Evaluation of α-fetoprotein-L3 and Golgi protein 73 detection in diagnosis of hepatocellular carcinoma

Zhiling Jia, Li Wang, Chang Liu, Zhonghe Yu, Lina Chai, Mancang Zhao

Department of Oncology, General Hospital of Beijing Command of PLA, Beijing, PR China

Aim of the study: Hepatocellular carcinoma (HCC) is common throughout the world. Most HCCs are diagnosed at an advanced stage. There is an urgent need to find new methods for screening and surveillance of individuals at risk for HCC. The aim of this study was to evaluate serum α-fetoprotein (AFP)-L3 and serum Golgi protein 73 (GP73) detection in diagnosis of HCC with different AFP concentration.

Material and methods: One hundred and eighty one patients were involved, including 102 with HCC and 79 with benign liver disease. The serum AFP-L3 and GP73 was measured by a liquid-phase binding assay and quantitative enzyme-linked immunosorbent assay, respectively.

Results: Of the 102 HCC patients, 53 were positive for AFP, 77 were positive for AFP-L3, and 79 were positive for GP73. The maximum area under the curve for AFP-L3% and for GP73 was significantly different from the AUC of 0.5525 for total AFP ($p < 0.01$). AFP-L3% was not detected for AFP < 20 ng/ml. However, elevated GP73 was detected in 87.50% of the patients. In the HCC patients with total AFP 20–400 ng/ml, elevated AFP-L3 was detected in 26 patients, whereas in 23 patients elevated GP73 could be detected. In the HCC patients with a total AFP > 400 ng/ml, AFP-L3% > 10% was present in 96.23%, and GP73 was detected in 87.50%.

Conclusions: The determination of AFP-L3% and GP73 in combination with AFP can increase the sensitivity and specificity in diagnosis of HCC. α-fetoprotein-L3% and GP73, in combination with AFP, are useful biomarkers to confirm the diagnosis of HCC.

Key words: α-fetoprotein, lectin, reactive α, fetoprotein, hepatocellular carcinoma.

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Introduction

Hepatocellular carcinoma (HCC) is common throughout the world, and represents the fifth most common cancer in the world and the third most frequent cause of mortality amongst oncological patients [1]. It is responsible for more than 500 000 deaths with over 600 000 new cases yearly worldwide [2]. The high mortality associated with HCC is partly due to unresponsiveness to treatment, with a 5-year survival rate of < 5% after diagnosis [3]. When diagnosed at an early stage, treatment of HCC with surgical resection or liver transplantation can be curative. In fact, most HCCs are diagnosed at an advanced stage. Therefore, there is an urgent need for improved methods of screening and surveillance of individuals at risk for HCC.

α-fetoprotein (AFP) has served as a diagnostic test for HCC since the 1970s, due to there being a correlation between elevated levels of AFP and the occurrence of HCC. However, AFP as a sole indicator of HCC is of limited value. The diagnostic sensitivity of AFP for small HCCs is relatively low. Furthermore, AFP levels are elevated both in patients with HCC and in those with chronic liver diseases, and there is a wide overlap between the two groups. α-fetoprotein is often elevated in patients with chronic hepatitis C virus infection in the absence of HCC and not elevated in as many as 50% of HCC [4]. Although it has relatively poor sensitivity and specificity as a surveillance test, serum AFP, used in combination with serial liver ultrasound examination, is a standard means for risk stratification, screening and surveillance for HCC.

The chemical structure of AFP shows that different sugar moieties of the bonds determine their binding capacity to lectin *Lens culinaris* agglutinin (LCA) [5]. α-fetoprotein-L3, as the LCA-bound fraction, is the major glycoform in the serum of HCC patients [6–8]. The AFP-L3 isoform has been reported to be more specific for the diagnosis of HCC than total AFP. It has also been shown to be associated with more aggressive HCCs, and to predict a worse outcome [9]. Recent reports also suggest that it is useful for predicting the risk of development of HCC in patients with chronic liver disease.

Another useful biomarker for HCC is Golgi protein 73 (GP73). Golgi protein 73 (also called GOLPH2) is a Golgi type II transmembrane protein of unknown function that is constitutively expressed in biliary epithelial cells in normal livers. It has been reported that GP73 is up-regulated at high levels in hepatocytes of patients with acute and chronic hepatitis and HCC [10–12]. Golgi protein 73 is highly expressed in hepatocytes of patients with HCC, and secreted into the serum of HCC patients following furin-mediated proteolytic cleavage of its *N*-terminus [13], a feature that can be exploited for the diagnosis of HCC [14]. Although its function has yet to be elucidated, its expression profile suggests that GP73 may serve as a biomarker of HCC.

In this study, we detected the serum AFP, AFP-L3% and GP73 in patients with HCC and benign liver diseases and compared their diagnostic utility in HCC.

Material and methods

Patients

We collected serum samples from 181 adult patients (158 men and 23 women, age ranged from 25 to 78 years, median age 52 years) evaluated for HCC or chronic liver disease between September 2009 and July 2010. The samples were stored at –70°C.

Of the 181 patients, 102 had HCC and 79 had benign liver disease. One hundred serum samples were from healthy volunteers (71 men and 29 women, age ranged from 25 to 60 years, median age 50 years). This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the General Hospital of Beijing Command of PLA. The patients of this series were all from the General Hospital of Beijing Military Area Command of PLA. Written informed consent was obtained from all participants.

Cases were selected for whom a specimen was available that had been drawn after diagnosis of HCC but before any therapy. The diagnosis of HCC was based on either histopathology or on non-invasive criteria. The non-invasive diagnosis of HCC was established based on the following criteria revised by the Society of Liver Cancer of the Chinese Anti-Cancer Association in September, 2001.

1) Total AFP ≥ 400 μg/l, excluding pregnancy, germinal and embryonal neoplasm, active liver disease and metastatic liver cancer, rigid liver mass could be palpated, or typical occupying lesion of HCC ascertained by imaging modalities (including ultrasound, contrast CT, contrast MRI, and angiography);
2) Total AFP < 400 μg/l, excluding pregnancy, germinal and embryonal neoplasm, active liver disease and metastatic liver cancer, typical occupying lesion of HCC ascertained by two imaging modalities (including contrast CT, contrast MRI, and angiography) or two HCC biomarkers (including DCP, GGT II, AFU and CA19-9) elevated, and typical occupying lesion of HCC ascertained by one imaging modality (including contrast CT, contrast MRI, and angiography).
3) Clinical manifestation in accordance with HCC and confirmed metastatic tumor except liver mass (including bloody pyoperitoneum or tumor cells found in pyoperitoneum), excluding metastatic liver cancer.

Clinical and laboratory information at the time of diagnosis were retrospectively ascertained from the medical records. Laboratory findings abstracted included liver biochemistries (AST, ALT, alkaline phosphatase, total and direct bilirubin, albumin), platelet count, prothrombin time, creatinine, hepatitis B and C serologies. Histopathology slides for patients who underwent surgical resection or needle biopsy were reviewed by a liver pathologist.

Detection of serum α-fetoprotein and α-fetoprotein-L3

Total serum AFP levels were determined by enzyme immunoassay using commercially available kits (ABBOTT AXSYM). AFP-L3 was measured by a liquid-phase binding assay. Briefly, AFP-L3 in the samples was isolated via lectin-affinity adsorption using the tube coupled with antibody-affinity blotting (Hotgen Co., Ltd.), after elution, and then quantified using chemiluminescence on the automated platform as AFP. The ratio of LCA-reactive AFP to total AFP (AFP-L3%) was calculated. The typical inter-assay variance for this test, expressed as the coefficient of variance, is between 2.8% and 13.4% for AFP-L3% and 2.6% and 4.6% for AFP concentration. The lower limit of detection for total AFP concentration was 0.8 ng/ml and for AFP-L3% 0.5%. If AFP-L3 was detected in the sample, the system only provided a reliable value of AFP-L3% for samples when the total AFP concentration was 10 ng/ml. For samples with total AFP of > 300,000 ng/ml, we elected not to assess AFP-L3% because of the need for multiple sequential dilutions of the sample. The recommended cut-off points for AFP and AFP-L3 were 400 ng/ml and 10%, respectively.

Detection of serum Golgi protein 73

The serum concentration of Golgi protein 73 (GP73) was detected by a quantitative enzyme-linked immunosorbent assay kit (Hotgen Co., Ltd.). The standard substance of GP73 with concentration of 50 ng/ml, 120 ng/ml, 300 ng/ml and 500 ng/ml was measured, and a standard curve was produced graphically. The optical density (OD) of the samples was compared to the standard curve. For the measured quantity (a given Y-axis value), the substance concentration (X-axis value) could be calculated correspondingly. The cut-off point of GP73 was 150 ng/ml.

Statistical analysis

For correlations between tumor markers, Pearson's correlation coefficient was used, with values of –0.4 to 0.4 considered as having no correlation. Data for marker concentrations are reported as the median with 25th and 75th percentiles.

Comparison of continuous and dichotomous variables between the two groups was performed using the Mann-Whitney *U*-test, the χ^2 test, or Fisher's exact test. The statistical analysis was performed using the SPSS13.0 software package. To compare abilities of tumor markers in diagnosis of HCC, receiver operator characteristic (ROC) curves, which correlate true- and false-positive rates [sensitivity and (1-specificity)], were constructed using the ROCKIT program. Additionally, areas under the ROC curve (AUC) were calculated for each marker. The statistical significance of differences between the two AUCs also was determined.

Results

Detection of serum α-fetoprotein and α-fetoprotein-L3 and Golgi protein 73

The comparative analysis of AFP, AFP-L3 and GP73 in patients with different liver diseases and healthy persons is shown in Table 1. The recommended cut-off points for

Table 1. Comparison of AFP, AFP-L3 and GP73 detection in patients with HCC

Group	Cases	AFP positive (%)	AFP-L3 positive (%)	GP73 positive (%)
HCC	102	53 (51.96)	77 (75.50)	79 (77.45)
benign liver diseases	79	39 (49.37)	5 (6.33)	20 (25.32)
healthy persons	100	0	0	0

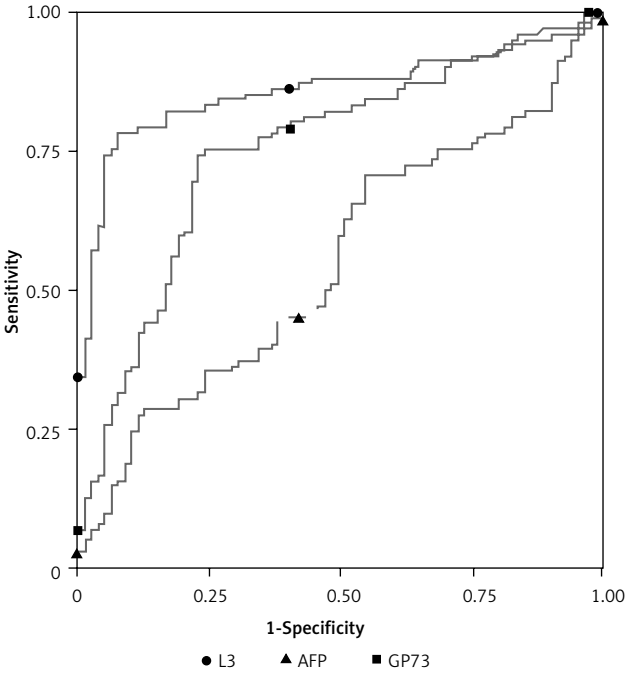

Fig. 1. ROC curves for the diagnosis of HCC using total AFP, AFP-L3% and GP73

Table 2. Comparison of AFP-L3 and GP73 in HCC patients with different AFP concentrations

AFP (ng/ml)	Cases	AFP-L3 positive (%)	GP73 positive (%)
0–20	16	0 (0)	14 (87.50)
20–400	33	26 (78.79)	23 (69.70)
≥ 400	53	51 (96.23)	40 (75.47)

AFP, AFP-L3 and GP73 were 400 ng/ml, 10%, and 150 ng/ml respectively. The AFP, AFP-L3 and GP73 levels were significantly elevated in the HCC group over the benign liver disease and healthy persons groups. Of the 102 samples from patients with hepatocellular carcinoma, 53 samples (51.96%) were positive for AFP, 77 samples (75.50%) were positive for AFP-L3, and 79 samples (77.45%) were positive for GP73. There were significant differences in the rate between AFP and AFP-L3, AFP and GP73.

Diagnostic utility of α-fetoprotein and α-fetoprotein-L3% and Golgi protein 73

Receiver operator characteristic curves for the diagnosis of HCC using total AFP, AFP-L3% and GP73 are shown in Fig. 1. The maximum area under the curve (AUC) for distinguishing between HCC and benign liver disease was 0.8625 for AFP-L3% and 0.7538 for GP73, almost significantly different from the AUC of 0.5525 for total AFP ($p < 0.01$). With the recommended AFP-L3% cut-off point of = 10%, the sensitivity with GP73 cut-point of = 150 ng/ml, the sensitivity of the GP73 for HCC was 71% and the specificity was 63% (Table 2).

Comparison of α-fetoprotein-L3 and Golgi protein 73 detection in hepatocellular carcinoma patients with different α-fetoprotein concentrations

In all patients, a small majority of HCC patients had a total AFP < 20 ng/ml (16/102; 15.69%), 32.35% (33/101) had a total AFP 20–400 ng/ml, and 51.96% (53/102) had a total AFP > 400 ng/ml.

Since AFP-L3% is typically not detected for AFP < 20 ng/ml, AFP-L3% was not relevant for the diagnosis of HCC for individuals with a total AFP < 20 ng/ml. However, an elevated level of GP73 can be detected in 87.50% (14/16) of the patients in this subset, and the maximum area under the curve (AUC) for distinguishing between HCC and benign liver disease was 0.9766 for GP73 (Fig. 2A).

Twenty-six (78.79%) of the HCC patients with total AFP 20–400 ng/ml showed an elevated level of AFP-L3, whereas 23 (69.70%) of the HCC patients in this subset showed an elevated level of GP73. The AUC for distinguishing between HCC and benign liver disease was 0.9063 for AFP-L3% and 0.7803 for GP73 (Fig. 2B).

On the other hand, AFP-L3% > 10% was present in 96.23% (51/53) of the patients with HCC and for total AFP > 400 ng/ml, GP73 could be detected in 87.50% (14/16) of the patients in this subset. The AUC for distinguishing between HCC and benign liver disease was 0.9690 for AFP-L3% and 0.6425 for GP73 (Fig. 2C).

Discussion

High mortality rates for HCC have significantly increased worldwide. Regular follow-up of chronic liver disease patients is indispensable for early detection of HCC [15, 16]. Serum AFP is the most widely used biomarker for diagnosis of HCC. The normal range for serum AFP levels is 10–20 ng/ml and a level > 400 ng/ml is usually regarded as of diagnostic value. However, two thirds of HCC patients with the nodule less than 4 cm have serum AFP levels less than 200 ng/ml and up to 20% of HCC patients do not produce AFP. Mild elevation of AFP also occurs in viral hepatitis and other liver diseases [17, 18]. It has limited utility of differentiating HCC from benign hepatic disorders.

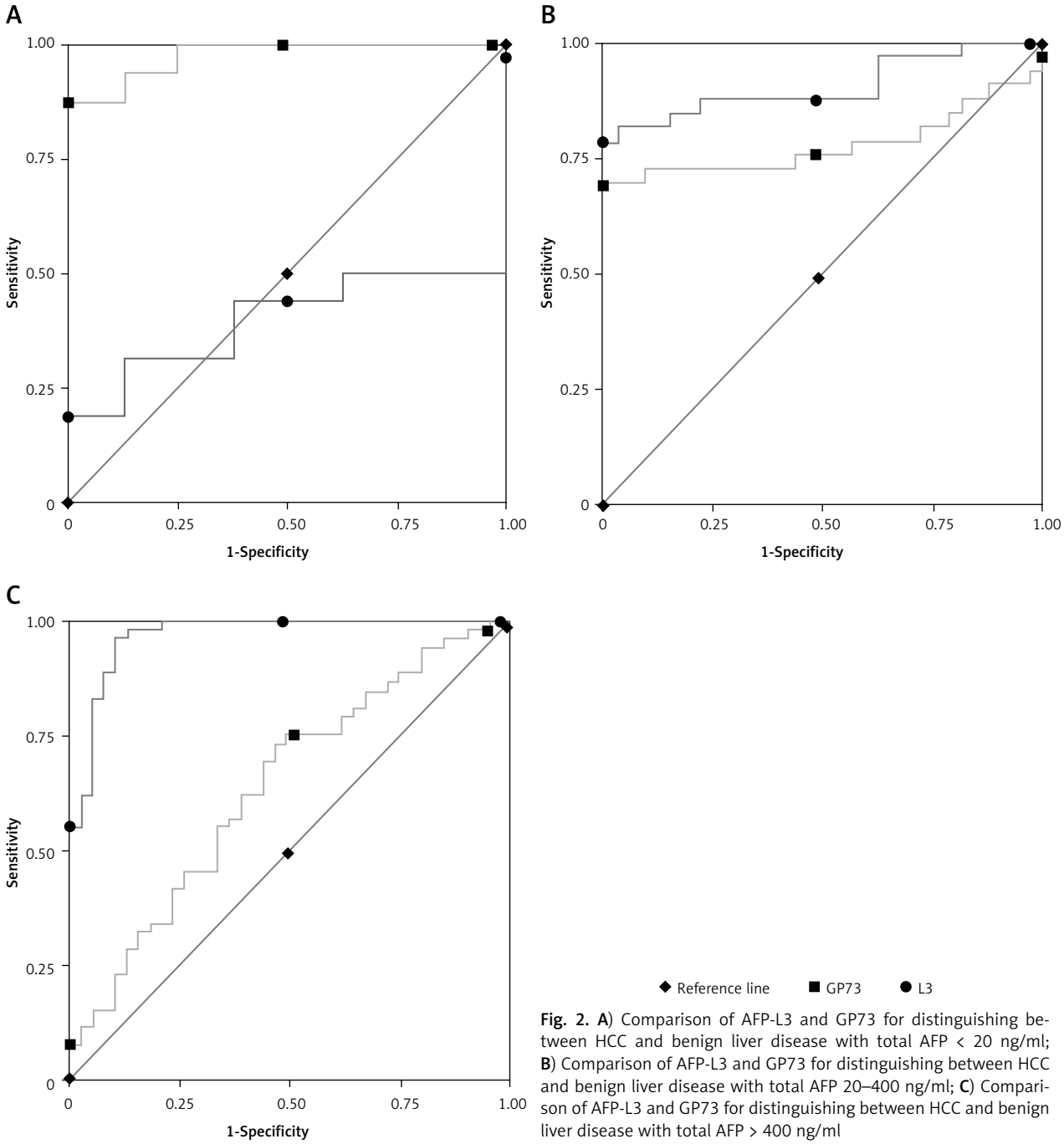

Fig. 2. A) Comparison of AFP-L3 and GP73 for distinguishing between HCC and benign liver disease with total AFP < 20 ng/ml; **B**) Comparison of AFP-L3 and GP73 for distinguishing between HCC and benign liver disease with total AFP 20–400 ng/ml; **C**) Comparison of AFP-L3 and GP73 for distinguishing between HCC and benign liver disease with total AFP > 400 ng/ml

α-fetoprotein-L3, as the LCA-bound fraction in total AFP, is also widely used as a tumor marker for HCC, and has been shown to be more valuable than AFP in differentiating HCC from nonmalignant hepatopathy, detecting small HCC, and predicting the prognosis [19]. In this study, with the recommended cut-off point of AFP-L3% = 10, the sensitivity of AFP-L3% for HCC was 71% and the specificity was 63%, significantly higher than total AFP. Since AFP-L3% is typically not reported for an AFP < 20 ng/ml, AFP-L3% was not relevant for the diagnosis of HCC for individuals with a total AFP < 20 ng/ml. On the other hand, the patients with a total AFP > 400 ng/ml and typical liver mass could be diagnosed with HCC; therefore the AFP-L3% again provided little benefit for the diagnosis of HCC in these patients. However, the patients with total AFP in the intermediate 20–400 ng/ml range, frequently individuals with chronic hepatitis B or C virus infection, often present a diagnostic dilemma, as they often have false-positive elevations of total AFP. For the total AFP range of 10–10,000 ng/ml, the AUC for distinguishing between HCC and benign liver disease was 0.80 for AFP-L3% and 0.78 for total AFP (p = 0.754). For the total AFP range of 10–400 ng/ml, the AUC for diagnosis of HCC using AFP-L3% was 0.76, almost significantly different from the AUC of 0.59 for total AFP (p = 0.074). α-fetoprotein-L3% > 40 is seldom observed in patients with benign liver disease. Thus, AFP-L3% is more valuable in differentiating HCC and benign liver disease in patients with a total AFP range of 10–200 ng/ml.

Golgi protein 73 is a newly introduced biomarker for HCC. The correlation between serum GP73 levels and a diagnosis of HCC in people has been confirmed recently. Total GP73 levels were shown to have a positive predictive value equal to or greater than AFP [20, 21]. In this study, with the recommended cut-off point of GP73 = 150 ng/ml, the sensitivity of GP73 for HCC was 71% and the specificity was 63%, significantly higher than total AFP. For diagnostic utility in all patients, GP73 > AFP-L3 > AFP in sensitivity, and AFP-L3 > GP73 > AFP in specificity.

In this study, a small majority of HCC patients (63/166; 38%) had a total AFP < 20 ng/ml, whereas 56% of benign liver disease patients had a total AFP < 20 ng/ml. It is reported that AFP is not elevated in as many as 50% of HCC cases, especially in its early stage [8]. Because AFP-L3% is negative in these patients, AFP-L3 detection could not help to distinguish HCC from benign liver disease in this group. However, serum GP73 elevated in % HCC patients with a total AFP < 20 ng/ml, elevated in % benign liver disease patients. For the total AFP range of 10–100 ng/ml, the AUC for diagnosis of HCC using AFP-L3% was 0.76, almost significantly different from the AUC of 0.59 for GP73. Golgi protein 73 is more useful for distinguishing between HCC and benign liver disease in patients with a total AFP range of 10–100 ng/ml, especially total AFP < 20 ng/ml.

In conclusion, our data suggest that the determination of AFP-L3% and GP73 in combination with AFP increases the sensitivity and specificity in diagnosis of HCC, especially in individuals with total AFP < 400 ng/ml. Thus, AFP-L3% and GP73 should be useful biomarkers, in combination with AFP, to confirm the diagnosis of HCC.

The authors declare no conflict of interest.

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Address for correspondence

Mancang Zhao
Department of Oncology
General Hospital of Beijing Command of PLA
No. 28 Fuxing Road Haidian District
Beijing 100700, China
e-mail: jzwlcn@126.com

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