Gastroenterology Report, 2024, **12**, goae031
https://doi.org/10.1093/gastro/goae031
Review Article

Review Article

Current advances and future directions in combined hepatocellular and cholangiocarcinoma

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Abstract

The low incidence of combined hepatocellular cholangiocarcinoma (cHCC-CCA) is an important factor limiting research progression. Our study extensively included nearly three decades of relevant literature and assembled the most comprehensive database comprising 5,742 patients with cHCC-CCA. We summarized the characteristics, tumor markers, and clinical features of these patients. Additionally, we present the evolution of cHCC-CCA classification and explain the underlying rationale for these classification standards. We reviewed cHCC-CCA diagnostic advances using imaging features, tumor markers, and postoperative pathology, as well as treatment options such as surgical, adjuvant, and immune-targeted therapies. In addition, recent advances in more effective chemotherapeutic regimens and immune-targeted therapies were explored. Furthermore, we described the molecular mutation features and potential specific markers of cHCC-CCA. The prognostic value of Nestin has been proven, and we speculate that Nestin will also play a role in classification and diagnosis. However, further research is needed. Moreover, we believe that the possibility of using machine learning liquid biopsy for preoperative diagnosis and establishing a scoring system are directions for future research.

Keywords: cHCC-CCA; epidemiological features; classification; molecular mutation characteristics; diagnosis, treatment

Introduction

According to 2020 statistics, primary liver cancer (PLC) ranks seventh in global cancer incidence and second in terms of mortality. Across most regions, PLC demonstrates higher mortality and incidence rates for men than for women [1]. East Asia has the highest PLC incidence and mortality [1]. Combined hepatocellular cholangiocarcinoma (cHCC-CCA), a rare form of PLC, has an incidence ranging from 0.4% to 14.2% among PLC cases. Notably, the incidence, morbidity, and mortality of cHCC-CCA have been steadily rising in recent years [2, 3]. cHCC-CCA displays remarkable heterogeneity, characterized by both hepatocyte and cholangiocyte differentiation within the same tumor.

Moreover, cHCC-CCA exhibits multiple coexisting and overlapping features [4, 5]. In the most recent classification, the World Health Organization (WHO) classified cHCC-CCA into two distinct types. Apart from the "classical" type, which exhibits two clearly differentiated types, "intermediate cell carcinoma" (IMC), characterized by the presence of single intermediate cells, is a distinct subtype of cHCC-CCA [4, 5]. Since its initial description by Wells [6] in 1903, the definition, associated terminology, and classification of cHCC-CCA subtypes have evolved. Furthermore, cHCC-CCA exhibits notable intratumoral heterogeneity and research on this aspect is limited. All of these factors make diagnosis more challenging. The impact of different subtype characteristics on diagnosis and prognosis remains inconclusive, necessitating further research to explore methods for enhancing diagnosis and selecting appropriate treatments. This review offers a comprehensive retrospective analysis of the epidemiological features, classification, molecular mechanisms, diagnosis, and treatment of cHCC-CCA and a forward-looking perspective on future advancements in diagnosis and treatment.

Epidemiological features

Due to the low prevalence, most studies of cHCC-CCA have been limited by small sample sizes. To obtain the precise epidemiological characteristics of cHCC-CCA and utilize them for diagnosis, we established the largest available cohort of patients with cHCC-CCA to date. We collected data from 78 articles published between 1993 and 2021, involving 5,742 patients with cHCC-CCA, 159,038 patients with hepatocellular carcinoma (HCC), and 23,992 patients with intrahepatic cholangiocarcinoma (ICC) and including information such as epidemiological characteristics (mean age, sex, body mass index (BMI), and history of hepatitis B virus (HBV) infection, cirrhosis, alcoholic liver disease, chronic

Received: 26 July 2023. **Revised:** 17 March 2024. **Accepted:** 24 March 2024

hepatitis, diabetes, and hypertension), clinicopathological features, and the expression of cHCC-CCA markers (Tables 1–3).

Patient characteristics

The highest prevalence of cHCC-CCA was observed among men aged 60–64 years and women aged 75–79 years [3, 5, 7]. Our analysis revealed that the mean age of patients with cHCC-CCA was 56.51 years, which was slightly lower than that of patients with HCC and ICC. We identified a significant male predominance for cHCC-CCA ($P < 0.001$). Our findings revealed a higher prevalence of HBV infection in patients with HCC and cHCC-CCA (65.34% and 61.80%, respectively) but a lower prevalence in patients with ICC (26.65%). Moreover, most patients with cHCC-CCA exhibited cirrhosis (52.75%) (Table 1). This finding is consistent with prevalence profiles reported in several Asian studies [3, 5, 7]. However, Western studies have shown that a less pronounced male predominance and a relatively low prevalence of HBV or cirrhosis background for cHCC-CCA tend to align more closely with the characteristics observed in ICC rather than HCC [7, 8]. In practice, the profile of these patients remains ambiguous and highly dependent on the geographic region.

Clinical features

cHCC-CCA exhibits overlapping clinical and biological patterns among its malignant components [8, 9]. In the early stages, it often presents with asymptomatic expansion, along with signs and symptoms, such as painless jaundice, pruritus, abdominal discomfort, weight loss, fever, fatigue, ascites, hepatomegaly, palpable gallbladder, and acute cholangitis [10]. Compared with HCC and ICC, cHCC-CCA had the smallest mean tumor diameter; however, it was more likely to be multifocal. Additionally, cHCC-CCA was associated with a high risk of vascular invasion (38.50%) (Table 2). This is consistent with other findings indicating that cHCC-CCA was inclined to develop multifocal liver lesions, which may be related to hepatocyte behavior [11, 12]. The percentage of lymphatic metastases (14.79%) was lower than that of ICC (22.04%) but much higher than that of HCC (2.24%) (Table 2). In addition, 27.62% of cHCC-CCA developed intrahepatic metastasis, and nearly half (46.52%) exhibited distant metastasis (Table 2). Hilar lymph node metastasis was common, with a frequency ranging from 12% to 33%. The incidence of extrahepatic metastasis varied, and sites of metastasis included the lungs, bones, brain, and adrenal glands [10].

cHCC-CCA classification evolution

In 1903, Wells [6] first described the combined type of HCC. Allen and Lisa [13] formalized the classification of cHCC-CCA in 1949. They categorized it into three types: i) separate tumor nodules of HCC and cholangiocarcinoma; ii) two tumor nodules, one with HCC features and the other with ICC features, that may combine with growth, with a transition zone at their point of convergence; and iii) a single tumor nodule exhibiting both HCC and ICC features.

In 1985, Goodman *et al.* [14] proposed a new classification for cHCC-CCA, aligning types I and II to the first two types proposed by Allen and Lisa [13]. Type III (fibrous lamellar type) was a new subtype, wherein the entire tumor showed a mixed differentiation of HCC and ICC without separate areas, with massive fibrin production. The fibrous lamellar type of cHCC-CCA was a more specific type with a better prognosis and rare cirrhotic background that was potentially linked to the younger age of patients at presentation.

In the 2010 WHO classification, cHCC-CCA was divided into the classical type and subtypes based on stem cell characteristics [15, 16], which included the typical, intermediate cell, and cholangiolocellular subtypes. Detailed information regarding each subtype is provided in Table 4. Jung *et al.* [17] showed that compared with the classical subtype, subtypes with stem cell characteristics demonstrated improved survival outcomes. However, a separate study indicated that patients with more stem cell features experienced worse overall survival (OS) [18].

Due to conflicting statements on cHCC-CCAs, an international panel of experts standardized their classification in 2018 [19]. In addition to removing subtypes with stem cell characteristics, they divided this heterogeneous cancer component into three categories: (i) cHCC-CCA, in which different components of hepatocellular and cholangiocellular differentiation coexist; (ii) IMC; and (iii) fine cholangiocarcinoma (CLC).

Whether IMC and CLC should be treated as subtypes of cHCC-CCA or classified as distinct and separate entities at that time was not fully determined. The consensus gave the following recommendations [19]: (1) the terminology for primary HCC with hepatocellular and cholangiocellular differentiation within the same tumor was cHCC-CCA; (2) the diagnosis of cHCC-CCA relied on routine histochemical staining, and immunohistochemistry played only an ancillary role; (3) if a combination of PLCs was present, the recommended diagnostic terminology included the form of PLC, e.g. cHCC-CCA, ciCCA-CLC, cHCC-CCA-CLC, cHCC-CCA-IMC, etc; and (4) if stem cell characteristics were present,

Table 1. Epidemiological characteristics of cHCC-CCA, HCC and ICC

Epidemiological characteristic	cHCC-CCA ($n = 5,742$)	HCC ($n = 159,038$)	ICC ($n = 23,992$)	*P* values*,[a]	*P* values*,[b]
Mean age, years (No. of cases)[c]	56.51 (2,348)	63.16 (60,096)	64.51 (8,061)		
Sex, No. of male/female patients (ratio)	4,117/1,625 (2.53)	121,366/37,672 (3.22)	12,205/11,787 (1.04)	<0.001	
BMI, kg/m^2 (No. of cases)[c]	29.30 ± 6.60 (208)	NA	NA		
HBV infection, No. of Patients/Total (%)[c,d]	1,631/2,639 (61.80)	10,287/15,743 (65.34)	472/1,771 (26.65)	<0.001	0.001
HCV infection, No. of Patients/Total (%)[c,d]	244/1,816 (13.44)	2,683/15,499 (17.31)	79/1,406 (5.60)	<0.001	0.257
Cirrhosis, No. of Patients/Total (%)[c,d]	1,324/2,510 (52.75)	10,265/15,206 (67.50)	345/1,569 (22.00)	0.003	0.001
Alcoholic liver disease, No. of Patients/Total (%)[c,d]	76/538 (14.13)	88/1,490 (5.90)	19/230 (8.26)	0.303	0.078
Chronic hepatitis, No. of Patients/Total (%)[c,d]	26/69 (37.68)	188/522 (36.00)	30/84 (35.70)	0.147	0.609
Diabetes, No. of Patients/Total (%)[c,d]	26/292 (8.90)	NA	5/21 (25.00)		0.0004
Hypertension, No. of Patients/Total (%)[c,d]	19/147 (12.90)	NA	NA		

[a] Differences between cHCC-CCA group and HCC group.
[b] Differences between cHCC-CCA group and ICC group.
[c] The patients who were counted in this indicator were not all of the patients included in the study, but only a subset of them.
[d] "Patients" refers to cHCC-CCA or HCC or ICC patients with prevalent disease and "Total" refers to all cHCC-CCA or HCC or ICC patients with this index detected.
* Statistical analysis was done using the chi-square test with SPSS 17.0. A *P*-value of < 0.05 was considered statistically significant.
cHCC-CCA = combined hepatocellular-cholangiocarcinoma; HCC = hepatocellular carcinoma; ICC = intrahepatic cholangiocarcinoma; BMI = Body Mass Index; HBV = hepatitis B virus; HCV = hepatitis C virus; NA = not available.

Table 2. Clinicopathological features of cHCC-CCA, HCC and ICC

Clinicopathological feature	cHCC-CCA ($n = 5,742$)	HCC ($n = 159,038$)	ICC ($n = 23,992$)	*P* values*,[a]	*P* values*,[b]
Tumor number, No. of multiple/single tumor (ratio)[c]	824/2,070 (0.40)	1,681/6,633 (0.25)	168/810 (0.21)	0.0001	0.5236
Tumor size, cm (No. of cases)[c]	5.58 (1,039)	6.38 (43,349)	6.43 (7,582)		
Capsular formation, No. of Presents/Total (%)[c,d]	55/164 (33.54)	245/368 (66.58)	32/128 (25.00)	0.0001	0.2396
Lymphatic metastasis, No. of Presents/Total (%)[c,d]	333/2,252 (14.79)	217/9,700 (2.24)	303/1,375 (22.04)	0.0001	0.0524
Vascular invasion, No. of Presents/Total (%)[c,d]	723/1,878 (38.50)	1,540/9,242 (16.66)	289/1,168 (24.74)	0.0001	0.0001
Intrahepatic metastasis, No. of Presents/Total (%)[c,d]	29/105 (27.62)	54/368 (14.67)	23/189 (12.17)	0.0425	0.3657
Distant metastasis, No. of Presents/Total (%)[c,d]	127/273 (46.52)	NA	NA		
Biliary invasion, No. of Presents/Total (%)[c,d]	19/221 (8.60)	19/509 (3.73)	NA	0.9961	
Tumor thrombosis, No. of Presents/Total (%)[c,d]	70/486 (14.40)	28/392 (7.14)	74/128 (57.81)	0.0373	0.0001

[a] Differences between cHCC-CCA group and HCC group.
[b] Differences between cHCC-CCA group and ICC group.
[c] The patients who were counted in this indicator were not all of the patients included in the study, but only a subset of them.
[d] "Presents" refers to cHCC-CCA or HCC or ICC patients with this pathological conditions and "Total" refers to all cHCC-CCA or HCC or ICC patients with this index detected.
* Statistical analysis was done using the chi-square test with SPSS 17.0. A *P*-value of < 0.05 was considered statistically significant.
NA = not available.

Table 3. Tumor markers of cHCC-CCA, HCC and ICC

Tumor marker[a]	cHCC-CCA ($n = 5,742$)	HCC ($n = 159,038$)	ICC ($n = 23,992$)	*P* values*,[b]	*P* values*,[c]
AFP, No. of Elevated/Total (%)[d,e]	1,057/1,888 (55.99)	5,533/8,951 (61.81)	106/1,149 (9.23)	0.0001	0.0001
CA-199, No. of Elevated/Total (%)[d,e]	572/2,770 (20.65)	102/991 (10.29)	401/692 (57.95)	0.0001	0.0001
AFP + CA-199, No. of Elevated/Total (%)[d,e]	57/283 (20.14)	6/236 (2.54)	6/159 (3.77)	0.1234	0.0123
CEA, No. of Elevated/Total (%)[d,e]	132/775 (17.03)	3/184 (1.63)	13/73 (17.81)	0.0078	0.0321
DCP, No. of Elevated/Total (%)[d,e]	73/160 (45.63)	NA	NA		

[a] Because different studies have different cut-offs for marker elevation, we have included all of them in the database as elevated.
[b] Differences between cHCC-CCA group and HCC group.
[c] Differences between cHCC-CCA group and ICC group.
[d] The patients who were counted in this indicator were not all of the patients included in the study, but only a subset of them.
[e] "Elevated" refers to cHCC-CCA or HCC or ICC patients with elevated markers and "Total" refers to all cHCC-CCA or HCC or ICC patients with this index detected.
* Statistical analysis was done using the chi-square test with SPSS 17.0. A *P*-value of <0.05 was considered statistically significant.
AFP = alpha-fetoprotein, CA-199 = Carbohydrate antigen 199, CEA = carcinoembryonic antigen, DCP = des-gamma-carboxy prothrombin, NA = not available.

they could be described in the report but not as a separate classification. In addition, the consensus proposed tumors not applicable to cHCC-CCA (Table 5).

Several deficits in the 2010 WHO classification gradually became apparent: (i) the observation of stem/progenitor cell features in various hepatocellular and cholangiocellular carcinomas with a primitive typical morphology; (ii) the coexistence of three tissue types with presumed stem cell/progenitor cell features; and (iii) the recognition of CLC as cHCC-CCA only when associated with the hepatocellular fraction [4, 16, 19]. The WHO cHCC-CCA classification (Table 5) was significantly updated in 2019. The intermediate cell subtype was referred to as IMC and represented a distinct subtype of cHCC-CCA. Conversely, the cholangiolocellular subtype was classified as a specific subtype of ICC rather than cHCC-CCA; it was categorized as cHCC-CCA only when HCC components were present. The typical subtype was HCC exhibiting stem cell features [5, 20].

Molecular mutation characteristics

The molecular mechanisms underlying the coexistence of HCC and ICC components in a single tumor remain elusive. Unraveling the molecular mutational characteristics of cHCC-CCA may help classify the tumor, select targeted therapies, and explore its origin. cHCC-CCA has been shown to harbor recurrent alterations in *TERT* (80%), *TP53* (80%), cell cycle genes (40%; *CCND1*, *CCNE1*, and *CDKN2A*), receptor tyrosine kinase/Ras/PI3-kinase pathway genes (55%; *MET*, *ERBB2*, *KRAS*, and *PTEN*), chromatin regulators (20%; *ARID1A* and *ARID2*), and Wnt pathway genes (20%; *CTNNB1*, *AXIN*, and *APC*), among which *TP53*, *AXIN1*, *RB1*, *PTEN*, *ARID2*, and *BRD7* were significantly mutated [21, 22].

Several studies have shown that *TP53* and *TERT* are the most frequently mutated genes in cHCC-CCA, with *TP53* mutations occurring significantly more frequently in cHCC-CCA than in pure HCC and ICC [21, 22]. Ito *et al.* [23] found that all tumors with diameters < 3 cm had *TP53* mutations, and six of seven tumors with diameters ≥ 3 cm did not have *TP53* mutations. The *TERT* promoter mutation rate in cHCC-CCA was much lower than that in HCC, and the mutation was absent in ICC. The much lower mutation frequency of *CTNNB1* and *KRAS* was also a unique feature of cHCC-CCA that was not biased by etiologic or ethnic factors, unlike HCC and ICC, respectively [22]. When comparing the two subtypes of HCC and ICC from the perspectives of gene expression and epidemiological characteristics, the conclusions of different studies are conflicting. In a study by Xue *et al.* [22], the classical type showed strong ICC-like features, whereas IMC showed HCC-like features. However, Joseph *et al.* [21] found that classical genetics were distinct from ICC (even in cirrhosis) and similar to HCC. Further explorations with larger sample sizes are needed, as they are associated with accurate diagnosis and treatment.

The mutational frequencies of *TP53* (44% vs 53%; $P = 0.35$) and the *TERT* promoter (25% vs 23%; $P = 0.83$) were comparable between the classical type and IMC. Interestingly, *AXIN1* mutations were significantly enriched in IMC ($P < 0.001$). Consistently, the expression of *AXIN1* in the classical type was significantly higher than that in IMC ($P < 0.05$). Genes for the epithelial-mesenchymal transition were the most enriched in the classical type, whereas

Table 4. 2010 WHO classification of cHCC-CCA

Dyeing method	Classical types		Subtypes with stem cell characteristics		
	HCC component	ICC component	Typical subtype	Intermediate cell subtype	Cholangiolocellular subtype
HE	-Typical HCC -Well to poorly differentiated type with scarce stroma	-Typical adenocarcinoma -Well to poorly differentiated type with intermediate-abundant stroma	-Nests of mature looking hepatocytes with peripheral clusters of small cells -High nucleus: cytoplasm ratio -Hyperchromatic nuclei with abundant stroma	-Features intermediate between hepatocytes and cholangiocytes -Strands, solid nests and/or trabeculae of small, uniform cells with scant cytoplasm -Hyperchromatc nuclei with intermediate-abundant stroma	-Admixtures of small monotonous glands, antler-like anastomosing patterns. -Cuboidal, smaller in size than normal hepatocytes -High nucleus: cytoplasm ratio -Distinct nucleoli with abundant stroma
IHC	HerPar-1: high expression	CK7, CK19, EMA: high expression	-CK7, CK19, EMA, CD56, c-kit, EpCAM: positive (hepatocytes and peripheral small cells) -EpCAM: circumferential staining HepPar-1: positive (nests)	-CK7, CK19, EMA, c-kit: high expression -EpCAM, CD56, vimentin: medium expression -HerPar-1, CD133: low expression	-CK7, CK19, EMA, EpCAM: high expression -CD56, vimentin: medium expression

cHCC-CCA = combined hepatocellular-cholangiocarcinoma, HCC = hepatocellular carcinoma, ICC = intrahepatic cholangiocarcinoma, IHC = immunohistochemistry, HE = hematoxylin-eosin staining, HerPar-1 = hepatocyte paraffin-1, CK = cytokeratin, EMA = epithelial membrane antigen, EpCAM = epithelial cell adhesion molecule, CD = cluster of differentiation.

xenobiotic and bile acid metabolism genes were prominent in IMC. Moreover, for immune clusters, the high immune group was significantly enriched in IMC, whereas the medium immune group was significantly enriched in the classical type [22]. Therefore, these two subtypes could be targeted for different therapies.

Despite these distinct characteristics, several studies have suggested a monoclonal origin for both subtypes based on numerous shared ubiquitous mutations [22–24]. In IMC, *TP53* and *GPR114* mutations were validated in 100% of single cells, indicating their presence in founding clones. In contrast, *SYNE1* and *PTPRT* mutations, validated in 80% of single cells, were clustered into a subclone [22]. For the classical type, the percentage of shared mutations in each case ranged from 27% to 95% [22] or 33.1% to 86.4% [24]. However, whether cHCC-CCA is a source of progenitor cells requires further exploration.

Diagnosis

Tumor markers

Carbohydrate antigen 19–9 (CA19-9) and alpha fetoprotein (AFP) are primary tumor markers for diagnosing ICC and HCC, respectively. Simultaneous elevation of these markers, coupled with dual phenotypic pathological characteristics and clinical experience, may indicate the presence of cHCC-CCA. In our data, 55.99% of cHCC-CCA cases exhibited elevated AFP expression, which was lower than that in HCC but significantly higher than that in ICC (Table 3). Similarly, the proportion of cHCC-CCA patients with elevated CA19-9 levels (20.65%) was between that of patients with HCC (10.29%) and ICC (57.95%). Simultaneous detection of elevated AFP and CA19-9 levels strongly indicated the diagnosis of cHCC-CCA (cHCC-CCA vs HCC vs ICC: 20.14% vs 2.54% vs 3.77%). Nevertheless, this diagnostic approach exhibited high specificity but low sensitivity (Table 3).

Imaging features

cHCC-CCA may exhibit varying degrees of radiological features from both HCC and ICC [8]. The American College of Radiology published the Contrast-Enhanced Ultrasound (CEUS) LI-RADS guidelines, which categorized the ultrasonographic features of liver nodules as CEUS LR-1 to LR-5, with the addition of LR-5V and CEUS LR-M Analysis of magnetic resonance imaging (MRI) and CEUS features in patients with cHCC-CCA revealed that most patients fell into the LR-M category [25, 26]. Regarding the staging characteristics of CEUS images, the prevalent enhancement pattern observed in cHCC-CCA was hyperenhancement (homogeneous or heterogeneous) in the arterial phase, followed by substantial washout in the delayed phase. The second most common enhancement pattern was peripheral hyperenhancement in the arterial phase, with significant washout in the delayed phase [27].

Li *et al.* [28] analyzed the preoperative CEUS features and utilized significant washout in the late phase to distinguish cHCC-CCA from HCC. The study reported a sensitivity, specificity, and accuracy of 78%, 90%, and 83%, respectively. Similarly, Zhang *et al.* [27] demonstrated a sensitivity of 82.2% and specificity of 60.0%. In contrast, when employing over-enhancement in the arterial phase and significant washout in the late phase to differentiate cHCC-CCA from ICC, the study reported a sensitivity, specificity, and accuracy of 55%, 78%, and 66%, respectively [28]. Yang *et al.* [29] developed a radiological model incorporating a $\geq$50% hypovascular component and delayed enhancement. This study quantitatively assessed the hypovascular components within the tumor and qualitatively evaluated the LI-RADS

Table 5. 2019 WHO classification of cHCC-CCA and inapplicable tumors

2019 WHO classification	Not applicable to tumor type (International Consensus Panel 2018)
- cHCC-CCA, classical (with well-defined, closely mixed hepatocyte and cholangiocyte differentiation, with possible intermediate areas of excess) - Intermediate cell carcinoma (primary liver cancer consisting solely of intermediate cells)	- Distinct (multifocal) HCC and ICC; - Collision tumors of HCC and ICC arising separately in the same liver; - Any form of hepatoblastoma or variants, such as those with cholangiocytic or ductal plate components; - The pediatric "transitional liver cell tumor" or variants; - Morphologically typical HCCs with only immunohistochemical expression of keratin19 or other cholangiocytic or stem/progenitor cell markers; - Morphologically typical ICCs with only immunohistochemical expression of hepatocytic or stem/progenitor cell markers, or ICC with in situ hybridization markers for hepatocytic differentiation (i.e., albumin); - Sclerosing/scirrhous HCC, a rare variant of HCC with some areas that may be suggestive of ICC (adenocarcinoma in sclerotic stroma).

cHCC-CCA = combined hepatocellular-cholangiocarcinoma; HCC = hepatocellular carcinoma; ICC = intrahepatic cholangiocarcinoma; IHC = immunohistochemistry.

features and other aggressive characteristics. These findings highlight the significance of quantitatively assessing the hypovascular component in effectively identifying cHCC-CCA.

Different dominant components in cHCC-CCA may lead to differences in imaging and prognosis [30–32]. Sheng *et al.* [31] categorized cHCC-CCA into HCC- and ICC-predominant groups based on histopathological features bound by 50%. Observed MRI features in different groups, including arterial phase hyperenhancement (APHE) ($P < 0.001$), washout ($P < 0.001$), an enhancing capsule ($P = 0.015$), and arterial hypovascular component < 50% ($P < 0.001$), were more prevalent in HCC-predominant cHCC-CCA. In contrast, a targetoid appearance ($P < 0.001$), rim APHE ($P < 0.001$), arterial peritumoral enhancement (APE) ($P < 0.001$), vascular invasion ($P = 0.003$), and lymph node metastasis ($P = 0.013$) were more common in ICC-predominant cHCC-CCA. Xiao *et al.* [32] reached a similar conclusion, bounded by 65%. Notably, in Sheng's study [31] when compared to HCC, the presence of a targetoid appearance ($P = 0.001$), along with the absence of an enhancing capsule ($P = 0.001$) and APE ($P = 0.003$), were independent predictors suggestive of HCC-predominant cHCC-CCA. In addition, the presence of an enhancing capsule ($P < 0.001$) and intratumoral hemorrhage ($P = 0.004$), as well as the absence of a targetoid appearance ($P = 0.005$) and liver surface retraction ($P = 0.021$), were independent predictors suggestive of ICC-predominant cHCC-CCA compared to ICC. However, our patients showed the opposite results. ICC-predominant (90%) cHCC-CCA had an absence of APE (Figure 1), whereas HCC-predominant (90%) cHCC-CCA showed significant APE and the presence of an enhancing capsule (Figure 2). However, a larger database is required to verify the accuracy and generality of the conclusions. Additionally, the HCC-predominant group had a significantly better prognosis than the ICC-predominant group and patients with ICC that was not significantly associated with HCC. For the MRI LI-RADS [31, 32], LR-4/5 nodules were more prevalent in the HCC-predominant group, whereas LR-M nodules were more common in the ICC-predominant group. Interestingly, Mao *et al.* [33] analyzed the primary components of cHCC-CCA tumors using histopathological and imaging methods and revealed a moderate consistency of 66.7% between these classifications. Their study indicated that imaging-based categorization had greater prognostic significance for patients than histopathological grouping. These studies were retrospective, and prospective studies with larger sample sizes are urgently needed.

Combined diagnosis

Integrating imaging techniques (CT, MRI, or CEUS) with complementary approaches could mitigate misdiagnosis and enhance the diagnostic accuracy for cHCC-CCA [34, 35]. Combining imaging with serum tumor markers like AFP and CA19-9 is a potentially specific diagnostic method for cHCC-CCA. Li *et al.* [36] reported that when elevated tumor markers (AFP or CA19-9) conflicted with the presumed imaging results (ICC or HCC pattern), cHCC-CCA should be proposed. Yang *et al.* [26] analyzed the feasibility and efficacy of CEUS LI-RADS in conjunction with tumor biomarkers for identifying cHCC-CCA. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of this method were 40.0%, 89.9%, 1.6%–39.6%, 90.1%–99.7%, and 76.9%, respectively. Other comparable studies have reported similar findings [35, 37].

Limited data are available regarding biopsy utility in the diagnosis of cHCC-CCA. Gigante *et al.* [37] discovered that amalgamating imaging and biopsy was an effective diagnostic approach, yielding a sensitivity of 60% and specificity of 82%. For comprehensive coverage of distinct tumor regions within the nodule, extensive tumor sampling should be conducted, encompassing all observable areas, with a minimum of one tissue block/cm during visual analysis [5].

A recent multicenter study on radiomics showed that the arterial phase-based clinicoradiomics model was a feasible technique to distinguish cHCC-CCA from HCC before surgery, with an AUC of 0.863 and a specificity and sensitivity of 0.918 and 0.738, respectively [38].

Postoperative pathological diagnosis

Conventional staining (hematoxylin-eosin [HE] staining) is the primary method for the histopathological diagnosis of cHCC-CCA, while immunohistochemistry (IHC) serves as a secondary approach that provides complementary evidence [19].

Histological characteristics

HCC tumor hepatocytes are polygonal with eosinophilic granular cytoplasm and round nuclei with prominent nucleoli. The main histological features resemble those of the normal liver in terms of plate-like growth and cytology [39]. Conventional ICC is an adenocarcinoma with different morphological features, including a tubular structure, vesicle formation, and microscopic structures [40]. In contrast, classical cHCC-CCA has a closely mixed

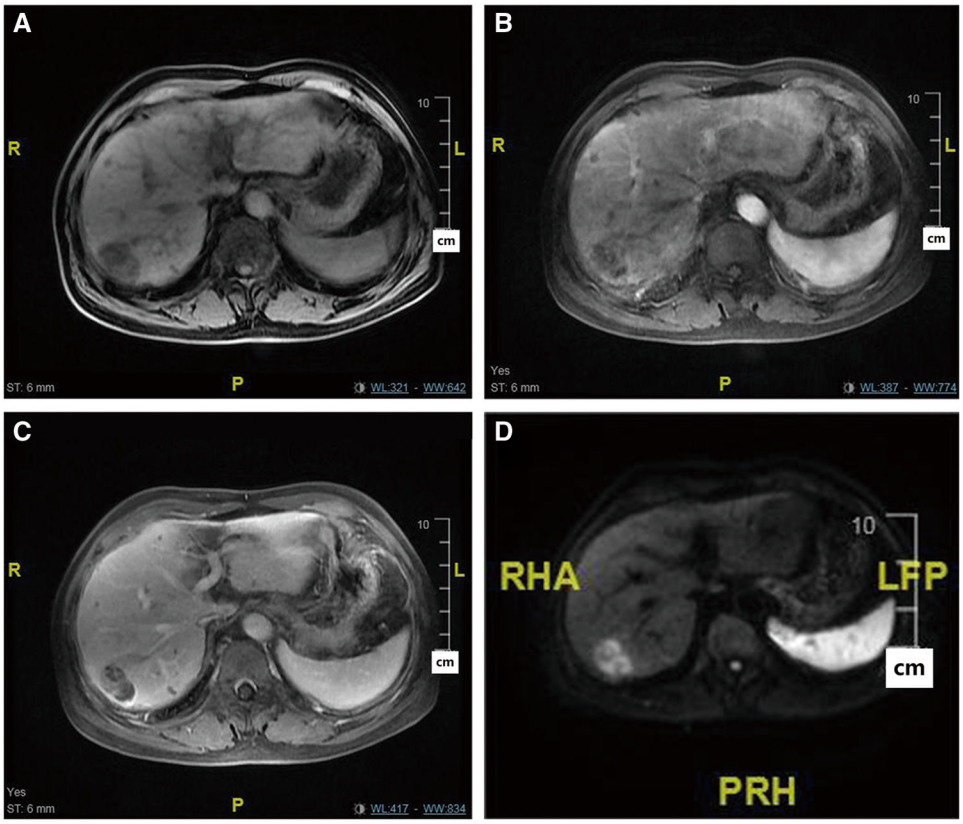

Figure 1. Combined hepatocellular cholangiocarcinoma in a 69-year-old man. (A) T1-weighted image shows a hypointense tumor. Contrast-enhanced (B) arterial phase image shows rim hyperenhancement without arterial peritumoral enhancement; (C) delayed image shows a non-washout (degressive) enhancement pattern with enhancing capsule. (D) Difusion-weighted image shows peripheral hyperintensity and central hypointensity.

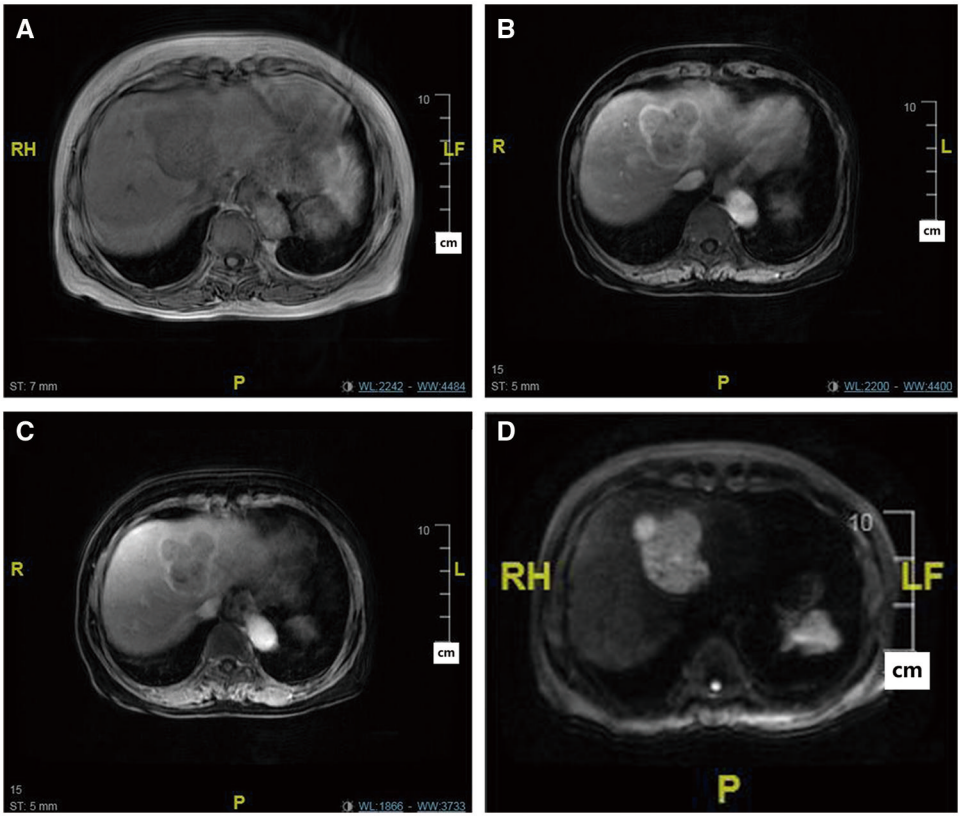

Figure 2. Combined hepatocellular cholangiocarcinoma in a 74-year-old woman. (A) T1-weighted image shows a hypointense tumor. Contrast-enhanced (B) arterial phase image shows rim hyperenhancement with arterial peritumoral enhancement; (C) delayed image shows a non-washout (degressive) enhancement pattern with enhancing capsule. (D) Difusion-weighted image shows peripheral hyperintensity and central hypointensity.

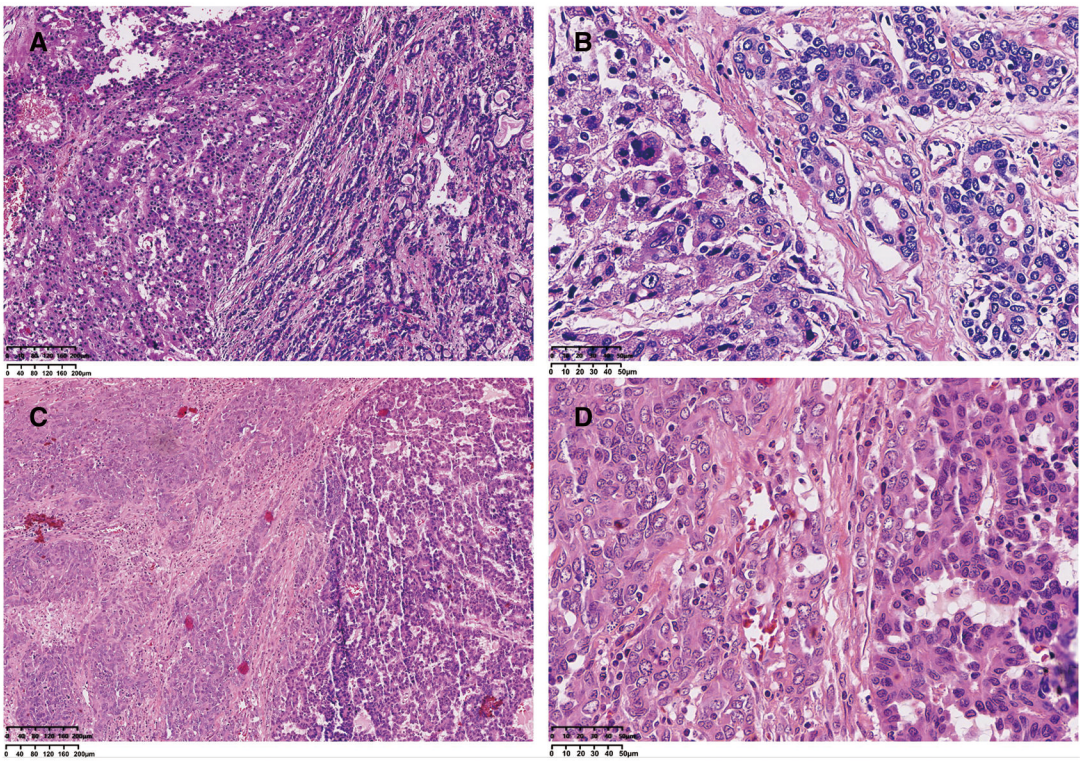

Figure 3. Hematoxylin and eosin stains for two patients with combined hepatocellular cholangiocarcinoma (cHCC-CCA). First patient, A (×10) and B (×40), shows a classical type of cHCC-CCA, HCC (left), and CCA (right). Second patient, C (×10) and D (×40), shows a classical type of cHCC-CCA, HCC (left), and CCA (right).

interface of variably differentiated adjacent (i.e. not separate foci) HCC and ICC regions and two well-defined components (Figure 3) [8, 41]. Hepatocytes are arranged in a trabecular or pseudo-glandular pattern within sparse stroma with a granular eosinophilic cytoplasm, which can be recognized by the appearance of bile-producing cells (and tubules).

In contrast, the epithelium in the ICC component forms true glandular structures, mucin production, or distinct connective tissue proliferative stomas. Detecting mucin by staining may be helpful [8, 41–43]. Intermediate tumor cells are very small and exhibit characteristics similar to HCC and ICC [8]. They are predominantly oval, have large, deeply stained nuclei and relatively abundant nucleoplasms, and lack mucin in their cytoplasm [44, 45].

Immunohistochemical characteristics

The main immunophenotypic markers of HCC include hepatocellular paraffin 1 (HepPar-1), AFP, glypican-3, polyclonal carcinoembryonic antigen (pCEA), CD10, cytokeratin 8 (CK8), and CK18 [39, 46]. ICC often expresses CK7, CK19, and epithelial membrane antigens (EMA) [40]. In contrast, cHCC-CCA has mixed ICC and HCC features. The immunohistochemical markers of the classical cHCC-CCA subtype include CK7/19, EMA, CD56, c-KIT, epithelial cell adhesion molecule (EpCAM), mucin, pCEA, CD10, AE1, and MOC31 [8, 39, 41, 42]. Hepatocyte components stain positive for HepPar-1, CD10, or pCEA; biliary components stain positive for CK7/19 and EMA. The intermediate cell subtypes express CK7/19, EMA, HepPar-1, CD56, CD133, vimentin, and EpCAM [16, 39]. However, CD133 and vimentin are expressed only in intermediate cell types [16]. In addition, Nestin is a characteristic diagnostic and prognostic marker of cHCC-CCA. Sasaki *et al.* [46] showed that Nestin expression in patients with cHCC-CCA (66.7%) was significantly higher than that in patients with large catheters and ICC (5%) ($P < 0.01$) or HCC (2.9%) ($P < 0.01$). Nestin and CK19 effectively distinguish between cHCC-CCA and HCC and are useful biomarkers of the subset of cHCC-CCAs associated with the worst clinical outcomes [47]. The median survival of patients with Nestin-positive tumors was 18.7 months, which was much lower than the median survival of 46.6 months for patients with Nestin-negative tumors ($P < 0.0001$) [22]. Therefore, Nestin has important diagnostic value, not only to identify the cHCC-CCA subgroup associated with the worst clinical outcomes but also to improve the treatment allocation of patients with this malignant tumor, which is closely related to poor prognosis.

Treatment

Surgical treatment

Hepatectomy

We have summarized the literature cited in the treatment section of this paper and the related clinical trials (Supplementary Tables S1 and S2). Surgical resection remains the primary curative treatment option for patients with cHCC-CCA, and lymph node dissection has the potential to improve survival outcomes [48, 49]. Studies have reported that the 3- and 5-year survival rates in patients with cHCC-CCA undergoing tumor resection are slightly lower than those in patients with HCC (38% and 24% vs 54% and 37%, respectively) or peripheral ICC (58% and 35%, respectively) [50].

Additionally, achieving free surgical margins of < 2 cm is crucial for enhancing prognosis [48]. Song *et al.* [51] compared laparoscopic and open hepatectomy and concluded that the former resulted in less hepatic impairment, making it a safe and effective treatment approach for select patients with cHCC-CCA, particularly those with smaller tumors. Also compared to open hepatectomy, Huang *et al.* [52] showed that robotic-assisted

radical resection of pCCA may get a larger total number of lymph nodes.

Transplantation

Analysis of the Surveillance, Epidemiology, and End Results Program (SEER) database from 1988–2009 revealed 5-year OS rates of 41.1%, 67%, and 29.0% for patients with cHCC-CCA, HCC, and ICC, respectively, who underwent liver transplantation ($P < 0.001$) [53]. Similarly, a subsequent retrospective analysis of the UNOS database reported 5-year survival rates of 40%, 62%, and 47% for patients with cHCC-CCA, HCC, and ICC, respectively [54]. Yang *et al.* [55] found liver transplantation was superior to resection for patients with HCC within the Milan criteria with a predicted high or low risk of microvascular invasion. However, cHCC-CCA lacks such comparative studies.

Adjunctive therapy

Locoregional therapy

In a small retrospective study [56], locoregional therapy (LRT) resulted in a median survival of 16 months, significantly surpassing the median survival of 5.6 months observed with systemic chemotherapy. Moreover, the study indicated that hepatic artery infusion chemotherapy (HAIC) achieved superior results compared to hepatic artery embolization chemotherapy (TACE) and transarterial radioembolization (TARE), with a partial response rate of 66% for HAIC, compared with response rates of 20% and 50% for TACE and TARE, respectively. However, the sample size for each treatment was limited to only six patients [54]. Kim *et al.* [57] evaluated 50 patients with cHCC-CCA who underwent TACE and found that 35 (70%) demonstrated a favorable response. This response was characterized by either a partial response or stable disease with successful (> 50%) tumor necrosis following TACE and a median patient survival period of 12.3 months.

However, hypovascular tumors may exhibit reduced susceptibility to the ischemic effects of TACE [58]. Considering that cHCC-CCA vascularity can vary depending on the relative predominance of HCC or ICC within an individual tumor, yttrium-90 (Y90) radioembolization has emerged as a potentially more favorable alternative [59]. Studies by Chan *et al.* [60] and Malone *et al.* [61] concluded that Y90 radioembolization is a safe and effective treatment option for unresectable cHCC-CCA. In a study by Chan *et al.* [60], the median OS for the first retreatment and initial diagnosis were 10.2 and 17.7 months, respectively, surpassing the results of systemic chemotherapy and demonstrating mild clinical toxicity. In a larger study, the disease response rate based on liver imaging was 55%, the disease control rate was 65%, the median survival was 9.3 months, and nearly all clinical toxicities occurring within 3 months were mild [61].

Chemotherapy

Combining local and systemic therapy is an alternative treatment option for advanced unresectable cHCC-CCA [62]. However, due to the rarity and complexity of cHCC-CCA, studies investigating systemic therapy options are scarce, making selecting agents with proven efficacy challenging. Standard systemic therapies for cHCC-CCA include gemcitabine and platinum-based agents (first-line chemotherapy for ICC), sorafenib and lentiviruses (standard therapies for advanced HCC), and 5-fluorouracil (5-FU) [62, 63].

Kobayashi *et al.* [64] demonstrated that platinum-containing regimens (e.g. gemcitabine plus cisplatin) were more beneficial than sorafenib monotherapy in treating unresectable cHCC-CCA (median OS: 11.9 vs 3.6 months). Similar observations were reported by Trikalinos *et al.* [62], who showed a significantly higher disease control rate (DCR) with gemcitabine, cisplatin, or oxaliplatin compared with that of gemcitabine with or without 5-FU (78.4% vs 38.5%) [62]. However, Kim *et al.* [65] argued that the objective response rate (ORR) (9.7% vs 21.6%, $P = 0.14$), median progression-free survival (PFS) (4.2 vs 2.9 months, $P = 0.52$), and median OS (10.7 vs 10.6 months, $P = 0.34$) were not significantly different between sorafenib and cytotoxic chemotherapy in cHCC-CCA. Hepatic arterial infusion of oxaliplatin plus raltitrexed may be efficacious in patients with unresectable HCC with or without PVTT but its effect on cHCC-CCA remains unclear [66]. Sorafenib may exhibit better efficacy in treating cHCC-CCA where HCC is predominant [67].

Notably, among the seven cases reported by Rogers *et al.* [68], a patient received gemcitabine and cisplatin as first-line treatments, followed by intensity-modulated radiation therapy. This was followed by fluorouracil, calcium folinic acid, and irinotecan as second-line treatments. The patient's OS was 32.8 months, which was significantly longer than that of the other six cases.

Immunotherapy

Immune checkpoint inhibitors (ICPIs) have proven effective in reversing immune failure in PLC [69]. Combination therapy with ICPIs has revolutionized the treatment of patients with advanced HCC, demonstrating efficacy in ICC; however, data on the use of ICPIs in the treatment of cHCC-CCA are still lacking, and standard systemic therapy for patients with relapsed or advanced cHCC-CCA disease has not yet been established [5, 69, 70]. Therefore, decisions regarding cHCC-CCA treatment are often extrapolated from HCC and ICC experiences [5]. The four case reports we examined involved nine patients; two had a complete or near-complete response, three had partial responses, two had stable disease, and two showed no improvement resulting from treatment discontinuation due to adverse effects [71–74]. Although immune combination therapy shows promising prospects for the combined treatment of cHCC-CCA, the number of cases is notably small.

In contrast, reports with poor efficacy have not been published, and further exploration is necessary. Nguyen *et al.* [75] identified two major immune subtypes of cHCC-CCA using cluster analysis: the “immune high” (IH) subtype (57% of cases) and the “immune low” (IL) subtype (43% of cases). In IH cHCC-CCA, the activation of genetic markers was correlated with the response to immunotherapy in patients with HCC, providing potential guidance for determining the suitability of immunotherapy for patients with cHCC-CCA.

Targeted therapy

Novel therapeutic targets and therapies continue to emerge and expand potential treatment options. Su *et al.* [76] proposed *BRCA2* mutations as potential therapeutic targets in patients with cHCC-CCA. Recent studies [77] demonstrated that a combination of 5FU and CD13 inhibitors (Adriamycin) effectively suppressed the proliferation of CD13-positive and liver cancer stem cells (LCSC), leading to a reduction in the overall tumor burden. Hence, LCSC may be promising therapeutic targets for PLC, and the potential of proton therapy in combination with other LRTs is currently being evaluated.

Rosenberg *et al.* [78] demonstrated that interleukin-6 (IL-6) trans-signaling had distinct effects on the development of liver tumors in Mdr2-KO$^{Foxl1\text{-}Cre;\ RosaYFP}$ mice, enhancing cHCC-CCA tumors while suppressing HCC growth. By targeting IL-6 using an anti-IL-6 antibody and blocking IL-6 trans-signaling, cHCC-CCA tumors were suppressed. Furthermore, inducing apoptosis in senescent cells, i.e. the source of IL-6, and using a senolytic agent

also led to the suppression of cHCC-CCA tumors. Importantly, ongoing early clinical studies are evaluating the use of senolytic agents, potentially introducing a new therapeutic approach for cHCC-CCA [79]. Most mutations discovered in cHCC-CCA, such as *TP53* or *TERT* mutations, cannot be targeted. Nonetheless, certain mutations are targetable (e.g. *EGFR* fusions or *IDH1*, *AXIN*, or *CTNNB1* mutations), and clinical trials of specific inhibitors for treating ICC or HCC are underway. Targeted therapies for HCC can also be applied to the different cHCC-CCA subtypes [80]. To date, no gene mutation-based therapies have been approved for the treatment of cHCC-CCA, emphasizing the need for further exploration of targeted therapies.

Nestin has recently emerged as a potential prognostic indicator of poor patient outcomes [22, 47]. Studies have demonstrated that treatment with *BRAF* and *MEK* inhibitors can effectively eradicate Nestin-expressing melanoma cells in human tumors [81]. Consequently, targeted therapy directed against Nestin is a promising avenue for future research.

Future perspectives and conclusion

Although cHCC-CCAs have a monoclonal origin, intratumoral heterogeneity has been observed [22]. Several studies have proposed that cHCC-CCAs may originate from the malignant transformation of hepatic progenitor cells [78]; however, transdifferentiation events cannot be dismissed [82]. Classical-type cHCC-CCA exhibits prominent ICC-like features, while IMC exhibits HCC-like features [22, 83]. Further investigations are needed to determine whether these two subtypes should be treated differently.

Despite recent advancements in the molecular characterization of cHCC-CCA, little progress has been made in the preoperative diagnosis, and significant challenges persist. Nestin has been investigated as a potential specific marker for cHCC-CCA. Differences in Nestin expression in different cHCC-CCA subtypes may be useful in distinguishing different subtypes of cHCC-CCA [84]. However, the diagnostic and prognostic value of Nestin requires further investigation. Several studies have suggested that the utility of liquid biopsy should be further explored for cHCC-CCAs [85, 86], and recent studies have demonstrated its diagnostic efficacy for HCC and ICC [87–89]. Varying results have been reported regarding EpCAM. Akiba *et al.* [16] demonstrated low EpCAM expression in the classical subtype but high expression levels in IMC.

In contrast, Wang *et al.* [24] arrived at the opposite conclusion, reporting high EpCAM expression in 80% of classical subtypes. Further investigations are required to explore these divergent findings. Characteristic mutations specific to cHCC-CCA, which are distinct from those in HCC and ICC, have not been identified [22, 24, 90], highlighting the need for further investigation of using circulating tumor DNA as a potential liquid biopsy tool.

Based on the characteristics of our dataset, imaging findings, and discussed tumor markers, we expect to build a scoring system in the future to identify patients with highly suspected cHCC-CCA in PLC. Notably, the substantial variations in sample sizes across certain datasets can potentially result in imprecise *P*-value calculation. Future advancements may be driven by emerging technologies such as deep learning algorithms [85]. However, further studies are necessary to compare the performance of these technologies with that of the existing diagnostic, staging, and predictive systems.

cHCC-CCA is a unique subgroup of primary liver malignancies. Given the distinctive HE characteristics, we recommend adopting the latest 2019 WHO categorization as a framework for subclassifying cHCC-CCA. Our database has a sufficiently large sample size that allows for comprehensive insights into the multifaceted attributes of cHCC-CCA. The distinct molecular mutations of cHCC-CCA, which distinguish it from HCC and ICC, require further investigation. The difference mutational patterns observed across different cHCC-CCA subtypes suggest that tailored therapeutic modalities may be necessary to different subtypes. MRI characteristics of cHCC-CCA remain contentious and necessitate elucidation through the augmentation of a larger clinical database. CEUS may serve as a beneficial adjunct in the diagnostic armamentarium. While the combined diagnosis enhances diagnostic precision, the possibility of missed diagnoses persists. Liquid biopsy and the application of deep learning algorithms are projected to be pivotal in the future of diagnostic hepatology. The combination of locoregional therapies with systemic chemotherapy appears to be a promising therapeutic paradigm. Present research efforts are concentrated on the exploration and development of immunological and targeted therapeutic strategies for the management of cHCC-CCA.

Supplementary Data

Supplementary data is available at *Gastroenterology Report* online.

Authors' Contributions

B.L. contributed to the review and editing, supervision, and funding acquisition. Y.Z., Y.L., J.S. and Y.H. contributed to the data collection and analysis, manuscript writing and resources. Y.Z., Y.L., and T.S. contributed to the project administration and validation. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China [82160578], the Natural Science Foundation of Jiangxi Province [20212BCJ23024], and the Health Department of Jiangxi Province [202130346].

Acknowledgements

We are thankful to all the laboratory members of the Department of General Surgery and Pathology, the Second Affiliated Hospital of Nanchang University for their helpful discussion. We thank the editage (https://www.editage.cn) for its linguistic assistance during the preparation of this manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest in this study.

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Gastroenterology Report, 2024, 12, 1–12
https://doi.org/10.1093/gastro/goae031
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