



UNIVERSITÀ DEGLI STUDI “G. d’Annunzio” Chieti-Pescara

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**From Sudan to Paris: Epidemiology of Liver Tumors and Survival
Outcomes After Surgery for Intrahepatic Cholangiocarcinoma**

Dottorando

Dott. Michele Fiordaliso

Coordinatrice

Prof.ssa Antonia Patruno

Relatore/Tutor

Prof. Nicola Tinari

Co-Tutor

Prof. Pierluigi Lelli Chiesa

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A chi mi ha sostenuto in ogni passo: la mia famiglia.

Preface

This doctoral thesis originates from the “Sudan Project,” an initiative conceived with the aim of directly assessing healthcare challenges in developing countries and contributing to the improvement of medical services through field experience. The original plan envisaged a period of work in Sudan, where I intended to analyze the existing healthcare system, identify its critical issues, and explore practical strategies to enhance the delivery of care.

Unfortunately, due to the outbreak of war, it was not possible to carry out the planned stay in Sudan. This was not only a logistical obstacle, but also a personal disappointment, as the field experience represented a fundamental part of the original vision of this work. Rather than abandoning the project, I chose to reshape it.

Thanks to the support and availability of Sudanese colleagues, and through an extensive review of the literature, the focus gradually shifted toward an epidemiological study of liver tumors in Sudan. Although different from what I had initially imagined, this new direction allowed me to remain connected to the country and to its healthcare challenges, while still pursuing a meaningful scientific objective.

At the same time, I tried to make the most of the opportunities offered by this doctoral journey. As a surgeon, I deepened my knowledge of hepatic oncology by training in highly specialized centers across Europe, including institutions in Istanbul, Milan, Strasbourg, Frankfurt and Paris. These experiences were essential for my professional growth and provided the clinical and scientific basis for much of the work presented in this thesis. I pursued this training to share and transfer the knowledge and skills I acquired with my Sudanese colleagues.

Moreover, despite geographical barriers, collaboration remained a central element of the project. Together with Sudanese colleagues, we organized web-based conferences that enabled multidisciplinary discussions of liver tumor cases. These exchanges not only enriched the scientific value of this research but also reinforced the importance of international cooperation in addressing complex medical challenges.

Looking back, this project took a different path than the one I had originally planned. Nevertheless, it became an experience of adaptation and learning.

This thesis reflects that journey: a path shaped by unexpected events, but also by collaboration, commitment, and the desire to contribute—however modestly—to a broader understanding of healthcare challenges in resource-limited settings.

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Part I

Introduction

Sudan, located in North Africa, is one of the largest countries on the continent, with a land area estimated at approximately 1,765,000–1,886,000 km² according to international sources, placing it among the twenty largest countries worldwide by territorial extent (1). The country is longitudinally divided by the Nile River into eastern and western regions (Fig. 1).

The Sudanese population reflects a combination of indigenous Nile Valley communities and descendants of migrants from the Arabian Peninsula. This vast territorial extension results in marked heterogeneity in access to healthcare services and in the level of epidemiological surveillance. Large rural areas remain underserved, with limited diagnostic facilities and incomplete cancer case registration.

The national population is estimated to exceed 50–52 million inhabitants in 2025–2026, with a relatively low average population density (Fig. 2) of approximately 29–30 inhabitants per km². Demographically, Sudan is characterized by a very young population, with a median age of around 18–19 years and a comparatively small proportion of elderly individuals when compared with high-income countries. Moreover, less than 36% of the population lives in urban areas, highlighting a predominantly rural population and potential inequalities in access to healthcare and early cancer diagnosis.

These geographic and demographic characteristics, together with limited diagnostic capacity and infrastructural constraints, provide essential context for critically interpreting cancer incidence patterns and their geographic distribution. They also help to explain the intrinsic limitations of data derived from existing cancer registries in the country.

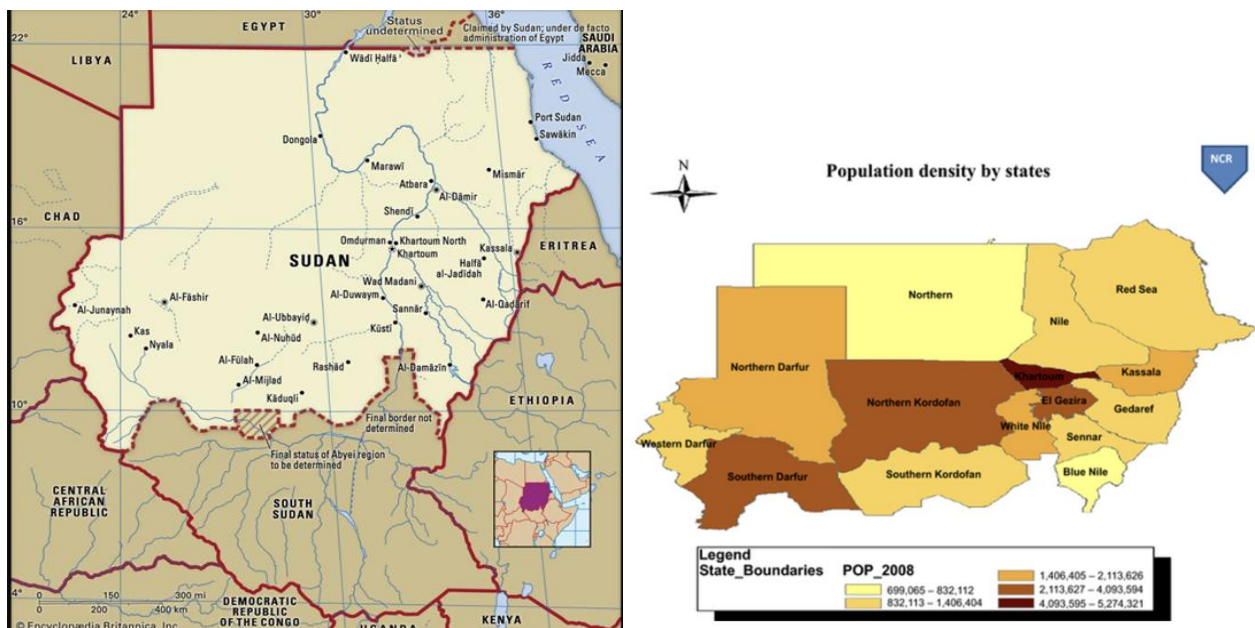


Fig. 1 and 2. Map showing the geographical location and its population density of Sudan in Northeast Africa.

1. Hepatocellular carcinoma (HCC) in Sudan

Liver cancers are a major cause of morbidity and mortality in developing countries, including Sudan, where they pose a significant challenge to health systems due to late diagnosis and limited available treatment resources. The 10 most common cancer (2) sites in Khartoum are shown in Fig. 3. Among all registered cancer cases with available information ($N = 6548$, 96.7%), breast cancer was the most common cancer with an incidence rate of 25.1 per 100,000, followed by leukemia (rate = 10.0 per 100,000), lymphoma (rate = 8.2 per 100,000), prostate cancer (rate = 7.3 per 100,000), colorectal cancer (rate = 7.1 per 100,000), oral cancer (rate = 6.1 per 100,000), cancer of the esophagus (rate = 5.8 per 100,000), liver cancer (rate = 4.2 per 100,000), stomach cancer (rate = 4.0 per 100,000), and cancer of the cervix (rate = 4.0 per 100,000).

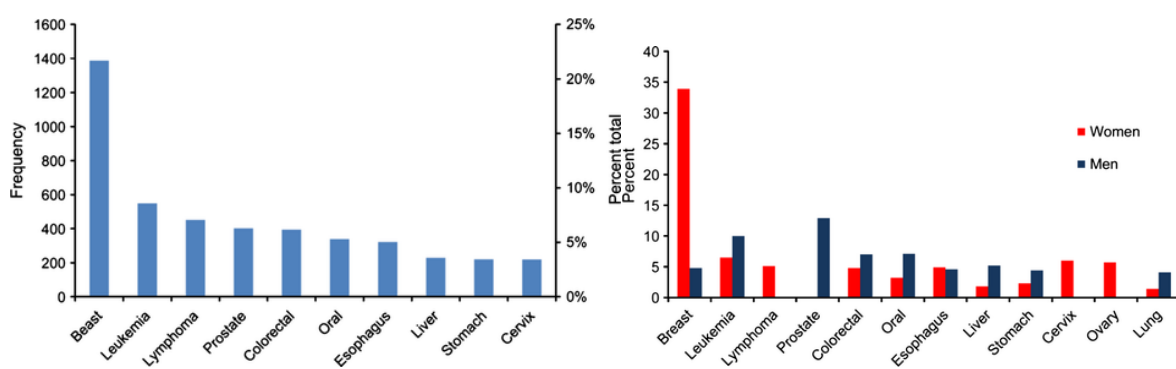


Fig. 3 and 4 Top ten cancers in Khartoum in terms of incidence and sex ratio according to the Cancer Registry 2009-10

In Sudan, hepatocellular carcinoma (HCC) is the most common type of liver cancer, followed by cholangiocarcinoma and secondary liver metastases from gastrointestinal tumors.

The high incidence of HCC in Sudan is closely related to the prevalence of chronic hepatitis B (HBV) and hepatitis C (HCV) infections, which are the main risk factors for the development of primary liver cancer. Furthermore, exposure to mycotoxins such as aflatoxin, frequently present in poorly stored cereals, contributes to the increased risk of liver cancer in the Sudanese population.

According to local data and regional studies, liver cancer tends to affect mainly working-age individuals, with a male predominance (Fig. 4), reflecting the significant socioeconomic impact of the disease on the country's social fabric. Limited access to screening programs, lack of early diagnosis, and limited treatment options contribute to the generally poor prognosis of patients with liver cancer in Sudan.

Furthermore, liver metastases from secondary tumors, particularly colorectal and gastric cancer, represent a significant proportion of liver cancers encountered in clinical practice, posing further challenges in integrated oncology management, given the diagnostic difficulties and limitations of surgical and systemic treatments.

Data from the Khartoum Cancer Registry for the period 2009–2010 identify breast carcinoma as the most frequently diagnosed malignancy, followed by leukemias. This distribution, which differs markedly from the epidemiological patterns observed in high-income countries, should be interpreted in light of the structural limitations of the cancer registration system and the local healthcare context. The registry, which is predominantly hospital-based and focused on urban settings, is likely affected

by substantial selection and information biases, favoring the inclusion of clinically evident or relatively easy-to-diagnose malignancies—such as advanced breast cancer and hematological neoplasms—while underrepresenting solid tumors that require more complex diagnostic pathways and technological resources that are not always available.

Moreover, the young demographic structure of the Sudanese population and the relatively low life expectancy contribute to shaping the observed cancer distribution, reducing the apparent incidence of cancers typically associated with older age and increasing the relative burden of leukemias. Taken together, these factors suggest that the available data are more reflective of access to healthcare services and diagnostic capacity than of the true cancer incidence in the general population. This highlights the need for cautious interpretation of estimates derived from registries that are not fully population-based and underscores the urgency of strengthening cancer surveillance systems in order to obtain more representative and internationally comparable data.

1.1 Incidence, mortality and demographic characteristics of HCC in Sudan

There is no functional national cancer registry in Sudan; however hospital sources indicate an increase in hepatocellular carcinoma (HCC) cases between 2000 and 2018, with an estimated incidence of approximately 4.2 per 100,000 inhabitants. HCC represents between the eighth and tenth most common cancers in the country (3-4).

In a study of 24 patients from Khartoum: HCC accounted for 1.97% of medical admissions and 40% of cancers, with a mean age of 54 years, a male to female ratio of approximately 6:1; higher prevalence in the west of the country. In a cohort of 102 patients (2003-05): mean age 44 years, 80% male; advanced stage and mean survival of 5 to 6 months. HCC tends to affect relatively young people (30-50 years), as frequently observed in sub-Saharan Africa. Furthermore, the incidence of HCC in Sudan between 2000 and 2018 has continuously increasing, in line with the increase in HBV/HCV infections (Fig. 5).

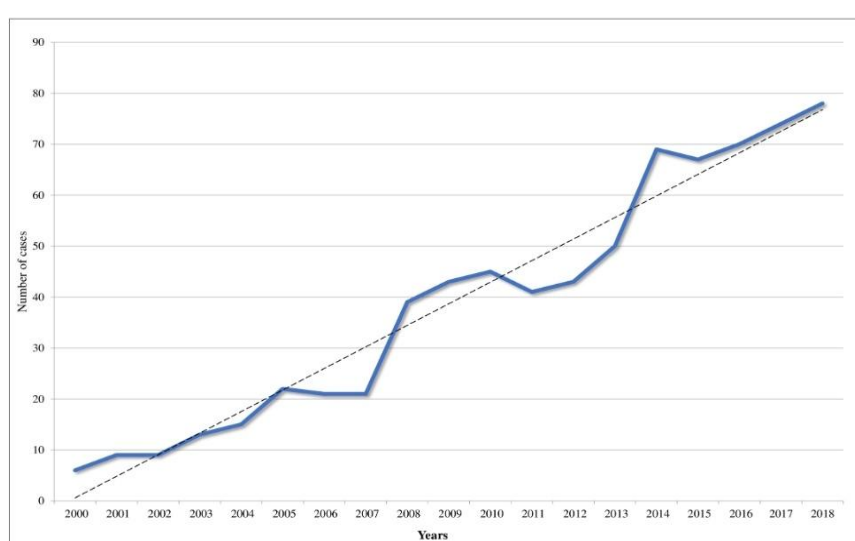


Fig. 5. HCC incidence trend in Sudan (2000-2018) The graph shows an increasing trend in HCC incidence in Sudan between 2000 and 2018

1.2 Risk factors and etiopathogenesis

1.2a Hepatitis B and C

Chronic hepatitis B (HBV) and hepatitis C (HCV) infections are the leading causes of liver cancer worldwide, and Sudan is no exception. Sudan is highly endemic for HBV (>8% HBsAg), with regional variations: from 6.8% in the centre to 26% in the south. The prevalence of HBV is estimated at 10 to 15% in certain regions, making it a significant public health concern.

Studies indicate that up to 60% of HCC cases in Sudan are attributable to chronic HBV infection, while HCV accounts for an additional 20-30% of cases (4). HBV infection is highly prevalent in rural areas due to limited vaccination coverage, unsafe healthcare practices, and perinatal transmission.

While approximately 12% of liver cancer deaths are attributed to hepatitis B virus, hepatitis C virus is responsible for 10% of these deaths (5).

The implementation of screening for hepatitis B virus and hepatitis C virus in all blood banks nationwide, along with the inclusion of hepatitis B virus vaccination to the expanded immunization program in 2006, represent significant milestones in the country's efforts to combat viral hepatitis.

1.2b Aflatoxins

Aflatoxins are mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*, which commonly contaminate foods such as cereals, nuts, and spices (6-7). Among the different aflatoxins, aflatoxin B1 (AFB1) is the most toxic and carcinogenic. It is classified by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen. At the molecular level, aflatoxin B1 is bioactivated in the liver by the cytochrome P450 enzyme system into a highly reactive epoxide that binds to DNA, forming adducts primarily with guanine. This process induces point mutations, including the characteristic TP53 mutation at codon 249 (AGG→AGT, arginine to serine), which is considered a biomarker of aflatoxin exposure and is strongly associated with hepatocellular carcinoma (HCC) in endemic areas. Aflatoxins also promote oxidative stress and disrupt cell-cycle regulation, further contributing to genomic instability and liver carcinogenesis.

Chronic exposure to aflatoxins is one of the major environmental risk factors for the development of hepatocellular carcinoma (HCC), particularly in synergy with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. The environmental conditions that favor fungal growth and toxin production include temperatures between 24 and 35 °C and a moisture content above 7% (or above 10% with ventilation). Aflatoxin contamination mainly affects regions located between 40° north and 40° south of the equator. As a result, this problem is especially prevalent in developing countries in tropical regions.

In Sudan, as in most countries of sub-Saharan Africa, high temperatures and humidity create particularly favorable conditions for the growth of *Aspergillus* species and the production of mycotoxins.

Younis and Malik investigated aflatoxin contamination in Sudanese groundnuts and groundnut products (excluding oil). They discovered that the percentage of aflatoxin contamination was as follows: 2% for kernels, 64% for butter, 14% for cake, and 11% for roasted groundnuts. Their findings showed that aflatoxin B1 predominated in all samples (8).

A study conducted on vegetable oil samples collected in Khartoum State reported that nearly all samples (98.8%) were contaminated with aflatoxins, with an average level of 57.5 µg/kg. In particular, all sesame oil samples were found to contain aflatoxin levels exceeding the acceptable limit of 20 µg/kg set by the U.S. Food and Drug Administration (FDA) (9-10).

Similarly, another study in Sudan aimed to assess aflatoxin levels in peanut, sesame, and cottonseed oils. Aflatoxins were detected in 14.3% of the samples. The highest incidence of contamination was observed in sesame oil, followed by peanut oil, while no aflatoxin contamination was detected in cottonseed oil. All contaminated samples were unrefined, highlighting the importance of refining processes in removing aflatoxins from edible oils. The higher frequency of aflatoxin contamination in sesame oil warrants further investigation, given its widespread consumption in Sudan in unrefined form.

In their study, Mariod et al. reported that aflatoxin B1 concentrations in peanut oil samples collected from different regions of Sudan ranged from 0.5 to 70 µg/kg. Similarly, sunflower oil samples showed aflatoxin B1 levels ranging from 0.7 to 35 µg/kg. The study also demonstrated that unrefined samples had significantly higher contamination levels. Consequently, the authors concluded that poor practices during crop cultivation, harvesting, handling, and storage were key factors contributing to the elevated levels of aflatoxin B1 detected in oil samples.

Omer et al. (11) investigated the combined effects of hepatitis B virus infection and aflatoxin B1 exposure on the development of HCC in Sudan. They found that Hepatitis B virus and aflatoxins exposure account for approximately 80% of HCC cases, whether alone or in combination.

1.2c Other causes

Hepatitis B virus (HBV) infection is highly prevalent in Sudan, where it is considered hyperendemic, affecting an estimated 6–12% of the general population. **Hepatitis D virus (HDV)** is also endemic in the country and frequently occurs as a co-infection in individuals who are already HBV-positive, substantially increasing the risk of progression to cirrhosis and hepatocellular carcinoma (HCC) (12-13).

Long-term HBV infection is a well-established risk factor for HCC. Its carcinogenic role is mediated both by persistent liver injury—characterized by chronic inflammation and continuous hepatocyte regeneration—and by the integration of HBV DNA into the host liver genome. When HDV infection occurs concurrently or as a superinfection, the course of liver disease is markedly accelerated. Patients with HBV/HDV co-infection progress to cirrhosis more rapidly than those infected with HBV alone, leading to an earlier and higher risk of HCC. Although HDV is not directly oncogenic, it amplifies HBV-related carcinogenesis by intensifying liver necroinflammation and increasing hepatocyte turnover.

In Sudan, observational studies indicate that patients **co-infected with HBV and HDV** tend to develop HCC at a younger age and have a poorer prognosis compared with patients infected with HBV alone. Mudawi et al. (14) reported that HBV infection was present in approximately 80% of HCC cases in Sudan, with HDV co-infection identified in 14% of HBV-positive HCC patients. The presence of HDV is strongly associated with faster progression to cirrhosis, which represents the main pathological background for the development of hepatocellular carcinoma.

Schistosomiasis, a parasitic disease endemic to Sudan, has been linked to liver fibrosis and portal hypertension, which can ultimately lead to liver cancer. *Schistosoma mansoni*, the species responsible

for intestinal schistosomiasis, is prevalent in the Nile River basin, affecting millions of Sudanese people, especially in rural communities with poor access to clean water and sanitation.

Although **alcohol consumption** is not widespread due to cultural and religious practices in Sudan, it is a risk factor for liver cancer among specific populations. Total per capita alcohol consumption (liters of pure alcohol, projected estimates, age 15+) in Sudan was reported to be approximately 0.51 liters/year in 2018 (15). Smoking, which is more prevalent in urban areas, has been identified as a cofactor that exacerbates liver damage in individuals with HBV or HCV infection, contributing to the development of HCC.

1.3 Diagnosis of liver tumors

The diagnosis of hepatic tumors in Sudan is challenged by multiple structural and resource-related factors that influence both the timeliness and accuracy of detection. The majority of hepatocellular carcinoma (HCC) cases are diagnosed at an advanced stages due to limited access to imaging modalities such as ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI), particularly in rural and peripheral regions. Laboratory support is also constrained, with alpha-fetoprotein (AFP) testing is inconsistently available across healthcare facilities, limiting its utility as a reliable biomarker for early detection. Furthermore, a lack of population-based screening programs and incomplete registry coverage results in underreporting and delays in case identification, introducing significant ascertainment bias in epidemiological data. Socioeconomic factors, including low health literacy, cultural barriers, and cost-related obstacles, further impede early presentation to healthcare services. Collectively, these constraints highlight the urgent need for integrated diagnostic strategies that combine targeted surveillance of high-risk populations, capacity building for imaging and laboratory diagnostics, and strengthening of tumor registries to enable timely detection and more accurate epidemiological assessment of hepatic malignancies in Sudan.

1.4 Therapy

Medical management of hepatocellular carcinoma (HCC) in Sudan faces major challenges, largely due to late diagnosis, limited resources, and restricted access to advanced treatment options. Many patients are diagnosed at an advanced stages of the disease, mainly because of the lack of effective screening programs and the high prevalence of risk factors such as hepatitis B and C, which remain endemic in the country. Curative treatments, including surgical resection and liver transplantation, are available only in a small number of centers, where access to experienced surgeons and adequate infrastructure is limited. As a result, palliative medical therapy is often the only realistic option. Sorafenib is used as a systemic treatment, but its high cost makes it inaccessible for most patients.

In some hospitals, transarterial chemoembolization (TACE) is offered to patients with preserved liver function; however, this procedure is not widely available across the country. Symptomatic management and palliative care therefore play a central role, focusing on the control of pain, ascites, and other symptoms related to disease progression. Patient follow-up is frequently irregular due to economic and logistical barriers, which further compromises effective disease management. Although there is growing interest in introducing targeted therapies and immunotherapy, their high cost and the need for close monitoring limit widespread use. Some centers collaborate with international organizations to improve access to medications and to adopt updated treatment protocols

in line with EASL and NCCN guidelines, yet a clear gap remains between recommended standards and real-world practice. Public education on early symptom recognition and primary prevention, particularly through hepatitis B vaccination, is considered essential to reducing the future burden of HCC in Sudan. Ongoing initiatives also aim to strengthen local healthcare capacity through multidisciplinary training, with the goal of improving access to medical therapies and reducing HCC-related mortality.

In a study by Salim and Mohamed (16), the long-term outcomes of radiofrequency ablation (RFA) as a first-line treatment for HCC were evaluated, along with prognostic factors affecting survival. The study included 36 patients treated between 2012 and 2020 at the National Ribat University Hospital in Khartoum, Sudan, who had been referred from different hospitals for further management. Eligible patients had absent or mild chronic liver disease (Child–Pugh class A or B) and were diagnosed with HCC presenting either as a single lesion larger or smaller than 6 cm, or up to three lesions each measuring 3 cm or less.

One notable finding was that 69.4% of patients with viral hepatitis had never received any form of treatment prior to enrollment. More than two-thirds of the study population had liver cirrhosis (71.4%) and elevated alpha-fetoprotein levels (AFP >100 U/L) were observed in 77.8% of cases. Nearly half of the patients were classified as Child–Pugh A (47.6%), while a similar proportion belonged to Child–Pugh B (47.6%); only one patient was classified as Child–Pugh C. With regard to tumor burden, the majority of patients (76.2%) presented with a single lesion, whereas a smaller proportion (23.8%) had two or more lesions.

Patients who underwent treatment had a survival of more than one and a half years, and nearly 20% of the study population survived for more than two years.

2. Cholangiocarcinoma (CCC)

2.1 Epidemiology and risk factors

There is no national cancer registry in Sudan that clearly distinguishes between hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCC). Although CCC appears to be less common than HCC, it is likely underdiagnosed due to limited access to diagnostic tools such as ultrasound, CT scans, and specific tumor markers like CA19-9. In sub-Saharan Africa, CCC is estimated to account for about 10–15% of primary liver malignancies, and a similar distribution is presumed in Sudan. In the absence of a national registry, it is difficult to accurately identify local risk factors, and available information is often inferred from data reported in neighboring countries, assuming comparable epidemiology and exposures. For example, an Egyptian study identified several potential risk factors for hilar cholangiocarcinoma, including male sex (male-to-female ratio of 1.7:1), agricultural work and rural residence, cirrhosis, hepatitis C infection (54%), schistosomiasis (66.5%), chronic typhoid infection (52%), and gallstone disease.

Possible risk factors relevant to the Sudanese context include:

- Infections with *Opisthorchis viverrini* or *Clonorchis sinensis*, which are major risk factors in Asia, have not been reported in Sudan.
- The prevalence of primary sclerosing cholangitis (PSC) in Africa remains unknown.
- Chronic hepatitis B and C infections, which some reviews suggest may play a role, particularly in intrahepatic cholangiocarcinoma.

- A possible contribution of aflatoxin exposure, although this is more strongly associated with HCC.
- Congenital malformations of the biliary tract, which are rarely reported.

2.2 Management and outcomes

Chemotherapy treatment was mainly used for palliative treatment and was reported in three studies (17-18-19). The chemotherapy was also administered as either neoadjuvant or adjuvant therapy . In most cases, the first-line chemotherapy used was gemcitabine-based (17-18-19).

Only two studies reported the use of percutaneous transhepatic SEMS (Percutaneous transhepatic self-expanding metal stents) for palliation of malignant biliary obstruction (20-21). The type of PBD reported in a single study includes percutaneous transhepatic biliary drainage (PTBD), and endoscopic retrograde cholangiopancreatography (ERCP) with stent placement (22).

Six studies reported surgery performed with curative intent (22). However, not all surgery was for curative intent, in some cases palliative bypass surgery was done to relieve patient of jaundice secondary to malignant obstruction .

The best median patient survival time reported was 36 months in patients who underwent surgery with curative intent. This was in patients that underwent major hepatectomy with caudate lobectomy. Patients who underwent palliative bypass surgery had a reported survival rate of 54.5% at one year and the longest survival period of 28 months (21). Palliative chemotherapy was used in patients whose CCC was unresectable. Two studies on palliative chemotherapy with a combined study population of 68 patients had the same median survival period of 9 months (17-18).

3. The Current Situation in Sudan

The conflict in Sudan (24) has severely disrupted medical supply chains, leading to a critical shortage of essential cancer medications, including chemotherapy drugs, hormonal therapies, and targeted therapies. This disruption has drastically affected patient outcomes and the ability of healthcare professionals to provide necessary treatments. The National Medical Supplies Fund in Khartoum, crucial for distributing these supplies across Sudan, has been vandalized and looted, with significant losses estimated at \$500 million US dollars in medical stocks. These events have hampered the delivery and distribution of medical supplies, severely affecting cancer treatment protocols across the country. Many hospitals have been forced to close or has been destroyed by the conflict. The only ones centers still functioning (Fig. 6) are located in the southern and eastern parts of the country, leaving many of Sudan's provinces, some with high population densities (Fig. 2), such as North and South Darfur and Kordofan, thousands of kilometers from the nearest medical center.

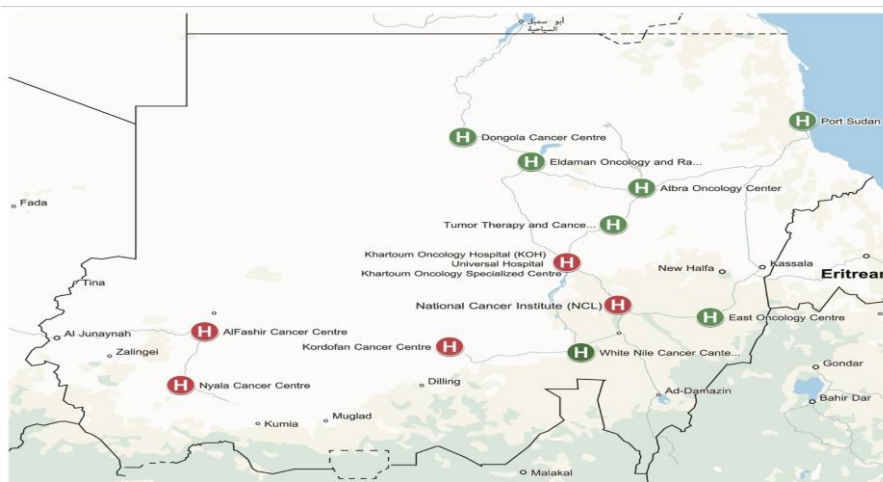


Fig.6 Map of cancer centers in Sudan. Functional: green. Nonfunctional: red. (24) This map depicts the location of cancer centers in Sudan, with color-coding indicating their current operational status. Green markers represent functional centers, while red markers represent centers that are nonfunctional because of the ongoing conflict.

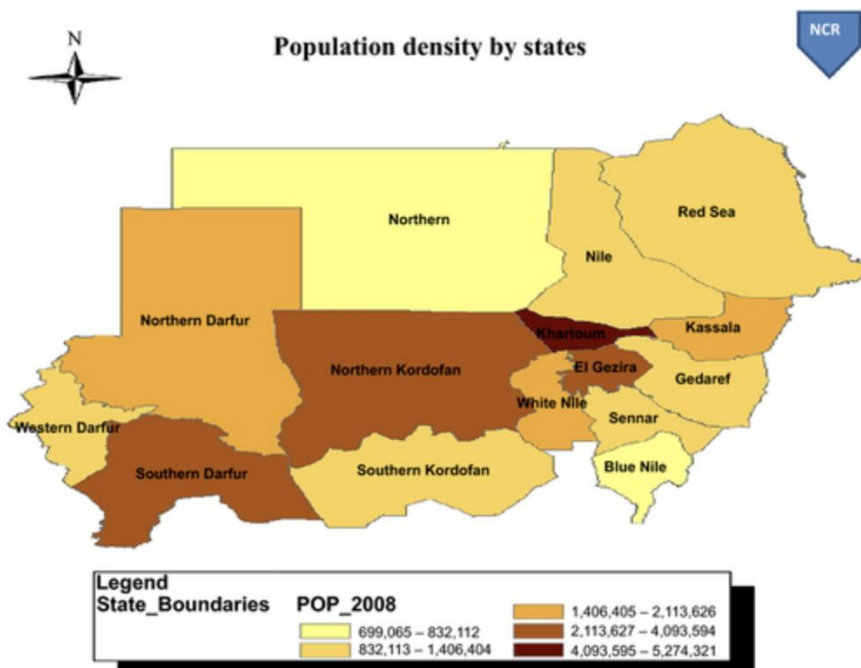


Fig. 2 Population density map of Sudan. The map illustrates a strong concentration of population along the Nile Valley and in major urban centers, while vast areas, particularly in the desert regions, exhibit very low population density.

The conflict has caused a catastrophic collapse of essential health care infrastructure, severely disrupting oncology services (Table 1). Key institutions such as the KOH and the NCI in Wad Medani are now nonfunctional, drastically limiting cancer care capabilities, including chemotherapy, radiotherapy, and surgery.

Oncology Center	Location	Type	Services	Current Status
Khartoum Oncology Hospital (KOH)	Khartoum	Public	Comprehensive oncology services including radiotherapy	Nonfunctional
Universal Hospital	Khartoum	Private	Radiotherapy and chemotherapy	Nonfunctional
Khartoum Oncology Specialized Centre	Khartoum	Private	medical oncology	Nonfunctional
National Cancer Institute (NCI)	Wad Medani Gezira State	Public	Comprehensive oncology services including radiotherapy	Nonfunctional
Eldaman Oncology and Radiotherapy Centre	Merowe, Northern State	Private	Radiotherapy and chemotherapy	Functioning with increased patient load
Tumour Therapy and Cancer Research	Shendi, River Nile State	Public	Limited radiotherapy and medical oncology	Increased patient load because of displacement
East Oncology Centre	El-Gadarif	Public	Chemotherapy and diagnostics	Increased patient load because of displacement
Atbra	Atbra	Private	Chemotherapy and diagnostics	Private consultation and chemotherapy
Port Sudan Oncology Centre	Port Sudan Red Sea State	Public	Cancer treatment unit providing primarily chemotherapy services	Increased patient load because of displacement
White Nile Cancer Centre	Kosti	Public	Cancer treatment unit providing primarily chemotherapy services	Increased patient load because of displacement
Dongola Cancer Centre	Dongola, Northern State	Public	Cancer treatment unit providing primarily chemotherapy services	Significant increase in patients' number because of displacement and patients on their way to Egypt
Nyala Cancer Centre	Nyala, South Darfur State	Public	Cancer treatment unit providing primarily chemotherapy services	Nonfunctional
AlFashir Cancer Centre	AlFashir, North Darfur State	Public	Cancer treatment unit providing primarily chemotherapy services	Nonfunctional
Kordofan Cancer Centre	El Obeid, North Kordofan State	Public	Cancer treatment unit providing primarily chemotherapy services	No chemotherapy available. High-risk area

Table 1. The Current Treatment Facilities in Sudan in the Governmental and Private Sectors (22). According to the latest information provided by Dr. Ibrahim Abbkar, the first two hospitals listed are now functioning again.

The suspension or significant reduction of chemotherapy and radiotherapy services, combined with widespread population displacement, has resulted in a sharp increase in both new and returning cancer patients at the few functioning centers located in relatively safer regions, which are themselves inadequately resourced. Treatment delays, known to be associated with increased cancer-related mortality, have particularly affected pediatric patients, while adult patients have also experienced major interruptions in care. These challenges are further compounded by substantial financial and logistical barriers, including the high costs and risks of travel and prolonged treatment, forcing some patients to discontinue therapy altogether. Overall, the crisis has placed cancer patients in an extremely vulnerable position and highlights the urgent need for sustained structural and financial support to ensure the continuity of oncology services in Sudan.

4. Strategies for Reducing the Incidence of Hepatitis and Hepatocellular Carcinoma: The Role of Early Antigen Detection, Aflatoxin Control, and Health Education

4.1 Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and represents a major public health burden worldwide. Its incidence is strongly associated with chronic infection by hepatitis B virus (HBV) and hepatitis C virus (HCV), as well as exposure to environmental carcinogens such as aflatoxins. Globally, an estimated 820,000 new cases of HCC are diagnosed each year, with the highest burden observed in sub-Saharan Africa and East Asia. The natural history of HCC underscores the critical role of primary prevention (vaccination, antiviral therapy, reduction of toxin exposure) and secondary prevention (early detection of viral hepatitis and premalignant liver disease). Among these, early detection of HBV infection through precise antigen-based diagnostic kits is an essential step in reducing disease progression and ultimately the incidence of HCC.

The proposed solutions emphasize the need for a multi-level approach to address the oncology crisis in Sudan. Key priorities include strengthening public awareness and international advocacy to mobilize political and financial support, drawing on successful interventions in other conflict settings. Medical evacuations are essential for the most vulnerable patients requiring urgent care, but these must be complemented by the reinforcement of local health systems through infrastructure rebuilding, decentralization of services to safer areas, healthcare worker training, and the provision of essential medical supplies. International cooperation and the integration of cancer care into humanitarian responses, in collaboration with organizations such as WHO, play a central role. Finally, the engagement of displaced healthcare professionals and local volunteer initiatives are identified as critical components for developing sustainable solutions and ensuring equitable and continuous access to oncology care in crisis contexts.

4.2 HBV Prevention and the Role of Antigen Detection

The main form of primary prevention is still HBV vaccination, which has dramatically decreased HBV transmission rates in areas where it has been used. However, vaccination rates are still below ideal in many countries with limited resources, and a significant percentage of the populace is chronically infected without being aware of it. The availability of precise, cost-effective, and rapid diagnostic kits to identify hepatitis B surface antigen (HBsAg) is essential for these people.

The earliest and most accurate serological indicator of HBV infection is HBsAg, which can be detected weeks after exposure and remains present in chronic infection cases. Rapid diagnostic tests (RDTs) for HBsAg that are commercially available can be conducted at the point of care and yield results in a matter of minutes. Although they require laboratory infrastructure, more complex chemiluminescence immunoassays (CLIAs) and enzyme-linked immunosorbent assays (ELISAs) offer greater sensitivity and specificity. Case detection can be significantly increased in underserved and rural areas by implementing inexpensive, field-validated HBsAg RDTs.

Hepatitis B e antigen (HBeAg) detection and HBV DNA quantification, in addition to HBsAg, offer vital insights into viral replication and contagiousness. HBV DNA testing measures the viral load and informs treatment choices, whereas HBeAg positivity signifies high viral replication. However, low-

income nations frequently lack access to molecular testing. RDTs for initial screening, followed by confirmatory ELISA/CLIA or HBV DNA testing in referral centers, are the suggested tiered approach.

Who Should Be Tested?

To optimize the efficacy of antigen detection techniques, target populations for HBV screening must be defined. The following groups should be given priority in accordance with recent data and WHO recommendations:

- Pregnant women: to stop mother-to-child transmission (MTCT), which is one of the main ways that HBV spreads in endemic regions. Timely neonatal vaccination and antiviral prophylaxis are made possible by early identification.
- Due to the high risk of horizontal transmission, household and sexual contacts of HBV-infected individuals.
- Healthcare personnel and high-risk occupational groups: to prevent the spread of nosocomial infections and to guarantee protection through immunization or therapy.
- Patients have high-risky behaviors, such as injecting drugs, having several sexual partners, or needing blood transfusions frequently.
- General population in high-prevalence countries – in regions where HBV prevalence exceeds 2%, population-level screening can be cost-effective.
- Patients with liver disease or abnormal liver function tests – as chronic HBV often presents asymptotically until advanced stages.

Thousands of undiagnosed cases can be found by implementing community-based screening campaigns using portable antigen-detection kits. In particular, field research conducted in Africa has shown that using HBsAg RDTs to screen over 1,000 people in rural villages over brief periods of time is feasible. In order to lower the long-term risk of HCC, these programs can be connected to referral systems for confirmatory testing and the start of antiviral therapy. Early detection of HBV infection allows for treatments that dramatically reduce the risk of developing cirrhosis and HCC. Antiviral treatments reduce the risk of HCC by up to 70% by achieving persistent viral suppression, especially with nucleos(t)ide analogues like entecavir or tenofovir. Widespread HBsAg testing, in conjunction with immunisation campaigns for those who are not infected, has the potential to change the epidemiology of HBV-related HCC.

4.3 Aflatoxin Exposure and HCC Risk

In many African and Asian countries, HBV infection coexists with high exposure to aflatoxins—mycotoxins produced by *Aspergillus flavus* and *A. parasiticus* contaminating staple foods such as maize and groundnuts. Chronic aflatoxin ingestion (11) synergistically increases the risk of HCC in HBV-infected individuals, often through the p53 gene mutation at codon 249 (R249S). Strategies to reduce aflatoxin exposure include:

- Improving post-harvest food storage to limit fungal contamination.

- Promoting the use of biological control agents such as Aflasafe, which competitively inhibits aflatoxin-producing fungi.
- Encouraging diet diversification to reduce dependence on contaminated staples.
- Establishing regulatory monitoring systems for aflatoxin levels in food products.

Reducing aflatoxin exposure in developing countries relies largely on controlling contamination in food and minimizing environmental risk factors. Rapid test kits and ELISA-based assays are available for detecting aflatoxins in food, even in low-resource settings, allowing contaminated products to be identified and removed before they reach consumers. Improving farming, harvesting, and storage practices, along with promoting proper food preservation methods, is essential to limit fungal growth. Public education campaigns that emphasize the importance of clean water, safe food handling, and hygiene further support prevention efforts. While it is technically possible to measure aflatoxin exposure in humans through biomarkers in blood or urine, these tests are currently confined to research settings or specialized laboratories and are not yet used as routine screening tools. Public health interventions that combine HBV vaccination and aflatoxin control have demonstrated synergistic reductions in HCC incidence, particularly in China and The Gambia.

4.4 Health Education as a Preventive Tool

Community education plays a critical role in sustaining prevention efforts. Effective health promotion should address:

- Transmission routes of HBV and HCV (blood exposure, unsafe injections, unprotected sex, MTCT).
- Benefits of vaccination and the need for neonatal immunization.
- Importance of early diagnosis through screening programs.
- Safe food storage and preparation practices to reduce aflatoxin contamination.
- Lifestyle modifications, such as reducing alcohol consumption and avoiding unnecessary medications that can injure the liver.

Schools, community centers, and local healthcare facilities provide suitable platforms for health education campaigns. Culturally adapted messages, delivered in local languages, enhance community engagement and acceptance.

4.5 Future Directions: Toward Integrated Screening and Prevention Programs

To reduce the worldwide impact of HCC, a multimodal approach is required to target both viral hepatitis and environmental carcinogens (Fig.7). Accurate, cost-effective HBsAg antigen detection kits can be used to identify infections early, link patients to care, and prevent the development of cancer and cirrhosis when combined with targeted screening of high-risk populations.

Working with international organizations could make it possible to supply basic antiviral medications even in areas where hospital services are not fully operational. In addition, training community health workers to identify suspected cases and support prevention efforts would strengthen local capacity. Setting up small, temporary health points in relatively safe areas, including the use of school buildings, could help ensure continuity of essential care. The use of telemedicine, supported by satellite connections, could also assist healthcare providers in diagnosing and managing more complex cases. It is equally important to discourage risky traditional practices, such as the use of non-sterile injections and scarification, through targeted public health messaging.

It would be helpful to launch awareness campaigns through local radio stations to inform communities about the importance of clean water and safe food storage in order to reduce exposure to aflatoxins, a significant risk factor for liver cancer. Promoting food production in non-contaminated areas and encouraging proper storage methods would help limit the growth of toxic molds. Even during times of conflict, distributing low-cost water filters could reduce infections that negatively affect liver health. Monitoring risk factors through locally collected data would support better planning of targeted interventions. Actively involving women in community-based prevention activities would help spread information more effectively.

Encouraging the adoption of basic hygiene practices can also reduce other infections that worsen liver disease. International aid should include supplies for vaccination programs and liver-related testing. Collaboration with local universities could support small research initiatives to identify high-risk areas, even in the context of ongoing conflict. Providing financial support to farmers to improve crop handling and storage practices would further reduce aflatoxin exposure. Finally, a long-term health system recovery plan is needed, with a strong focus on strengthening liver disease prevention services to reduce the future burden of liver cancer in Sudan.

Together, these strategies can dramatically lower the prevalence of hepatitis-associated HCC, particularly in countries with high burdens.

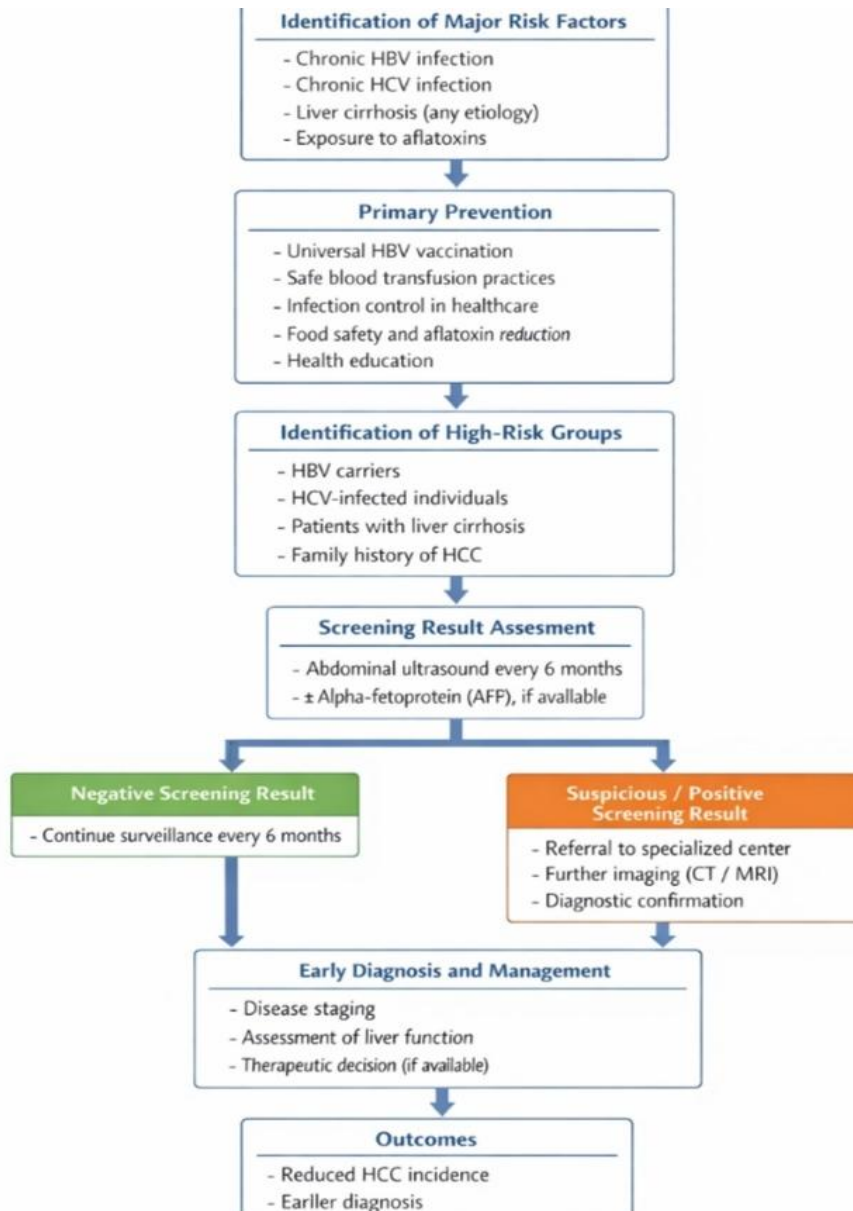


Fig.7 The flowchart summarizing the key factors and interventions aimed at reducing the incidence of hepatocellular carcinoma (HCC) and improving early diagnosis in Sudan. The flowchart integrates preventive strategies, risk factor control, and healthcare system–level actions, emphasizing the role of vaccination programs, public health awareness, screening of high-risk populations, and improved access to diagnostic and surveillance services.

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Part II

Introduction

Cholangiocarcinoma (CCA) originally described by Durand-Fardel in 1840 is a malignant neoplasm of the biliary epithelium characterized by an extremely poor prognosis, both because it is often diagnosed at an advanced stage and because it has a poor response to available treatment options. CCA is the second most frequent primary liver malignancy after hepatocellular carcinoma (1), and it is responsible for 3% of the malignant gastrointestinal neoplasms nowadays (2). The current anatomic classification of CCA (Fig.1) considers intrahepatic carcinoma (iCCA) as the subtype arising between the bile ductules and the second-order bile ducts (i.e. segmental bile ducts), perihilar cholangiocarcinoma (pCCA or Klatskin tumor) as the subtype arising in the right and/or left hepatic duct and/or at their junction and distal cholangiocarcinoma (dCCA) as the subtype involving the common bile duct. Recent consensus statements and guidelines agree that this classification is more accurate than the prior classification of CCA as either iCCA or extrahepatic CCA (eCCA), since this eliminates the difficulties in classifying pCCA as iCCA or eCCA (3).

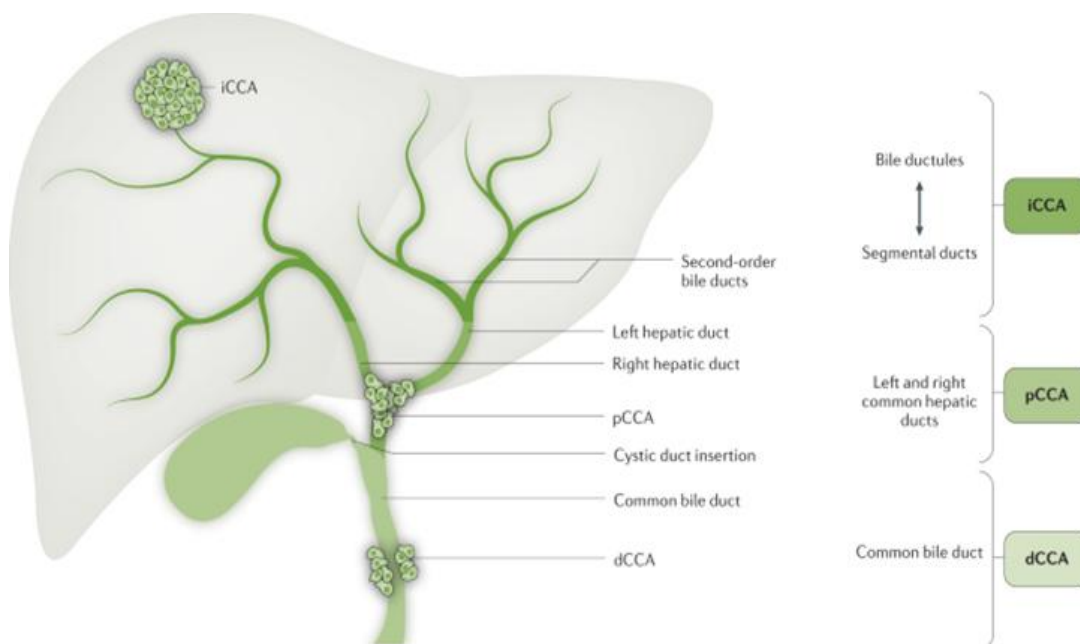


Fig. 1 Classification of Cholangiocarcinoma

Regarding pCCA, the Bismuth-Corlette classification (4) based on the longitudinal extent of the tumor has been developed, particularly identifies four types of pCCA: type I, tumor confined to the common hepatic duct; type II, tumor limited to the confluence of the hepatic ducts, without involvement of the secondary ducts; type III, tumor involving the confluence with extension to the right (III A) or left (III B) hepatic duct; type IV, tumor involving the biliary confluence with involvement of secondary intrahepatic ducts on both sides.

1. Epidemiology

Cholangiocarcinoma is a rather rare neoplasm, with an incidence of 1 to 2 cases per 100,000 inhabitants, although it represents 10-20% of primary malignant liver tumors, with a male to female ratio of 1.5:1 (5). The age of onset is higher than that of HCC; it affects individuals over 65 years old with a peak incidence in the eighth decade. (5) It is estimated that there are between 3,000 and 4,500 new cases of cholangiocarcinoma per year in the USA, of which 30-60% are Klatskin tumors. In an important review conducted by Nakeeb (6) on 294 cholangiocarcinomas, 66% of patients had a hilar tumor, in 27% of cases the tumor was located in the middle or distal portion of the bile duct, and in the remaining 6% of cases the cholangiocarcinoma was intrahepatic (iCCA). This data are also supported by several subsequent studies in which more than 50% of extrahepatic cholangiocarcinomas appear to be located at the bifurcation. (7-8) It also seems that some ethnic groups have a higher predisposition. For example, a higher incidence has been found in Israel, Japan, and among American Indians compared to the average American population. Globally, the area with the highest incidence of CCA is certainly Southeast Asia (Fig.2), particularly Thailand (9-10-11-12), where the age-standardized incidence is 100 per 100,000 male individuals and 50 per 100,000 female individuals. In the western regions of the globe, the incidence of CCA is around 0.5 to 2 per 100,000 individuals. This discrepancy seems to be due to exposure to different risk factors such as endemic liver infections in eastern areas (10). Regardless of the site, the prognosis is poor, with survival rates of about 15% at 2 years from the diagnosis of extrahepatic tumors. In recent years, the frequency of extrahepatic CCA (eCCA) has stabilized, while that of iCCA seems to be increasing, and the mortality of the latter also seems to have increased (Figs. 3 and 4) (8).

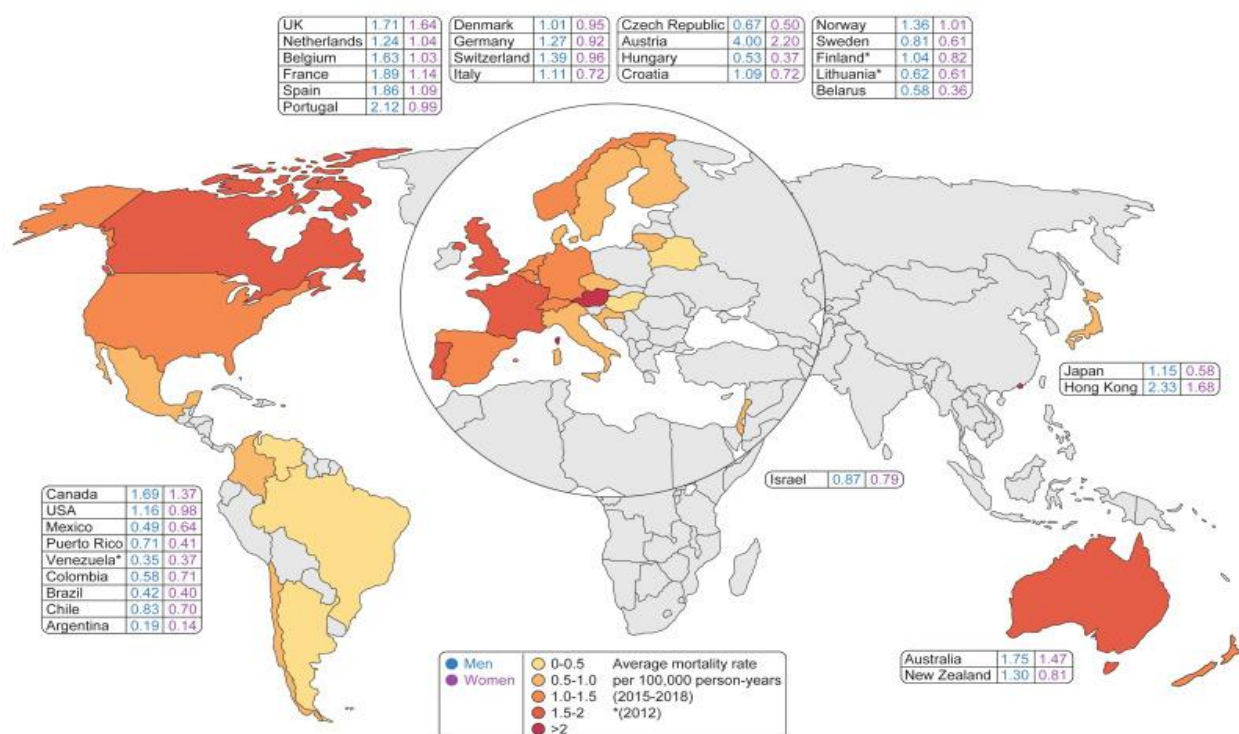


Fig. 2 Mortality associated with iCCA in different geographic areas. European Association for the Study of the Liver Journal of Hepatology 2023

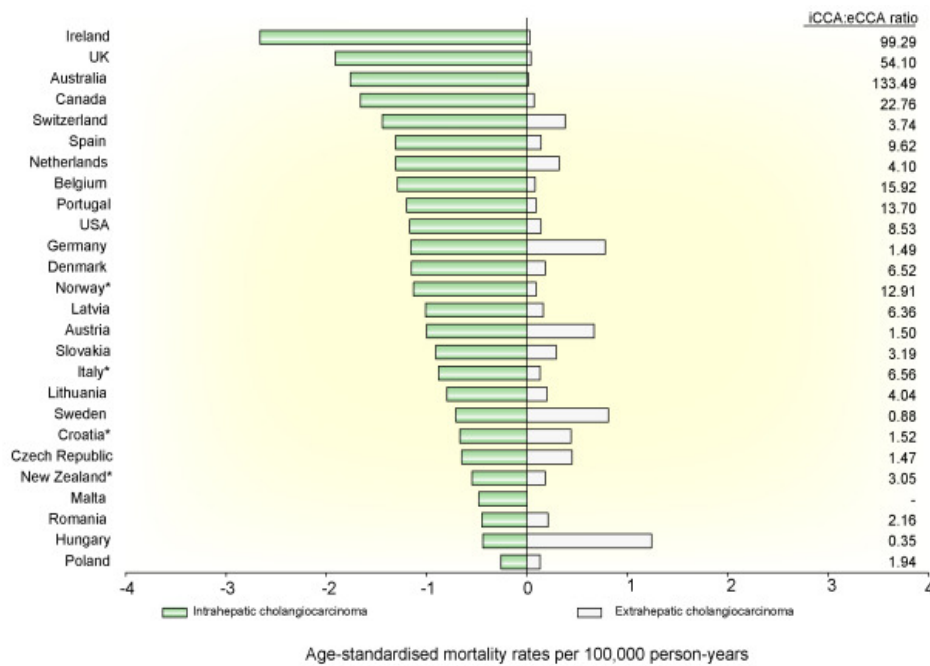


Fig. 3 Age-standardised mortality rates for intrahepatic and extrahepatic cholangiocarcinoma per 100,000 person-years for males from Western countries for 2018.

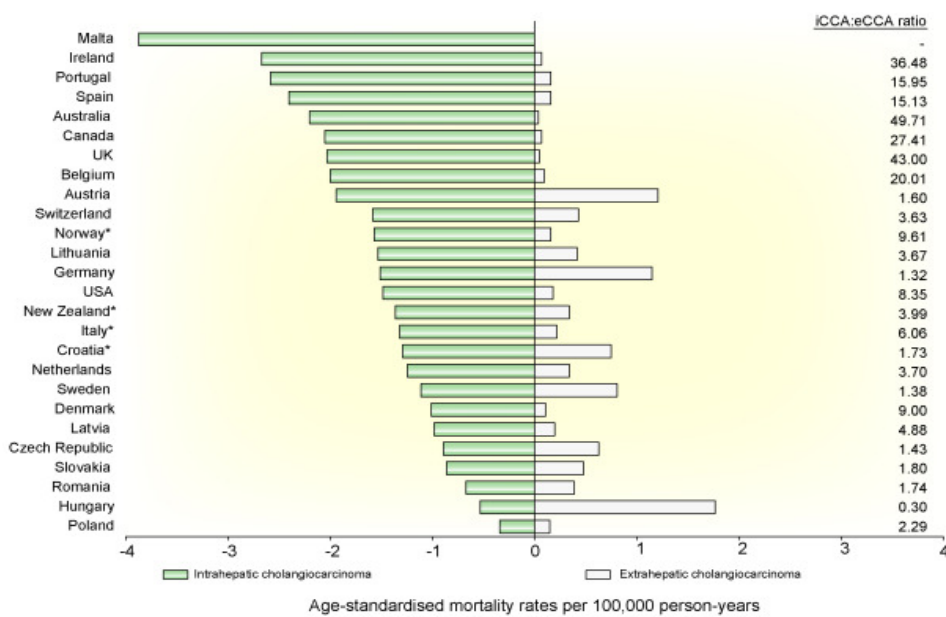


Fig. 4 Age-standardised mortality rates for intrahepatic and extrahepatic cholangiocarcinoma per 100,000 person-years for females from Western countries for 2018.

Previous studies have demonstrated that mortality rates for iCCA are rising. There are several drivers underlying the differing trends in iCCA and eCCA, including misclassification of CCAs, varying risk factors, early diagnosis and surveillance, and access to specialist care. Differences among studies, registries, and classification of iCCA and eCCA may account for some of the temporal variations observed in CCA. For example, Klatskin tumors were not given a unique code in Version 1 of the ICD-O (International Classification of Diseases for Oncology) (1973–1991); therefore, it could have been characterized topographically as iCCA or eCCA. In Version 2 of the ICD-O (1992–2000), it was given a unique histology code that could be linked to iCCA rather than eCCA. In Version 3 of the ICD-O (2001-present), the histological code could be linked to either iCCA or eCCA (13-14). In addition to the misclassification of Klatskin tumors, there are other possible reasons for the misclassification of CCA include the detection of CCAs at an advanced stage which makes it difficult to determine the anatomical origin, and the histological variation of CCAs which can result in their classification as other hepatobiliary malignancies. Given that CCA is a relatively rare liver cancer in most world regions, misclassifications can substantially impact the findings of epidemiological studies.

2. Risk Factors for Cholangiocarcinoma

Most CCA are sporadic, but there are risk factors that show some correlation with the onset of these tumors. Conditions that cause chronic inflammation, primary sclerosing cholangitis, and cholestasis can promote the occurrence of mutations, an example could be the presence of choledochal cysts that seem to be correlated with both iCCA and eCCA (Table 1). Liver cirrhosis is more closely related to iCCA, while choledocholithiasis is more strongly associated with eCCA. Depending on the region of the world we are in, risk factors can vary and be more or less strong; for example, in the East, tumors related to HBV infection and cirrhosis are more common.

People who have chronic (long-standing) inflammation of the bile ducts have an increased risk of developing bile duct cancer. Certain conditions of the liver or bile ducts can cause this. These include:

- **Primary sclerosing cholangitis (PSC)**, an autoimmune disease in which inflammation of the bile ducts (cholangitis) leads to the formation of scar tissue (sclerosis). People with PSC have an increased risk of bile duct cancer. The majority of PSC-associated CCAs develop within the first few years after PSC diagnosis. Chronic inflammation, proliferation of biliary epithelium, production of endogenous bile mutagens, and bile stasis are postulated mechanisms of carcinogenesis. The cause of the inflammation is not usually known. Primary sclerosing cholangitis (PSC) is often associated with inflammatory bowel disease (ulcerative colitis), which is clinically evident in 50-80% of people with PSC (15).
- **Hepatolithiasis**, can also cause inflammation of the biliary epithelium that increases the risk of bile duct cancer. Additionally, infestation with parasites such as *Clonorchis sinensis* and *Ascaris lumbricoides* has been shown in up to 30% of patients with hepatolithiasis (16).
- **Choledochal cyst disease**, also known as congenital biliary dilation, presents as the dilation of extrahepatic bile ducts. The etiology of Choledochal cyst (CC) remains unclear. There are two main theories, “pancreaticobiliary maljunction” and “congenital stenosis of bile ducts”, proposed for the pathogenesis of CC (17). Bile-duct cysts are thought to develop from an abnormal pancreatobiliary junction, a congenital malformation, in which the pancreatic and biliary ducts join outside the duodenum and are typically associated with a long common channel (> 10mm) (18). In patients with pancreatobiliary maljunction, the sphincter of Oddi fails to regulate the function of the pancreatobiliary junction, resulting in a two-way regurgitation and a mixing of pancreatic juice and bile, which activates the pancreatic enzymes in the pancreatic juice, leading to bile duct damages and inflammation, which subsequently result in a dilation of the bile duct as seen in CC. Congenital stenosis of bile ducts is characterized by a reduced number of ganglia and neurons of the common biliary duct (CBD), which causes a dysregulated contraction and increased intraluminal pressure. According to Todani’s classification (19), CC can be anatomically classified into 5 major types (type I–V) (Fig.5). Type I, a dilation of CBD, and type IV, a dilation of both intrahepatic bile duct and extrahepatic CBD, are the two most common types, accounting for about 50–80% and 15–35% of all the Cholangiocarcinoma cases, respectively. Type II, a diverticulum of CBD, and Type III, a dilation of the duodenal portion of the CBD that bulges into duodenal lumen, are rarely seen in infants.

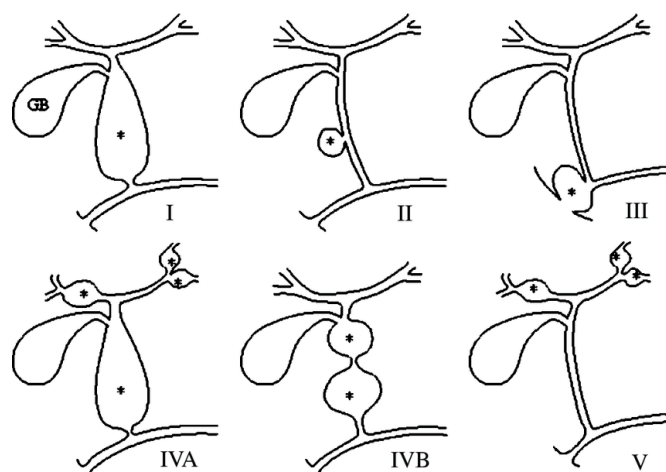


Fig. 5 Todani classification of bile duct cysts

- Liver fluke infections**, which occur in some Asian countries when people eat raw or poorly cooked fish that are infected with these tiny parasite worms. In humans, these flukes live in the bile ducts and can cause bile duct cancer. There are several types of liver flukes. The ones most closely related to bile duct cancer risk are *Clonorchis sinensis* and *Opisthorchis viverrini*. Liver fluke infection is rare in the US, but it can affect people who travel to Asia. They are flat worms that inhabit the bile ducts and, occasionally, the gallbladder and pancreatic duct of mammals. Eggs laid by the adult worms are passed in feces, which may be ingested by snails, where they hatch and then mature into cercariae and subsequently penetrate the flesh of freshwater fish, where they develop into metacercariae. Infection in humans occurs via ingestion of raw, pickled or undercooked fish (20,21). Both parasites increase the susceptibility of cholangiocytes to endogenous and exogenous carcinogens via chronic irritation and increased cellular turnover (20).
- Virus Infections:** Recent studies have reported a few additional risk factors including HIV infection, smoking, diabetes, cirrhosis and hepatitis C (HCV) and B (HBC) infection (19-20). The association between HCV and iCCA has gained special attention since the incidences of both are on the rise. A cohort study of a large group of US veterans suggested that HCV infection conferred a >2-fold rise in the risk of iCCA (21).
- Obesity and Non-alcoholic fatty liver disease** can increase the risk of cancers of the gallbladder and bile ducts. This could be because obesity increases the risk of gallstones and bile duct stones, as well as the risk of non-alcoholic fatty liver disease. But there may be other ways that being overweight can lead to bile duct cancers, such as changes in certain hormones. People with diabetes (type 1 or type 2) have a higher risk of bile duct cancer. This increase in risk is not high, and the overall risk of bile duct cancer in someone with diabetes is still low (22).
- Other rare diseases of the liver and bile duct that may increase the risk of developing bile duct cancer include **polycystic liver disease** and **Caroli syndrome**, a congenital dilatation of the intrahepatic bile ducts .

- **Exposure to Thorotrast:** A radioactive substance called Thorotrast (thorium dioxide) was used as a contrast agent for x-rays until the 1950s. It was found to increase the risk for bile duct cancer, as well as some types of liver cancer, and is no longer used.

2.1 Other possible risk factors

Studies have found other factors that might increase the risk of bile duct cancer, but the links are not less clear (23-24-25). These include:

- Smoking
- Chronic pancreatitis (long-term inflammation of the pancreas)
- Exposure to asbestos
- Exposure to radon or other radioactive chemicals
- Exposure to dioxin, nitrosamines, or polychlorinated biphenyls (PCBs)

Risk factors	iCCA	eCCA
Choledochal cyst	++++	++++
Caroli syndrome	++++	++++
Cholangitis	++++	++++
Cirrhosis	+++ / +++++	++ / +++++
Hepatolithiasis	+++ / +++++	No
Choledocal lithiasis	++ / +++++	++++
Hepatitis B (HBV)	++ / +++++	+
Hepatitis C (HCV)	++ / +++++	++ / +++
Chronic Pancreatitis	++	+++
Obesity	+	+
Alcohol	++	No
Smoke	+	+

Table 1 Association between risk factors and iCCA and eCCA.

+, weak or modest correlation; ++, modest correlation; +++, strong correlation; +++++, very strong correlation.

3. Molecular pathogenesis

The molecular pathogenesis of iCCA is a complex issue involving several signal transduction pathways and molecular events (Fig. 6) (26). iCCA likely results from malignant transformation of cholangiocytes, and in a subset of cases from progenitor cells, although this paradigm has been challenged. Recent data indicate common genomic traits between iCCA and HCC (Fig.7), supporting the hypothesis of common cell of origin in specific molecular subclasses: (1) Transcriptome analysis suggests that the poor prognostic subclass of iCCA shares genomic traits and signatures of poor prognosis of HCC (26-27), which are associated with stem-like molecular signatures ; (2) iCCA and HCC share common copy number variations including gains (1q, 8q, and 17q) and losses (4q, 8p, 13q, and 17p) of arms and high-level amplifications of 11q 13 (26-27); and (3) iCCA shares dominant risk factors associated with HCC development, including cirrhosis, HBV and HCV infections, and metabolic syndrome due to diabetes and/or obesity .

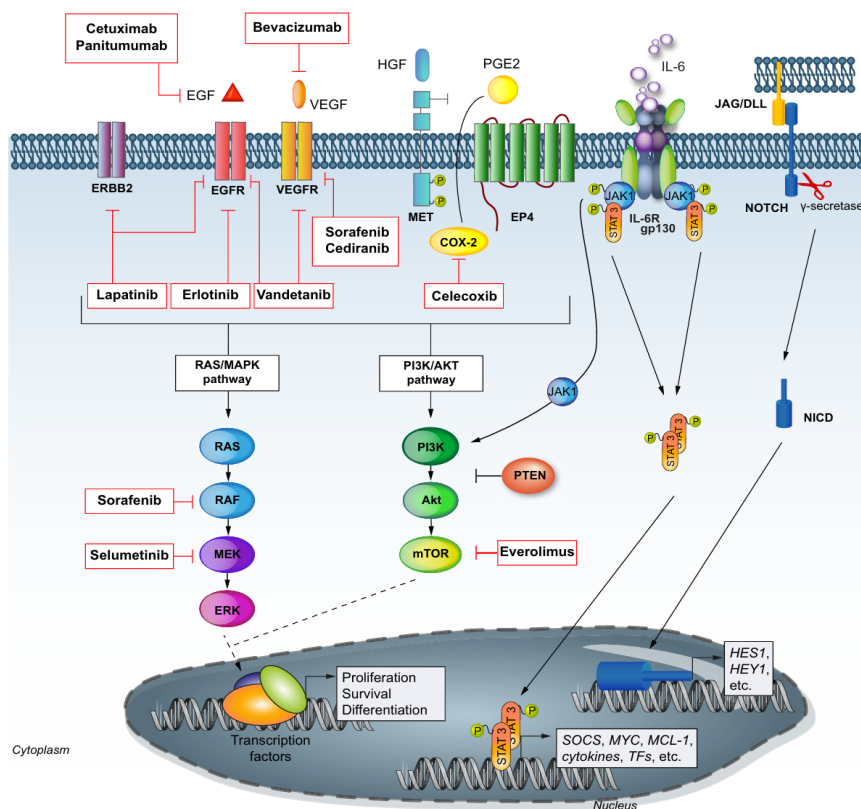


Fig.6 Signaling pathways and molecular therapies in intrahepatic cholangiocarcinoma. (Bridgewater J. Journal of Hepatology 2014)

The pathway of CCA development is linked to the NOTCH gene, which physiologically regulates embryonic development and proliferation of the bile ducts. Notch signalling mechanism is regarded as an important regulator for differentiation of cholangiocytes. Additionally, various studies highlight that the Notch signalling is essential for the maintenance of homeostasis of liver tissue in post-natal life, due to its involvement in the regulation of hepatic glucose and lipid content production. During chronic liver disease, liver cells have been observed to be capable of converting into biliary progenitor cells. In the case of mutations or epigenetic alterations, there may be a deviation from its normal

function, resulting in the activation of the malignant pathological process (28). In the pathogenic process of CCA, various factors can play an important role: Inflammation is one of these; it promotes an increase in cytokines, bile acids, and growth factors, which act by altering apoptotic processes, senescence, and cell cycle regulation, promoting proliferation. In particular, inflammatory cytokines induce an increase in nitric oxide synthase enzyme activity, resulting in an increase in nitric oxide, an important mediator of single and double-strand DNA damage; furthermore, it alters the DNA repair function of certain enzymes. Interleukin 6 (IL-6) produced by both activated inflammatory cells and tumor cells, plays an important role in carcinogenesis both directly and indirectly by stimulating the production of anti-apoptotic mediators such as Mcl-1 (myeloid cell leukemia-1).

3.1 Genetic alterations in iCCA

A discrete number of mutations, chromosomal aberrations, deregulated signaling pathways, and epigenetic changes have been reported in iCCA (Table 2).

Activating mutations of KRAS represent one of the most frequent genetic mutations found in iCCA (5–54%) (26). The KRAS gene has been shown to be a bona fide oncogene inducing iCCA in genetically engineered mouse models, and these mutations have been associated with a more aggressive phenotype. BRAF, NRAS, PI3K, EGFR, and MET mutations are rare events involving <5% of cases (26). Genes encoding isocitrate dehydrogenase enzymes (IDH1, IDH2), are among the most common genetic lesions in iCCA (25%) and they lead to an accumulation of 2-hydroxyglutarate (2-HG), an oncometabolite inhibiting members of the family of α -ketoglutarate-dependent dioxygenase enzymes, resulting in epigenetic and genetic dysregulation and oncogenesis (29). Interestingly, FGFR2 rearrangements and IDH1 mutations seem to be mutually exclusive (30).

The fibroblast growth factor (FGF) pathway is involved in several biological processes including angiogenesis, cell proliferation, migration, differentiation and wound healing. A deregulation of FGF signaling has been described in a number of epithelial cancers including CCA. In particular, a variety of FGFR2 rearrangements with gene fusion have been found in about 10% to 16% of iCCA, but much rarer in pCCA/dCCA (31,32).

Both amplifications and mutations in the epidermal growth factor receptor (EGFR) gene have been found in CCA. EGFR overexpression occurs in 16% of CCAs, with a slight prevalence in iCCA (11–27%) compared with pCCA/dCCA (5–19%) (33–34). On the other hand, EGFR mutations have been reported in the 10–15% of CCA, but without a clear predominance between iCCA and pCCA/ dCCA (35–36). Human epidermal growth factor 2 (HER 2) amplification has been reported in up to 11–20% of pCCA/ dCCA while it is rare in iCCA.

Alterations of the TP53 gene affecting DNA repair mechanisms, which are commonly present in gallbladder cancer (59%), are also frequent in CCA, being more prevalent in pCCA/dCCA (40%) than in iCCA (27%) (37). The RAS/RAF/MEK and the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway is defective in a large number of epithelial tumors including CCAs.

Another factor related to the development of CCA is the microenvironment, particularly carcinogenesis leads to an alteration of the stroma, recruitment of fibroblasts, modification of the extracellular matrix, alteration of the migration pattern of immune cells, and an increase in

neoangiogenesis. In CCA, there is a dense and reactive desmoplastic stroma associated with the presence of cancer-associated fibroblasts (38).

	iCCA	pCCA/dCCA	
Genetic Variants	Prevalence	Prevalence	Gene significance
IDH1/IDH2 mutations	25%		Metabolic enzymes involved in oxidative stress, glutathione metabolism, and citric acid cycle; IDH defects induce accumulation of (D)-2-hydroxyglutarat
FGFR2 aberrations/ rearrangement	10–16%		Tyrosine kinase receptor for FGF is involved in cell proliferation, differentiation, and angiogenesis
EGFR overexpression	16%		Tyrosine kinase receptor for EGF regulating cell proliferation, cell-cell interactions, and cell morphogenesis
TP53 mutatio	27%	40%	Tumor suppressor gene involved in cell cycle arrest, cell apoptosis, cell senescence, and DNA repai
KRAS mutations	22%	42%	Proto-oncogene encoding for a protein belonging to the small GTPase family
CDKN2A mutation	47%		Tumor suppressor gene inhibiting cell cycle and proliferation
MET amplification	2%		Proto-oncogene member of the tyrosine kinase receptor family
HER2 amplification		11–20%	Tyrosine kinase receptor belonging to the EGF receptor family, regulating cell morphogenesis, development, and proliferation
PRKACA/PRKACB rearrangement		rare	Member of the protein kinase C family is involved in several cellular processes such as cell cycle, adhesion, transformation, angiogenesis and agin

Table 2 Genetic mutations in different types of cholangiocarcinoma

4. Macroscopic type of intrahepatic cholangiocarcinoma

The classification proposed by the Liver Cancer Study Group of Japan defines three categories according to macroscopic appearance: the mass-forming type, with a nodular shape; the periductal infiltrating type, characterized by arboriform growth along the branches of the ductal walls; and the intraductal growth type (Fig.7). However, the intraductal growing type has been reclassified as intraductal papillary neoplasm in the 4th World Health Organization (WHO) classification. The mass-forming type, when not associated with intraductal infiltration and lymphovascular invasion, has a better prognosis than the other forms; on the other hand, there is also a pathological classification, proposed by Yamamoto in 1998, recently updated by the Liver Cancer Group of Japan (2003), which associates the morphostructural characteristics of the different histotypes of iCCA with the different prognosis after R0 surgical resection (Fig. 7*) (39).

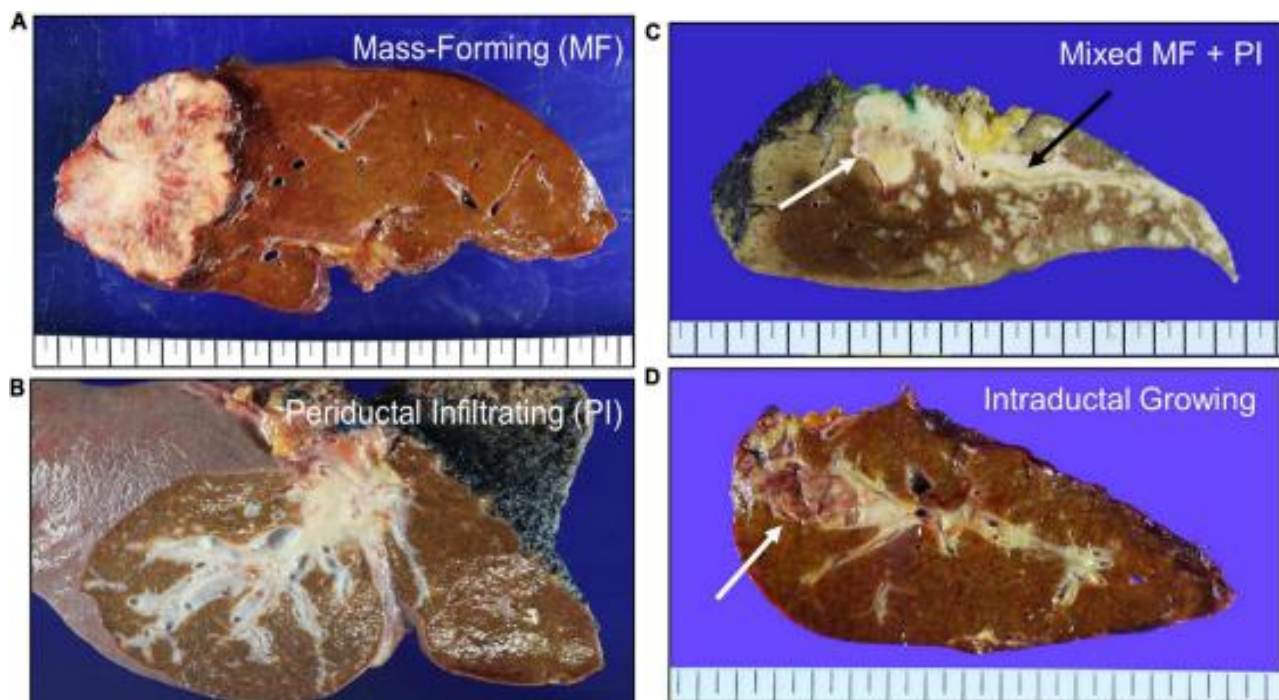
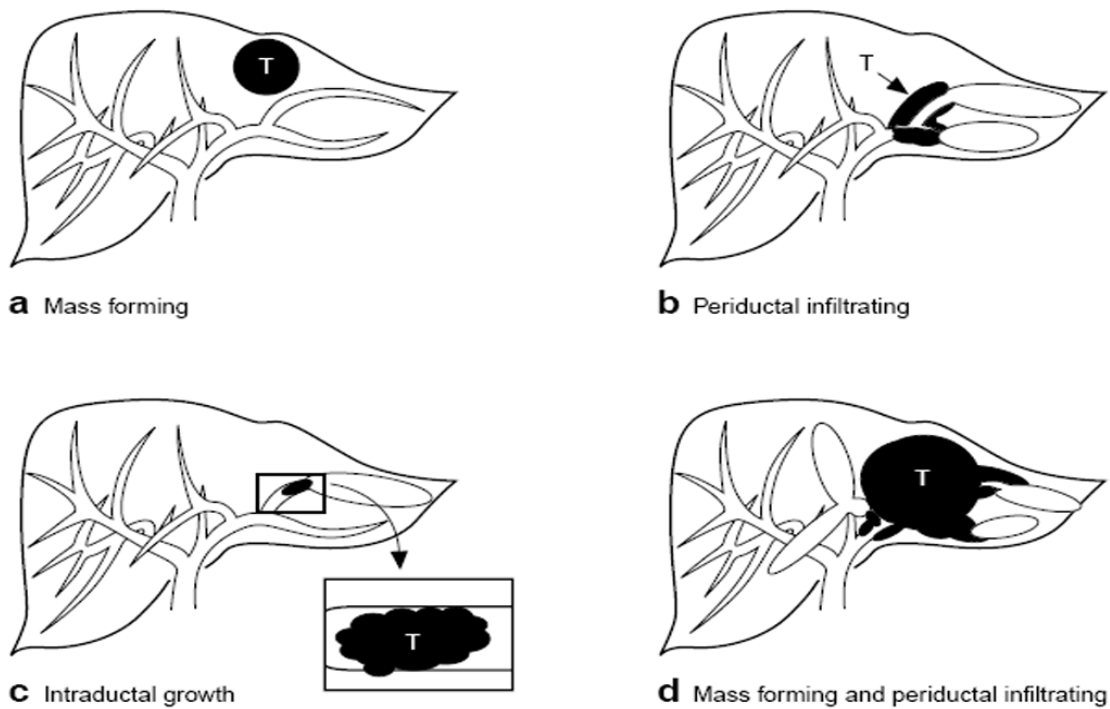


Fig. 7. Macroscopic appearance of various types of intrahepatic cholangiocarcinoma. A,B,C,D : the mass-forming type; the periductal infiltrating type, characterized by arboriform growth along the branches of the ductal walls; the mixed type (MF+PI) and the intraductal growth type.



- a) Mass forming with a 5-year survival: 39%
- b) Periductal infiltrating type with a 5-year survival: 17%
- c) Intraductal growth type with a 5-year survival: 80%
- d) Mass forming and periductal infiltrating types with a 5-year survival : 12%

Fig. 7*. Intrahepatic cholangiocarcinoma: Prognostic classification/stratification (Liver Cancer Study Group of Japan -2003-)

5. Histological classification

In 90% of cases, the intrahepatic neoplasm consists of an adenocarcinoma with glandular or tubular structures lined by non-bile-producing cuboidal epithelium, surrounded by markedly desmoplastic stroma; the remaining 10% is distributed among minor histological variants such as squamous, adenosquamous, mucinous, papillary, small cell, clear cell, signet-ring, or undifferentiated. The microscopic organization follows nodular, papillary, or sclerosing patterns. Characteristically, there is a tendency for lymphatic, perineural, and vascular invasion, with frequent findings of intrahepatic satellitosis.

Recently, a histopathologic classification of small duct type and large duct type iCCA has been introduced (Fig.8).

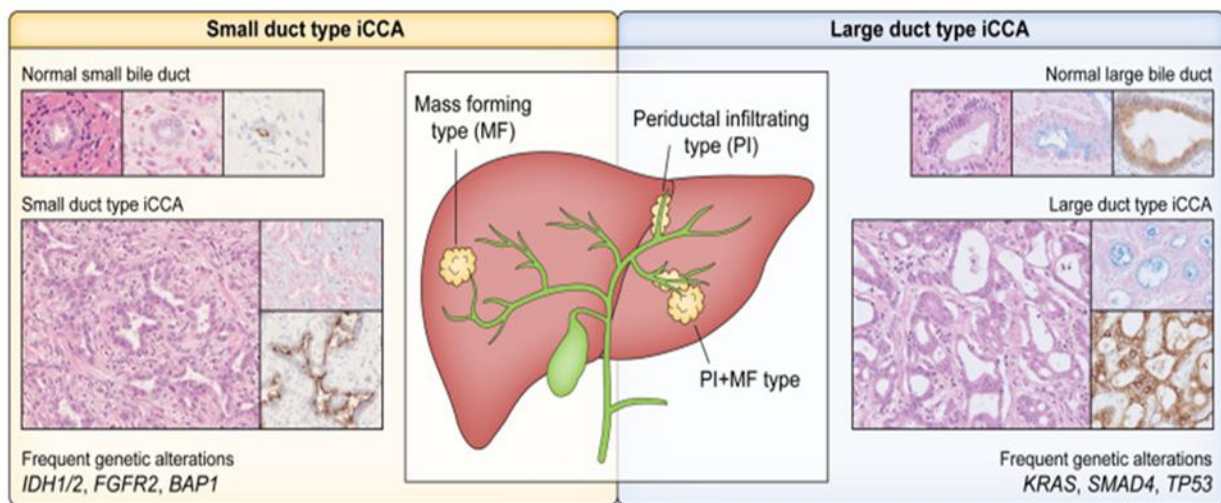


Fig.8 Histological classification of iCCA, according to the 5th WHO classification (European Association for the Study of the Liver Journal of Hepatology 2023).

Both these types of tumors exhibit differences in clinicopathological features, mutational profiles, and prognosis. Small duct type iCCA is composed of non-mucin-producing cuboidal cells, whereas large duct type iCCA is composed of mucin-producing columnar cells, reflecting different cells of origin. Large duct type iCCA shows more invasive growth and poorer prognosis than small duct type iCCA. The background liver of small duct type iCCA often shows chronic liver disease related to hepatitis B or C viral infection, or alcoholic or non-alcoholic fatty liver disease/steatohepatitis, in contrast to large duct type iCCA that is often related to hepatolithiasis and liver fluke infection. [Aishima 2014]. Another classification divides intrahepatic cholangiocarcinoma into a ductal type (conventional type), a bile ductular type and a intraductal type [Nakanuma 2015]. Intraductal papillary neoplasm of the bile duct (IPNB) is defined as a grossly visible premalignant neoplasm showing intraductal papillary or villous growth of biliary-type epithelium (39). It is considered the counterpart of a similar tumor arising in the pancreas, the so-called intraductal papillary mucinous neoplasm (IPMN). IPNB is divided into low-grade and high-grade based on the highest degree of cytoarchitectural atypia. When invasive carcinoma develops in this lesion, it is diagnosed as IPNB with associated invasive carcinoma. Biliary intraepithelial neoplasia (BilIN), a precursor lesion of CCA, occurs at the epithelium of intra- and extrahepatic bile ducts and in the peribiliary glands. Large

duct type iCCA, but not small duct type, is often accompanied by BilIN . BilIN is virtually invisible upon gross examination, although it may be associated with subtle changes such as mucosal thickening. Microscopically, BilIN consists of flat or micropapillary (less than 3 mm in height) epithelial lesions that are graded as low-grade or high-grade (carcinoma *in situ*) based on the highest degree of cytoarchitectural atypia (39-40). This two-tiered classification replaces the former three-tiered classification, wherein the former BilIN-1 and BilIN-2 are now classified as low-grade, and the former BilIN-3 is now classified as high-grade.

On the immunohistochemical level, positivity for CK7 and CK19 and negativity for HepParl and AFP differentiate cholangiocarcinoma from hepatocellular carcinoma [Durnez 2006], although mixed phenotype forms can rarely be observed due to the derivation of the two cell lineages from a common progenitor.

The two types of intrahepatic cholangiocarcinoma, in addition to having differences in terms of etiological factors and prognosis, also present differences in terms of therapy, in fact the small duct type is known to harbour isocitrate dehydrogenase (IDH)-1 and -2 mutations and fibroblast growth factor receptor (FGFR) fusions which are treatable with currently available targeted therapies. Moreover, the efficacy of these treatments is promising. On the other hand, the large duct type often presents with KRAS and SMAD4 mutations, also observed in the pCCA and dCCA subtypes. These data indicate the utility of subtyping iCCA in terms of clinical outcome and treatment choice. Since there is a clear correlation between pathological iCCA subtypes and genetic alterations (41-42-43) iCCA subtyping is useful to predict genetic alterations in IDH1/2 and/or FGFR2 (Fig.9). Therefore, iCCA subtypes should be determined before proceeding with genetic investigations. Certainly, given the typical heterogeneity of iCCA, the accuracy of subclassification is higher in surgical than biopsy specimens. Therefore, the subclassification of iCCA into large duct type and small duct type should be considered on the pathology report, given its potential clinical utility, and this subclassification may be used to guide future trial design.

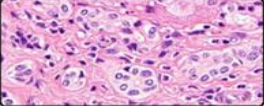
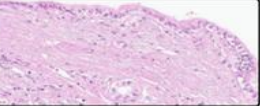
Intrahepatic Cholangiocarcinoma			
Classification	Small Duct Type		Large Duct Type
Gross Type	Mass-forming	Mixed	Periductal Infiltrating
Cell of Origin			
	Canal of Hering Bile ductule		Columnar cholangiocytes Peribiliary glands
Main Etiology	Chronic hepatitis HBV / HCV Alcoholic / Metabolic		Hepatolithiasis Liver fluke PSC
Immuno- histochemistry & Mucin stain	NCAM N-cadherin CRP		S100P Mucin
Frequent Mutations	BAP1 IDH1/2 FGFR2 fusion		KRAS TP53 SMAD4
Suggested Molecular Classification*	Inflammation Class		Proliferation Class
Patient Outcome	Favorable		Poor

Fig.9 The pathological, clinical, and molecular characteristics of iCCA (Sia D, *Gastroenterology*. 2013).

5.1 Correlation between the macroscopic and histological aspects

The distinction between the MF and MF+PI subtype is important as it directs the decision on the type of surgery, as well as reflecting post-surgical outcomes (44-45-46). In brief, the MF+PI subtype is associated with a worse prognosis than the MF subtype because of more frequent lymphatic invasion and perineural invasion in the portal tract (45-46). However, being able to distinguish between them is not straightforward as the PI component is not always clearly detectable on imaging. Importantly, macroscopic classification has a clear correlation with iCCA subtype; the iCCA large duct type clearly demonstrates the PI and MF+PI subtypes, whereas the small duct type exclusively presents with the MF subtype. Thus, combining macroscopic classification with the pathological iCCA subtype is useful to differentiate MF from MF+PI, as MF+PI presents exclusively in large duct type iCCA. In other words, if large duct iCCA is seen in a MF subtype biopsy, it is almost certainly MF+PI.

6. Clinical and laboratory manifestations

The diagnosis of CCA is most often late, particularly in iCCA, because clinical manifestations appear at an advanced stage of disease, which contributes to a poor prognosis. Clinical presentations differ between eCCA and iCCA, in the first case cholestasis signs dominate the symptomatic picture, consequently there will be jaundice, acholic stools, dark urine, and possible itching; other less specific accompanying signs are: general malaise, weight loss, and asthenia. iCCA, on the other hand, is most often pauci-symptomatic, it can present as abdominal mass associated with nonspecific symptoms.

6.1 Laboratory analysis

In the diagnostic process of biliary tract stenosing lesions, it is also important to evaluate tumor markers, CA 19-9 is a glycoprotein present on the cell surface, it is formed by a tetrasaccharide with a transmembrane part and a glycosylated extracellular part. This marker is produced by pancreatic cells, bile ducts, but also by cells of the stomach, colon, uterus, and salivary glands. CA 19-9 is elevated in 85% of patients, however, having low specificity, it can also rise in other situations such as pancreatic tumors, chronic pancreatitis, and cirrhosis. A CA 19-9 value > 100 U/mL in a biliary tract stenosis is highly suspicious for pCCA. It has also been found that a cut-off of 100 U/mL in a biliary tract stenosis has a sensitivity of 53% and a negative predictive value of 92% in predicting a CCA. Further studies have evaluated the ability of CA19-9 to distinguish between CCA and hepatocarcinoma; however, this marker seems to have moderate accuracy (63%-67%) (*Tao LY Am Surg 2010*).

Coelho et al (*GE Port J Gastroenterol 2017*) reported that patients with CA 19-9 levels ≥ 103 U/L were associated with a higher probability of metastasis ($p = 0.001$) and lower rates of treatment with curative intent ($p = 0.024$). In a multivariate analysis, CA 19-9 levels < 103 U/L and surgery were independent predictors of survival.

Another marker that has been considered in CCA is CEA, but it also has low specificity, therefore its elevation is not pathognomonic of the disease. In eCCA, there may be an increase in conjugated bilirubin, gamma-GT, and alkaline phosphatase levels. Furthermore, we could evaluate IgG4 levels to exclude possible IgG4-positive cholangitis.

7. Pathologic diagnosis

A pathological diagnosis of iCCA obtained through biopsy is often necessary and is required in most patients, particularly those with cirrhosis and small liver lesions, and in all inoperable patients who are candidates for chemotherapy or radiotherapy. In most cases, it is an adenocarcinoma showing tubular and/or papillary structures, surrounded by fibrous stroma (44-45). The biopsy allows differentiation of iCCA from benign biliary lesions such as peribiliary glands, reactive ductular proliferation, biliary micro hamartomas (von Meyenburg complexes), and biliary duct adenomas (peribiliary tract hamartomas), particularly in the presence of inflammation that can cause reactive cellular atypia (26). The histological appearance of iCCA is similar to that of metastatic adenocarcinoma derived from extrahepatic primary tumors and particularly from those of gastrointestinal origin such as lung, pancreas, esophagus, and stomach (26). The differentiation between iCCA and mixed HCC tumors may require the use of immunohistochemistry with the

detection of certain markers such as Hep-Par-1, GPC3, HSP70, glutamine synthetase, EpCAM, and CK19. Positivity for CK19 is not specific for iCCA. The expression of cytokeratin 7 and cytokeratin 20 can be useful for establishing a biliary origin (46).

8. Diagnosis using imaging studies

Usually, the first suspicion of iCCA is raised on ultrasound, where iCCA appears as a solid mass with aspecific variable echogenicity (mixed, hypo or hyperechogenic) with possible dilatation of bile ducts peripheral to the mass.

On CT scanning, the typical appearance is of a hypodense hepatic mass in the unenhanced phase with irregular margins, peripheral rim enhancement in the arterial phase, and progressive hyperattenuation on venous and delayed phases. CT can also detect the level of biliary obstruction, capsular retraction or hepatic atrophy. Dynamic CT scanning can help distinguish between iCCA and HCC. Up to 81% of iCCA are characterized by a progressive contrast uptake from the arterial to the venous and especially in the delayed phase. This effect may reflect fibrosis which is slow to enhance but retains the intravenous contrast agent. In contrast, HCC is characterized by rapid enhancement during the arterial phase and relative washout (hypoattenuation) in the venous or delayed phases. However, some small mass-forming iCCA are arterially enhancing and may mimic hepatocellular carcinoma (47).

On MRI, iCCA appear hypointense on T1-weighted and hyperintense on T2-weighted images (48); T2-weighted images may also show central hypointensity corresponding to areas of fibrosis. Dynamic images show peripheral enhancement in the arterial phase followed by progressive and concentric filling-in of the tumor with contrast material. Pooling of contrast on delayed images is indicative of fibrosis and suggestive of an iCCA in the right clinical setting. MRI with cholangiopancreatography (MRI/MRCP) can be helpful to visualize the ductal system and vascular structures and thereby to determine the anatomic extent of tumor.

Fluorodeoxyglucose positron emission tomography (FDG-PET scan) can detect cholangiocarcinomas. Mass forming iCCA as small as 1 cm can be detected with a reported sensitivity of 85–95%. However FDG-PET is less useful for infiltrating tumors (49). There is limited clinical utility of CT/PET for diagnosis of iCCA in the liver when CT or MRI imaging has been performed. A presumed diagnosis of iCCA can be made on radiological criteria such as venous phase contrast enhancement on dynamic imaging in the absence of other extrahepatic primary malignancies and cirrhosis. Radiological criteria are insensitive for the diagnosis of iCCA in the presence of cirrhosis. Radiological studies cannot reliably differentiate between cirrhou HCC and iCCA, or metastatic adenocarcinoma and iCCA.

The usefulness of CT/positron emission tomography (PET) is of relevance for lymph node metastasis. Most papers look at the role of high-quality MRI or describe the role of CT scanning but very few studies include a head-to-head comparison. The only recent paper using current imaging modalities which addresses this issue was published by Kim et al. in 2021; (50) this was a retrospective multicentre study in Korea. Based on these data, MRI appears to be superior to CT scanning in staging iCCA within the liver. CT imaging is superior for the detection of vascular involvement and thus for the assessment of resectability.

9. Preoperative evaluation

Actually the rate of perioperative mortality after major hepatectomy (≥ 3 segments) is less than 5% (51). One of the main goals of major liver surgery is represented by avoiding post-hepatectomy liver failure (PHLF), which still remains the main cause of mortality after major liver resection. PHLF is unequivocally related to quality of liver and to quantity of future remnant liver (51). In recent era, the development of even more precise tools (morphologic and functional) allow to calculate the correct value of future liver remnant (FLR), the rates of PHLF is quite low (51-52). Generally, in relation with the quality of liver parenchyma, we consider safe a FRL of $\geq 30\%$ of volume in case of normal liver, $\geq 30-40\%$ for severe steatosis and cholestasis, and $\geq 50\%$ for cirrhotic patients. Another important correlation has been determined among FRL and Body weight, called Remnant Liver Volume to Body Weight Ratio (RLV-BWR) determining a ratio that could indicate if the future remnant liver is enough. Actually, in case of normal liver, the minimal value of RLV-BWR of liver is 0.5%, in case of steatosis and cholestasis is 0.75%, and in case of cirrhosis can be considered 1% (52). Hayashi et al (53) report in their study that a FLR/BW $\geq 0.65\%$ may serve as a volumetric basis to reduce posthepatectomy liver failure after extended hepatobiliary resection.

Major hepatic resections increase the possibility of radical treatments and thus curative interventions, but they increase the risk of complications, mainly represented by liver failure. To reduce this risk, two strategies have been adopted in particular: decompressive biliary drainage and preoperative portal embolization (PVE). After PVE, two parameters are essentially measured: liver volumetry and liver function. Liver volumetry is calculated by a contrast-enhanced CT scan performed 4-6 weeks after PVE. Liver function is measured through the Indocyanine green clearance test (ICG test). The test is performed 1-3 days before PVE and then 11-13 days after PVE. Indocyanine green is administered intravenously at a dose of 0.5mg/Kg and a blood sample is taken at 5, 10, and 15 minutes after injection and then analyzed with a spectrophotometer at 805 nm. The disappearance of Indocyanine green from the plasma (Indocyanine green clearance: KICG or PDR: Plasma Disappearance Rate) follows a linear function. The most important parameter is the retention percentage of Indocyanine green after 15 minutes (retention rate at 15 minutes: R15. Normal value: R15 < 10%). The measurement of KICG of the future residual liver is obtained from the following formula: $KICG \times (\% \text{ of residual liver volume}) / 100$. It is suggested that the serum total bilirubin level and ICG 15 value are sufficient for deciding on the extent of hepatic resection, and that many other examinations are not always required to determine the resection volume of the liver (Table 3).

Another tool available for the assessment of liver function is hepatobiliary scintigraphy with ^{99m}Tc -mebrofenin (HBS). Mebrofenin is an iminodiacetic acid (IDA) agent that, once absorbed by hepatocytes, transits through the bile canaliculi without any biotransformation. The first 6 minutes of the test allow the calculation of the extraction rate of ^{99m}Tc -mebrofenin (expressed in $\%/ \text{min}/\text{m}^2$) for the entire liver, which is correlated to the underlying parenchymal state (55) and ICG clearance (sharing the same OATPB1/B3 transporters) (56). Then, a short acquisition of less than ten minutes is performed with SPECT (single-photon emission computed tomography) to evaluate the distribution of the radiotracer at the regional and segmental levels, calculating the extraction rate of ^{99m}Tc -mebrofenin in the volume of interest, particularly in the FRL (57). In patients with hyperbilirubinemia, false results can occur because mebrofenin competes with bilirubin. de Graaf et al. showed that a cut off of $2.7\%/ \text{min}/\text{m}^2$ is of high negative predictive value (98%) to exclude the risk of PHLF in patients planned for large hepatectomy (55).

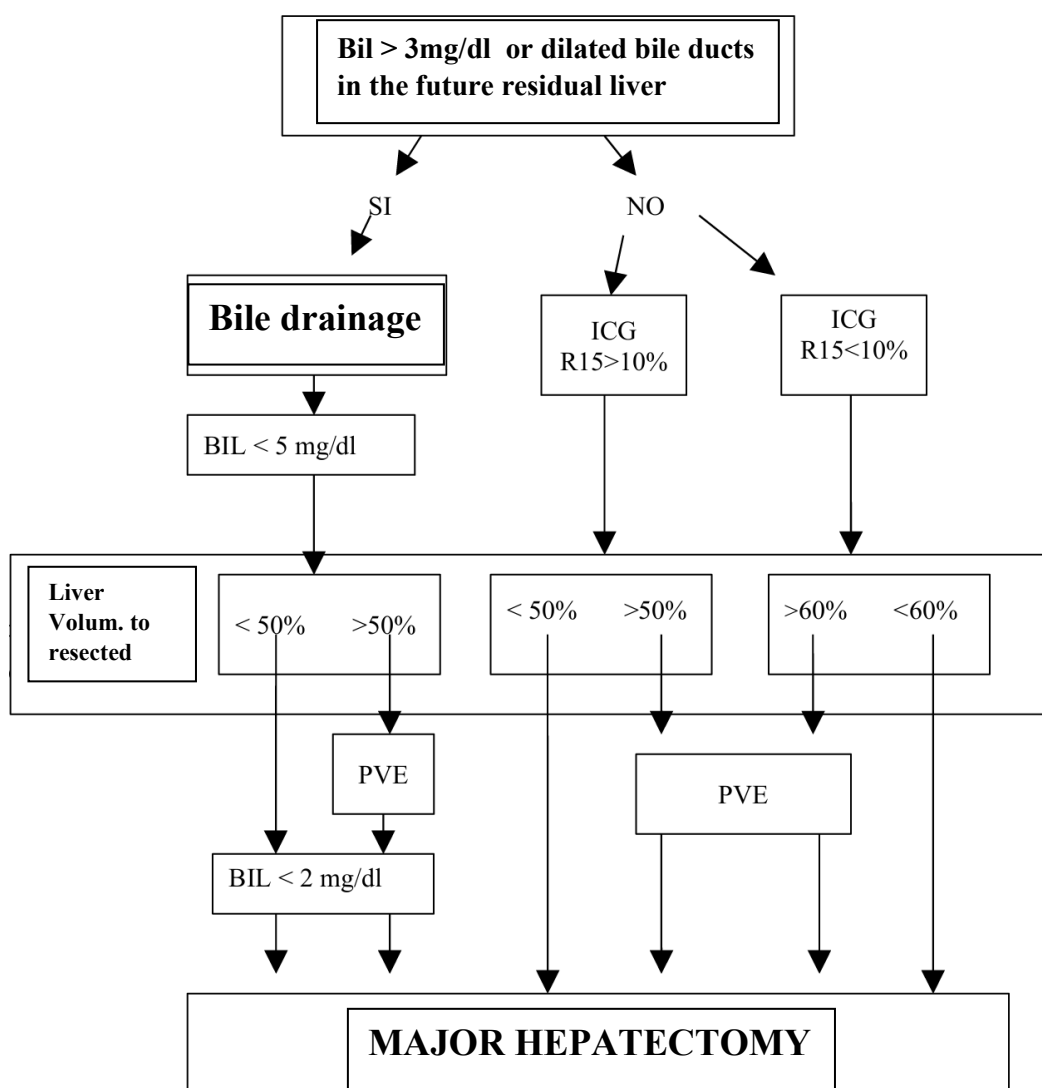


Table 3 Pre-operative algorithm in the evaluation of patients to undergo major hepatectomy.

10. Surgery

It is not advisable to systematize the surgical management of all iCCA, as these can be located in any liver segment, with sizes varying from a few millimeters to, sometimes, more than 15 or 20 cm. It should be noted that, unlike HCC, anatomical resection is not mandatory. However, for large tumors or centro hepatic tumors with close glissonian contact, anatomical resection (segmentectomy, bisegmentectomy, major hepatectomy) is often necessary for vascular excision reasons. Attention should also be paid to the parenchymal, biliary, and/or vascular extension, which generally exceeds the lesion limits visible on instrumental examinations. Therefore, unlike metastases of colorectal origin for which a "vascular" R1 resection may be acceptable (58), this type of resection should be formally avoided for iCCA. This implies, for example, when the lesion is in contact with the inferior vena cava or the cavo-suprahepatic confluence, performing venous excisions and reconstructions.

This surgical intervention should be performed in expert centers because complex techniques of liver vascular exclusion may be required, with or without associated caval clamping (and extracorporeal circulation), and, if necessary, also cooling. Cooling for hepatectomy and vascular reconstruction, limiting the risk of liver failure caused by repeated clamping. It is accepted that, in case of a predictable clamping duration of more than 60 minutes, cooling is necessary (59-60). The jaundice presentation may also be due to either compression/infiltration of the biliary convergence or an obstructive endobiliary tumor. In addition to the possible need for preoperative endoscopic biliary drainage, the surgical treatment is similar to that of pCCA, with right or left hepatectomy, if necessary extended to segments 1 and 4 or 5 and 8, respectively. Unlike pCCA, common hepatic duct resection is not systematic but must be performed "on request" based on intraoperative (invasion) and instrumental findings. For example, it is possible to perform a right hepatectomy extended to segments 1 and 4 without vascular or biliary resection. Attention must be paid during the transection of the floor of segment 4 to avoid injuries and/or devascularization of the terminal left hepatic duct. For large lesions, an anterior approach (parenchymal transection without liver mobilization) may be recommended, as for HCC (61). The hanging maneuver (passing a tape inter-hepato-cavally) can also facilitate hepatectomy, bringing the deep parenchyma to the surface and guiding the axis of the hepatectomy, provided there is no invasion of the inferior vena cava (62).

10.1 Role of Lymphadenectomy

The role of lymphadenectomy is still much debated. Many centers do not perform routine lymphadenectomy in the presence of macroscopically and radiologically negative lymph nodes, as they believe it does not offer survival benefits but is useful only for disease staging and preventing local recurrence. The approach changes in the presence of clinically pathological lymph nodes; in these cases, lymphadenectomy can improve the survival of these patients. These results were confirmed in a multicenter retrospective analysis in Korea and Japan (63). A total of 1138 patients with iCCA who underwent liver resection were included. Lymphadenectomy was performed in 413 patients. Surgical removal of more than four (≥ 4) lymph nodes improved survival outcomes in resected iCCA with positive lymph node metastasis (13 months vs. 30 months, $p = 0.045$). The AJCC and the National Comprehensive Cancer Network recommend a lymphadenectomy consisting of all fibroadipose and lymphatic tissue within the hepatoduodenal ligament between the hilar plate and the head of the pancreas, inclusive of a minimum of six nodes, to be performed during hepatectomy in patients with iCCA, as recommended for all patients who have biliary tract malignancies. Specifically, lymphadenectomy should include both station 8 (common hepatic artery) and station 12 (hepatoduodenal ligament) nodes because 82% of nodal metastatic disease manifests in these sites.

10.2 TNM Classification

No distinction was made in the sixth edition of the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) staging manual between iCCA and HCC, in part because of the relative rarity of the disease. The sixth edition AJCC/UICC (Table 4) staging system used tumor size, tumor number, and presence of vascular invasion as major prognostic criteria to establish the T-category subgroups. However, combining iCCA and HCC into a single staging system may be problematic, because iCCA and HCC have distinct mechanisms of carcinogenesis, underlying

risk factors, and biologic and clinical behaviors. The seventh edition of the AJCC/UICC staging manual has incorporated a new distinct staging system for iCCA based on prognostic factors including tumor number and vascular invasion but not tumor size. In the eighth edition, factors that has been analyzed separately in previous editions-- namely tumour size above or below 5 cm and the presence or absence of vascular inasion –were combined.. Like other staging systems for solid malignancies, the eighth edition AJCC/UICC staging system also requires ascertainment of nodal status. However, the role of lymphadenectomy for iCCA remains controversial, with many surgeons not performing lymph node (LN) evaluation (63).

sixth edition		seventh edition	
T1	Solitary tumour without vascular invasion	T1	Solitary tumour without vascular invasion
T2	Solitary tumour with vascular invasion or multiple tumours, none more than 5 cm	T2a	Solitary tumour with vascular invasion
T3	Multiple tumours more than 5 cm or tumour involving a major branch of the portal or hepatic veins	T2b	Multiple tumours, with or without vascular invasion
T4	Tumour(s) with direct invasion of adjacent organs other than the gallbladder or with perforation of visceral peritoneum	T3	Tumour(s) perforating the visceral peritoneum or involving the local extra hepatic structures by direct invasion
		T4	Tumour with periductal invasion
N0	no regional lymph node metastases	N0	no regional lymph node metastases
N1	regional lymph node metastases	N1	regional lymph node metastases
M0	no regional lymph node metastases	M0	no regional lymph node metastases
M1	regional lymph node metastases	M1	regional lymph node metastases
Stage		Stage	
I	T1 N0 M0	I	T1 N0 M0
II	T2 N0 M0	II	T2 N0 M0
IIIa	T3 N0 M0	III	T3 N0 M0
IIIb	T4 N0 M0	IVa	T4 N0 M0, Any T N1 Mo
IIIc	Any T N1 M0	IVb	Any T, Any N, M1
IV	Any T, Any N, M1		

8 th edition	
T1a:	solitary tumor ≤5 cm without vascular invasion
T1b:	solitary tumor >5 cm without vascular invasion
T2:	solitary tumor with intrahepatic vascular invasion or multiple tumors, with or without vascular invasion
T3:	tumor perforating the visceral peritoneum
T4:	tumor involving local extrahepatic structures by direct invasion
N0:	no regional lymph node metastasis
N1:	regional lymph node metastasis present
IA:	T1a N0 M0
IB:	T1b N0 M0
II:	T2 N0 M0
IIIA:	T3 N0 M0
IIIB:	T4 and/or N1, M0
IV:	any T, any N, M1

Table 4. Classification for intrahepatic cholangiocarcinoma (from sixth to eighth edition of the AJCC/UICC).

11. Therapy

Surgical resection remains the only potentially curative therapeutic option and the central cornerstone of treatment. Adjuvant systemic treatment is recommended after resection or in the palliative setting. Knowledge of the genetic-molecular alterations of CCA has significantly increased in recent years and has enabled the development of targeted therapies and has renewed interest in neoadjuvant treatment. In particular, several driver mutations offering therapeutic intervention opportunities have been identified in about 40% of CCAs, particularly in intrahepatic CCA. The most frequent driver alterations (Fig. 10) are mutations of IDH1/2 (10-20%), FGFR2 fusions (7-16%) and NTRK (2%), microsatellite instability (MSI, 2%), BRAF mutations (5%), PIK3CA (7%) and BRCA 1/2 (3%) mutations, and ERBB2 (10%) and MET (2%) amplifications. A drug for patients with CCA and FGFR2 fusions has been approved by the EMA (pemigatinib), while a drug for patients with IDH1 mutations has been approved by the FDA (ivosidenib) as second-line therapies. Other agents are available in clinical trials. Given the high number of molecular targets present in CCA, international (Mosele et al. Ann Oncol, 2019) and national (Pinto et al. Future Oncol, 2021) guidelines recommend that advanced/metastatic CCA be analyzed from the time of diagnosis, with next-generation sequencing (NGS) panels that cover all major driver mutations for which molecularly targeted agents are available.

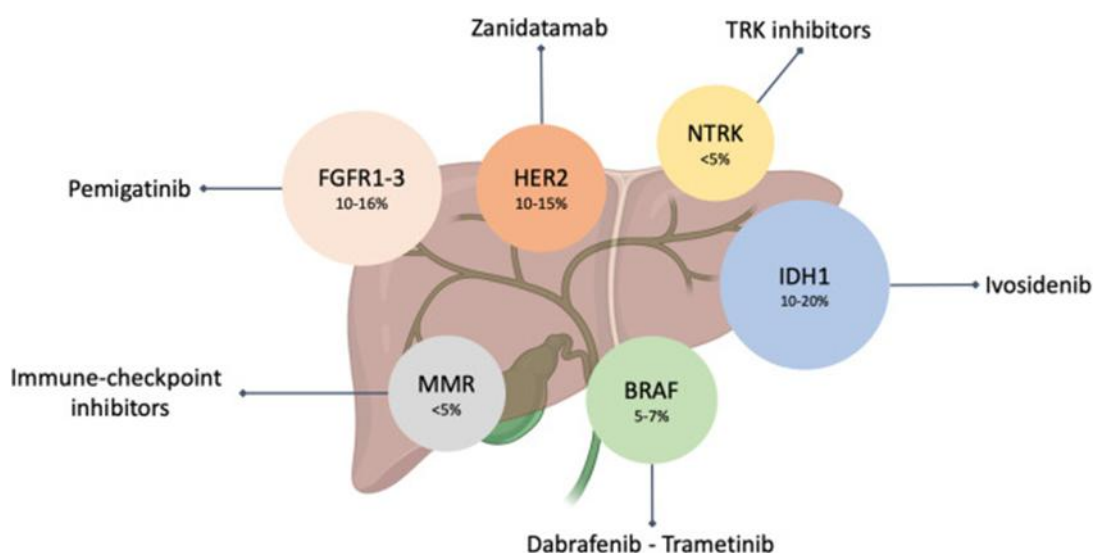


Fig.10 Molecular alterations in intrahepatic cholangiocarcinoma with related target therapies.

Chemotherapy based on Capecitabine or Gemcitabine/cisplatin regimens, with or without immunotherapy, is mainly used as adjuvant therapy (Fig. 11). Due to the inadequate success of systemic therapy, there is no recommendation for neoadjuvant treatment in resectable iCCA.

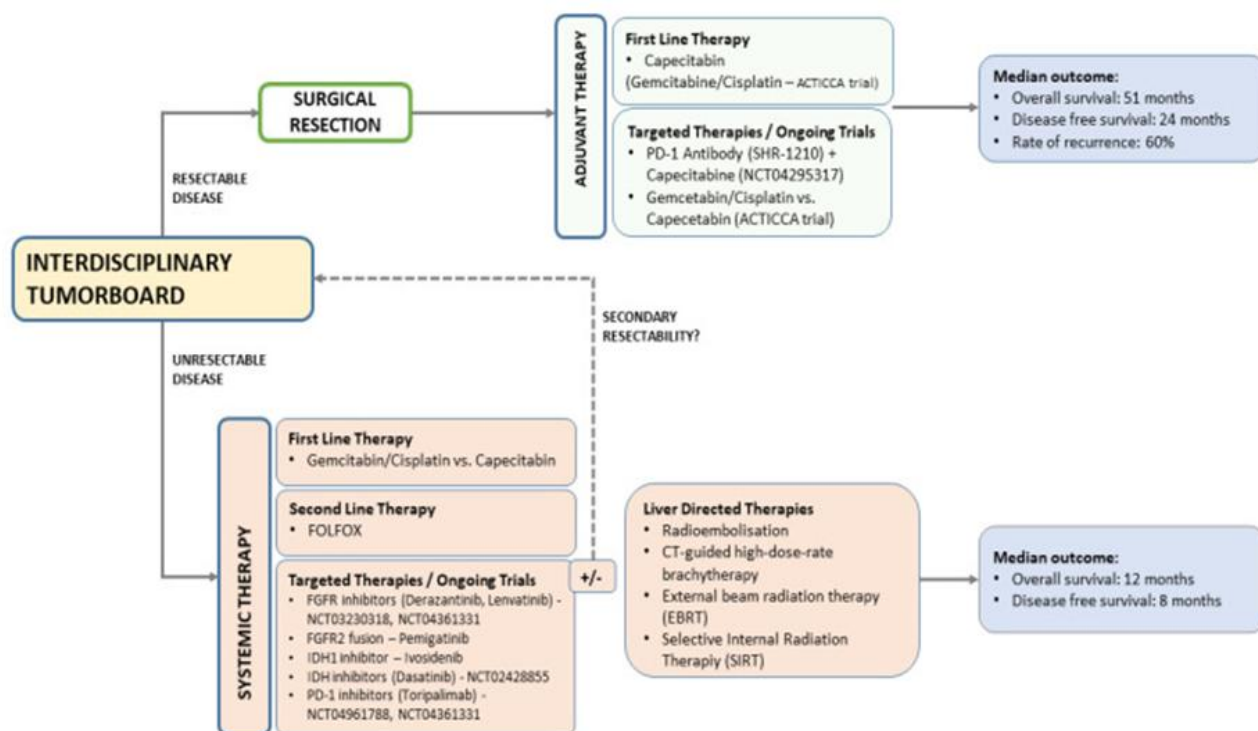


Fig.11 Decision algorithm in patients with intrahepatic cholangiocarcinoma.

12. Materials and Methods

A retrospective, single-center observational study was conducted based on the medical records of 55 consecutive patients with a diagnosis of intrahepatic cholangiocarcinoma (ICCA) treated at the Department of Hepatopancreatobiliary Surgery and Liver Transplantation of Paul-Brousse Hospital, Paris, over a 5-year period.

Inclusion criteria were histologically confirmed ICCA, age ≥ 18 years, and availability of complete clinical and follow-up data. At diagnosis, tumor staging was assessed using abdominal computed tomography (CT) and/or magnetic resonance imaging (MRI) with cholangiopancreatography (MRCP). Preoperative evaluation of vascular involvement and resectability was performed using CT and/or MRI. All treatment decisions were made within a multidisciplinary team including surgeons, gastroenterologists, radiologists, and oncologists.

The primary endpoint was overall survival (OS), defined as the time from surgical resection to death from any cause. Long-term survival was defined as survival ≥ 5 years.

Secondary endpoints included disease-free survival (DFS), defined as the time from surgery to tumor recurrence or death, recurrence patterns (intrahepatic versus extrahepatic), and postoperative outcomes, including morbidity and mortality within 30 and 90 days.

The following variables were collected: age, sex, date of diagnosis, presenting symptoms, serum CA 19-9 and CEA levels, platelet count, albumin levels, presence of metastases, tumor resectability, type and date of surgery, use of chemotherapy or radiotherapy, recurrence (date and site), and survival status. Pathological evaluation included tumor size, number, histological differentiation, vascular invasion, perineural invasion, and resection margin status (R0/R1).

Patients were considered unsuitable for curative resection in the presence of metastatic disease, extensive vascular invasion, or severe comorbidities. In eligible patients, macroscopic curative-intent

liver resection was performed. The extent of hepatic resection was determined according to tumor burden, and major hepatectomy was defined as resection of ≥ 3 liver segments. In selected cases, extrahepatic bile duct resection with Roux-en-Y reconstruction was performed.

All patients undergoing surgery had lymph node assessment, including dissection of the hepatoduodenal ligament and the porta hepatis, unless unresectable disease or peritoneal spread was identified intraoperatively. Patients with lymph node metastases or positive resection margins were considered at high risk of recurrence and were offered adjuvant therapy. Doublet chemotherapy with gemcitabine and cisplatin was considered the standard first-line regimen.

Table 5 Clinical data.	
Characteristic	N = 55
Age	64 (55, 70)
Sex	
Females	35 (64%)
Males	20 (36%)
Lenght Operation	340 (262, 450)
Unknown	1
Major hepatectomy>3 segm: yes 1; no 0	
0	7 (13%)
1	48 (87%)
Pre-op Platlets	233 (196, 289)
Unknown	4
Pre-op CA 19-9 (U/mL)	13 (3, 83)
Unknown	7
Pre-op CEA (U/mL)	3 (1, 4)
Unknown	7
PreOP Albumina	40.7 (34.5, 43.3)
Unknown	17
IO blood loss ml	450 (100, 725)
Unknown	35
lenght of Pringle min	60 (44, 70)
Unknown	31
Vascular resection: yes 1; no 0	
0	39 (71%)
1	16 (29%)
Biliary resection: yes 1; no 0	
0	45 (82%)
1	10 (18%)
Biledigestive anastomosis : yes 1; no 0	
0	46 (84%)
1	9 (16%)
Biliobiliary anastomosis : yes 1; no 0	
0	54 (98%)
1	1 (1.8%)
Histopathologic Well 0; Moderate 1; Poor 2	
0	24 (45%)
1	25 (47%)

2	4 (7.5%)
Unknown	2
Number lymph posit.	
0	43 (78%)
1	5 (9.1%)
2	6 (11%)
5	1 (1.8%)
Number of lymph. resected	4 (2, 7)
Unknown	8
Surgical Status R0: 0; R1: 1	
0	35 (64%)
1	20 (36%)
Vascular invasion: yes 1; no 0	
0	16 (36%)
1	28 (64%)
Unknown	11
Perineural invasion: yes 1; no 0	
0	23 (68%)
1	11 (32%)
Unknown	21
Weight of specimen gr.	580 (385, 996)
Unknown	15
Margin cm.	0.10 (0.00, 0.50)
Unknown	6
Size cm.	7.0 (5.0, 9.5)
Unknown	5
Tumor multiple: yes 1; no 0	
0	49 (89%)
1	6 (11%)
Therapy : -1 NeoCHT; 1 Adjuv. ; 0 NoCHT	
-1	15 (27%)
0	11 (20%)
1	29 (53%)
Recurrence: yes 1; no 0	
0	17 (33%)
1	34 (67%)
Unknown	4
Time recurrence month	12 (7, 23)
Unknown	22
Death: yes 1; no 0	
0	30 (58%)
1	22 (42%)
Unknown	3
Time survival (months)	33 (26, 58)
Unknown	3
¹ Median (IQR); n (%)	

12.1 Statistical analysis

Statistical analysis was performed using Jamovi v.2.4 software. Specifically, for the qualitative variables the absolute and percentage frequencies were calculated, while for the quantitative variables the numerical summary indices such as mean, median, standard deviation and IQR. At an inferential level, the Kruskal-Wallis test and the Wilcoxon test for independent samples were applied as bivariate tests for the numerical variables, and the Pearson Chi-square test for the categorical ones.

Overall survival (OS) was defined as the time from surgical resection to death from any cause or last follow-up. Disease-free survival (DFS) was defined as the time from surgery to tumor recurrence or death. Survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test. To identify factors associated with survival, univariable analyses were initially performed. Variables with a p-value <0.10 in univariable analysis were subsequently included in a multivariable Cox proportional hazards regression model to identify independent prognostic factors. Results were expressed as hazard ratios (HRs) with 95% confidence intervals (CIs). A two-sided p-value <0.05 was considered statistically significant.

Missing data were assessed for all variables. Given the limited proportion of missing values, a complete-case analysis was performed, and no imputation methods were applied. The number of missing observations for each variable is reported in the corresponding tables.

12.2 Results

Table 5 lists the clinicopathological features of the 55 patients with pathologically confirmed iCCA who were included in the study. Preoperatively, tumor markers CA 19-9 and CEA and albumin and platelet values were measured. We operated on 55 patients, including 20 men (36%) and 35 women (64%), with a median age of 64 years (range: 55-70 years). A majority of patients presented with a solitary tumor (n 49;89%), and the median size of the largest lesion was 7 cm (range : 5 to 9,5 cm). In all patients a lymphadenectomy was performed with a median of at least 4 (range: 2-7) lymphnodes removed. In 9 (16%) patients, given the tumor extension to the main bile duct, this was also resected. Continuity was re-established with biliary digestive anastomosis. At the time of surgical resection, in most cases a major hepatectomy was performed (n: 48;87%). A vascular resection was performed in 16 (29%) patients, respectively patients with portal resection and patients with caval resection. An hepatic artery resection was performed in only one patient. During follow-up, 22 (42%) patients died with a median survival time of 33 months (range 26-58).

From the following bivariate analyses (Table 6), it can be seen that the factors that statistically significantly correlate with mortality are the number of preoperative platelets (equal to a median value of 214.5 in the group of survivors and 272 in the group of deceased; $p=0.01$), pre-operative albumin (equal to a median value of 42.9 in the group of survivors and a median value of 37.6 in the group of deceased; $p=0.04$), vascular invasion (12 out of 25 survivors had vascular invasion vs 14 of 17 deceased; $p=0.02$) and recurrence (14 of 29 in the survivor group and 20 of 21 in the deceased group; $p<0.01$).

Bivariate Analysis

Cross Table 6 - Death No 0, Yes 1

Cross Table for Dependent death_no_0_yes_1

	N	0	1	Test Statistic
		(N=30)	(N=22)	
Age	55	50.8 65.5 70.1	57.8 62.5 74.1	F _{1,50} =0.01, P=0.92 ³
Sex : Males	55	0.3 8/30	0.5 11/22	X ₂₁ =2.98, P=0.08 ²
PreOp Platlets	51	186.4 214.5 256.7	223.8 272.0 313.0	F _{1,46} =8.68, P=0.01 ³
Preop CA 19-9 (U/mL)	48	2.1 10.1 36.0	8.3 34.0 1460.9	F _{1,43} =3.10, P=0.09 ³
Preoperative CEA (U/mL)	48	1.4 2.7 3.8	1.5 3.8 7.4	F _{1,43} =1.73, P=0.20 ³
PreOP Albumina	38	37.9 42.9 44.1	33.2 37.6 41.2	F _{1,35} =4.36, P=0.04 ³
Histopathologic Well 0; Moderate 1; Poor 2	53			X ₂₂ =0.06, P=0.97 ²
0		0.5 13/28	0.5 10/22	
1		0.5 13/28	0.5 10/22	
2		0.1 2/28	0.1 2/22	
Size cm	50	5.0 8.0 10.0	4.8 6.5 9.2	F _{1,46} =0.19, P=0.67 ³
Weight of specimen	40	385.7 593.0 1004.6	384.5 597.0 1032.8	F _{1,36} =0.24, P=0.63 ³
Multiple Tumor yes 1 : 1	55	0.1 4/30	0.1 2/22	X ₂₁ =0.22, P=0.64 ²
Major>3 segm: 1 ; 0 : 1	55	0.8 25/30	1.0 21/22	X ₂₁ =1.83, P=0.18 ²
Margin	49	0.0 0.1 0.3	0.1 0.2 0.8	F _{1,44} =3.48, P=0.07 ³

	N	0	1	Test Statistic
		(N=30)	(N=22)	
Surgical Status R0 0; (R1) 1 ; R2 2 : 1	55	0.4 13/30	0.3 6/22	X ₂₁ =1.41, P=0.23 ²
Number of Lymph.	47	2.0 5.0 8.1	2.0 4.0 5.3	F _{1,42} =0.36, P=0.55 ³
number lymph pos	55	0.0 0.0 0.0	0.0 0.0 1.0	F _{1,50} =3.04, P=0.09 ³
Perineural invasion Yes 1 No 0 : 1	34	0.2 3/15	0.4 6/16	X ₂₁ =1.15, P=0.28 ²
Vascular invasion Yes 1 No 0 : 1	44	0.5 12/25	0.8 14/17	X ₂₁ =5.06, P=0.02 ²
Lenght of Operation	54	273.7 343.0 455.2	266.7 345.0 466.7	F _{1,49} =0.02, P=0.89 ³
IO blood loss ml	20	116.7 400.0 783.3	333.3 700.0 783.3	F _{1,16} =0.24, P=0.63 ³
lenght of Pringle min	24	49.3 61.0 73.0	42.3 60.0 79.3	F _{1,20} =0.75, P=0.40 ³
Vascular resection yes/no : 1	55	0.3 8/30	0.4 8/22	X ₂₁ =0.56, P=0.45 ²
Biliary resection : 1	55	0.2 7/30	0.1 3/22	X ₂₁ =0.77, P=0.38 ²
Biliodigestive anastomosis yes no : 1	55	0.2 6/30	0.1 3/22	X ₂₁ =0.36, P=0.55 ²
Biliobiliary : 1	55	0.0 1/30	0.0 0/22	X ₂₁ =0.75, P=0.39 ²
Adjuvant_therapy	55			X ₂₂ =5.29, P=0.07 ²
-1 NeoCHT		0.2 7/30	0.3 7/22	
0 No CHT		0.3 9/30	0.0 1/22	
1AdjuvantCHT		0.5 14/30	0.6 14/22	

	N	0	1	Test Statistic
		(N=30)	(N=22)	
Recurrence yes 1/no 0 : 1	51	0.5 14/29	1.0 20/21	X21=12.34, P<0.01 ²
PreOP Albumina - PreOPAlbuminadummy : >35	38	0.8 16/20	0.6 11/17	X21=1.09, P=0.30 ²

N is the number of non-missing value. ¹Kruskal-Wallis. ²Pearson. ³Wilcoxon.

From the analysis by recurrence (Table 7), it can be seen that the factors that correlate in a statistically significant way with recurrences are the size (median value of 6 cm in the group without recurrences, and 8 cm in the group with recurrences; p=0.04) and the chemotherapy (among those who survived, 10% underwent neoadjuvant chemotherapy, 60% did not undergo therapy and the remaining 30% underwent therapy adjuvant; among those who died, 40% underwent neoadjuvant chemotherapy and the remaining 60% adjuvant chemotherapy; p<0.01). The number of positive lymph nodes also appears to be correlated with recurrence (p=0,01).

Cross Table 7 - Recurrence yes 1/no 0

Cross Table for Dependent recurrence_yes_1_no_0

	N	0	1	Test Statistic
		(N=17)	(N=34)	
Age	55	61.0 68.0 71.7	50.8 62.0 70.2	F _{1,49} =1.77, P=0.19 ³
Sex : Males	55	0.2 4/17	0.4 14/34	X21=1.55, P=0.21 ²
Pre op platlets	51	202.5 217.0 287.5	187.8 236.5 287.5	F _{1,45} =0.00, P=0.98 ³
Preop CA 19-9 (U/mL)	48	1.9 7.8 34.5	6.9 23.7 121.4	F _{1,42} =3.19, P=0.08 ³
Preoperative CEA (U/mL)	48	2.2 3.4 4.0	1.4 2.7 4.2	F _{1,42} =0.06, P=0.80 ³
PreOP Albumina	38	38.1 42.6 44.1	32.9 40.6 43.1	F _{1,33} =2.26, P=0.14 ³
Histopathologic Well 0; Moderate 1; Poor 2	53			X22=0.42, P=0.81 ²
0		0.4 6/15	0.5 17/34	
1		0.5 8/15	0.4 15/34	
2		0.1 1/15	0.1 2/34	
Size cm	50	3.1 6.0 9.0	5.3 8.0 10.0	F _{1,45} =4.62, P= 0.04 ³
Weight of specimen	40	229.6 472.0 926.7	461.0 777.0 1019.2	F _{1,35} =2.71, P=0.11 ³
Multiple Tumor yes 1 : 1	55	0.1 2/17	0.1 3/34	X21=0.11, P=0.74 ²

	N	0	1	Test Statistic
		(N=17)	(N=34)	
Major>3 segm: 1 ; 0 : 1	55	0.8 13/17	0.9 32/34	X21=3.40, P=0.07 ²
Margin	49	0.0 0.3 0.9	0.0 0.1 0.5	F _{1,43} =0.62, P=0.43 ³
Surgical Status R0 0; (R1) 1 ; R2 2 : 1	55	0.3 5/17	0.4 13/34	X21=0.39, P=0.53 ²
Number of Lymph.	47	0.3 4.0 7.0	3.0 4.0 9.0	F _{1,41} =0.88, P=0.35 ³
number lymph pos	55	0.0 0.0 0.0	0.0 0.0 1.0	F _{1,49} =7.67, P= 0.01 ³
Perineural invasion Yes 1 No 0 : 1	34	0.3 3/10	0.3 6/21	X21=0.01, P=0.93 ²
Vascular invasion Yes 1 No 0 : 1	44	0.5 6/11	0.6 19/30	X21=0.26, P=0.61 ²
Length of operation	54	224.7 300.0 386.0	278.3 345.0 479.3	F _{1,48} =1.95, P=0.17 ³
IO blood loss ml	20	66.7 200.0 866.7	341.7 650.0 800.0	F _{1,15} =0.80, P=0.39 ³
length of Pringle min	24	51.7 69.0 73.0	44.8 60.0 61.6	F _{1,19} =1.20, P=0.29 ³
Vascular resection : Yes	50	0.3 4/14	0.3 11/32	X21=0.15, P=0.70 ²
Biliary resection : 1	55	0.1 2/17	0.2 8/34	X21=1.00, P=0.32 ²
Biliodigestive anastomosis yes no : 1	55	0.1 2/17	0.2 7/34	X21=0.61, P=0.44 ²
Biliobiliary : 1	55	0.0 0/17	0.0 1/34	X21=0.51, P=0.48 ²
CHTherapy	55			X22=21.02, P<0.01 ²
-1 NeoCHT		0.1 2/17	0.4 12/34	
0 No CHT		0.6 10/17	0.0 1/34	
1 Adjuvant CHT		0.3 5/17	0.6 21/34	
PreOP Albumina - PreOPAlbuminadummy : >35	38	0.9 11/12	0.7 15/23	X21=2.89, P=0.09 ²

N is the number of non-missing value. ¹Kruskal-Wallis. ²Pearson. ³Wilcoxon.

A multivariate analysis was therefore conducted (Table 8) from which it emerges that, for the 27 patients for which data are available, there are no statistically significant HRs at the univariate level but preoperative albumin appears to be statistically significant (HR = 0.89; p=0.030) indicating that higher levels of this parameter are associated with lower levels of mortality.

Table 8. Multivariable Survival Analysis

Dependent: Surv(mytime, myoutcome)		all	HR (univariable)	HR (multivariable)
Recurrence yes 1/no 0	0	6 (22.2)	-	-
	1	21 (77.8)	326334429.94 (0.00-Inf, p=0.998)	778520038.09 (0.00-Inf, p=0.999)
Vascular invasion Yes 1 No 0	0	14 (51.9)	-	-
	1	13 (48.1)	2.69 (0.74-9.82, p=0.134)	1.75 (0.42-7.25, p=0.438)
PreOP Albumina	Mean (SD)	38.9 (6.3)	0.93 (0.86-1.01, p=0.091)	0.89 (0.81-0.99, p=0.030)
PreOp Platlets	Mean (SD)	227.7 (72.3)	1.00 (1.00-1.01, p=0.376)	1.00 (0.99-1.01, p=0.488)

Model Metrics: Number in dataframe = 27, Number in model = 27, Missing = 0, Number of events = 13, Concordance = 0.755 (SE = 0.077), R-squared = 0.421(Max possible = 0.928), Likelihood ratio test = 14.774 (df = 4, p = 0.005)

In order to investigate this significant relationship between pre-operative albumin and mortality, a survival analysis for continuous variables (Table 9-Cox model) was also carried out for all 37 patients for whom the value of this parameter and the indication were available of death or not. This association, however, is not statistically significant at the univariate level (p=0.106).

Survival Analysis for Continuous Variable

Cox Regression Summary and Table 9- PreOP Albumina

Cox Table- PreOP Albumina

Explanatory	Levels	all	HR (Univariable)	HR (Multivariable)
.	Mean (SD)	39.0 (5.8)	0.93 (0.86-1.01, p=0.106)	0.93 (0.86-1.01, p=0.106)

Model Metrics: Number in dataframe = 37, Number in model = 37, Missing = 0, Number of events = 17, Concordance = 0.624 (SE = 0.070), R-squared = 0.064(Max possible = 0.936), Likelihood ratio test = 2.460 (df = 1, p = 0.117)

PreOP Albumina

Cut Point	Statistic
41.300	2.621

Since the sample is small, this lack of evidence in favor of the alternative hypothesis could be due to a reduced power of the study and therefore it was considered at an exploratory level to report the cut-off data (Table 10) and related distribution that emerge from the analyses.

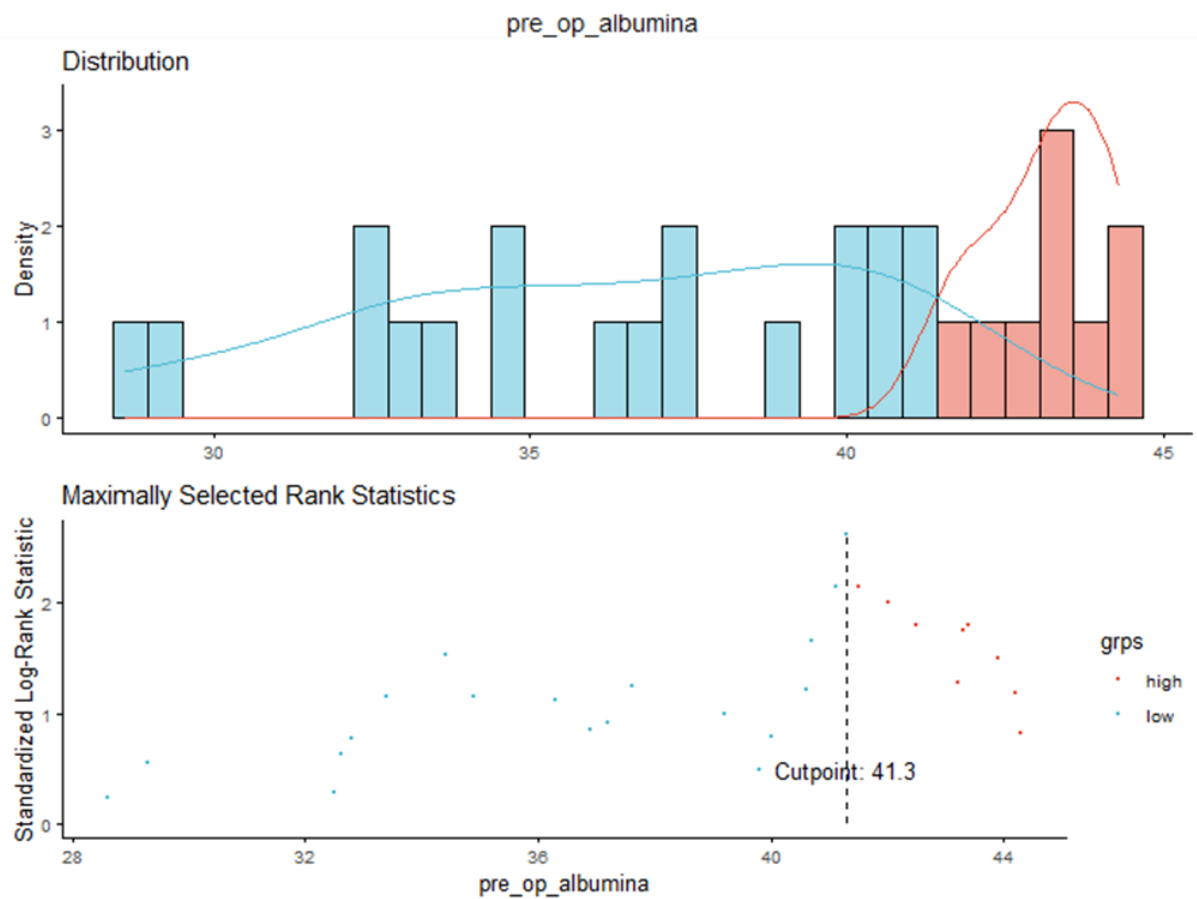
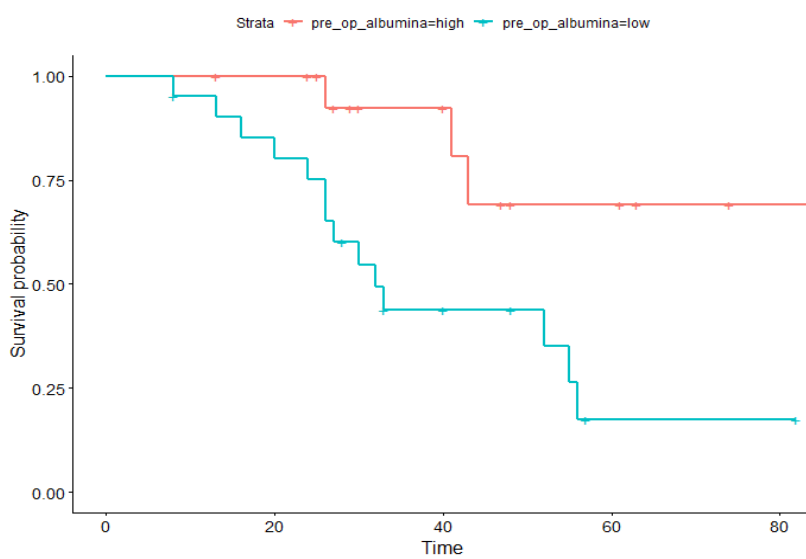


Table 10. Survival Plot - PreOP Albumina Grouped with New Cut-Off



1, 3, 5 year Survival - PreOP Albumina						
					95% Confidence Interval	
Levels	time	Number at Risk	Number of Events	Survival	Lower	Upper
high	12	16	0	100.0 %	100.0 %	100.0 %
high	36	9	1	92.3 %	78.9 %	100.0 %
high	60	4	2	69.2 %	45.0 %	100.0 %
low	12	19	1	95.2 %	86.6 %	100.0 %
low	36	7	10	43.7 %	26.3 %	72.7 %
low	60	1	3	17.5 %	5.3 %	57.4 %

Fig. 12 Survival curves as a function of the value of pre Op-Albumina. It is highlighted that the patients who survive the longest are those who have a preop albumin value greater than 41.3 g/l.

Similarly to what was done for albumin, at an exploratory level the same was done for the preoperative number of platelets (Table 11), reporting the cut-off data (223×10^9 /L) and the relative distribution.

Survival Analysis for Continuous Variable

Cox Regression Summary and Table 11 - pre op platlets

Cox Table- pre op platlets

Explanatory	Levels	all	HR (Univariable)	HR (Multivariable)
.	Mean (SD)	246.4 (89.9)	1.00 (1.00-1.01, p=0.258)	1.00 (1.00-1.01, p=0.258)

Model Metrics: Number in dataframe = 48, Number in model = 48, Missing = 0, Number of events = 22, Concordance = 0.531 (SE = 0.065), R-squared = 0.024(Max possible = 0.948), Likelihood ratio test = 1.145 (df = 1, p = 0.285)

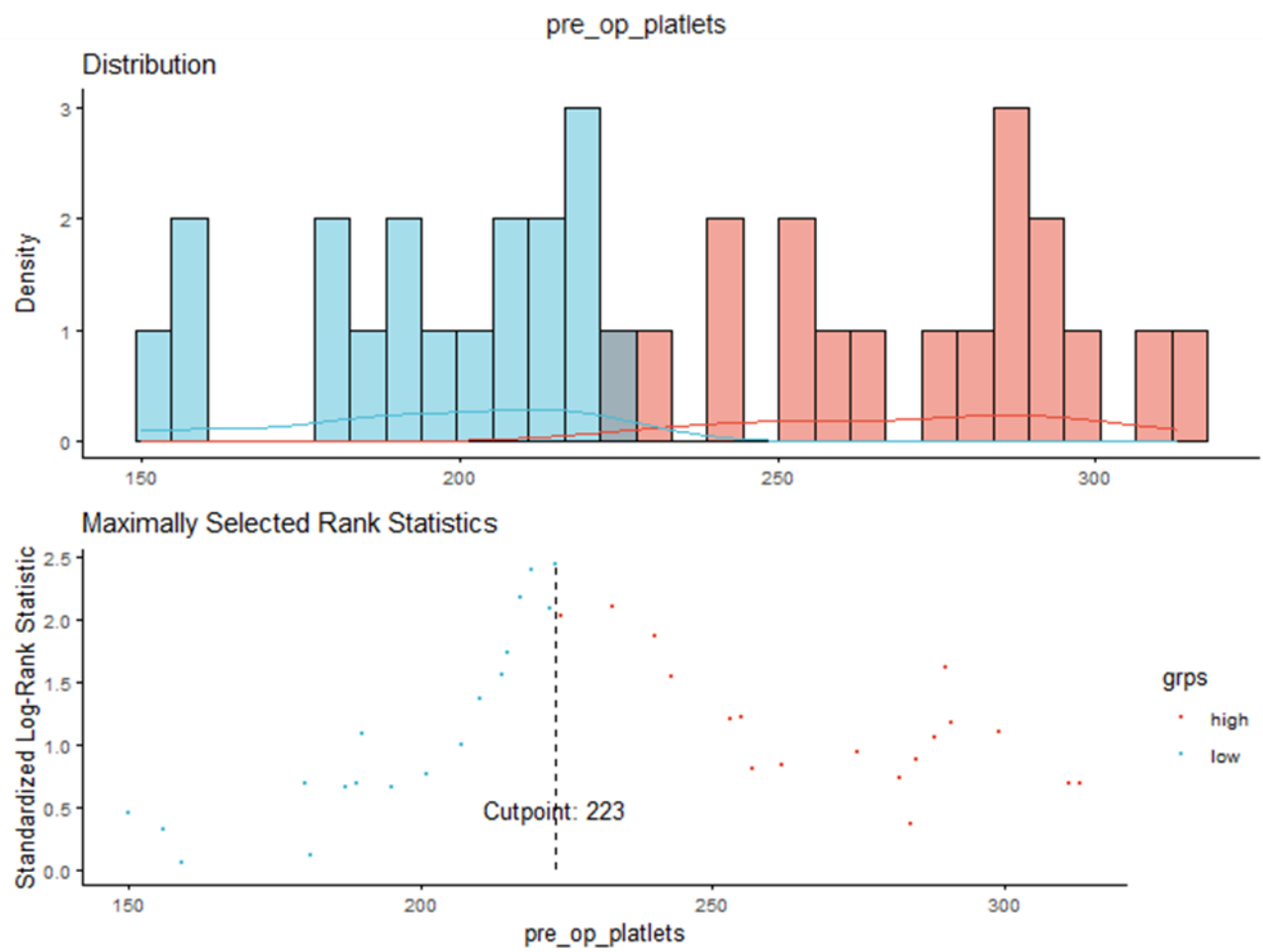
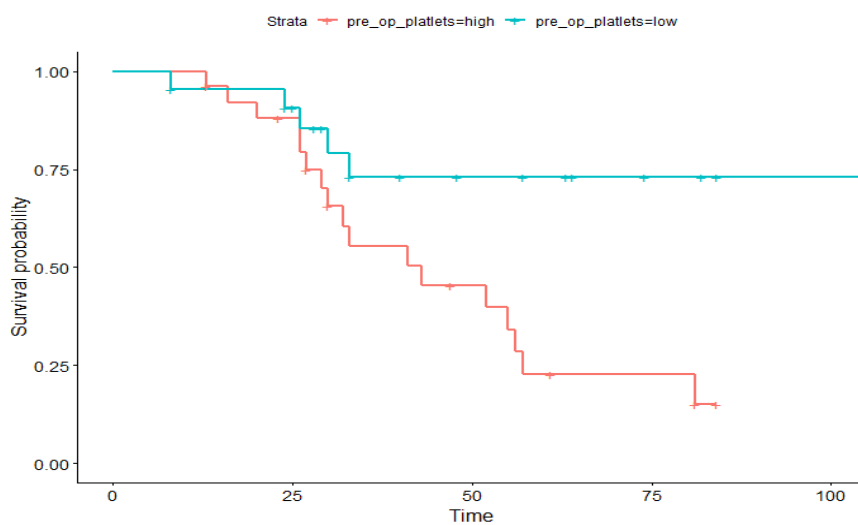


Table 12. Survival Plot - PreOP Platlets Grouped with New Cut-Off



1, 3, 5 year Survival - pre op platlets						
					95% Confidence Interval	
Levels	time	Number at Risk	Number of Events	Survival	Lower	Upper
high	12	26	0	100.0 %	100.0 %	100.0 %
high	36	11	10	55.5 %	38.0 %	80.9 %
high	60	4	6	22.7 %	9.8 %	52.4 %
low	12	20	1	95.5 %	87.1 %	100.0 %
low	36	11	4	73.2 %	55.3 %	96.8 %
low	60	6	0	73.2 %	55.3 %	96.8 %

Fig. 13. Survival curves as a function of the value of pre Op-Platlets. It is highlighted that the patients who survive the longest are those who have a preop Platlets number less than $223 \times 10^9/L$.

Finally, the relationship between therapy and mortality was evaluated, divided by vascular invasion. As can be seen from the tables below, the relationship is statistically significant ($p < 0.05$) only in the group with vascular invasion (Table 13). The percentage of deaths in the group without vascular invasion is 18.8% (with percentages ranging from 0% of those who did not undergo therapy to 33.3% of those who underwent NeoadjuvantChemotherapy), while in the group with vascular invasion it was of 53.8% (with line percentages between 0% in the group that did not undergo therapy and 75% in the group that did therapy Neoadjuvant).

Contingency Tables 13.

			Death No 0, Yes 1		
Vascular invasion Yes 1 No 0	Chemotherapy		0	1	Total
0	-1	Observed	4	2	6
		% within row	66.7 %	33.3 %	100.0 %
	0	Observed	3	0	3
		% within row	100.0 %	0.0 %	100.0 %
	1	Observed	6	1	7
		% within row	85.7 %	14.3 %	100.0 %
1	Total	Observed	13	3	16
		% within row	81.3 %	18.8 %	100.0 %
	-1	Observed	1	3	4
		% within row	25.0 %	75.0 %	100.0 %
	0	Observed	6	0	6
		% within row	100.0 %	0.0 %	100.0 %

Contingency Tables 13.

			Death No 0, Yes 1		
Vascular invasion Yes 1 No 0	Chemotherapy		0	1	Total
		% within row	100.0 %	0.0 %	100.0 %
	1	Observed	5	11	16
		% within row	31.3 %	68.8 %	100.0 %
	Total	Observed	12	14	26
		% within row	46.2 %	53.8 %	100.0 %
Total	-1	Observed	5	5	10
		% within row	50.0 %	50.0 %	100.0 %
	0	Observed	9	0	9
		% within row	100.0 %	0.0 %	100.0 %
	1	Observed	11	12	23
		% within row	47.8 %	52.2 %	100.0 %
	Total	Observed	25	17	42
		% within row	59.5 %	40.5 %	100.0 %

χ^2 Tests				
Vascular invasion Yes 1 No 0		Value	df	p
0	χ^2	1.621	2	0.4445
	N	16		
1	χ^2	9.150	2	0.0103
	N	26		
Total	χ^2	7.803	2	0.0202
	N	42		

13. Discussion

The main objective of this study was to observe the influence of different factors on recurrence and survival in patients with iCCA who underwent surgery. In addition to vascular invasion and tumor size, all factors that have already emerged from other studies in the literature, from our study, two interesting parameters emerged as prognostic factors: preoperative albumin and platelet values. Preoperative albumin is important in evaluating both the patient's nutritional status and liver function. In early epidemiological studies, lower serum albumin concentrations were associated with an increased risk of cancer-related mortality; further, in gastrointestinal malignancies such as colorectal, gastric and hepatocellular carcinoma, low albumin has been found to be a poor prognostic indicator of survival.

Fu X et al (64) analyzed the role of albumin in invasion and metastasis in patients with hepatocellular carcinoma. In fact, they have demonstrated an interaction of albumin with some components of the cellular matrix such as metalloproteases: MMP2, MMP9 and uPAR. Urokinase-type plasminogen activator receptor (uPAR) serves as the receptor for uPA and the uPA-uPAR complex initiates the extracellular matrix degradation cascade. In cancer, aberrantly elevated uPAR expression is associated with invasion and metastasis. Therefore, the suppressive action carried out by albumin would be lost in patients with low albumin levels.

As also demonstrated in Waghray's study (65) an albumin level >3.0 g/dL at presentation is an independent predictor of overall survival in patients with hilar cholangiocarcinoma. Gani et al (66) concluded that resection prolongs survival for patients with cholangiocarcinoma. Survival duration was reduced if patients had low pre-operative serum albumin levels, R1 resection, or histologically positive lymph nodes.

Huang XT et al (67-68) evaluate the nutritional status of the patient, using the prealbumin (PA) marker, and the inflammatory state of the patient, respectively with the number of neutrophils. Studies reported that patients with lower PA have poorer outcomes in iCCA. On the other hand, inflammation was associated with tumor development. By calculating the ratio of neutrophil and prealbumin (NPRI), a higher value reflects existence of inflammation or/and poor nutritional status, and both of them are potentially related to poor prognosis. Furthermore, the multivariate analysis identified that $\text{NPRI} \geq 1.74$ ($p = 0.015$), $\text{CA19-9} \geq 35\text{U/mL}$, multiple tumor, vascular invasion, local extrahepatic invasion, poor differentiation, lymph node metastasis were independent predictors for OS in iCCA. Interestingly, this study also found that higher NPRI was correlated with longer postoperative hospitalization and higher incidence of complications.

PLT-based models have been reported to have significant value on the recurrence of malignant hepatic tumors. There are several mechanisms by which platelets can interact with the tumor. Platelets would play a role in cancer associated-inflammation; in fact they would recruit T cells and activated chemokines, which play a role in tumor growth and metastasis (68). Furthermore, the tumor can cause an activation of platelets which surround it, protecting it from the immune system. PLTs may release growth factors that stimulates cellular growth, proliferation, healing, and cellular differentiation, such as transforming growth factor- β and fibroblast growth factor, which increases invasive capacity and proliferation of cancer cells (69-70)

Pavlovic et al (71) reported that platelets promote HCC cell proliferation and invasion, but their involvement goes beyond the direct effect on tumor cells, but indirectly by interfering with the pro-fibrinogenic signaling and the hepatic immune response.

Li B et al (72) describe that platelet distribution width (PDW) may have clinical significance in predicting OS after surgery in hilar cholangiocarcinoma patients.

We also carried out a review of the literature to see, in larger samples, which other factors negatively affected the prognosis of patients with iCCA. de Jong et al, (73) report in their study that the presence of vascular invasion was associated with worse survival. Tumor size was also associated with survival on univariate analysis. On multivariate analysis, tumor number and presence of vascular invasion remained associated with poor outcome. In contrast, tumor size had no impact on survival after surgical resection of ICC; In conclusion, data from the current study demonstrate that although tumor size provides no prognostic information, tumor number, vascular invasion, and LN metastasis were associated with survival after surgical resection of ICC.

Wang et al (74) analyze the genetic mutations of a total of 714 ICC patients from three cohorts (Eastern, $n=407$; Western, $n=209$; and mixed, $n=98$). Among Eastern populations, *TP53* and *KRAS* have been recognized as the most important driver genes with the highest mutation rates. In contrast, *BAP1* and *IDH1/2* mutations, *FGFR2* fusion, and *CDKN2A* deletion are more prevalent among Western populations. In the present study, they found that all *TP53* mutation subtypes were associated with poorer OS, while only the *KRAS*^{G12} mutation rather than the *KRAS*^{non-G12} mutation demonstrated a negative prognostic value in ICC.

In the multivariable analyses, nine factors were identified as independent predictors for OS, which included tumor size, tumor number, vascular invasion, lymph node metastasis, adjacent invasion, CA19-9 greater than 100, CEA greater than 10, *TP53* mutation and *KRAS*G12 mutation (75).

Raoof et al (76) describe that age greater than 60 years, high-grade tumors, node positivity, multifocality, and extrahepatic extension to contiguous organs were independently associated with lower overall survival. Tumor size, extent of surgery, vascular invasion, adjuvant chemotherapy or radiotherapy, and surgical complications were not associated with overall survival. Although node positivity was independently associated with lower overall survival, performing portal lymphadenectomy did not affect overall survival in the multivariate model.

Yoh T et al (77) evaluate also the influence of lymphadenectomy in patients with clinically node-negative intrahepatic cholangiocarcinoma and conclude that lymphadenectomy can be associated with better long-term outcomes in patients with clinically node-negative intrahepatic cholangiocarcinoma.

Luo et al (78) report that patients with CA19-9 >1,000 U/ml and/or CEA >100 ng/ml had significant worse survival and they also add that R1 resection had a profound negative impact on survival. On univariate analysis in this study, both the number and the size of tumors were factors that significantly influenced the OS; however, only the number of tumor independently influenced patients' survival on multivariate analysis. Moreover, they also showed that among patients with multiple tumors, those with more than three tumors had poor survival as compared with those with two to three tumors. They showed that LN metastasis was a significant independent variable that unfavorably influenced the prognosis of iCCA. Patients with LN metastasis had significantly poorer survival compared with those without LN metastasis. They also found that, in the presence of LN metastasis, patients had similar survival whether they obtained R0 or R1 resection. This result might partly explain the reported conflicting results about the influence on patient survival of resection margins status (R0 or R1), which had a significant influence on survival only in the patients without LN metastasis.

Dhanasekaran R et al (79) describes that the AJCC stage of the tumor, the presence of microvascular invasion, perineural invasion, nodal positivity and positive margins were factors influencing survival.

On multivariate analysis, advanced AJCC stage and presence of microvascular invasion were found to be significant and independent predictors of poor survival.

Ohira M. et al (80) evaluates the best therapy in case of patients with iCCA recurrence. Surgical resection was the best treatment option if the patient condition was favorable. The favorable factors in patients with recurrent iCCA after curative resection were surgical treatment at recurrence, gross appearance in MF type, and the absence of bile duct invasion in the initial pathology. Bile duct invasion is also one of the poor prognostic factors for iCCA surgery. Bile duct invasion tended to increase the perihilar or systemic recurrence. Regarding gross appearance of iCCA, MF type was related with intrahepatic recurrence after surgery. The lymph node metastasis is considered as one of the most important prognostic factors in iCCA. However; lymph node metastasis did not affect the prognosis after iCCA recurrence in this cohort.

Li T et al (81) reported that regional lymph node metastasis, tumour number and vascular invasion, serum GGT level was an independent prognostic factor for OS of patients after R0 resection. The incidence of biliary and vascular invasion was significantly higher in high GGT group than in normal GGT group. Factors predictive of recurrence were multiple tumours and regional lymph node metastasis.

Murakami et al (82) reported in an univariate analysis that elevated CA19-9 is an important prognostic factor after surgical resection. Another factor predicting survival was pathological differentiation. If the histological diagnosis based on preoperative biopsy is not well differentiated adenocarcinoma, patients could be a candidate for neo-adjuvant chemotherapy. They examined surgical margin width in patients with R0 resection because the influence of resection margin on outcome has also been controversial. Patients with narrow or wide negative margins showed similar recurrence patterns. In contrast, a recent paper reported that margin width and prognosis correlated linearly in patients without lymph node metastasis, and 10 mm could be considered a safe surgical margin.

Chen LP et al (83) analyze a total of 64 iCCA patients who underwent resection with negative margin and showed that lymph node metastasis, perineural invasion and total tumor size greater than 5 cm were prognostic factors in multivariate analysis.

This study has several limitations that should be acknowledged. First, its retrospective, single-center design introduces inherent selection and information biases. Although data were collected from a prospectively maintained database, some variables had missing values, and treatment decisions were not standardised beyond institutional protocols.

Second, the relatively small sample size ($n = 55$) and the limited number of events restrict the statistical power, particularly for multivariable analyses. Consequently, the identification of independent prognostic factors should be considered exploratory and may not be generalizable to larger populations.

Finally, variations in surgical technique, adjuvant therapy, and follow-up may also have influenced outcomes. Despite these limitations, the study provides valuable insights into long-term survival and prognostic factors after curative-intent resection of intrahepatic cholangiocarcinoma

14. Conclusion

Surgical resection is a key factor in improving overall survival in patients with iCCA. As shown from our study but also from the literature review, there are several factors that negatively affect the survival of patients with iCCA. These can depend on both the characteristics of the tumor, including its macroscopic appearance, histology, and biology, as well as patient characteristics and those related to surgery. These factors, as reported in Table 14, should not be considered individually but evaluated as a whole. We have already seen for pancreatic tumors (84) that an elevated preoperative value of (CA 19-9 >120 U/ml) can turn a tumor defined as radiologically resectable into "biological borderline resectability" and thus deserving of a neoadjuvant approach. Also for iCCA, to improve postoperative outcomes, a broader evaluation that also considers tumor biology is required. Therefore, the determination of resectability cannot depend solely on the radiological characteristics of the tumor, but factors related to the patient and the biology of the tumor must also be evaluated (Table 14). Further studies are necessary to understand the extent to which these factors influence survival in order to develop the optimal treatment strategy and to improve the outcomes of iCCA.

Tumor Biology	Tumor characteristics	Patients characteristics	Surgery
CA19-9 ≥ 35 U/mL	vascular invasion	Age > 60 years	R1
NPRI ≥ 1.74	multiple tumor	HBV	lymphadenectomy
CA19-9 > 1,000 U/ml	lymph node metastasis	Cirrhosis	
CEA > 100 ng/ml	local extrahepatic invasion	high GGT	
TP53 mutation	Tumor size > 5cm	ratio of neutrophil and prealbumin ≥ 1.74	
KRAS ^{non-G12} mutation	Tumor number	PreOp-Albumin	
CEA > 200 ng/ml	Pathological differentiation (not Well)	PreOp-Platelets	
	perineural invasion		

Table 14. Factors associated with the survival of all patients with intrahepatic cholangiocarcinoma.

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