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Original Paper

Clinical Application of Serum Tumor Markers CEA, CA199 Combined with Serum Biochemical indicators ALT, ALP, GGT in the Diagnosis of the Hepatocellular Carcinoma

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Abstract

Objective: *To investigate the clinical application value of the combined detection of serum tumor markers carcinoembryonic antigen (CEA), glycan antigen 199 (CA199), serum alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), and alkaline phosphatase (ALP) for the early diagnosis of hepatocellular carcinoma.* ***Methods:*** *Fifty-one patients with hepatocellular carcinoma diagnosed for the first time in our hospital from September 2022 to November 2024 were collected as the hepatocellular carcinoma group, and 24 patients with cirrhosis of admitted to our hospital during the same period were selected as the cirrhosis group, and 26 patients were selected as the hepatitis group. CEA and CA199 were detected using chemiluminescence, and ALT, GGT and ALP were detected using enzyme assay, and the differences in the levels of the five test items were compared among the hepatocellular carcinoma group, cirrhosis group and hepatitis group, and the value of the combined item tests for the differential diagnosis of early-stage hepatocellular carcinoma was predicted by using the receiver operating characteristic (ROC).* ***Results:*** *The positive rates of serum biochemical indexes ALT, GGT and ALP in the hepatocellular carcinoma group were higher than those in the cirrhosis group and hepatitis group, and the differences were statistically significant (P<0.05). In the comparison of serum ALT, GGT and ALP enzyme activity levels among the three groups, the difference was statistically significant (P<0.05), and*

in the comparison of CEA and CA199 levels, the difference was not statistically significant (P>0.05). Under the ROC of subjects, the AUC area of combined detection of CEA, CA199, ALT, GGT, ALP was larger than that of separate detection, and the specificity and sensitivity of combined detection were 96.77% and 96.08% in turn, which were higher than that of separate detection, and the difference was statistically significant (P<0.05). ***Conclusion:*** *Combined detection of CEA, CA199, ALT, GGT, ALP can improve the diagnostic ability of the hepatocellular carcinoma.*

Keywords

hepatocellular carcinoma, Tumor Markers, Biochemical Test, Combined Detection

1. Introduction

Among the most common malignant tumors in the world, hepatocellular carcinoma (HCC) ranks the sixth[1, 2], and is also a tumor with relatively high mortality[3]. According to the survey of global Cancer deaths by the American Cancer Society ASC in 2020, about 10 million people died of cancer, among which HCC accounted for about 0.83% of the total number of deaths[4]. Non-alcoholic fatty liver disease caused by obesity[1], chronic viral infection, especially HBV and HCV infection[5] and consumption of aflatoxin contaminated food[6] are the most important risk factors for patients with HCC. Abdominal pain and jaundice[7] are the most common clinical manifestations of HCC, but they are easy to be ignored by patients in the early stage, which is one of the reasons why patients cannot be diagnosed early. According to surveys, HCC caused by chronic liver disease accounts for four out of five of all patients, most of which are caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) infection[8, 9]. Tumor marker CEA is a glycoprotein involved in cell adhesion and widely exists on the surface of tumor cells differentiated from human endodermal cells. CEA is expressed in a variety of digestive system tumors, especially in colorectal cancer, and is often used as a screening marker for a wide range of tumors[10, 11]. CA199, a glycan antigen containing sialic acid, is particularly important in the diagnosis of pancreatic cancer[12]. In addition, it has been reported that CA199 can be highly expressed in hepatocellular carcinoma[13]. ALT, GGT and ALP are mainly used to evaluate the enzymatic indicators of liver cell function and the condition of liver function in clinical practice, and their elevated values are positively correlated with the severity of liver function damage[14-16].

Therefore, in this study, serum tumor markers CAE and CA199 and biochemical routine test items ALT, ALP and GGT were combined to explore the value of the combined application of the two in the diagnosis of hepatocellular carcinoma, especially early hepatocellular carcinoma.

2. Materials and Methods

2.1 General Data: A total of 51 patients with early-stage HCC initially diagnosed in our hospital from September 2022 to November 2024 were collected and grouped as the HCC group, including 42 males, accounting for 82.35% of the total cases, aged (60.76±12.33) years old; Nineteen females, or 17.65% of the total, had an average age of 58.78±10.69 years. Seventeen males, making up 70.8% of the total cases,

had an average age of (55.47±12.53) years, while seven females, making up 29.2% of the total cases, had an average age of (70.14±12.57) years. During the same period, our hospital admitted 24 additional patients with liver cirrhosis, who were chosen as the liver cirrhosis group. The hepatitis group consisted of 26 patients; 13 of these were men, making up 50% of the total cases; their ages ranged from 43.12±9.14 to 57.25±14.38 years. The study was given the green light by our hospital's ethics committee (Ethics number: 2024LC045).

2.2 Inclusion Criteria and Exclusion Criteria: Inclusion criteria: (1) patients diagnosed with early-stage HCC at the first visit in our hospital; (2) no drug treatment. Exclusion criteria: (1) patients with confirmed HCC or combined with other tumors; (2) complicated with other diseases that may cause abnormal liver function; (3) Patients were newly diagnosed, but clinical data were not easy to obtain.

2.3 Method: The patient should be ready and informed about the necessary precautions the day prior to blood collection. Five milliliters of fasting venous blood was drawn from each of the three groups—HCC, liver cirrhosis, and hepatitis—first thing in the morning on the day of blood collection. Serum samples were then separated using a low-speed centrifuge set to 3500 revolutions per minute for ten minutes before detection.

2.4 Detection System: CEA and CA199 were detected by Roche e801 automatic chemiluminescence analyzer and its matching reagents Roche CEA assay kit (electrochemiluminescence method, batch number 779117), Roche CA199 assay kit (electrochemiluminescence method, batch number 729262), Roche CEA assay kit (electrochemiluminescence method, batch number 729262). ALT, GGT and ALP were measured by Hitachi LST008AS(S)ISE automatic biochemical analyzer and its supporting reagents Sichuan Mike ALT determination kit (alanine substrate method, batch number 240206), Sichuan Mike GGT (GCANA substrate method, batch number 240206). Batch number 241011), Sichuan Mike ALP (NPP substrate -AMP buffer method, batch number 241021).

2.5 Judgment Criteria: the results of each test item exceeding the upper limit of the reference range were judged as positive.

1.6 Statistical processing: This study used SPSS 22.0 software for data analysis. The data was presented as (x±s) when the conditions for normal distribution were satisfied, and as M(P25,P75) when they were not. The two groups were compared using the Mann-Whitney U test. The predictive value of each indicator was assessed using ROC curve, and a P-value less than 0.05 was deemed statistically significant.

3. Results

3.1 Comparison of positive rates of test results among patients in the HCC group, liver cirrhosis group and hepatitis group

The HCC group had greater frequencies of CEA, CA199, GGT, and ALP positivity than the liver cirrhosis and hepatitis groups, at 19.6%, 37.3%, 94.1%, and 82.3%, respectively. The hepatitis group had the greatest percentage of ALT positivity, at 77.9%. Check out the first table. Check out the first table.

Table 1. Comparison of the Positive Rates of Test Items among the HCC Group, Liver Cirrhosis Group and Hepatitis Group

Groups	CEA(%)	CA199(%)	ALT(%)	GGT(%)	ALP(%)
The HCC group	19.6	37.3	47.50	94.10	82.30
The Cirrhosis group	16.8	29.1	41.6	41.6	50
The hepatitis group	10.2	18.4	77.9	89.3	69.9

3.2 Comparison of serum biochemical indexes among HCC group, liver cirrhosis group and hepatitis group

When the activities of ALT, GGT, and ALP were compared, the HCC group had considerably greater enzyme activities compared to the other two groups, and there were statistically significant differences ($P<0.05$) between the three enzyme activities. No statistically significant variation in CEA and CA199 content was seen between the groups when comparing their levels ($P>0.05$). Refer to Table 2.

Table 2. Comparison of Test Results among HCC Group, Liver Cirrhosis Group and Hepatitis Group [M(P25,P75)]

Groups	n	CEA	CA199	ALT(U/L)	GGT(U/L)	ALP(U/L)
The HCC group	51	3.17(1.76,4.56)	26.5(16.9,58.95)	50.6(36.1,115.4)	262(152.25,409.56)	218.45(138.46,334.29)
The Cirrhosis group	24	2.15(1.84,3.74)	19.3(12.21,82.75)	34.75(21.07,70.85)	45.72(28.18,118.12)	119.07(73.43,145.22)
The hepatitis group	26	3.81(1.27,5.14)	17.2(13.6,26.5)	62.68(34.7,128.2)	154.32(44.07,336.9)	97.79(73.7,138.9)
U		1.663	4.263	28.422	26.891	6.73
P		P>0.05	P>0.05	P<0.05	P<0.05	P<0.05

3.3 Diagnostic value of combined detection of biochemical tests and tumor markers for HCC

The receiver operating curve (ROC) showed that the area under the ROC curve (AUC) of CEA, CA199, ALT, GGT, ALP and the combined detection (CEA, CA199, ALT, GGT, ALP) were: 0.557, 0.625, 0.604, 0.809, 0.853, 0.991. The sensitivity of CEA, CA199, ALT, GGT and ALP were 66.67%, 84.31%, 94.12%, 70.59% and 74.51%, respectively. The specificities were 54.84%, 48.39%, 35.38%, 83.87% and 90.32%, respectively. Combined detection has the best sensitivity (96.08%) and specificity (96.77%). Based on the statistically significant difference ($P<0.05$), it can be inferred that combination detection can enhance diagnostic efficiency. Refer to Figure 1 and Table 3.

Table 3. Evaluation of the Effect of Combined Detection of Tumor Markers and Biochemical Tests

Index	sensitivity%	specificity%	95% confidence interval	AUC	Yoden Index	Optimal decision value	P
ALP	74.51	90.32	0.757-0.921	0.853	0.648	150.5	<0.05
ALT	94.12	35.48	0.490-0.710	0.604	0.296	26.5	<0.05
CA199	84.31	48.39	0.511-0.729	0.625	0.327	15.6	<0.05
CEA	66.67	54.84	0.443-0.627	0.557	0.215	2.53	<0.05
GGT	70.59	83.87	0.707-0.877	0.809	0.545	171.77	<0.05
Combined testing	96.08	96.77	0.938 - 1.000	0.991	0.928	-	-

Note. "-" indicates blank space.

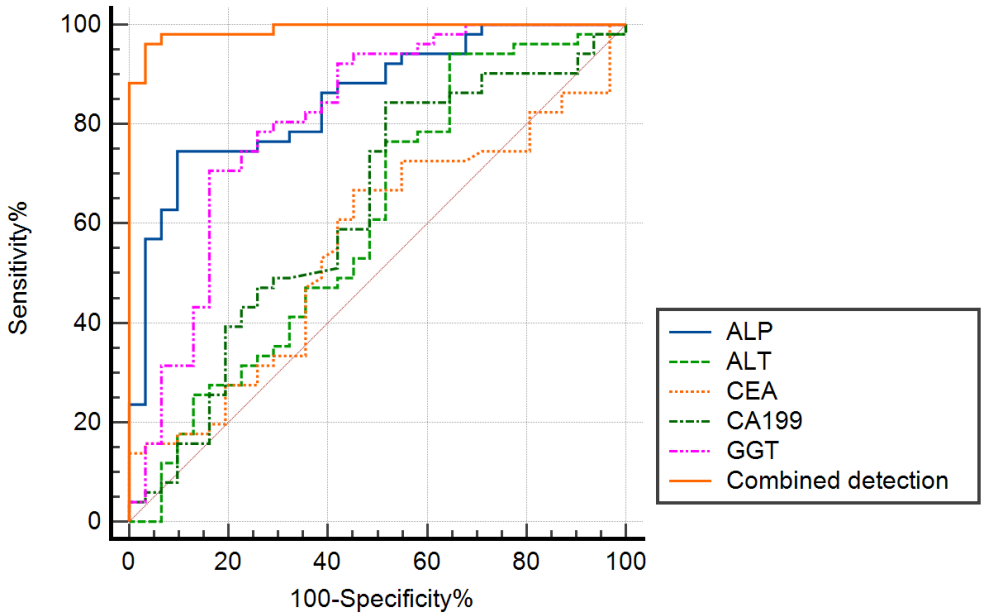

Figure 1. Receiver Operating Characteristic Curve

4. Discussion

Studies have shown that long-term chronic infection of hepatitis B virus is one of the important factors for the development of cirrhosis and eventual progression to hepatocellular carcinoma in patients with hepatitis B[17, 18]. At present, there are about 296 million people infected with hepatitis B virus in the world[19]. In 2019, hepatitis B cirrhosis and hepatitis B-related HCC caused about 331,000 and 192,000 deaths worldwide, respectively, and the number is still increasing[20]. At present, the gold standard for clinical confirmation of HCC mainly relies on pathology and virology for diagnosis[21, 22]. In addition, it also includes imaging techniques such as ultrasound, CT, MRI and detection methods such as Aptameric Fluorescent Biosensors[5, 23, 24]. Levels of serum tumor markers, which are proteins generated by tumor cells' interactions with the host, are inversely proportional to the frequency and progression of

malignancies. Thus, in tumor prevention, treatment, and prognosis, dynamic tumor marker identification is crucial[25, 26]. Serum CEA is a glycoprotein with low specificity in the diagnosis of tumors, and its content is increased in various tissue tumors such as lung cancer and colorectal tumors[27]. At the same time, according to a study, the CEA content of smokers is also increased compared with non-smokers[28]. The glycan antigen containing sialic acid, called carbohydrate antigen CA199, is currently mainly used as an auxiliary examination index for digestive system tumors such as pancreatic cancer, but its content will also change in some non-tumor patients[12]. Therefore, the increase of its content can not only be considered as tumor, but also need to consider the possibility of other diseases. As traditional liver biological indicators, serum biochemical indicators ALT, GGT and ALP are mainly used to evaluate liver function, but they are not unique to liver cells. A variety of diseases such as bone tumors and bile duct diseases can cause changes in the above indicators[25]. As a traditional biochemical indicator of hepatocyte damage, serum ALT expression is increased in viral hepatitis and fatty liver[29]. Recent studies have shown that ALT can be a strong predictor of metabolic diseases such as coronary heart disease, atherosclerosis and thrombosis and type 2 diabetes[13]. Studies have shown that serum GGT is closely related to HCC, liver fibrosis and chronic liver cell injury[4]. Serum ALP is a heterogeneous enzyme widely present in all tissues and cells of the human body, and its activity is increased in hepatogenic diseases: hepatitis, cholestatic diseases, and non-hepatogenic diseases such as bone and endocrine diseases[30, 31]. Therefore, we sought for a possible link between tumor markers and biochemical indicators in the diagnosis of HCC to provide effective guidance for clinicians.

According to the current research status at home and abroad, serum alpha-fetoprotein (AFP) is currently the most commonly used tumor marker for primary hepatocellular carcinoma[32]. Despite the fact that AFP-L3%, which combines serum AFP with the percentage of alpha-fetoprotein variation, can predict the risk of HCC following liver transplantation[25, 33], it is obviously unreasonable to use a single index such as AFP or AFP-L3% for the diagnosis of hepatocellular carcinoma[21, 23]. According to the current research status, AFP has certain defects in the diagnosis of HCC, as follows:

1. Insufficient sensitivity (high false negative rate): Not all HCCS secrete AFP. About 30% of HCC patients (especially early-stage, well-differentiated HCC or some specific molecular subtypes) can have serum AFP levels within the normal range (<20 ng/mL)[13]. For these patients, AFP detection can not play a diagnostic or screening role, and it is easy to be missed.

2.Insufficient specificity (high false positive rate): elevated AFP is not unique to HCC. Many diseases can also cause varying degrees of elevated AFP, causing false positive results, leading to unnecessary anxiety and further testing. Common diseases include: active liver disease: acute and chronic viral hepatitis (especially acute onset of hepatitis B and C), drug-induced liver injury, alcoholic hepatitis and other liver cells with massive necrosis and regeneration, liver cirrhosis[34]; Embryonal tumors: such as yolk sac tumors (endodermal sinus tumors), some teratomas[35]; Pregnancy: The fetus will produce AFP, and maternal serum AFP will physiologically increase during pregnancy[36].

3. The diagnostic value of early and small hepatocellular carcinoma is limited: the ability of early HCC or small hepatocellular carcinoma to secrete AFP is relatively weak, and the increase of serum AFP level may not be obvious or not increased, resulting in the reduction of its sensitivity in early diagnosis. In its 2010 guidelines for the diagnosis and monitoring of HCC, the American Association for the Study of Liver Diseases (AASLD) removed AFP from the criteria because of its reduced diagnostic sensitivity[37]. Compared to the liver cirrhosis and hepatitis groups, the HCC group had a higher positive rate of tumor markers CEA (19.6% vs. 37.3%), although there was no statistically significant difference in CA199 or CEA among the three groups (P>0.05). Specific analysis found that:

1. The biological characteristics of tumor markers CEA and CA199 are weakly related to HCC: hepatocellular carcinoma is mostly hepatogenic, while CEA is secreted by intestinal epithelial cells in the embryonic stage, and CA199 is derived from bile duct epithelial cells, both of which do not fit the molecular origin of HCC.

2.Interfering factors in benign disease group: in cirrhosis, the clearance rate of CEA decreased due to liver dysfunction, and the content of CA199 increased due to bile duct inflammation or obstruction (such as cholestasis); In hepatitis, the increase of intestinal permeability leads to the absorption of CEA into the blood, which leads to the non-specific increase of CEA and CEA.

To sum up: The levels of CEA and CA199 did not differ significantly (p>0.05) between the groups with HCC, cirrhosis, and hepatitis. The rationale for this is that CEA and CA199 have a weak relationship to HCC biology and can be easily elevated in benign illnesses without specificity. Thus, CEA and CA199 did not differ significantly across the HCC, liver cirrhosis, and hepatitis groups in this investigation. According to the aforementioned research, tumor markers are still a valuable reference indicator for the accurate and trustworthy laboratory diagnosis of liver malignant tumors. This is consistent with the study of Zhu HQ[38] et al.

A patient's ability to use their liver depends on the extent of their liver damage. When looking at the positive rates of serum biochemical markers, the group with liver cirrhosis had much lower rates of ALT, GGT, and ALP. The three enzyme activities mentioned earlier showed a statistically significant difference (P<0.05) with the HCC group greatly outperforming the other two groups. The results of this study suggest that liver function is severely impaired in patients with HCC, and the severity of impairment increases with the progression of HCC, and there is a clear trend change. This suggests that we should be alert to the possibility of tumorigenesis when the patient's serum enzyme activity is persistently increased.

The investigation indicated that in result 2.3, the area under the receiver operating curve (AUC) for CEA, CA199, ALT, GGT, and ALP were 0.557, 0.625, 0.604, 0.809, and 0.853, respectively. There were statistically significant differences among the three groups (P<0.05). The AUC of combined detection of the above five items was 0.991, which was higher than that of single detection, and the sensitivity and specificity were 96.08% and 96.77%, respectively, which were also higher than those of single detection of the above five items, and the difference was statistically significant (P<0.05). The combined detection

of CEA, CA199, ALT, GGT and ALP can effectively improve the diagnostic ability of HCC.

The innovation of this study is to combine the common tumor marker CEA with CA199 and biochemical indicators to detect HCC, through complementary mechanisms to improve diagnostic performance.

1. Covering different pathological types: metastatic HCC (especially liver metastasis from colorectal cancer), cholangiocarcinoma (ICC) or mixed HCC, both of which provide some complementary diagnostic clues for AFP-negative HCC (especially non-HCC types).

2. Improve sensitivity and negative predictive value: studies have found that about 30% of patients with HCC are AFP-negative [13], so the missed diagnosis can be reduced by combined detection (especially AFP-negative HCC).

3. Auxiliary differential diagnosis: if CEA and CA199 are significantly increased or continuously increased but AFP is normal, metastatic HCC or cholangiocarcinoma should be suspected, but not HCC.

Therefore, the combined detection assay can cover different cancer types and complementary AFP blind areas, which can be used as a supplementary method for AFP, especially for AFP-negative HCC or non-HCC types, but it still needs to be judged comprehensively in combination with imaging and clinical background. Studies have shown that multi-dimensional joint detection is necessary. At present, a multidisciplinary joint detection mode including medical laboratory science, pathology, imaging, etc., has been formed to jointly improve the screening and diagnosis ability of HCC.

Our study is limited by the fact that we collected all the participants in a specific location, consecutively, over a specific period of time, so our participants are well representative of the HCC patients in our hospital; however, the lack of large-scale validation at multiple sites and regions may lead to some deviations from the general HCC population. Future studies will collect and investigate a large number of samples in multiple locations and hospitals to understand the overall correlation between tumor markers and biochemical indicators and HCC, or explore regional differences in included patients. At the same time, more multidisciplinary projects including imaging and pathology should be added to explore the early detection, diagnosis and treatment of HCC patients.

In conclusion, the combined application of serum tumor markers CEA and CA199 and serum biochemical markers ALT, ALP and GGT can significantly improve the diagnostic efficiency and play a positive role in the detection and diagnosis of early liver malignant tumors due to the low sensitivity and specificity of single detection of five items in this study.

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