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Diagnostic value of combining serum CA19-9, CA125, CEA, and ferritin with contrast-enhanced CT features for cholangiocarcinoma

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► ***Original article***

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ABSTRACT

Background: To probe the diagnostic value of combining serum tumor markers - Cancer Antigen 19-9 (CA19-9), carbohydrate antigen 125 (CA125), carcinoembryonic antigen (CEA), and Ferritin - with contrast-enhanced computed tomography (CT) for cholangiocarcinoma (CCA). ***Materials and Methods:*** A total of 114 patients from Anhui No.2 Provincial People's Hospital from February 2020 to October 2025 were divided into a cholangiocarcinoma group (n=60) and a benign biliary lesion group (n=54). Serum levels of CA19-9, CA125, CEA, alpha-fetoprotein (AFP), and Ferritin were measured in both groups. CT imaging features of patients in both groups were analyzed. Furthermore, the diagnostic value of the serum markers alone, contrast-enhanced CT alone, and their combination was assessed. ***Results:*** Serum concentrations of CA19-9, CA125, CEA, and Ferritin were markedly enhanced in the cholangiocarcinoma group compared to the benign group. Cholangiocarcinoma typically presented as circular high-density shadows within the common bile duct or hepatic duct, accompanied by duct wall infiltration, invasion of the common bile duct, hepatic lobe atrophy, and lymph node enlargement. In contrast, benign lesions primarily appeared as circular high-density shadows with minimal duct wall invasion or lymphadenopathy. The combined approach demonstrated superior sensitivity, specificity, and accuracy for identifying cholangiocarcinoma compared to any single method. The area under the curve (AUC) (0.922) for the combined model was higher than the AUC of 0.865 for the serum marker alone. ***Conclusion:*** The integration of serum CA19-9, CA125, CEA, and Ferritin with contrast-enhanced CT findings shows promising diagnostic efficacy for cholangiocarcinoma and offers significant insights for evaluating tumor invasiveness.

INTRODUCTION

Cholangiocarcinoma (CCA) refers to a malignant tumor arising from the biliary epithelium, representing a digestive system malignancy with poor prognosis and increasing global incidence [1, 2]. The common sites of occurrence include the left and right hepatic ducts [3], with a peak incidence in individuals aged 50–70 years [4]. Due to its complex anatomical location and early propensity for perineural and local tissue invasion, complete surgical resection - the only curative option - is often unachievable at diagnosis. Consequently, early and accurate diagnosis is critical for optimizing treatment strategies and improving patient outcomes [5].

Currently, the diagnosis of CCA heavily relies on imaging. Contrast-enhanced computed tomography (CECT) is a first-line modality due to its widespread availability and rapid acquisition, enabling visualization of bile duct wall thickening, strictures, dilation, and metastatic disease, which are essential for staging and assessing resectability [6, 7]. However, the imaging features of CCA can overlap with those of benign biliary conditions such as primary sclerosing cholangitis or inflammatory strictures, leading to diagnostic ambiguity and a non-negligible risk of misdiagnosis [8].

Serum tumor markers offer a complementary, non-invasive approach. Carbohydrate Antigen 19-9 (CA19-9) is the most widely used biomarker for CCA [9], yet its elevation is also frequently observed in pancreatic cancer, other gastrointestinal malignancies, and, importantly, in benign biliary obstruction and cholangitis, compromising its specificity [10, 11]. Carcinoembryonic Antigen (CEA) and Carbohydrate Antigen 125 (CA125) show elevated expression in a subset of CCA patients but suffer from low sensitivity and specificity when used alone [12]. Recently, Ferritin, an iron-storage protein implicated in inflammation and tumor progression, has emerged as a potential diagnostic adjunct for CCA, though its standalone diagnostic performance remains to be fully defined [13]. Given the limitations of individual markers, clinical guidelines increasingly

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suggest multi-marker panels to improve diagnostic accuracy [14]. Recognizing the complementary strengths and weaknesses of imaging and serology, the integration of multiparameter serum markers with detailed CECT morphological analysis presents a promising strategy to enhance diagnostic precision [15]. Preliminary studies have suggested the potential of combined models, but a systematic evaluation of a panel encompassing CA19-9, CA125, CEA, and Ferritin alongside CECT features is lacking.

This study therefore, aims to systematically evaluate the clinical diagnostic value of combining serum CA19-9, CA125, CEA, and Ferritin with CECT features for cholangiocarcinoma. We hypothesize that this integrated model will outperform either approach alone. By assessing its diagnostic efficacy, we endeavor to establish a more reliable and optimized diagnostic strategy, providing robust evidence-based support for the early identification and differential diagnosis of this challenging malignancy.

MATERIALS AND METHODS

Clinical data

The cholangiocarcinoma group comprised sixty patients diagnosed with cholangiocarcinoma and admitted to Anhui No.2 Provincial People's Hospital from September 2020 to August 2025. Inclusion criteria: (1) All patients were diagnosed with primary cholangiocarcinoma by pathology; (2) Complete medical records; (3) All were first-time cases; (4) Informed consent has been obtained. Exclusion criteria: (1) Presence of concurrent malignancies in other organs; (2) A history of radiotherapy or chemotherapy prior to surgery; and (3) Perioperative mortality. The final cholangiocarcinoma group consisted of 60 patients (38 males and 22 females), with a mean age of 65.58±10.25 years (range: 42-87). Tumor distribution included 24 intrahepatic and 36 extrahepatic cases. According to TNM staging, there were 4, 10, 14, and 32 cases in stages I through IV, respectively. Pathological grading revealed 21 well-differentiated, 26 moderately differentiated, and 13 poorly differentiated tumors. 54 patients with benign biliary conditions were concurrently enrolled as controls (29 males, 25 females; mean age 65.39±16.37 years; range: 18-94). This group included 16 intrahepatic and 38 extrahepatic lesions, with underlying diagnoses of gallstones (n=25), bile duct cysts (n=3), Benign Biliary Tract Tumors (n=5), obstructive jaundice (n=27), cholangitis (n=19), and biliary infection (n=2). This study was approved by the Medical Ethics Committee of Anhui No.2 Provincial People's Hospital, with registration Number:2020-126.

Detection of serum tumor markers

Specific markers closely associated with cholangiocarcinoma were selected for detection. In the morning, peripheral venous blood was collected from patients after an overnight fast. Following centrifugation, the serum was collected from supernatant and stored in a -80°C refrigerator. Prior to analysis, the serum was pretreated to remove any impurities and interfering factors, ensuring the accuracy of marker detection. Serum tumor markers, such as CA19-9, CA125, CEA, Alpha-fetoprotein (AFP), and Ferritin were then detected using Siemens Atellica IM 1600 Immunoassay Analyzer using commercial enzyme-linked immunosorbent assay (ELISA) kits (CA19-9: #hz-H0488c, eBioscience; CA125: # hz-H0487c, eBioscience; CEA: # hz-R0108c, eBioscience; AFP: # CL01235, Shanghai Yaji Biotechnology Co., LTD; Ferritin: #11202, Shanghai Toujing Life Technology Co., Ltd) according to the manufacturers 'instructions.

CT examination

Contrast-enhanced CT examination was conducted at hospital admission. CT scans were performed using Siemens Healthineers' Dual Source CT with a conventional plain scan plus three-phase enhanced scan. The scanning parameters were 120 kV, 250 mA, and pitch 1. The contrast agent for the enhanced scan was ioversol at 100 mL. The injection rate of the high-pressure injector was 2.5 - 3.0 mL/s. The structures of the bile duct and surrounding organs were observed.

CT image analysis

Two radiologists, each with over five years of clinical experience, independently reviewed the CT images in a double-blind manner. The imaging features were extracted based on visual assessment. The following CT morphological features were evaluated and recorded for each patient: (1) lesion morphology (circular high-density shadow, irregular nodule); (2) bile duct wall characteristics (infiltration, irregularity); (3) tumor boundary (clear or blurred with surrounding tissues); (4) bile duct involvement (dilation, stenosis, level of obstruction); (5) local invasion (invasion of the common bile duct, cystic duct, or hepatic arteries); (6) secondary signs (hepatic lobe atrophy, enlarged pancreatic head); and (7) lymph node status (enlargement). A total of seven major feature categories encompassing the above specific findings were analyzed. Any disagreement between the two readers was resolved by consensus discussion. These assessed CT features, along with the serum marker levels, were subsequently used for the combined diagnostic analysis.

Statistical methods

All analyses were carried out using SPSS 22.0 (version 22.0; IBM Corp., Armonk, NY, USA). Continuous data are reported as mean ± standard deviation (SD) and analyzed by *t*-test, with

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categorical data presented as n (%). Significant difference was approved with a p-value below 0.05.

RESULTS

Serum marker levels

As indicated in table 1, the levels of serum CA19-9, CA125, CEA and ferritin in the cholangiocarcinoma group were higher than those in the benign lesions of common bile group (P<0.05). However, there was no significant difference in serum AFP between the two groups (P>0.05).

Table 1. Comparison of CA19-9, CA125, CEA, AFP and ferritin levels (`x±s).

Groups	n	CA19-9 (U/mL)	CA125 (U/mL)	CEA (U/mL)	AFP (μg/mL)	Ferritin (μg/L)
Cholangiocarcinoma	60	243.65 ± 31.45	126.48 ±17.52	88.69± 13.46	11.19± 3.76	841.32 ± 45.73
Benign lesions of common bile	54	63.45± 14.43	37.14± 4.55	18.12± 3.28	9.88± 1.17	162.45 ± 23.54
t		18.542	27.643	8.657	1.547	16.728
P		<0.001	<0.001	<0.001	0.253	<0.001

Note: cancer antigen 19-9 (CA19-9), carbohydrate antigen 125 (CA125), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP).

CT imaging features

Among the 54 patients with benign lesions of common bile, there were 43 cases of circular high-density shadows within the common bile duct, 31 cases of mild or moderate dilation of the bile ducts, 22 cases of "crescent sign", 16 cases of enlarged pancreatic head, 13 cases of biliary tract lower end stenosis, and 13 cases of enlarged gallbladder. See the details in figure 1A.

Among the 60 patients with cholangiocarcinoma, 31 cases presented with circular high-density shadows within the common bile duct accompanied by infiltration of the duct walls. 17 cases showed irregular nodules within the bile ducts, with unclear boundaries between the masses and the surrounding tissues. 16 cases had infiltration of the cystic duct. 16 cases had irregular duct walls of the left and right hepatic arteries. 14 cases had liver lobe atrophy. 25 cases had enlarged lymph nodes. See the details in figure 1B.

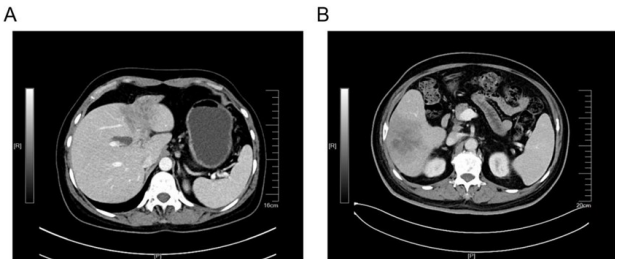


Figure 1. (A-B) Representative computed tomography (CT) images of patients with benign lesions of common bile and those with cholangiocarcinoma.

Positive detection rates

Using surgical pathology as the gold standard for the 60 cholangiocarcinoma patients, the diagnostic results were as follows. Serum tumor marker positivity was identified in 49 cases for CA19-9, 47 cases for CA125, 45 cases for CEA, and 45 cases for Ferritin. Enhanced CT yielded 48 positive cases. The combined diagnostic approach demonstrated superior performance, correctly identifying 54 positive cases.

Table 2. Combined detection in the diagnosis of cholangiocarcinoma.

Serum markers	Positive rate (cases)	Negative rate (cases)
CA19-9	81.67 (49)	18.33 (11)
CA125	78.33 (47)	21.67 (13)
CEA	75.00 (45)	25.00 (15)
ferritin	75.00 (45)	25.00 (15)
enhanced CT	80.00 (48)	20.00 (12)
Combination	90.00 (54)	10.00 (6)

Note: cancer antigen 19-9 (CA19-9), carbohydrate antigen 125 (CA125), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), computed tomography (CT).

Diagnostic performance

Based on pathological diagnosis, the combined detection of all serum biomarkers and contrast-enhanced CT yielded higher sensitivity, specificity, and accuracy for cholangiocarcinoma diagnosis than any of the tests used individually. See the details in table 3.

Table 3. Comparison of the diagnostic value for cholangiocarcinoma [%(n/m)].

Diagnostic methods	sensitivity (%)	specificity (%)	accuracy (%)
CA19-9	85.19 (46/54)	66.67 (4/6)	83.33 (50/60)
CA125	85.19 (46/54)	66.67 (4/6)	83.33 (50/60)
CEA	83.33 (45/54)	66.67 (4/6)	81.67 (49/60)
ferritin	81.48 (44/54)	83.33 (5/6)	81.67 (49/60)
enhanced CT	83.33 (45/54)	66.67 (4/6)	81.67 (49/60)
Combination	92.59 (50/54)	100.00 (6/6)	93.33 (56/60)

Note: cancer antigen 19-9 (CA19-9), carbohydrate antigen 125 (CA125), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), computed tomography (CT).

ROC curve analysis

As shown in figure 2, the combined detection has diagnostic value for cholangiocarcinoma. The AUC value was 0.922, which was higher than the AUC value of serum markers detection (0.865).

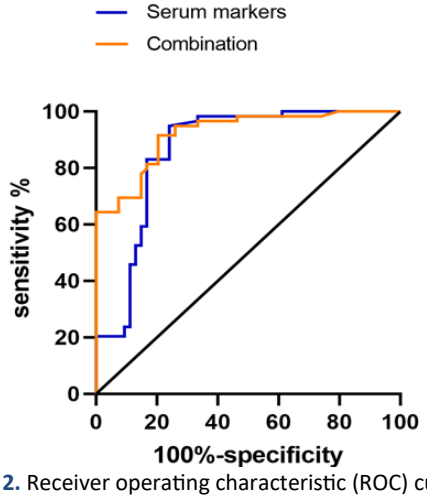


Figure 2. Receiver operating characteristic (ROC) curve of serum markers combined with contrast-enhanced computed tomography (CT) for predicting cholangiocarcinoma.

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DISCUSSION

Accurate diagnosis of cholangiocarcinoma remains a significant clinical challenge. This study evaluated an integrated diagnostic model combining a four-serum-marker panel (CA19-9, CA125, CEA, and Ferritin) with contrast-enhanced CT morphological features. Our principal finding is that this combined approach significantly outperforms either serology or imaging alone in diagnostic sensitivity, specificity, and overall accuracy (AUC 0.922), underscoring the synergistic value of multimodal assessment.

The elevated levels of CA19-9, CA125, and CEA in our CCA cohort align with their established, albeit limited, roles as biliary malignancy biomarkers [16]. The novelty of our panel lies in the inclusion of Ferritin. Recent meta-analyses have highlighted Ferritin's association with systemic inflammation and tumor progression across various cancers [17]. Our data corroborate its supplementary diagnostic value for CCA, suggesting that its elevation may reflect the underlying inflammatory tumor microenvironment [17]. The combined elevation of these markers likely captures heterogeneous aspects of tumor biology, potentially explaining the improved performance over CA19-9 alone, which is notoriously susceptible to false positives in benign biliary obstruction [18, 19].

On imaging, the CT features associated with malignancy in our study - such as duct wall infiltration, irregular margins, and concomitant lymphadenopathy - are consistent with established radiological signs of invasive carcinoma [20, 21]. These features provide critical anatomical and structural context that serum markers lack. The key advance demonstrated here is that the integration of this qualitative imaging information with quantitative serological data creates a more robust diagnostic filter. This aligns with the growing trend towards multiparametric diagnostic models in oncology [22]. While advanced radiomics or machine learning models are emerging [23, 24], our study demonstrates that a pragmatic combination of routinely available visual CT assessment and common serum tests can yield substantial diagnostic gain, enhancing its immediate clinical translatability.

The superior AUC of the combined model underscores a fundamental principle: serological markers (reflecting biological activity) and anatomical imaging (depicting structural consequences) provide complementary information [25]. One modality mitigates the weaknesses of the other - imaging helps confirm malignancy in the face of non-specific serological elevations, while positive serology can raise suspicion even when imaging findings are equivocal [26]. This synergy is crucial for differentiating CCA from benign strictures, a common diagnostic dilemma [27].

Limitations of this study must be acknowledged. First, its retrospective, single-center design may introduce selection bias and limit the generalizability of our findings. Second, the sample size, particularly of the benign control group, is moderate; validation in a larger, prospective multicenter cohort is necessary. Third, the assessment of CT features was qualitative and dependent on radiologist experience, introducing an element of subjectivity. Future studies could incorporate quantitative radiomic features from CT or explore the added value of other imaging modalities like MRI. Finally, while our marker panel was selective, other promising biomarkers or liquid biopsy components were not evaluated [28].

CONCLUSION

In conclusion, the combination of serum CA19-9, CA125, CEA, and Ferritin with standard contrast-enhanced CT features significantly improves the diagnostic accuracy for cholangiocarcinoma. This integrated, clinically practical model can serve as a more reliable tool for pre-operative evaluation, aiding in timely diagnosis and treatment planning. Future work should focus on prospective validation and refining the model with additional biomarkers and advanced imaging analytics.

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Conflict of Interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Ethical approval: This study was approved by the Medical Ethics Committee of Anhui No.2 Provincial People's Hospital, Number:2020-126. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Author contributions: All authors contributed equally throughout the work and preparation of the manuscript. All authors read and approved final version of the manuscript submitted for publication.

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