects of MDT

In the implementation of MDT, there should be a distribution mechanism to protect the income and rights of doctors and show respect for doctors' work, which can improve the enthusiasm of doctors in MDT and ensure the continuous operation of MDT.

Although MDT model runs through the whole process of diagnosis and treatment of pancreatic cancer, which can fully integrate the resources of various disciplines, give full play to the advantages of disciplines, and seek individualized diagnosis and treatment scheme for patients, how to break through the bottleneck of diagnosis and treatment of pancreatic cancer still depends on the progress of science and technology to improve the proportion of early diagnosis of pancreatic cancer. At the same time, the research on the treatment of pancreatic cancer still cannot stop, hoping to explore a more valuable treatment. With the help of MDT, patients will benefit more, especially those conditions with poor therapeutic effect, such as pancreatic cancer. As for how to choose a variety of treatment methods in the future, the expand of MDT still needs to think carefully.

We can try the Internet + MDT (e MDT) model for pancreatic cancer (102, 103). E-MDT should be based on the current perfect MDT model, combined with Internet, 5th-Generation (5G), Artificial Intelligence (AI) Technology and big data to build an internet medical consortium cloud platform integrating medical record data collection, imaging, laboratory, pathology, remote consultation, surgical demonstration and remote learning, providing remote consultation, joint outpatient service, mobile ward round, teaching and training and other remote services; Integrating convenient mobile medicine, the cloud platform will become a telemedicine platform that can support multi person, multi terminal (personal computer (PC), mobile phone, iPad, etc.) integration and multi scene applications; it can be moved forward to the consulting room, patients' bed, mobile phone terminal for online consultation, multi person multidisciplinary consultation and mobile consultation at any time, which will facilitate the development of consultation business between different medical institutions.

Author contributions

QS and WY conceived the project. All authors collected and analyzed the data. QS and XC prepared the figure. QS wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Impact of endobiliary radiofrequency ablation on survival of patients with unresectable cholangiocarcinoma: a narrative review

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Cholangiocarcinoma (CCA) is a rare cancer originating from the biliary epithelium and accounts for about 3% of all gastrointestinal malignancies. Unfortunately, the majority of patients are not eligible for surgical resection at the time of diagnosis, because of the locally advanced stage or metastatic disease. The overall survival time of unresectable CCA is generally less than 1 year, despite current chemotherapy regimens. Biliary drainage is often required as a palliative treatment for patients with unresectable CCA. Recurrent jaundice and cholangitis tend to occur because of reobstruction of the biliary stents. This not only jeopardizes the efficacy of chemotherapy, but also causes significant morbidity and mortality. Effective control of tumor growth is crucial for prolonging stent patency and consequently patient survival. Recently, endobiliary radiofrequency ablation (ERFA) has been experimented as a treatment modality to reduce tumor mass, and delay tumor growth, extending stent patency. Ablation is accomplished by means of high-frequency alternating current which is released from the active electrode of an endobiliary probe placed in a biliary stricture. It has been shown that tumor necrosis releases intracellular particles which are highly immunogenic and activate antigen-presenting cells, enhancing local immunity directed against the tumor. This immunogenic response could potentially enhance tumor suppression and be responsible for improved

survival of patients with unresectable CCA who undergo ERFA. Several studies have demonstrated that ERFA is associated with an increased median survival of approximately 6 months in patients with unresectable CCA. Furthermore, recent data support the hypothesis that ERFA could ameliorate the efficacy of chemotherapy administered to patients with unresectable CCA, without increasing the risk of complications. This narrative review discusses the results of the studies published in recent years and focuses on the impact that ERFA could have on overall survival of patients with unresectable cholangiocarcinoma.

KEYWORDS

cholangiocarcinoma, malignant biliary strictures, endobiliary radiofrequency ablation, ERCP, PTC, biliary drainage, biliary stent patency, overall survival

Introduction

Cholangiocarcinoma (CCA) is a rare cancer originating from the biliary epithelium and accounts for about 3% of all gastrointestinal malignancies (1, 2). The tumor is classified as intrahepatic, perihilar and distal, according to its anatomical location (1–3). Perihilar tumors represent 50-60% of all cholangiocarcinomas, intrahepatic CCA accounts for 10-20% of cases and extrahepatic cancers involving the main bile duct are diagnosed in 20-30% of patients (3, 4). Surgery offers the best outcome, but the majority (approximately 70%) of patients are not eligible for surgical resection at the time of diagnosis, because of the locally advanced stage or metastatic disease (2–4). The survival time of patients with unresectable CCA undergoing chemotherapy is generally less than 1 year (10.6-11.7 months), while best supportive care is associated with a median overall survival of 5 (2.8-7.7) months (2–4).

Since the majority of patients with unresectable CCA present with malignant biliary obstruction, biliary drainage is a crucial palliative treatment for patients with hilar or distal CCA. This can be obtained either by means of ERCP (Endoscopic Retrograde ColangioPancreatography) or PTC (Percutaneous Transhepatic Colangiography), placing one or more biliary stents (plastic or metal) which relieve jaundice, without changing patients prognosis (5, 6). Unfortunately, recurrent jaundice and cholangitis tend to occur because of reobstruction of the biliary stents due to tumor growth, despite the use of self expandable metals stents (SEMS), which have replaced plastic biliary stents in clinical practice to reduce the occurrence of recurrent jaundice (7, 8). This not only jeopardizes the efficacy of chemotherapy, but also causes significant morbidity and mortality (3, 4). Effective control of tumor growth is crucial for prolonging stent patency and consequently patient survival.

Recently, endobiliary radiofrequency ablation (ERFA) has been experimented as a treatment modality to reduce the tumor mass and delay tumor growth, extending stent patency (9–12). Several studies have demonstrated that ERFA is associated with an increased median survival of approximately 6 months in patients with CCA, without increasing the risk of complications (13–19). However, the improved overall survival could be simply secondary to the effect of ERFA on stent patency, which is usually prolonged by approximately 2 months (20–23). Both the prolonged patency of biliary stents and the delayed tumor growth could be strictly connected and allow a prompt recovery with prolonged jaundice free status, which avoids discontinuation of chemotherapy (9–12, 16).

This narrative review summarizes the results of the studies published in recent years and focuses on the impact that ERFA could have on the overall survival of patients with unresectable cholangiocarcinoma.

Overview of endobiliary therapy for unresectable cholangiocarcinoma

Endobiliary therapy of the tumor complementing chemotherapy for treatment of patients with unresectable CCA is appealing and it has been evaluated in clinical practice. The majority of patients with unresectable CCA require biliary drainage because of obstructive jaundice. Biliary stenting improves the quality of life but does not extend overall survival of these patients (18). At the same time of biliary drainage, endobiliary locoregional therapy can be administered and the combination of chemotherapy and endobiliary therapy has shown to improve the overall survival and the quality of life in patients with unresectable CCA (9, 24–26). It seems that local control of the tumor growth is crucial and this could be achieved by using different ablative techniques. These can be extrabiliary, like irreversible electroporation (IRE), or endobiliary such as intraluminal brachytherapy (ILBT), photodynamic therapy (PDT), and radiofrequency ablation (RFA) (9, 24–29).

IRE is a non-thermal tumor ablation technique which is mainly indicated for the treatment of locally advanced pancreatic cancer (27). IRE generates high-voltage electric current which induces cell apoptosis, because it alters the permeability of the cell membrane, without damaging the surrounding structures (27–29). Therefore, IRE can be used safely for the treatment of lesions near vascular and biliary vessels (30). Based on these findings, IRE has been used for the treatment of patients with unresectable CCA resulting in prolonged biliary decompression and improvement in both quality of life and

overall survival (28, 29). The main limitation of IRE is related to the technique itself which requires surgery (open VS laparoscopic) or percutaneous approach, always performed under ultrasound guidance (27–29). ILBT requires the insertion of iridium-192 (192Ir) or iodine-125 (125I) seeds contained in an impregnated wire which is advanced into the lumen of a nasobiliary tube or an external biliary catheter previously placed at the time of ERCP or PTC, respectively (24, 26). The radioactive seeds are placed inside the biliary stricture under fluoroscopic guidance using the markers present on the wire and high dose radiation (10-20 Gy) is locally delivered reducing the tumor mass, as well as controlling its growth by means of DNA damage, inhibition of cellular replication, and induction of tumor cells apoptosis (9, 24–26). Contiguity of the radiation source to the tumor allows the delivery of a higher dose of radiation, with less adverse effects on the surrounding structures (25). The efficacy and safety of ILBT has been evaluated in several heterogeneous small clinical studies, whose results do not allow to draw final conclusions on its effect in prolonging overall patient survival and stent patency (24, 26). An increased overall survival of the patients has been reported after ILBT in combination with external beam radiation therapy with or without chemotherapy (9, 24–26). The complexity of the procedure, the logistic problems of managing the radioactive material properly and some delayed serious adverse events (duodenal stenosis, gastrointestinal bleeding and hemobilia) have limited the use of ILBT in clinical practice (9, 24, 26).

Endobiliary PDT requires the administration of an intravenous photosensitizing agent (porfimer sodium) which concentrates in malignant biliary cells and is activated by a laser light of a specific wavelength delivered by a laser fiber placed into the biliary tree at the level of the stricture by means of ERCP or PTC (24). Subsequent generation of radical oxygen species with photoperoxidation of cellular membranes leads to apoptosis and necrosis of the neoplastic tissue which is also favored by inflammatory and antiangiogenic pathways locally activated by PDT (9, 24–26, 31). Moreover, the laser light refracting within the bile is transmitted through the biliary system and allows PDT to treat peripheral and unreachable lesions (24). After PDT, endoscopic biliary stenting is required because of tissue inflammation and edema. Plastic stents are preferred to metals stent because they allow repetition of PDT every 2-3 months at the time of stent exchange. However, there is no standardized protocol for endobiliary PDT regarding the number of sessions, interval between sessions, and bilateral vs unilateral endobiliary therapy. Numerous published studies, including several meta-analyses, reported a significant improvement of overall patient survival, and prolonged stent patency after endobiliary PDT (9, 24–26, 31, 32). The association of this ablative technique with chemotherapy has a beneficial effect, resulting in significantly longer overall survival and median progression-free survival of patients undergoing combined therapy (9, 24–26, 31, 32). According to the results of a systematic review and meta-analysis, endobiliary PDT is more effective than ERFA and stenting alone for the treatment of patients affected by unresectable CCA, with significantly prolonged overall patient survival as well as reduced mortality (32). Despite its reported therapeutic efficacy, endobiliary PDT has not become a standard of practice because of its side effects and pitfalls. Increased risk of bacterial cholangitis, liver abscess, and hemobilia are rare, but serious complications (25). Phototoxicity may result in pruritus, diffuse pain, skin erythema, and even blistering which may be prevented by avoiding direct sunlight for 4-6 weeks after PDT (9, 24–26, 31). This significantly affects the quality of life of patients who need to be carefully informed before undergoing PDT, especially if multiple sessions are predicted (9, 24, 26). Other practical downsides are the interval required between the administration of the intravenous photosensitizing agent and the execution of PDT as well as the time needed for each therapeutic session which is approximately 13 minutes (26). Finally, the high cost of each PDT session together with the need of a special laser contributes to the limited application of PDT for the treatment of patients with unresectable CCA (24, 26).

After preliminary experimental studies, in 2011 Steel et al. published the first report of a pilot study which evaluated feasibility, efficacy and safety of ERFA for the treatment of patients with malignant biliary obstruction (MBO) (17). The results of this study stimulated both experimental and clinical research with the objective of introducing ERFA in clinical practice for the management of patients with MBO and especially those with unresectable CCA for whom both ILBT and PDT do not represent the best therapeutic approach (Tables 1A, B) (9, 26).

Endobiliary radiofrequency ablation

ERFA is accomplished by means of a high-frequency alternating current which is released from an active electrode located in the

TABLE 1.A Comparison among ILBT, PDT and ERFA (from 24-26).

	ILBT	PDT	ERFA
INDICATIONS	- Perihilar U-CCA - Neoadjuvant therapy associated to chemoradion before liver transplantation in selected patients with CCA	- Perihilar U-CCA - U-CCA Bismuth IV - Neoadjuvant therapy associated to chemoradion before liver transplantation in selected patients with CCA	- Perihilar U-CCA - Distal U-CCA - Adjuvant therapy associated to chemotherapy - Occluded biliary metal stent - Intraductal residual tissue of resected ampullary adenomas

(Continued)

TABLE 1.A Continued

	ILBT	PDT	ERFA
CONTROINDICATIONS	- Poor clinical status (KPS < 50) - Severe liver insufficiency (PT ≤ 40%) - Severe kidney disease (CrCl < 10mL/min)	- Poor clinical status (KPS < 50) - Coagulopathy - Severe liver insufficiency (PT ≤ 40%) - Severe kidney disease (CrCl <10mL/min)	- Poor clinical status (KPS < 50) - Cardiac devices - Coagulopathy - Severe liver insufficiency (PT ≤ 40%) - Severe kidney disease (CrCl <10mL/min)
MECHANISM OF ACTION	- Localized delivery of high-dose radiation - Direct DNA damage - Inhibition of cellular replication - Induction of apoptosis of tumor cells	- Concentration of photosensitizing agent into the cancer cells - Activation of the photosensitizer by exposure to light of a laser fiber - Photoperoxidation of cell membranes - Cancer cells apoptosis	- Heat generation with local T> 50° - Coagulative necrosis and tumor cells death - Release of highly immunogenic intracellular particles - Enhancement of local immunity directed against the tumor
SPECIAL CONSIDERATIONS	- Insertion of iridium-192 (192Ir) or iodine-125 (125I) seeds into the biliary stricture - Need of ribbon or impregnated wire - Nasobiliary tube or external biliary catheter placed at ERCP or PTC - High-dose rate ILBT preferred - Shielded room - Recommended combination of ILBT with external beam radiation - Biliary stenting requiring a second procedure - Relatively expensive: about 14,000 USD $*	- Intravenous administration of photosensitizing agent - Diode laser system - Laser fiber with a cylindrical diffuser at the distal end and specific wavelenght (630 nm) - Delivery of PDT to malignant tissue away from the laser fiber - Recommended endoscopic biliary stenting = plastic vs metal - Repeatable (if plastic stent used) - Expensive: about 50,000 USD $ per PDT session	- Two endobiliary ERFA probe systems: HABIB and ELRA - Dedicated radiofrequency generator (ELRA) - Commercially available electrosurgical generator (HABIB) - Required direct tissue contact to obtain tissue destruction - Recommeded endoscopic biliary stenting = plastic vs metal - Repeatable (if plastic stent used) - Inexpensive: price of an ERFA probe is about 2,300 USD $

PDT, Photodynamic Therapy; ERFA, Endobiliary Radiofrequency Ablation; ILBT, Intraluminal Brachytherapy; U-CCA, Unresctable Cholangiocarcinoma; KPS, Karnofsky Performance Scale; CrCL, Creatinine Clearance; T, temperature; *from the WEB.

TABLE 1.B Comparison among ILBT, PDT and ERFA (from 24-26).

	ILBT	PDT	ERFA
OUTCOMES compared to stent alone	- Increased Stent Patency - Prolonged Survival	- Prolonged Survival - Longer Stent Patency - Improved KPS	- Improved Survival - Improved Stent Patency
ADVERSE EVENTS	- Cholangitis - Hemobilia - Gastrointesinal Bleeding - Duodenal Stenosis	- Phototoxicity - Abdominal Pain - Cholangitis - Liver abscess - Hemobilia	- Abdominal Pain - Cholangitis - Cholecystitis - Hemobilia - Liver infarction - Intraheptic Pseudoaneurysm
downsides	- Complexity of the procedure - Logistic problems - Challenging management of the radioactive material (handling, storing, devilering) - Radioprotection issues	- 3 day interval between the administration of the intravenous photosensitizing agent and the execution of PDT - Need to avoid direct sunlight for 4-6 weeks after PDT - Long therapeutic sessions (13 minutes)	- Often, more than one session - Low energy settings for ablation of intrahepatic stricture - Impairment of efficacy due to anatomical characteristics - Heat-sink effect

PDT, Photodynamic Therapy; ERFA, Endobiliary Radiofrequency Ablation; ILBT, Intraluminal Brachytherapy; KPS, Karnofsky Performance Scale.

middle portion of an endobiliary probe. This is placed inside the biliary stricture where the subsequent emission of thermal energy causes coagulative necrosis and cellular death when the temperature exceeds 50°C (11, 12). It has been shown that tumor necrosis releases intracellular particles which are highly immunogenic and activate antigen-presenting cells, enhancing local immunity directed against the tumor (33–36).

Immunogenic response

Radiofrequency ablation (RFA) has been shown to induce antigen-presenting cell infiltration and enhance systemic antitumor T-cell immune response as well as tumor regression in hepatocellular carcinoma (36). The tumor necrosis generated could be an antigen source for the immune system and it has been demonstrated that RFA determines a weak but detectable immune response which involves the activation of macrophages and the release of inflammatory cytokines (34). An early increase of cytokine IL6, followed by a delayed elevation of the serum levels of chemokines CXCL11, CXCL5, and CXCL1 was recently demontrasted in patients with pancreatic cancer and cholangiocarcinoma undergoing ERFA (37). However, the systemic immune response detected after ERFA was not specifically related to the endobiliary ablation and it was attributed to a general inflammatory response (37). Most likely, the immunogenic effects of RFA occur at the tumor site where the necrotic neoplastic tissue induces severe inflammation which can determine immune-mediated tumor destruction by neutrophils, macrophages, dendritic cells, natural killer cells together with B and T lymphocytes (33, 37). It has been speculated that the immune-mediated tumor destruction is not triggered by necrotic neoplastic tissue, but it is induced by the immunostimulatory and inflammatory factors present in the sub-vital tissue surrounding the ablated necrotic area (33). This could be particulary true for biliary strictures treated with ERFA where there is no certainty of complete tumor destruction since the width, the depth and the length of the ablation are not foreseeable, as demonstated experimentally (38–43). The local immunogenic response could potentially enhance tumor suppression and be responsible for the improved survival of patients with MBO and unresectable CCA who undergo ERFA (20–23) (Figure 1).

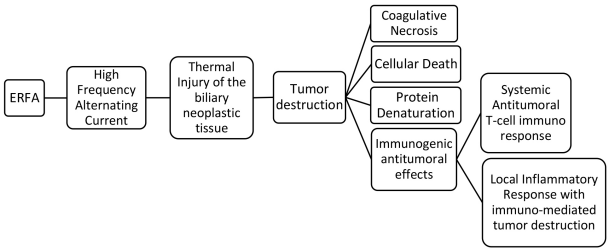

FIGURE 1
Pathophysiology of Endobiliary Radiofrequency Ablation (ERFA) (modified from 25).

Endobiliary probes

To date, there are two ERFA catheters which have been approved for clinical use (Table 2). The HABIB catheter (Boston Scientific, Marlborough, MA, USA) is a power-controlled 8-French bipolar probe, 180 cm long, with two circumferential electrodes placed 8-mm apart on the distal tip of the catheter to achieve an ablation length and depth of 20-25 mm and 3-5 mm, respectively (17, 43, 44). The HABIB catheter can be connected to different RFA generators, among which the most frequently used are the ERBE electrosurgical generators (Erbe,Tübingen, Germany). The recommended settings are effect 8 and 10 Watts (W) for ablation in the common bile duct and 8W for ablating strictures at the biliary bifurcation, near the cystic duct and the ampulla (38, 39). The ELRA catheter (STARmed, Goyang, Korea) is a 7-French temperature-controlled bipolar probe, 175 cm long, with two to four circumferential electrodes in its distal tip which are placed at different lengths. There are four distinct types of ELRA probes which accomplish diverse coagulation lengths (11, 18, 22, and 33 mm) along with circumferential ablation depths between 6 and 8 mm and median ablation depth of 4.0 mm (39, 40, 43, 45). The ELRA catheter operates only with the VIVA comboTM RFA generator (Taewoong Medical, South Korea), which allows presetting the target temperature and automatically stopping the procedure if this is exceeded during the ablation time (45). The recommended settings are a target temperature of 80°C and a power of 7 W or 10 W, on the basis of the type of catheter used as well as the location of the biliary stricture (39, 40, 45). The ELRA catheter differs from the Habib probe due to its different length and its temperature sensor on the tip of the device which provides a temperature-controlled ablation. Theoretically, these features offer the advantage to properly treat biliary strictures of different lengths and to reduce the risks of injuring the biliary ducts (45, 46).

Procedure

ERFA can be performed at the time of ERCP or PTC before biliary drainage in patients with MBO and strictures due to different neoplastic etiologies (11, 47, 48). Both approaches require cholangiography to properly visualize and measure both the length and the caliber of the stricture, before placing the wire-guided ERFA catheter inside it, under fluoroscopic monitoring (41, 45). The tip of the probe with the electrodes has to be positioned in direct contact with the target tissue. This is crucial for tissue destruction using either one of the devices, with a linear relationship between depth of ablation, preset power and established time of ERFA delivery (40, 49). Usually, each ablation lasts 60-120 seconds, with an average time of 90 seconds (38–41). In the case of long strictures (> 15 mm) the ablation needs to be repeated, without overlapping the treated segments when using the HABIB catheter; on the other hand a different length of the ELRA catheter can be choosen, avoiding repeated ablations for strictures up to 30 mm in length (41, 45). However, two or more ablations are always required when there is a

TABLE 2 ERFA Bipolar Catheters (modified from 9).

	HABIB Bipolar Catheter	ELRA Bipolar Catheter
Manufacturer	- Boston Scientific, Marlborough, MA, USA	- STARmed, Goyang, Korea
Diameter/Length	- 8-French (2.6 cm)/180 cm	- 7-French (2.31 cm)/175 cm
Distal Tip	- 24 mm long - two circumferential bipolar electrodes placed 8-mm apart	- 11 or 22 mm long - two circumferential bipolar electrodes - 18 or 33 mm long - four circumferential bipolar electrodes
Median Ablation Depth	- 4 mm	- 4 mm
Ablation Length	- 20-25 mm	- From 11 to 33 mm, depending on the type of probe used
RFA generator	- different RFA generators: - preferred VIO300D electrosurgical generator (Erbe,Tübingen, Germany)	- Only VIVA comboTM RFA generator (Taewoong Medical, South Korea)
Settings	- Power: - effect 8 and 10 W for common bile duct - effect 8 and 8 W for hilum, and ampulla	- Target temperature of 80°C - Power of 7 W or 10 W - type of catheter used - location of the biliary stricture
Energy Control	- YES	- YES
Temperature Control	- NO	- YES
Alarm, if insufficient electrode contact	- NO	- YES

ERFA, Endobiliary Radiofrequency Ablation.

complex hilar stricture, which requires separate treatment of both the right and left hepatic ducts (47). When ERFA is repeated, a 60 s resting period is recommended between applications. After removing the ERFA catheter, the bile duct is swept by using a retrieval balloon to remove residual necrotic tissue and a cholangiogram is obtained to rule out possible complications (24). Eventually, a biliary stent is placed to ensure long term biliary drainage, because of the stricture and the possibility of additional segmental biliary stenosis that ERFA can cause (40). Plastic stents are indicated if periodic ERFA sessions are planned at regular intervals, while metal stents are placed only when a single ERFA is forecasted and performed at the time of biliary drainage (50).

Technical issues

The therapeutic efficacy of ERFA could be affected by the morphology of the biliary stricture and its location (11, 47). Since the electrodes of the ERFA catheter need to be in strict contact with the target tissue, some features of the biliary stricture can affect the results of the ablation. The narrower the stricture, the higher the amount of energy delivered inside the stenosis: a powerful ablation not only causes deep and irregular coagulative necrosis, but also results in ineffective tissue ablation and increased risk of injury to the duct (18, 51). Similarly, when the biliary stricture is short, irregular or mushy and loose, ERFA could be unsuccessful because of uneven contact between the electrodes of the probe and the target tissue (22, 47, 52). Furthermore, the electrodes of the ERFA catheter can overlap the stricture, and ablate the normal bile duct beyond the biliary stenosis. This usually happens during the ablation of short strictures, but it can also occur with long stenosis when overlapping consecutive ablations are performed (22, 47, 52). In both cases ablation of the normal bile duct develops scar tissue, which expands the length of the original stricture and increases the risk of stent occlusion (40).

Anatomic characteristics of the intrahepatic and hilar biliary ducts might affect the efficacy of ERFA and/or increase the risk of complications. Bile ducts angulation in the hilum can impair ERFA treatment because straight and rigid endobliary catheters may not pass the angulations and/or the tip of the probe may not mantain the required tight contact with the target tissue (53). Hilar and intrahepatic biliary ducts have a subtle wall that is more susceptible to thermal damage, which can extend to surrounding structures, even in the presence of a tumor mass (42, 54). Strictures located in the hilum are close to both portal and hepatic veins as well as hepatic arteries: the blood flow acts as a cooling circuitry (heat-sink effect), which may prevent the ERFA catheter to deliver the proper energy needed to obtain effective tissue ablation (11, 49, 53). Intrahepatic strictures may be difficult to ablate completely because the ERFA catheter cannot pass easily them or the stenoses are too numerous to be all treated effectively (53). In these cases, selective ablation of dominant strictures is performed because complete treatment is not feasible (54).

Several possible solutions to the above mentioned technical issues have been proposed. A preoperative road map with abdominal MRI (magnetic resonance imaging) and MRCP (magnetic resonance cholangiopancreatography) is recommended to accurately assess the tumor surroundings and evaluate the relationship of the target biliary stricture with the surrounding vascular and biliary structures, especially for the treatment of hilar and intrahepatic stenosis (50). Before ERFA, both the length of the

stricture and the thickness of the biliary wall should be measured by using intraductal or endoscopic ultrasonography, especially if there is no apparent tumor mass on preoperative imaging (18, 55, 56). This information maximizes the efficacy of ERFA, reducing the risk of thermal injury by means of proper settings of the RFA generator and the duration of ablation, respectively (38–42, 45, 49). Patient-tailored settings may achieve better clinical outcomes for ERFA, which can be ultimately adapted to the native anatomy and the tumor mass (39). The temperature reached by the target tissue during ERFA correlates with the thermal damage of the bile ducts. Therefore, the novel temperature-controlled ERFA system could avoid unintended thermal injury of the biliary wall and the surrounding structures (45, 46). ERFA usually lasts 90-120 seconds. During this time the position of the electrodes may change inside the stricture provoking unintended thermal injury to the normal biliary wall. Therefore, it has been proposed to perform two consecutive 60 seconds ablations with an interval time of 60 seconds which is useful for checking the position of the electrodes by means of fluoroscopy and detecting the possible onset of adverse events, such as bleeding (53). The best way to correctly place the electrodes of the ERFA catheter inside the biliary stricture is to position the probe after direct visualization and evaluation of the stenosis using the peroral digital cholangioscope (57–59). Subsequently, another cholangioscopy evaluates the efficacy of the ablation and rules out possible immediate complications, such as bleeding and perforation (60). Placement of metal stents has been recommended to prevent bile duct injury, because they assure an immediate decompression of the biliary tree and a cooling effect on the ablated tissue by means of a copious biliary flow (23). Placement of fully covered SEMS has been suggested to avoid septic consequences of inadvertent bile duct injury (61, 62).A different technique can be considered for the local treatment of the biliary strictures if the risk of collateral damage induced by ERFA is classified as too high, at the time of preoperative road map (18).

Adverse events

The major advantages of ERFA are simplicity and low cost, without many major adverse events and very few controindications. The latter include the presence of cardiac devices, coagulation disorders, and ascites, as well as pregnancy (11, 63). There is considerable variability in the reported incidence of the adverse events after ERFA that can range from 7% to 48%, averaging the data of four previous published reviews (42, 56, 63, 64). This variability can be due to the fact that some adverse events after ERFA are not strictly related to it, but are the possible complications after ERCP or PTC, and therefore they might not have been reported (47). Other explanations reside in different etiology, location and morphology of the stricture; degree of thickness of the bile duct wall; contiguity of vascular structures with the biliary stenosis; variance in energy settings and duration of ablation; type of biliary stents (plastic or metal) placed after ERFA (64). The majority of patients complain of abdominal pain, which occurs in almost 50% of cases and it is self limited (10, 14, 65–67). Reported pancreatobiliary adverse events are pancreatitis, cholangitis, cholecystitis and minor bleeding (10, 14–16, 18–20, 22, 23, 44, 46, 50, 55, 61, 65, 66, 68–74). These are the typical adverse events that can occur after ERCP or PTC (5–8, 75). However, a higher number of cholecystitis and cholangitis have been reported, especially in patients with hilar strictures treated with ERFA (10, 22, 23, 50, 65, 68, 69, 73, 76). The incidence of cholecystitis requiring percutaneous drainage after ERFA has been estimated to be between 2%–4% and it is significantly higher than that reported after standard biliary drianage; similarly, cholangitis seems to occur more frequently after ERFA and subsequent placement of biliary stents (2-8%) (24). A possible explanation for the onset of suppurative cholecystitis could be the obstruction of the cystic duct, as a consequence of its thermal injury due to edema or tissue destruction when ERFA is delivered too close to the opening in the bile duct (10, 65). However, cholecysistis is not always reported after ERFA (18), and it has been hypothesized that the type (plastic vs metal) and the number (1-2 vs multiple) of biliary stents could be associated with a higher risk of cystic duct blockage (13). Since the reported total number of cases of cholecystis remains low and this complication resolves in few days with percutaneous gallbladder drainage and/or antibiotics, ERFA with subsequent biliary drainage is considered safe, even when the biliary stricture is close to the opening of the cystic duct (65). The high frequency of cholangitis has been initially attributed to necrotic debris which can remain in the bile ducts after ERFA with early subsequent obstruction of biliary stents; to avoid this possible complication the bile ducts are swept with an extraction ballon after ERFA and before stent placement (24, 48, 65, 77). Another possible cause of cholangitis is the ablation of the normal bile duct beyond the stricture, which sometimes occur because of technical and/or anatomical difficulties determining the onset of iatrogenic strictures of the bile ducts which could not be properly stented (40, 47). To reduce the risk of unintended strictures an accurate measurament of the stricture is required, especially if the biliary stenosis is long and requires ovelapping ERFA (18, 55, 56, 78). Few life-threathening adverse events have been reported (20, 36, 50, 62, 79–81). Therefore, it is important for the biliary endoscopist to be aware of these complications. Seven cases of biliary perforations occurred after endoluminal ablation of narrow biliary strictures, two of which were dilated before performing two overlapping ablations (20, 62, 73). After ERFA late severe melena developed in two patients and this was due to the rupture of a pseudoaneurysm originating from an artery, which was too close to the electrodes of the ablation catheter (79, 81). Six cases of delayed hemobilia were reported 4-6 weeks after ERFA and two of them were fatal because of hemorrhagic shock (41, 50). Liver infarction due to arterial thrombosis was diagnosed in a patient 3 days after ERFA of a stricture of the right hepatic duct: this complication was attributed to the proximity of the biliary stricture with a branch of the right hepatic artery (50). Vascular as well as biliary injuries ending in severe complications are mostly related to severe thermal injury of the bile duct inside and beyond the stricture which extends to the surrounding vascular and biliary stricture (50, 79, 81). Furthermore, aberrant angiogenesis after ERFA could explain delayed spontaneous hemobilia (41, 50). Placement of a SEMS after ERFA could be an effective method for preventing the onset of

late bleeding and biliary fistulas. It has been hypothesized that the high radial force of SEMS may have both a tamponade and hemostatic effect on the oozing from the necrotic tissue resulting after ERFA (25, 78). The rapid flow of bile through the strictures ensured by SEMS could have a cooling effect preventing deep bile duct injuries (23). Hyperkalemia was the cause of a sudden non-fatal cardiac failure in a patient with chronic kidney disease who underwent ERFA for the treatment of a biliary stricture at the time of biliary drainage (80). Another three cases of non-lethal heart failure occurred in two patients with a history of coronary heart disease and hypertension within 24 hours after ERFA (20, 74). Finally, a case of fatal hepatic coma, a left bundle branch block, and a few cases of liver abscess have been reported, especially after ERFA at the time of PTC (16, 19, 21, 22, 50, 54).

Strict patient-selection and ablation with customized settings (according to the location of the biliary stricture and the comorbidity of the patient) have been proposed to reduce the incidence of severe complications (20). Careful postoperative follow-up is necessary, and evaluation of the results of ERFA with cholangioscopy has been recommended (47, 60, 66).

Beneficial effects of ERFA in the care of unresctable cholangiocarcinoma

Patients with unresectable CCA have an overall survival of approximately 10 months if they undergo chemotherapy and about 4 months if they receive best supportive care (BSC) (3). The most common regimen of chemotherapy is based on the association between gemcitabine and cisplatin, which significantly reduces the risk of death compared to BSC or gemcitabine alone (1–3). In case of failure, modified FOLFOX should be used as second-line treatment, with a median progression free survival and median overall survival of 3.2 and 7.2 months, respectively (1, 2). Recently, a subset of patients showing isocitrate dehydrogenase isoenzyme 1 mutations (mIDH1) had been treated with ivosedinib, an oral small molecule inhibitor of mIDH1 with a median progression free survival of 2.7 months (2). Despite all these efforts, the prognosis of patients with unresctable CCA undergoing chemotherapy remains dismal (3, 4).

Among endobiliary therapies ERFA is the best option for its semplicity, low cost and relatively few serious side effects (24, 25, 31). At the time of biliary drainage of jaundiced patients with unresectable CCA, ERFA could be used as adjuvant therapy with the aim to control the biliary and peribiliary growth of the tumor (24). The majority of published studies have mainly evaluated the role of ERFA in the management of biliary obstruction due to bilio-pancreatic cancer, considering its impact on both stent patency and overall survival of the patients (10, 14, 17, 19–22, 37, 39, 41, 45, 46, 50, 51, 57, 66, 69, 71–73, 76, 78). The hypothetical beneficial effects of ERFA on palliative treatment of unresectable CCA has been investigated in the recent years (15, 16, 18, 23, 44, 52–55, 61, 67, 68, 70, 74). Three are single arm studies aimed to mainly assess both feasibility and safety of ERFA (Table 3) (44, 61, 74). Eight comparative studies, three of whom were randomized controlled trials (Table 4) and five were retrospective studies (Table 5), explored the impact of ERFA on stent patency, overall survival and improved functional status of the patients (15, 18, 23, 53, 54, 67, 68, 70). Finally, three studies evaluated the hypothesis that the combination between ERFA and chemotherapy could have a cumulative beneficial effect improving the overall survival as well as the quality of life in patients with locally advanced unresectable CCA (16, 52, 55). Recently, a meta-analysis evaluated the results of nine comparative studies, which had assessed both stent patency and overall survival in patients with unresectable CCA undergoing ERFA (65). The majority of these studies reached the conclusion that ERFA improves both stent patency and overall survival of patients with unresctable CCA. However, it is still unclear if these beneficial effects are related or independent, since the improved overall survival could be the consequence of prolonged stent patency.

ERFA and stent patency

Maintaining the patency of biliary stents guarantees the administration of chemotherapy without interruption. Despite the use of biliary SEMS, recurrent jaundice and cholangitis tend to occur because of reobstruction of the biliary stents due to tumor growth (7, 8). Several studies have demonstrated the beneficial effects of ERFA on stent patency, which is usually prolonged by approximately 2 months (20–23). The effects of ERFA on stent patency has been investigated by the majority of the cited comparative studies (15, 18, 23, 53, 54, 67, 68, 70). It seems that ERFA has the capability to prolong the patency of uncovered metal stents inducing a reduction in the tumor mass, which is associated with slowed endobiliary neoplastic growth and improved bile flow (52, 54). The decreased risk of sludge and/or biofilm formation could also explain the prolonged patency of plastic stents fter ERFA (54). As mentioned above, three single arm studies confirmed that ERFA can be performed safely at the time of biliary drainage either by means of ERCP or PTC and followed by placement of plastic or metal stents (44, 61, 74). The advantage of using plastic stents is that they permit repeated sessions of ERFA at scheduled times and this protocol seems to be beneficial for patients with unresectable CCA (15, 18, 44, 68). The impact of ERFA on biliary stent patency has been confirmed by a recent meta-analysis whose data demonstrated the superiority of ERFA plus stenting over stenting alone, independent of the stent type used (plastic vs metal) (10). However, these data are still controversial since it has been reported that ERFA has no effect on prolonging patency of both metal and plastic stents, respectively by a retrospective study and two randomized controlled trials (53, 54, 69). Similar doubtful and inconclusive results were obtained by a recent meta-analysis whose authors were unable to perform a pooled analysis of avalaible data and just reported that only three of five studies evaluated showed a beneficial impact of ERFA on stent patency (65). Biliary plastic stents need to be exchanged and this can be performed respectively, on schedule every three months or on demand (i.e. at the occurrence of signs and/or symptoms of obstruction) after the second session of ERFA scheduled at the time of first 3-month

TABLE 3 Single arm studies evaluating feasibility and safety of ERFA for NR-CCA (modified from 47).

Study	Study Design	#Patients	Localization and Bismuth Classification of NR-CCA	Procedure	# ERFA sessions	Type of biliary stent	Technical Success	Stent Patency median -range	Survival *mean - range median-range	Adverse events
44. Laquière A, et al. Surg Endosc (2015) (44)	Bicentric Case Series	12	Hilar = 12 4 Bismuth I 3 Bismuth II 2 Bismuth III 3 Bismuth IV	ERCP	19 (1-3 sessions) 5 pts = 2 sessions 1 pts = 3 sessions	Plastic	100%	NR	*12.3 mos (3-31)	1 cholangitis 1 Sepsis No mortality
62. Alis H, et al. Hepatobiliary Pancreat Dis Int (2013) (61)	Retrospective Single Arm	10	Distal = 6 Hilar = 4 4 Bismuth I	ERCP	10 1 session per pts	Metal = FC-SEMS	90%	9 mos (6-15)	NR	2 pancreatitis No mortality
76. Wang Y, et al. Oncotarget (2016) (74)	Retrospective Single Arm	9	Hilar = 9 2 Bismuth III 7 Bismuth IV	PTC	10 9 pts = 1 session 1 pts = 2 sessions	Metal = U-SEMS	100%	100 days (85-115)	5.3 mos (2.5-8.1)	3 abdominal pain 4 cholangitis 1 atrial fibrillation No mortality

In all the studies ERFA was performed using the HABIB bipolar probe with the following setting of RFA generator: 10 Watts.
NR-CCA, non resectable cholangiocarcinoma; ERFA, endobiliary radiofrequency ablation; NR, not reported; mos, months; pts, patients; U-SEMS, uncovered self expanding metal stent; FC-SEMS, fully covered self expanding metal stents.

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TABLE 4 Comparative randomized controlled studies evaluating the impact of ERFA on the management of NR-CCA (modified from 9, 47).

Study	#Patients		Localization and Bismuth Classification of NR-CCA		Proce-dure	ERFA Probe	RFA generator settings	# ERFA sessions	Type of biliary stent	Stent Patency Time median-mean range			Overall Survival Time median-mean range			Adverse Events (%) median-mean		
	ERFA + Stent	Stent	ERFA + Stent	Stent						ERFA + Stent	Stent	P	ERFA + Stent	Stent	P	ERFA + Stent	Stent	P
18. Yang J, et al. Endoscopy (2018) (18)	32	33	Distal = 22 Hilar = 10 Bismuth I-II	Distal = 24 Hilar = 9 Bismuth I-II	ERCP	HABIB	7-10 W	Repeated ERFA, every 6 months depending on IDUS results	Plastic	6.8 mos 3.6 -8.2	3.4 mos 2.4 -6.5	0.02*	13.2 mos 11.8-14.2	8.3 mos 7.3-9.3	<0.001*	6%	9%	>0.05
54. Kang H, et al. J Hepatobiliary Pancreat Sci (2022) (54)	15	15	Hilar = 15 2 Bismuth II 6 Bismuth III 7 Bismuth IV	Hilar = 15 3 Bismuth II 8 Bismuth III 4 Bismuth IV	ERCP	ELRA	7 W T = 80°C	Repeated ERFA and replacement of plastic stent with U-SEMS after 3 months	Plastic and then Metal = U-SEMS scheduled stent exchange at 3 months	178 days 96-260	122 days 111-139	0.154	230 days 77-383	144 days 0-323	0.643	60%	73%	>0.05
70. Gao D-J, et al. Gastrointest Endosc (2021) (68)	87	87	Distal = 62 (including ampullary cancer) Hilar = 25 8 Bismuth I 9 Bismuth II 8 Bismuth III	Distal = 65 (including ampullary cancer) Hilar = 22 10 Bismuth I 7 Bismuth II 5 Bismuth III	ERCP	HABIB	7-10 W	Repeated ERFA at scheduled stent exchange every 3 months	Plastic	3.7 mos	4.1 mos	0.674	14.3 mos	9.2 mos	<0.001*	28%	19%	0.21

NR-CCA, non resectable cholangiocarcinoma; ERFA, endobiliary radiofrequency ablation; IDUS, intraductal ultrasonography; NR, not reported; mos, months; U-SEMS, uncovered self expanding metal stent.
*statistically significant.

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TABLE 5 Comparative Retrospective Studies evaluating the impact of ERFA on the management of NR-CCA (modified from 9, 47).

Study	#Patients		Localization and Bismuth Classification of NR-CCA		Proce-dure	ERFA Probe	RFA generator settings	# ERFA sessions	Type of biliary stent	Stent Patency Time median-mean range			Overall Survival Time median-mean range			Adverse Events (%) median-mean		
	ERFA + Stent	Stent	ERFA + Stent	Stent						ERFA + Stent	Stent	P	ERFA + Stent	Stent	P	ERFA + Stent	Stent	P
15. Bokemeyer A, et al. Scientific Reports (2019) (15)	20	22	Hilar = 20 1 Bismuth III 19 Bismuth IV	Hilar = 22 2 Bismuth I 20 Bismuth II	ERCP	HABIB	8 Watts (22 sessions) 10 Watts (19 sessions) Others (5 sessions)	Repeated Sessions in 40.7% of cases	Plastic (85% vs 91%) Metal = U-SEMS (15% vs 9%)	NR	NR	–	342 days	221 days	0.046*	18.5%	NR	–
23. Liang H, et al. Journal of Cancer Therapy (2015) (23)	34	42	Distal = 22 Hilar = 12 All Bismuth I	Distal = 27 Hilar = 15 All Bismuth I	ERCP (29 vs 37) PTC (5 vs 5)	HABIB	10 Watts	Repeated Sessions in 11.8% of cases	Metal = U-SEMS (30 vs 36) FC-SEMS (4 vs 6)	9.5 mos 4.5-14	8.3 mos 4.9-11	0.024*	12.8	11.3	0.036*	26.5%	23.8%	>0.05
53 Oh D, et al. Journal of Gastroenterol and Hepatology (2022) (53)	28	51	Hilar = 26 1 Bismuth I 2 Bismuth II (GB cancer) 14 Bismuth III 11 Bismuth IV	Hilar = 36 1 Bismuth I 9 Bismuth II (GB cancer) 19 Bismuth III 22 Bismuth IV	ERCP	ELRA	7–10 W T = 80°C	NO	Metal = U-SEMS	192 days	140 days	0.41	311 days	311 days	0.73	7.1% (early)	2% (early)	0.25
69. Wu TT, et al. Cardiovasc Intervent Radiol (2017) (67)	35	36	Distal = 35	Distal = 36	PTC	HABIB	10 Watts	No	Metal = U-SEMS = 58	241 days (28 U-SEMS)	137 days (30 U-SEMS)	0.001*	245 Days (28 U-SEMS	209 days (30 U-SEMS	>0.05	0% severe events	16.6% severe events	–

(Continued)

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TABLE 5 Continued

Study	#Patients		Localization and Bismuth Classification of NR-CCA		Procedure	ERFA Probe	RFA generator settings	# ERFA sessions	Type of biliary stent	Stent Patency Time median-mean range			Overall Survival Time median-mean range			Adverse Events (%) median-mean		
	ERFA + Stent	Stent	ERFA + Stent	Stent						ERFA + Stent	Stent	P	ERFA + Stent	Stent	P	ERFA + Stent	Stent	P
72. Qi S, et al. Am J Transl Res (2021) (70)	60	60	NR	NR	PTC	NR	NR	No	Metal = U-SEMS	NR	NR	–	11.1 mos	8.2 mos	<0.001*	15%	25%	0.114
									FC-SEMS = 13							(28 U-SEMS)	(30 U-SEMS)	

NR-CCA, non resectable cholangiocarcinoma; ERFA, endobiliary radiofrequency ablation; NR, not reported; U-SEMS, uncovered self expanding metal stent; FC-SEMS, fully covered self expanding metal stent.
*statistically significant.

endoscopic follow-up (18, 68). When plastic stents are exchanged, a repeat ERFA session can be performed. The need of reintervention could be decided on the basis of the results of cholangiography and/or intraductal ultrasonography (IDUS), which can measure the caliber and the width of the bile duct (18, 48, 56, 66). ERFA should be repeated when IDUS detects a significant increase in tumor thickness and a reduction in the bile duct diameter at the site of the previously treated stricture (18). Another technique used to monitor the results of ERFA is cholangioscopy which can also guide the correct placement of the ERFA catheter inside the targeted biliary stricure (58–60). Ideally, plastic stents are indicated when multiple sessions of ERFA are scheduled in patients with a locally advanced CCA without metastases and in good functional status (24). On the other hand, SEMS are recommended when only a single session of ERFA is planned and their use has been advocated to reduce the risk of late bleeding and biliary fistulas (23, 25, 78). Moreover, SEMS are the preferred stents after the execution of ERFA at the time of PTC, which is usually performed to treat intrahepatic unresctable CCA (23, 48, 65, 67, 70).

Survival benefit of ERFA

The most valuable effect of ERFA is its impact on the overall survival of patients with unresectable CCA undergoing biliary drainage and stent placement. The above mentioned comparative studies as well as the cited meta-analysis investigated the impact of ERFA on overall survival and improved functional status of patients (15, 18, 23, 53, 54, 65, 68, 70). The overall survival of patients with unresectable CCA is significantly improved after ERFA plus stenting, with a pooled mean survival of 374 days vs 263 days of those treated only with stent placement at the time of biliary drainage (15, 18, 23, 68, 70). Similar data were obtained by the meta-analysis which reported a median survival of 294 days in patients undergoing ERFA vs 216 days in those who received only a biliary stent, independent from the type of stent placed (65). As already mentioned, the improved survival of patients undergoing ERFA could be due to the local immunogenic response, which potentially enhances tumor suppression and decreases the tumor burden delaying neoplastic progression inside as well as outside the bile duct (20–23). The direct action on the tumor and the induced local and systemic immune mechanisms could explain the favorable impact of ERFA on overall survival of patients undergoing endobliary ablation (65, 68). Only two retrospective studies reported no difference in overall survival between patients undergoing ERFA and those treated only with biliary stent placement (53, 54). There are several possible explanations for these controversial results: the anatomy of the biliary ducts, which could have been too angulated for adequate ablation; the cooling effect due to the blood flow of the surroundings vessels which could have prevented sufficient ablation of the tumor; the type of CCA treated, since Bismuth III and IV are characterized by the presence of multiple strictures, which could not be ablated as a whole, invalidating the efficacy of ERFA; the placement of SEMS which could have hidden the beneficial effects of ERFA; the use of different probes and generator settings which could have affected the

TABLE 6 Comparative studies evaluating the impact of ERFA plus Chemotherapy on the management of NR-CCA (modified from 9, 47).

Study	Design	#Patients		Localization and Bismuth Classification of NR-CCA		Proce-dure	ERFA Probe	RFA generator settings	# ERFA sessions	Type of biliary stent	Overall Survival Time median-mean			Progression Free Survival			Adverse Events (%) median-mean		
		ERFA + Stent + CHT	Stent + CHT	ERFA + Stent + CHT	Stent + CHT						ERFA + Stent + CHT	Stent + CHT	P	ERFA + Stent + CHT	Stent + CHT	P	ERFA + Stent + CHT	Stent + CHT	P
16 Gonzalez – Carmona MA et al. Scientific Reports (2022) (16)	Retrospective	40	26	Distal + Hilar Bismuth I-II = 9 Hilar Bismuth III-IV = 31	Distal + Hilar Bismuth I-II = 7 Hilar Bismuth III-IV = 19	ERCP	HABIB	10 Watts	Repeated Sessions in 55% of cases	Plastic Metal in case of early disfun-ction	17.3 mos	8.6 mos	0.004*	12.9 mos	5.7 mos	0.045*	72.5% Cholangitis Similar Hematologic toxic effects	53.8% Cholangitis Similar Hematologic toxic effects	0.031*
52. Inoue T, et al. Curr Oncol (2022) (52)	Retrospective	25	25	Distal = 4 Hilar = 21	Distal = 3 Hilar = 22	ERCP	HABIB	7-10 Watts	1 session	U-SEMS	17.1 mos	11.3 mos	0.017*	8.6 mos	5.8 mos	0.014*	8% 1 Cholangitis 1 Pancreatitis Similar Hematologic toxic effects	8% 1 Cholangitis 1 Bleeding Similar Hematologic toxic effects	1.000

NR-CCA, non resectable cholangiocarcinoma; ERFA, endobiliary radiofrequency ablation; NR, not reported; U-SEMS, uncovered self expanding metal stent; FC-SEMS, fully covered self expanding metal stent.
*statistically significant.

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outcomes, especially in patients with hilar CCA undergoing ERFA (53, 54). Besides overall survival, ERFA seems to have also a beneficial effect on the functional status of the patients undergoing endobiliary ablation. Several studies reported rapid improvement of the jaundice and increased albumin values which translated to a better functional status and higher Karnofsky Performance Scale (KPS) scores in comparison with patients treated only with stent placement (18, 23, 68, 70). ERFA, cancer stage, Bismuth type I-III, level of serum albumin near normal and the administration of adjuvant chemotherapy could be positive prognostic factors that have a beneficial cumulative impact on the overall survival of patients with unresectable CCA (23, 65, 68). Among these, adjuvant chemotherapy has been proven to be the most effective and its combination with ERFA could be the best option to improve the overall survival of patients with advanced CCA (65).

Impact of the combination of ERFA and adjuvant chemotherapy on overall survival

It has been postulated that the thermal cell injury induced by ERFA could increase the cytotoxic effect of chemotherapy, especially in the case of intrahepatic CCA where the endobiliary ablation is often sublethal (53). Moreover, some data suggest that stent patency is shorter after ERFA without chemotherapy, and failure of its administration can be considered a risk factor for stent occlusion in patients with unresectable CCA undergoing endobiliary ablation (23, 53). The possible advantage of the combination between ERFA and adjuvant chemotherapy has been investigated in three studies, two of which were retrospective and the other one was a randomized controlled trial (16, 52, 55). In the two retrospective studies patients undergoing combination therapy were compared with those treated only with chemotherapy after biliary drainage with the aim of evaluating the impact of combination therapy on both the overall survival and the progression free survival of patients with unresectable CCA (Table 6) (16, 52). The superiority of combination therapy over ERFA alone was then demonstrated by a randomized controlled trial which investigated the effect of the consecutive administration of ERFA and a novel anti-cancer drug in improving both overall survival and progression free survival of patients with locally advanced unresctable CCA (55). All the data presented in these studies support the efficacy of the additional effect of ERFA on chemotherapy, with an average median survival of 16.6 months compared to 10.3 months of patients undergoing only chemotherapy (16, 52, 55). Similarly, median progression free survival (PFS) was improved in patients undergoing chemotherapy after ERFA (16, 52). These advantages were clear for locally advanced CCA, but became less evident in patients with metastatic CCA, for whom the combination of ERFA and chemotherapy did not significantly increase both median survival and PFS in comparison to patients undergoing chemotherapy alone (16, 52). The combined therapy also had a beneficial impact on the functional status and the quality of life of patients with a prolonged high KPS scores after ERFA (55). No major side effects of both treatments and no increase in adverse events were reported with the combination of ERFA together with adjuvant chemotherapy (16, 52, 55). Therefore, the results of these three studies support our change of approach in patients with locally advanced CCA undergoing biliary drainage, especially if they have a life expectancy of at least 6 months: if possible, they should undergo ERFA before starting adjuvant chemotherapy (82).

Conclusions

Available literature data support the role of ERFA as adjuvant therapy which increases both stent patency and overall survival in patients with unresectable CCA. These beneficial effects could add up to those of chemotherapy, with a cumulative impact of combination therapy on functional status, PFS and overall survival of patients with unresectable CCA. In the light of its potential benefit, ERFA could become part of the management of patients with locally advanced unresectable CCA, Bismuth type I-III, with a prognosis of at least 6 months (Figure 2) (83). However, there are still some issues that need to be clarified regarding the settings of the RFA generator, the type of ERFA catheter (energy and temperature controlled vs energy controlled), the number of ablations, the frequency of sessions (if more than one), and the type of biliary stent (metal vs plastic) in order to develop a standardized protocol. All these questions require further research and some of

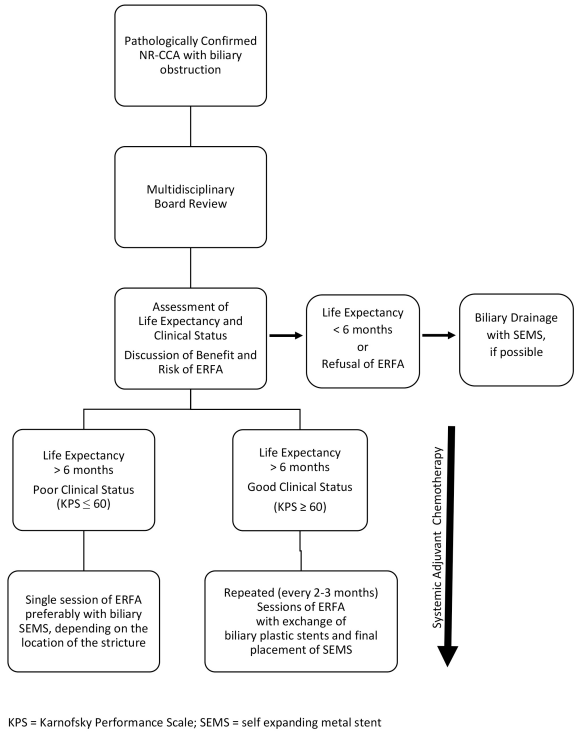

FIGURE 2
Proposed algorithm for Endobiliary Radiofrequency Ablation (ERFA) in the management of jaundiced patients with locally advanced unresectable cholangiocarcinoma (NR-CCA), Bismuth type I-III (modified from 8 and 23).

them could be answered in the three ongoing clinical trials which are investigating the role of ERFA for the treatment of unresectable CCA (84–86).

Author contributions

EDG and MDB managed the overall project and prepared the first draft. LT, FF, AO and AB reviewed the first draft. All other authors reviewed the manuscript and contributed to the final version of the article. EDG and MDB finalized the article on the basis of comments from the other authors. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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