BMC Biotechnology

RESEARCH

Open Access

Development of a chitosanase 3-like protein 1 assay kit and study of its application in patients with hepatocellular carcinoma

Min Liu[1,3†], Yanru Qiu[1,3†], Erfu Xie[4], Pu Qian[1,3], Shuxian Yang[4], Simin Zhao[1,3], Wenjun Yan[1,3], Xuan Huang[1,3*] and Shuang Han[2,3*]

Abstract

Objective The detection kit for plasma Chitinase-3-like Protein 1 was developed using the magnetic bead chemiluminescence method, in order to investigate the diagnostic value of DD, FDP, CHI3L1, AFP-L3 and PIVKA-II in hepatocellular carcinoma.

Method The CHI3L1 detection kit was developed using the chemiluminescence method. The luminescence value obtained from the chemiluminescence analyzer was utilized for sensitive detection of CHI3L1, and the performance of the kit was evaluated accordingly. Moreover, this study enrolled 200 patients with hepatocellular carcinoma who were treated at the Oncology Department of the Affiliated Hospital of Jiangnan University between August 2022 and November 2023 as study subjects, while 100 healthy individuals undergoing physical examinations during the same period served as a control group. The plasma CHI3L1 levels in these subjects were measured using our institute's developed kit. Simultaneously, DD, FDP, AFP-L3, and PIVKA-II levels were assessed in all subjects to investigate their relationship with general pathology in patients with hepatocellular carcinoma. Additionally, ROC curves were generated to evaluate both single and combined detections' diagnostic efficacy for hepatocellular carcinoma.

Result The serological index changes of DD, FDP, AFP-L3, PIVKA-II, and CHI3L1 were not associated with patient gender. The concentrations of AFP-L3 and PIVKA-II in the 45–59 age group were significantly higher than in other groups ($P < 0.05$). Additionally, DD, CHI3L1, and PIVKA-II levels were markedly elevated in patients with tumors > 5 cm, medium-to-high differentiation, nerve invasion, lymph node metastasis, or distant metastasis. In advanced liver cancer (stages III–IV), DD, FDP, and CHI3L1 concentrations were significantly higher than in early-stage patients (stages I–II). For single diagnostic analysis, the AUC for CHI3L1 was 0.923, while the combined AUC for all five indices was 0.961, indicating greater diagnostic value when used together. The CHI3L1 chemiluminescence detection kit had a minimum detection limit of 1.50 ng/mL, with precision and accuracy within 10%, and $R > 0.99$. Compared to a clinical reference kit, the correlation coefficient (R) was 0.994, meeting clinical performance evaluation criteria.

†Min Liu and Yanru Qiu shared as co-first authors.

*Correspondence:
Xuan Huang
xghx.2007@163.com
Shuang Han
han_frost@163.com

Full list of author information is available at the end of the article

Conclusion The CHI3L1 chemiluminescence kit developed meets clinical requirements. CHI3L1 can be used as an indicator for early screening of liver cancer, and the detection value of combined five indicators DD, FDP, AFP-L3, PIVKA-II and CHI3L1 is higher than that of single detection.

Keywords Chitinase-3-like Protein 1, Chemiluminiscence, Kit development, Hepatocellular carcinoma, Predictive value

Introduction

In recent years, the mortality rate of Hepatocellular Carcinoma (HCC) has continued to increase, and it currently ranks as the third leading cause of cancer-related deaths globally, accounting for approximately 800,000 fatalities annually [1]. A comprehensive review of relevant systematic studies reveals that early-stage HCC can be effectively managed and potentially cured; however, late-stage HCC is characterized by limited therapeutic options and a poor prognosis. The detection of HCC is intricately linked to early tumor identification, curative interventions, and an overall improvement in survival rates [2–5]. Current biomarker-based and imaging-driven technologies designed for identifying individuals at high risk of developing HCC and detecting the disease at its earliest stage are outdated and lack sensitivity [5]. Clinical symptoms, physical examinations, imaging screenings, and routine laboratory indicators alone are insufficient for the specific detection of HCC [6], often leading to missed opportunities for optimal treatment timing among patients [7, 8]. Consequently, the development of non-invasive serological detection markers or marker combinations that are safe, highly specific, and sensitive holds substantial clinical value for early detection, treatment optimization, and prognostic assessment of HCC.

Chitinase-3-like Protein 1 (CHI3L1), which plays a pivotal role in various diseases, exhibits particularly high expression levels in liver tissue and is involved in inflammatory responses and tissue remodeling. The abundant secretion of CHI3L1 has been observed in osteosarcoma cells MG63 [9], leading to increased attention on its involvement in tumor development. Relevant studies have demonstrated that CHI3L1 participates in the proliferation, invasion, and metastasis of liver cancer cells [10]. The utility of Alpha Fetoprotein (AFP), currently used as a routine screening indicator for HCC, has been challenged due to its elevation also being associated with other malignant diseases [11]. Lens Culinaris Agglutinin-reactive Fraction of AFP (AFP-L3), an isomer of A-fetoprotein, has shown promise for early detection of HCC but is presently only included in routine detection protocols within Japan [12]. Another important indicator recognized by the Whole Liver Society of Japan for early diagnosis of liver cancer is Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II), primarily synthesized by the liver when there is a deficiency of vitamin K within the human body [13]. Fibrin Degradation Product (FDP) and D-Dimer (DD), coagulation markers commonly used for routine coagulation tests, have been found to be related to cancer. Studies have indicated that DD can be utilized for diagnosing liver cancer patients while FDP has proven helpful in distinguishing between liver cancer and cirrhosis through combined detection methods [14–16]. These findings suggest that further investigation should explore the potential use of CHI3L1, AFP-L3, PIVKA-II, FDP, and DD as diagnostic indicators for liver cancer; however, no studies comparing their predictive value have been conducted yet. Additionally, their combined diagnostic value remains largely unexplored.

There are various methods available for the detection of serum CHI3L1 [17, 18]. Among these, enzyme-linked immunosorbent assay (ELISA) is commonly employed for quantitative measurement of serum CHI3L1; however, its clinical utility is limited due to prolonged processing time and complex procedures [6]. Subsequently, immunochromatography incorporating fluorescent microsphere labeling technology was developed as a simpler and cost-effective alternative for CHI3L1 detection [18]. Nevertheless, further improvements are required to enhance accuracy and specificity. Chemiluminescence immunoassay (CLIA), characterized by rapid detection time and high specificity, has gained increasing popularity in routine clinical applications.

Materials and methods

Main reagents and materials

The CHI3L1 antigen, antibody, calibrator and luminescent substrate were provided by Jiangsu Bming Biotechnology Co., LTD. (Wuxi, China), and the magnetic bead reagent was purchased from Thermo Mercer Technology Co., LTD. (China). The automatic chemiluminescence analyzer BM 1800 was purchased from Jiangsu Biming Biotechnology Co., LTD. (Wuxi, China), the board washing machine was purchased from Thermo Fisher Technology Co., LTD. (China), and the pH meter was purchased from METTler Toledo (Switzerland).For the preparation method of raw materials during the experiment, see Supplementary Material 1.

Reaction process

The reaction principle of CHI3L1 detection process is double antibody sandwich method, adding 30 μL samples, 80 μL HRP labeled antibodies, 80 μL biotinylated antibodies, mixing, incubating at 37 ℃ for 30 min,

adding 30 μL magnetic beads for 5 min, magnetic separation with magnetic field, cleaning for 3 times. Substrate was added to detect luminescence value (RLU).(Fig. 1)

Study population

Clinical and pathological data and blood samples of 200 patients who were pathologically confirmed to carcinoma and were treated at the Affiliated Hospital of Jiangnan University from August 2022 to November 2023 were collected for the study, which was approved by the Institutional Ethics Committee of the Affiliated Hospital of Jiangnan University. Their basic clinical information was collected, including 149 males and 51 females with an age range of 41–85 years. Blood samples were collected from 100 healthy medical checkups during the same period to compare the detection values of DD, FDP, AFP-L3, PIVKA-II, and CHI3L1 in HCC patients with hepatocellular carcinoma. The patient inclusion and exclusion criteria were as follows:

Inclusion criteria: (1) pathologically confirmed diagnosis of hepatocellular carcinoma; (2) patients with complete clinical data and follow-up data; and (3) patients' informed consent.

The exclusion criteria were as follows: (1) cholangiocellular carcinoma or mixed hepatocellular carcinoma; (2) a combination of severe heart, lung, kidney, and other vital organ diseases; and (3) the use of procoagulant or anticoagulant drugs for various reasons prior to sampling.

Data analysis

Data were analyzed and plotted using SPSS 26.0 and Graph Pad Prism 10.0. Normally distributed continuous variables are expressed as mean ± standard deviation, and non-normally distributed variables are expressed as median [quartiles (P25, P75)]. The Mann-Whitney U test was used to compare differences between groups. The diagnostic efficiencies of DD, FDP, AFP-L3, PIVKA-II, and CHI3L1 were evaluated using the subject's work characteristic (ROC) curve, and the maximum Jordon's index was used as the critical value. Statistical significance was set at $P < 0.05$.

Results of CHI3L1 chemiluminescence detection kit development

Optimization of antibody working concentration

When the paired antibodies were screened, the optimal working concentration needed to be determined. Based on the checkerboard method, different calibrator concentrations were used as samples for the test, and under the premise of guaranteeing the experimental effect, we chose a lower antibody concentration to reduce the cost. Under the condition that the concentration of the biotin antibody of CHI3L1 was 2 ng/mL and the concentration of the HRP antibody was 1.5 ng/mL,the signal values of each standard were the most appropriate, and the ratio between them was closer, as shown in Supplementary Table 1.

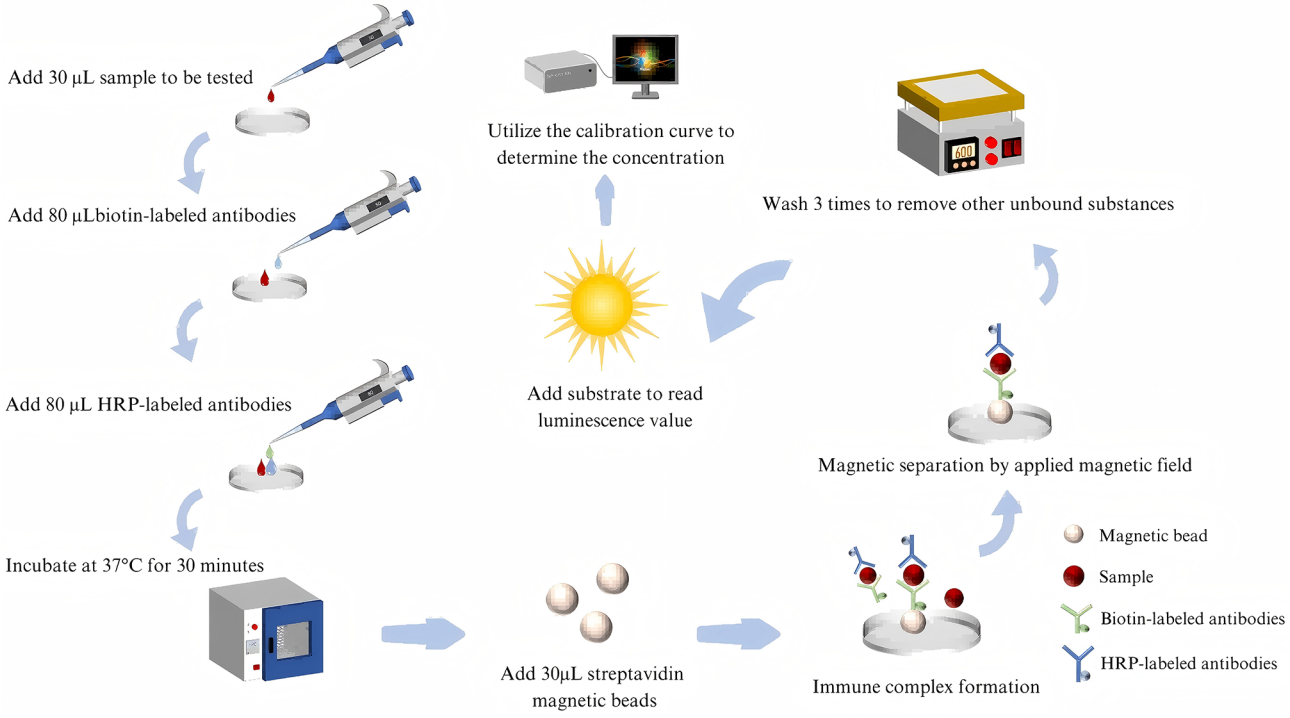

Fig. 1 CHI3L1 test flow chart

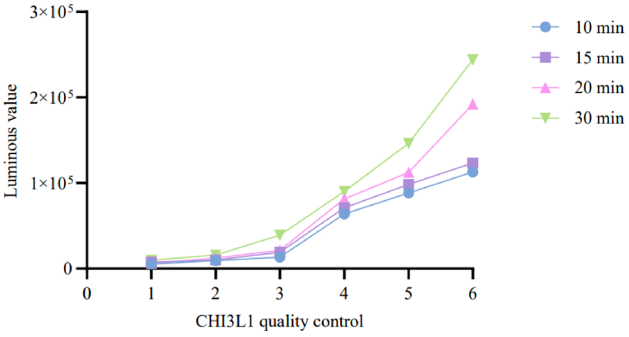

Fig. 2 Experimental results of CHI3L1 kit incubation time at 37 ℃

Table 1 Results of clinical performance evaluation of CHI3L1 kit

Minimum Detection Limit	Test 1	Test 2	Test 3
M	73695.70	72121.95	67874.05
SD	3564.94	4028.29	5985.90
M + 2SD	80825.57	80178.53	79845.85
LOD(ng/mL)	1.50	1.44	1.39
Accuracy	Concentration(ng/mL)	Average(ng/mL)	Bias(%)
	3.00	3.01	5%
	54.00	54.38	−2%
Precision	Average(ng/mL)	Fitted Concentration CV	Deviation of fitted concentration
	3.10	4.2%	3%
	56.01	2.4%	4%
Acceleration Stability	Bias		
Time	Test 1	Test 2	Test 3
1 Day	2%	4%	5%
	2%	−1%	2%
3 Day	−3%	3%	6%
	1%	−1%	2%
6 Day	−5%	3%	6%
	1%	−3%	3%
10 Day	2%	4%	−5%
	3%	2%	−2%
Real-time Stability	CV value		
Month	S2	S4	S6
3	5.91%	2.10%	5.41%
6	7.67%	3.92%	7.70%
9	7.53%	2.36%	7.01%
12	5.61%	1.38%	5.88%

Optimum incubation time

Different times had an effect on RLU. Controls 1–6 were unfolded and assayed at different time points at the same time, and the CHI3L1 results are shown in Supplementary Table 2. below and Fig. 2.When incubated at 37 ℃ for 30 min, the signal value of each calibrator of CHI3L1 was higher overall, which improved the overall detection efficiency. Therefore, the reaction conditions for CHI3L1 kit were set at 37 ℃ for 30 min.

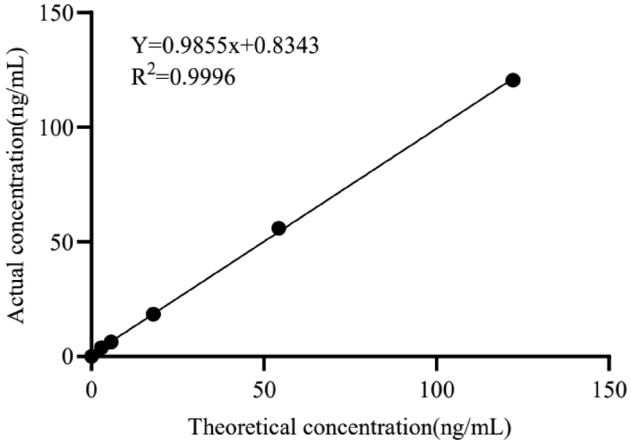

Fig. 3 Linear regression analysis of the CHI3L1 kit

Minimum detection limit, accuracy, precision, accelerated stability, real-time stability results

According to the performance evaluation conducted in this study, the developed CHI3L1 detection kit demonstrated a minimum detection limit of 1.50 ng/mL. The kits exhibited a relative deviation of less than 5%, with a CV coefficient of 4.2 % for low-value quality control batch and 1.8% for high-value quality control batch, indicating high precision and accuracy that meet clinical requirements. The luminescence values were measured three times at both 37 ℃ and 4 ℃ over different time intervals (1 day, 3 days, 6 days, and 10 days), yielding a calculated relative error (B%) within ± 10%, satisfying test requirements. Furthermore, real-time stability measurements revealed that the CHI3L1 kit could be effectively stored at room temperature for up to 12 months.The results of the performance evaluation tests of the CHI3L1 kit in this study are summarized in Table 1 below.

Linearity

The linear regression equation of the CHI3L1 test kit is y = 0.9855x + 0.8343, as depicted in Fig. 3. The high correlation coefficient (R = 0.9997) indicates a robust and significant linear relationship between the theoretical and actual concentration of the sample.

Comparison of clinical samples

In order to validate the detection efficacy of the kit developed in this study, plasma samples were collected from 150 patients (provided by the Affiliated Hospital of Jiangnan University). The CHI3L1 detection kit (model: CH-100Z) provided by Hangzhou Proprium Biotech Co., LTD. and affiliated with the hospital was employed for analysis, and the resulting data were exported. The measured values of plasma CHI3L1 using our self-made kit

were plotted on the horizontal axis, while those obtained from hospital measurements served as the vertical axis for comparative fitting. Linear regression analysis yielded a correlation coefficient $R^2 = 0.9875$, exceeding 0.95 (Fig. 4). These findings demonstrate that our self-made CHI3L1 kit exhibits excellent linear correlation with existing detection reagents and fulfills clinical detection requirements.

Results of serum markers in the detection of hepatocellular carcinoma

Status of research subgroups

This study was conducted on 200 patients with liver cancer, of whom 149 (74.5%) were male and 51 (25.5%) were female. The elderly accounted for a large proportion in this study. There were 144 (72%) people aged 60 years and above, 54 (27%) people aged 45–59 years, and only 2 (1.0%) people aged 44 years and below. The above data suggest that the elderly have a the high incidence of hepatocellular carcinoma, and the TNM staging data show that there are 28 cases of patients with the severity of disease reaching stage I, 45 cases of patients with the severity of disease reaching stage II, and 127 cases of patients with disease reaching stage III–IV. Refer to Supplementary Table 3 for details.

Relationship between age, gender and the five indicators

As shown in Supplementary Table 4, DD, FDP, and CHI3L1 did not differ with respect to age, and AFP-L3 and PIVKA-II had significantly higher values between the ages of 45–59 years than in the other age groups ($P < 0.05$).As shown in Supplementary Table 5, DD, FDP, CHI3L1, AFP-L3, and PIVKA-II did not differ significantly by sex ($P > 0.05$).

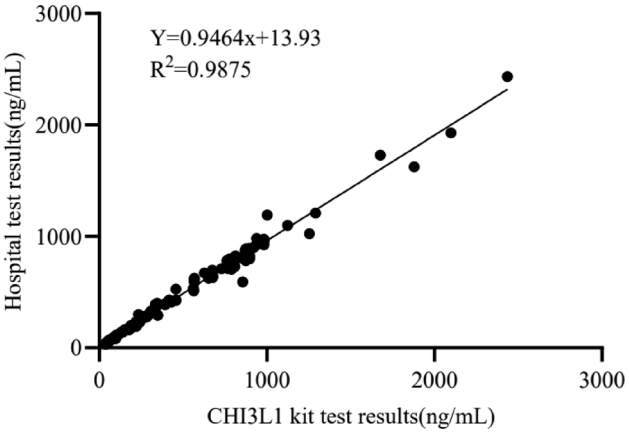

Fig. 4 Comparison of test results between CHI3L1 homemade reagents and listed products

Relationship of the five indicators to general pathology

Relationship between plasma DD levels and general pathologic features in patients with hepatocellular carcinoma

The correlation between serum DD concentration and its overall clinicopathological characteristics was investigated in 200 HCC patients using the rank-sum test. The results revealed significantly higher levels of DD in patients with tumor sizes > 5 cm, moderately and highly differentiated tumors, nerves with invasion, lymph node metastasis, and distant metastasis ($P < 0.001$ or $P < 0.01$). There was no significant difference in DD levels in patients with MVI and presence of peritoneum ($P > 0.05$). Please refer to Supplementary Table 6 for further details.

Relationship between plasma CHI3L1 levels and general pathological characteristics of hepatocellular carcinoma patients

The level of CHI3L1 was significantly higher in patients with a tumor size > 5 cm, moderate to high differentiation, nerve invasion, lymph node metastasis, and distant metastasis. The difference was statistically significant ($P < 0.001$). There was no significant difference in CHI3L1 in MVI and the presence or absence of peritoneum ($P > 0.05$), as shown in Supplementary Table 7.

Relationship between plasma PIVKA-II levels and general pathologic features in patients with hepatocellular carcinoma

The level of PIVKA-II was significantly higher in patients with only distant metastases, and the difference was statistically significant ($P < 0.001$ or $P < 0.01$). There was no significant difference in PIVKA-II in tumor size, MVI, presence or absence of peripheral medium and high differentiation, presence or absence of nerve invasion, and presence or absence of lymph node metastasis ($P > 0.05$). For details, please refer to Supplementary Table 8.

Relationship between plasma AFP-L3 levels and general pathologic features in patients with hepatocellular carcinoma

There was no significant difference in AFP-L3 levels among the various tumor size categories, presence of microvascular invasion (MVI), and peritumoral medium and high differentiation ($P > 0.05$). Please refer to Supplementary Table 9 for detailed information.

Relationship between plasma FDP levels and general pathologic features in patients with hepatocellular carcinoma

The level of FDP was significantly higher in patients with a tumor size > 5 cm, medium-high differentiation, nerve invasion, lymph node metastasis, and distant metastasis. The differences were statistically significant ($P < 0.001$ or

Table 2 Work characterization curve (ROC) analysis of subjects with hepatocellular carcinoma

Markers	Cut-off values	AUC(95%CI)	Sensitivity(%) (95%CI)	Specificity(%) (95%CI)	Youden Index	FPR(%)	FNR(%)
AFP-L3	17.28 ng/mL	0.849(0.804–0.893)	77.4(84.5–87.3)	87.0(50.1–60.7)	0.644	13	22.6
PIVKA-II	61.67 μg/L	0.582(0.517–0.647)	41.2(18.3–22.0)	88.0(64.3–71.4)	0.292	12	58.8
D-Dimer	0.600 mg/L	0.687(0.622–0.752)	70.4(64.3–71.4)	63.0(50.4–59.7)	0.334	37	29.6
FDP	2.35 mg/L	0.776(0.719–0.833)	92.5(34.8–46.8)	52.0(74.7–84.9)	0.445	48	7.5
CHI3L1	58.575 μg/L	0.923(0.890–0.957)	84.9(94.1–96.2)	89.0(46.9–57.2)	0.739	11	15.1

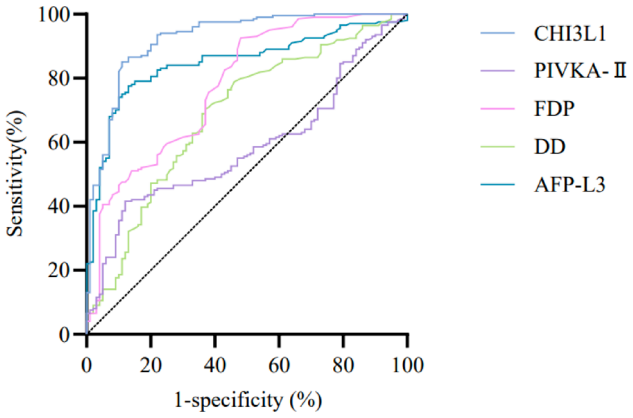

Fig. 5 Tumor marker single diagnostic ROC curves

P < 0.01). There was no significant difference in FDP in MVI and with or without peritoneum (P > 0.05). Please refer to Supplementary Table 10 for details.

Relationship between five indicators and staging of HCC patients

In this study, the correlation between the levels of DD, CHI3L1, FDP, AFP-L3, and PIVKA-II and their clinical stages and prognosis was analyzed using the non-parametric rank sum test with HCC as the study population. The results of the analysis revealed that the values of DD, FDP, and CHI3L1 were significantly higher in late-stage patients (stage III–IV) than in early-stage patients (stage I–II), with a statistically significant difference (P < 0.001). However, AFP-L3 and PIVKA-II did not show a significant difference in staging (P > 0.05). Please refer to Supplementary Table 11 for details.

Individual and combined diagnostic value of five index levels in patients with hepatocellular carcinoma

Individual diagnostic value

For the diagnosis of hepatocellular carcinoma, the area under the curve (AUC) of AFP-L3, PIVKA-II, DD, FDP, and CHI3L1 were 0.849, 0.582, 0.687, 0.776, and 0.923 respectively (Table 2 and Fig. 5). Among these markers, FDP exhibited the highest sensitivity at 92.5%, while CHI3L1 demonstrated the highest specificity at 89%.

Combined diagnostic value

For the combined diagnosis of hepatocellular carcinoma, the area under the curve (AUC) of DD + FDP, AFP-L3 + PIVKA-II + CHI3L1, and AFP-L3 + PIVKA-II + CHI3L1 + DD + FDP were 0.788, 0.958, and 0.961, respectively. For more details, please refer to Table 3 and Fig. 6.

Discussion

HCC is a common malignant tumor with low early diagnosis rates. This study recruited 200 HCC patients (3:1 male-to-female ratio) to evaluate novel biomarker combinations for early diagnosis and prognosis.

The onset of HCC is not age-limited. This study found that AFP-L3 and PIVKA-II levels were significantly higher in the 45–59 age group compared to other groups (P < 0.05), indicating a need for special attention to these markers in this age range. Prior studies showed that CHI3L1, DD, and FDP levels in liver cancer patients correlate closely with clinical stage, with significantly higher values in stages III–IV than in stage II (P < 0.001).

Currently, HCC early screening relies on color ultrasound and AFP markers, but their sensitivity for early detection is low. The sensitivity and specificity of ultrasound alone in diagnosing early HCC were 47% and 91% respectively. The sensitivity and specificity of AFP are 52.9% and 93.3% [19]. Afp-l3 is used for early HCC detection with a sensitivity of 77.0% and specificity of 87%, which is higher than traditional AFP [20]. AFP-L3 is mainly used for the early diagnosis of tumors < 2 cm in diameter, but its manifestations have not changed significantly with the change of stage, which is consistent with previous studies.CHI3L1 levels significantly increase in

Table 3 Analysis of subjects' work characteristic curves (ROC) for the co-diagnosis of five hepatocellular carcinoma markers

Markers	AUC (95%CI)	Sensitivity(%) (95%CI)	Specificity(%) (95%CI)	Youden Index	FPR(%)	FNR(%)
Combination of two indicators	59.0(0.729–0.846)	59.0(54.7–62.1)	94.0(66.8–72.8)	0.53	6	41
Combination of three indicators	86.0(0.935–0.981)	86.0(65.0–72.0)	95.0(76.0–82.7)	0.81	5	14
Combination of five indicators	84.0(0.939–0.982)	84.0(65.1–72.1)	97.0(76.2–82.9)	0.81	3	16

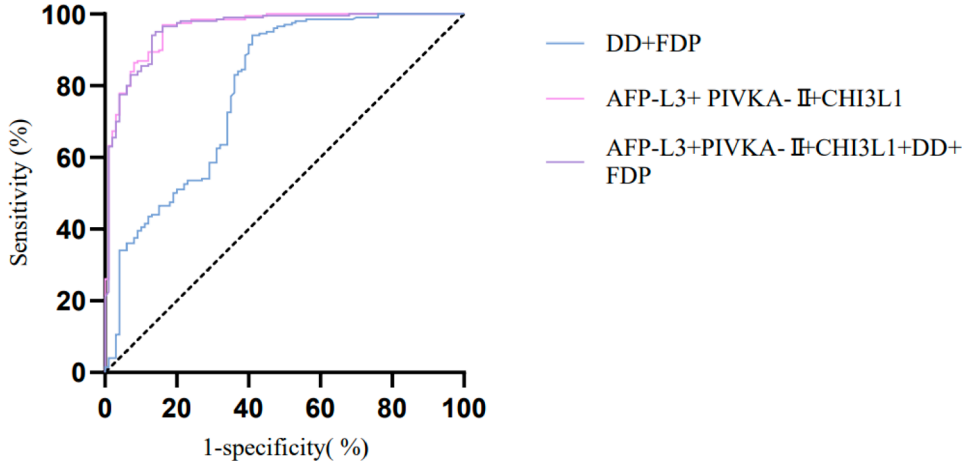

Fig. 6 Tumor marker co-diagnostic ROC curves

patients with tumors > 5 cm, lymph node metastasis, or nerve invasion, suggesting its potential as a prognostic indicator for liver cancer.

This study of serum marker in patients with liver cancer single ROC detection is analyzed, the results are as follows:

CHI3L1 is important to promote vascular growth factors, can promote the tumor blood vessel formation and regeneration. Liver cancer cell line BEL7404 transfected with CHI3L1 can inhibit liver cancer proliferation and angiogenesis [21]. ROC curve showed that the area under the curve of CHI3L1 was 0.923, sensitivity 84.9%, specificity 89.0%, which was better than AFP-L3(area under the curve 0.849, sensitivity 77.4%, specificity 87.0%), indicating that CHI3L1 was a better diagnostic indicator for liver cancer and could complement AFP-L3.

HCC patients may suffer from vitamin K deficiency or difficulty in absorption, and vitamin K enters cells through the endocytosis of F-actin. However, F-actin rearrangement in HCC patients leads to the abnormal absorption of vitamin K, resulting in a large amount of PIVKA-II expression [22]. In this study, the specificity of PIVKA-II in the diagnosis of liver cancer was 88%, which was better than the traditional index AFP-L3. Previous studies have shown that the efficacy of PIVKA-II in the diagnosis of liver cancer is better than that of AFP, DD and FDP [14], but the sensitivity of PIVKA-II in this study was the lowest (41.2%), which may be due to the lack of vitamin K-related screening in the early stage of this study. Combined with the literature and the results of this study, PIVKA-II as a single indicator has limitations in the diagnosis of liver cancer, and screening for vitamin K deficiency and the use of related drugs should be conducted before detection.

In conclusion, after combining the three indexes, the area under the curve (AUC) of AFP-L3 + PIVKA-II + CHI3L1 was 0.958, and the sensitivity and specificity were improved, and the combined diagnostic value was higher than the single diagnostic value.

Zhang Ying [12] et al. investigated DD and FDP changes in HCC patients with cirrhosis. Results showed that in HCC patients with cirrhosis and hepatitis B, DD and FDP levels increase with disease progression. This study combined DD and FDP with AFP-L3, CHI3L1, and PIVKA-II, key markers in HCC research, to evaluate their diagnostic value alone and in combination. The ROC curve analysis revealed AUC values of 0.788 for DD + FDP, 0.958 for AFP-L3 + PIVKA-II + CHI3L1, and 0.961 for the full combination. While combined detection outperforms single markers, adding DD and FDP to the three-marker panel does not significantly improve sensitivity or specificity.

According to the above results, HCC patients with coagulation abnormalities complicated with coagulation risk or a history of hepatitis B infection are recommended to combine five tests for liver cancer, and other patients are recommended to combine AFP-L3 + PIVKA-II + CHI3L1 three tests.

With advancements in testing technology, the CHI3L1 detection kit uses chemiluminescence for cost-effective and precise measurements. It shows high consistency with traditional reagents, maintaining stability within 10% at room temperature for up to 12 months. The kit reduces blood dilution steps, minimizing errors and lowering costs, which alleviates the economic burden on patients. Its performance meets clinical standards, ensuring quick detection times and offering significant value for clinical use.

The potential clinical challenges associated with this assay include the initial requirement to procure specialized instruments, which may impose an economic burden on resource-limited medical institutions. Additionally, the relatively low penetration of such instruments in primary hospitals and remote areas may necessitate reliance on external laboratories for testing, thereby extending the

diagnostic cycle. Clinicians, on the other hand, tend to prefer established detection algorithms, and their acceptance of novel tests is contingent upon robust evidence from evidence-based medicine. These challenges highlight specific directions for future research, such as the development of cost-effective, portable chemiluminescence equipment or the implementation of a centralized detection center model for cost-sharing. Furthermore, multi-center studies should be expanded to validate the diagnostic potential of CHI3L1 in liver cancer. The clinical significance of CHI3L1 should also be disseminated through academic conferences and guideline updates to foster multidisciplinary collaboration.

Conclusion

It is of great clinical significance to find new and reliable serological diagnostic and prognostic indicators for the diagnosis and detection of liver cancer. This study confirmed that AFP-L3, PIVKA-II, CHI3L1 combined with DD and FDP are early noninvasive detection methods for detecting coagulation abnormalities, hepatitis B infection and cirrhosis in HCC patients, and this method has good sensitivity and specificity. Considering the economic affordability of patients, the combination of AFP-L3, PIVKA-II and CHI3L1 can be used as early diagnosis indicators for patients with liver cancer, which is of great significance for early diagnosis and timely treatment of patients. For high-risk patients with coagulation abnormalities complicated with coagulation risk or a history of related hepatitis B infection, it is recommended to combine five tests for liver cancer. Given that CHI3L1 levels are elevated in HCC patients with tumors > 5 cm, lymph node metastasis, and neuroaggression, it may be used as a tool for prognostic assessment of liver cancer patients, and relevant studies on prognostic assessment can be further conducted in the future. At the same time, the performance of the CHI3L1 chemiluminescence kit developed in this study meets the clinical design requirements, no longer requires blood dilution, and can be directly detected on the machine to shorten the detection time and simplify the detection steps, which can meet the clinical detection needs.

Abbreviations

CHI3L1	Chitosanase 3-like Protein 1
AFP-L3	Alpha-fetoprotein Heterodimer 3
PIVKA-II	Protein Induced by Vitamin K Absence or Antagonist-II
D-D	D-Dimer
FDP	Fibrin Degradation Products
HCC	Hepatocellular Carcinoma
CLIA	Chemiluminescence Immunoassay
MVI	Microvascular Invasion
HRP	Horseradish Peroxidase
RLU	Relative Light Units
M	Mean
SD	Standard Deviation
LOD	Limit of Detection
CV	Coefficient of Variation
ROC	Receiver Operating Characteristic
AUC	Area Under the Curve
FPR	False Positive Rate
FNR	False Negative Rate

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s12896-025-00970-w.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7
Supplementary Material 8
Supplementary Material 9
Supplementary Material 10
Supplementary Material 11
Supplementary Material 12

Acknowledgements

We are grateful for the cooperation of medical colleagues and researchers in the Affiliated Hospital of Jiangnan University, as well as for the equipment and technical support provided by Jiangsu Baiming Biological Co., Ltd.

Author contribution

YR Q: Data management, survey administration, verification processes, data visualization, and initial draft composition; S Z, WJ Y,EF X, P Q and SX Y: Responsible for trial and clinical sample collection and management; S H and M L: conceptualization, methodological framework development, writing-review and editing, project supervision, and verification; X H: resource allocation, project management oversight, funding acquisition strategies, and supervisory roles.

Funding

This work was supported by the Wuxi Institute of Translational Medicine (No. YJZ202304), the Science and Technology Program of Wuxi Municipal Health and Family Planning Commission (T202237) and the Wuxi Medical Innovation Team (No. CXTDPY2021003) as well as the General Project of Open Subjects of Shanghai Key Laboratory of Molecular Imaging (No. KFKT-2023-35).

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki. The study was approved by Medical Ethics Committee of The Affiliated Hospital of Jiangnan University (Wuxi, Jiangsu, China) (Ethics: LS202409), and written informed consent was obtained from all participants before enrolment in the study.

Competing interests

The authors declare no competing interests.

Author details
[1]Department of Laboratory Medicine, Affiliated Hospital of Jiangnan University, No. 1000 Hefeng Road, Wuxi City, Jiangsu Province 214122, China
[2]Department of Pathology, Affiliated Hospital of Jiangnan University, No. 1000 Hefeng Road, Wuxi City, Jiangsu Province 214122, China
[3]Wuxi Medical College, Jiangnan University, Wuxi, Jiangsu, China
[4]Department of Laboratory Medicine, The First Afiliated Hospital with Nanjing Medical University, Nanjing City, Jiangsu, China

Received: 23 November 2024 / Accepted: 28 April 2025
Published online: 12 May 2025

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020:GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49.
2. Singal AG, Zhang E, Narasimman M, et al. HCC surveillance improves early detection, curative treatment receipt, and survival in patients with cirrhosis: a meta-analysis. J Hepatol 2022;77:128–39.
3. Singal AG, Pillai A, Tiro J.Early detection, curative treatment,and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. PLoS Med. 2014;11(4):e1001624.
4. McMahon BJ, Bulkow L, Harpster A, et al. Screening for hepatocellular carcinoma in Alaska Natives infected with chronichepatitis B: a 16-year population-based study. Hepatology 2000;32:842–46.
5. Khanna K, Barnes E, Benselin J, Culver E, Irving W, Innes H, Pavlides M, Consortium D. Prospective cohort for early detection of liver cancer (Pearl): a study protocol. BMJ Open. 2024 Oct 1;14(10):e085541. https://doi.org/10.1136/bmjopen-2024-085541.
6. Liao Y, Peng S, Huang L, Li Z, Hu J, Xu R, Tang W, Zhuang J. Analytical and clinical evaluation of a Chemiluminescent Immunoassay to Detect Serum Chitinase-3-like Protein 1 in HBV-Related Liver Diseases. Int J Anal Chem. 2024 Jan 24;2024:6688819. https://doi.org/10.1155/2024/6688819.
7. Piñero F, Dirchwolf M, Pessôa MG. Biomarkers in hepatocellular carcinoma: diagnosis, prognosis and treatment response assessment. Cells. 2020 Jun 1;9(6):1370. https://doi.org/10.3390/cells9061370.
8. Galle PR, Foerster F, Kudo M, Chan SL, Llovet JM, Qin S, Schelman WR, Chintharlapalli S, Abada PB, Sherman M, Zhu AX. Biology and significance of alpha-fetoprotein in hepatocellular carcinoma. Liver Int. 2019 Dec;39(12):2214–29. https://doi.org/10.1111/liv.14223.
9. Johansen JS, Williamson MK, Rice JS, Price PA. Identification of proteins secreted by human osteoblastic cells in culture. J Bone Miner Res. 1992 May;7(5):501–12. https://doi.org/10.1002/jbmr.5650070506.
10. Wang S, Hu M, Qian Y, Jiang Z, Shen L, Fu L, Hu Y. CHI3L1 in the pathophysiology and diagnosis of liver diseases. Biomed Pharmacother. 2020Nov;131:110680.https://doi.org/10.1016/j.biopha.2020.110680.
11. Salazar J, Le A. The heterogeneity of liver cancer metabolism. Adv Exp Med Biol. 2021;1311:127–36. https://doi.org/10.1007/978-3-030-65768-0_9.
12. Mohammed AF, Chen X, Li C. 2024. Clinical utility of biomarkers of hepatocellular carcinoma. Bratisl Lek Listy. 125(2):102–06. https://doi.org/10.4149/BLL_2024_016
13. Kudo M, Kawamura Y, Hasegawa K, Tateishi R, Kariyama K, Shiina S, Toyoda H, Imai Y, Hiraoka A, Ikeda M, Izumi N, Moriguchi M, Ogasawara S, Minami Y, Ueshima K, Murakami T, Miyayama S, Nakashima O, Yano H, Sakamoto M, Hatano E, Shimada M, Kokudo N, Mochida S, Takehara T. Management of hepatocellular carcinoma in Japan: JSH consensus statements and recommendations 2021 update. Liver Cancer. 2021 Jun;10(3):181–223. https://doi.org/10.1159/000514174.
14. Kim DY, Toan BN, Tan CK, Hasan I, Setiawan L, Yu ML, Izumi N, Huyen NN, Chow PK, Mohamed R, Chan SL, Tanwandee T, Lee TY, Hai TTN, Yang T, Lee WC, Chan HLY. Utility of combining PIVKA-II and AFP in the surveillance and monitoring of hepatocellular carcinoma in the Asia-Pacific region. Clin Mol Hepatol. 2023 Apr;29(2):277–92. https://doi.org/10.3350/cmh.2022.0212.
15. Kim AS, Khorana AA, McCrae KR. Mechanisms and biomarkers of cancer-associated thrombosis. Transl Res. 2020Nov;225: 33–53.https://doi.org/10.1016/j.trsl.2020.06.012..
16. Pabinger I, van Es N, Heinze G, Posch F, Riedl J, Reitter EM, Di Nisio M, Cesarman-Maus G, Kraaijpoel N, Zielinski CC, Büller HR, Ay C. A clinical prediction model for cancer-associated venous thromboembolism: a development and validation study in two independent prospective cohorts. Lancet Haematol 2018 Jul;5(7):e289–e298. https://doi.org/10.1016/S2352-3026(18)30063-2. Epub 2018 Jun 7. Erratum in: Lancet Haematol. 2018 Aug;5(8):e332. doi: 10.1016/S2352-3026(18)30095-4.
17. Schmalenberg M, Beaudoin C, Bulst L, Steubl D, Luppa PB. Magnetic bead fluorescent immunoassay for the rapid detection of the novel inflammation marker YKL40 at the point-of-care. J Immunol Methods. 2015Dec;427: 36–41. https://doi.org/10.1016/j.jim.2015.09.004.
18. Jing P. Performance evaluation of fluorescence immunochromatography for quantitative detection of CHI3L1 and its application in diagnosis of Liver Fibrosis[J]. China Med Device Inform. 2021;27(01):25–27.
19. Wenliang C, Mingxi X. 2023. New progress in the study of markers for the early diagnosis of liver cancer[J]. Chin Med Herald. 20(1):40–44. https://doi.org/10.20047/j.issn1673-7210.2023.01.08
20. Conti F, Dall'Agata M, Gramenzi A, Biselli M. 2015. Biomarkers for the early diagnosis of bacterial infection and the surveillance of hepatocellular carcinoma in cirrhosis. Biomarker Med. 9(12):1343–51. https://doi.org/10.2217/bmm.15.100
21. Qiu QC, Wang L, Jin SS, Liu GF, Liu J, Ma L, Mao RF, Ma YY, Zhao N, Chen M, Lin BY. CHI3L1 promotes tumor progression by activating TGF-β signaling pathway in hepatocellular carcinoma. Sci Rep. 2018 Oct 9;8(1):15029. https://doi.org/10.1038/s41598-018-33239-8.
22. NingNing L, YingHua Z, HaiYan W, WenFeng G, JiaSheng Z. 2017. Value of serum alpha-fetoprotein and protein induced by vitamin K absence or antagonist-II in pathological diagnosis of hepatocellular carcinoma[J]. J Clin Hepatol. 33(11):2162–65. https://doi.org/10.3969/j.issn.1001-5256.2017.11.022

Publisher's Note