Evaluation of Lens culinaris agglutinin-reactive α-fetoprotein for diagnosis of malignant tumors

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Research

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Abstract

Background: To assess the potential of AFP-L3% for the utility to diagnose malignant tumors.

Methods: The AFP-L3 was concentrated from clinically-collected serum samples via the Hotgen Biotech glycosyl capture spin columns and then measured through the protein microarrays. The levels of AFP and AFP-L3 were detected by electrochemiluminescence immunoassay. In this retrospective study, 266 patients with the level of serum AFP-L3 over 1ng/ml were recruited from December 2014 through April 2019,Among them, 155 patients were clinically diagnosed/confirmed with malignant tumors, including 101 hepatocellular carcinomas, 47 stomach malignant tumors, and 7 other malignant tumors; and the rest of 111 patients were nomalignant tumors.

Results: Patients with serum AFP-L3 level of greater than 1ng/ml were mainly detected in hepatic diseases, including hepatocellular carcinoma, cirrhosis and chronic hepatitis. In patients with no tumors, the levels of serum AFP-L3 over 1ng/ml were only observed in liver disease. The levels of AFP-L3 in blood were substantially greater in patients with HCC. Among the malignant tumor patients with the level of serum AFP-L3 over 1ng/ml, HCC accounted for 60%, gastric cancer for nearly 40%. The AFP, AFP-L3 and AFP-L3% in blood were increased significantly in patients with liver malignancy, chronic liver disease and cirrhosis. However, the elevation of AFP-L3 and AFP-L3% in the malignant cohort was more evident than that in the nonmalignant counterpart.

Conclusions: AFP-L3 is likely to contribute to differential diagnosis of HCC as well as other hepatic diseases, AFP-L3% is a reliable indicator for diagnosing benign and malignant tumors.

Keywords: Hepatocellular carcinoma; malignant tumors; liver cirrhosis; gastric cancer; non-neoplastic; AFP-L3%

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Background

Hepatocellular carcinoma (HCC) belongs to one of the most common malignant tumors(1-4). Once diagnosed, patients usually have reached the middle or late tumor stage, with poor therapeutic effect and high mortality rate. Previous studies illustrate that continuous serum α-fetoprotein (AFP) level with 400ng/ml or more exhibits a significantly high specificity to diagnose primary HCC(5), the levels of AFP were observed to correlate with the velocity of tumor growth(6). However, it turns out that in clinic the levels of AFP in blood within certain patients with hepatic diseases were greater than 400ng/ml, but their clinical diagnosis was nonmalignant diseases (e.g., chronic hepatitis and cirrhosis)(7-9). Studies also have found that AFP from different sources presented differential affinity to Lens culinaris agglutinin(10). Based on its difference, AFP is divided into 3 categories: AFP binding to Lens culinaris agglutinin_1 (AFP_L1), Lens culinaris agglutinin_2 (AFP_L2) and Lens culinaris agglutinin_3 (AFP-L3), respectively(8). It has demonstrated that AFP-L3 was closely related to primary HCC, which can contribute to the definite diagnosis of HCC(11-13). The percentage of AFP-L3 (AFP-L3%) has newly been proposed as a reliable indicator for diagnosing malignant tumors with a higher diagnostic value in HCC than AFP. Previous research has determined the serum AFP-L3 level of 10% to be a cut-off readout to diagnose HCC(14-16).

The concentration of serum AFP-L3 in healthy populations is remarkably low and cannot be detectable using the current common method in clinic which has a maximum sensitivity of 0.605ng/ml. This study investigated the patients with the level of serum AFP-L3 over 1ng/ml, aiming to understand the potential links of the AFP-L3 in blood to its related diseases, to explore the differences of AFP_L3% values in patients with or without malignant tumors, and to explore its potential clinical applications.

Methods

Research subjects

Clinically-collected blood samples were drawn from 266 patients assessed for hepatic tumors or other diseases at the First Affiliated Hospital of Anhui Medical University from December 2014 to April 2019 under the Institutional Review Board–approved protocol. The clinical and laboratory information on these recruited subjects was retrospectively ascertained from relevant medical records. All subjects in this study were informed consent and their medical documents were under critical review to confirm their medical history as well as physical checks and laboratory examinations.

Of the 266 patients, 202 males and 64 females both with the levels of AFP-L3 over 1ng/ml were included, with the age of 25 ~ 87 (54.94 ± 12.51) years old. Among them, 155 patients were clinically diagnosed with malignant tumors, including 101 HCCs, 47 stomach malignant tumors, and 7 other malignant tumors; while the other 111 patients were nomalignant tumors, including 69 with cirrhosis, 35 with chronic hepatitis B, and 7 with other types of hepatitis. These patients were chosen for whose specimens were available that had been collected after disease diagnoses yet before any therapies.

Measurement for AFP-L3 percentages

Blood samples were collected when diagnosed and before any interventions, then were frozen at -80°C before use. The levels of AFP and AFP-L3 were detected using AFP testing reagents supplied

by Roche (Germany) through an electrochemical luminescence analyzer (e601 module, Roche, Germany). Prior to AFP-L3 detection, the blood samples need to be pre-processed. Every AFP-L3 portion supernatant was split from the blood samples via a Hotgen Biotech glycosyl capture spin-column. The primary mechanism of the spin-column was that the spin-column first pre-loaded Lens culinaris agglutinin to have the fucosylated stuff thoroughly captured, followed by a simple sugar added to compete with the aforementioned captured fucosylated stuff to bind to Lens culinaris agglutinin. Hence, the fucosylated stuff in the sera, the AFP-L3 included, was consequently eluted from the spin-column. After cleaning, free AFP was removed, and AFP-L3 was obtained by elution and centrifugation. Then the Roche electrochemical luminescence analyzer was applied to detecting the contents of total AFP and AFP-L3 respectively. the AFP-L3 percentages were calculated accordingly, and the maximum sensitivity for this assay of AFP-L3 was set to 0.605ng/ml.

Statistical analysis

All data were performed using SPSS19.0 software and GraphPad Prism (GraphPad software). Results were statistically analyzed using Nonparametic Tests (Mann-Whitney U) to compare differences among the groups. Differences of $P<0.05$ were considered statistically significant.

Results

Subject features

HCC in patients with the level of serum AFP-L3 over 1ng/ml accounted for only 38%, as shown in Table 1. Other diseases were found to present 41.7% in non-neoplastic liver diseases in the total detected cases, including 25.9% cirrhosis, 13.2% chronic hepatitis B, and 2.6% other hepatitis; and other tumors presented 20.3% within all the detected subjects, among which the gastric cancer was predominant (17.7%). Similarly, these patients were mainly found in liver diseases, and males were significantly higher than females (76% vs. 24%).

Comparison of AFP, AFP-L3, as well as AFP-L3% in different groups of diseases

The serum levels of AFP-L3 were remarkably elevated in patients with HCC compared to that in those with other cancers or non-neoplastic diseases. However, the amount of serum AFP and AFP-L3 was significantly increased in a small number of patients with nonmalignant tumors. It was observed that the increase of AFP-L3 in patients with gastric cancer was not comparable to that in HCC patients, whereas the value of AFP-L3% was similar to that in patients with HCC (Figure 1).

Comparison of AFP, AFP-L3, as well as AFP-L3% in malignant and nonmalignant tumor groups

AFP-L3% was more pronounced and intuitive than AFP as well as AFP-L3 in distinguishing malignant and nonmalignant tumors. However, a small percentage of nonmalignant patients possessed the AFP-L3% value of more than 10% (Figure 2).

Table1. Primary characteristics of patients with the level of AFP-L3 over 1ng/ml in blood (n=266)

Variables	n (%)
Age (years)	
Mean (range)	55 (25-87)
Sex	
Male	202 (75.97)
Female	64 (24.06)
Disease	
Malignant tumor	155 (58.27)
Hepatocellular carcinoma	101 (37.97)
Gastric cancer	47 (17.67)
Other tumors	7 (2.63)
Nonmalignant tumor	111 (41.73)
Liver cirrhosis	69 (25.94)
Chronic hepatitis B	35 (13.16)
Non-hepatitis B	7 (2.63)

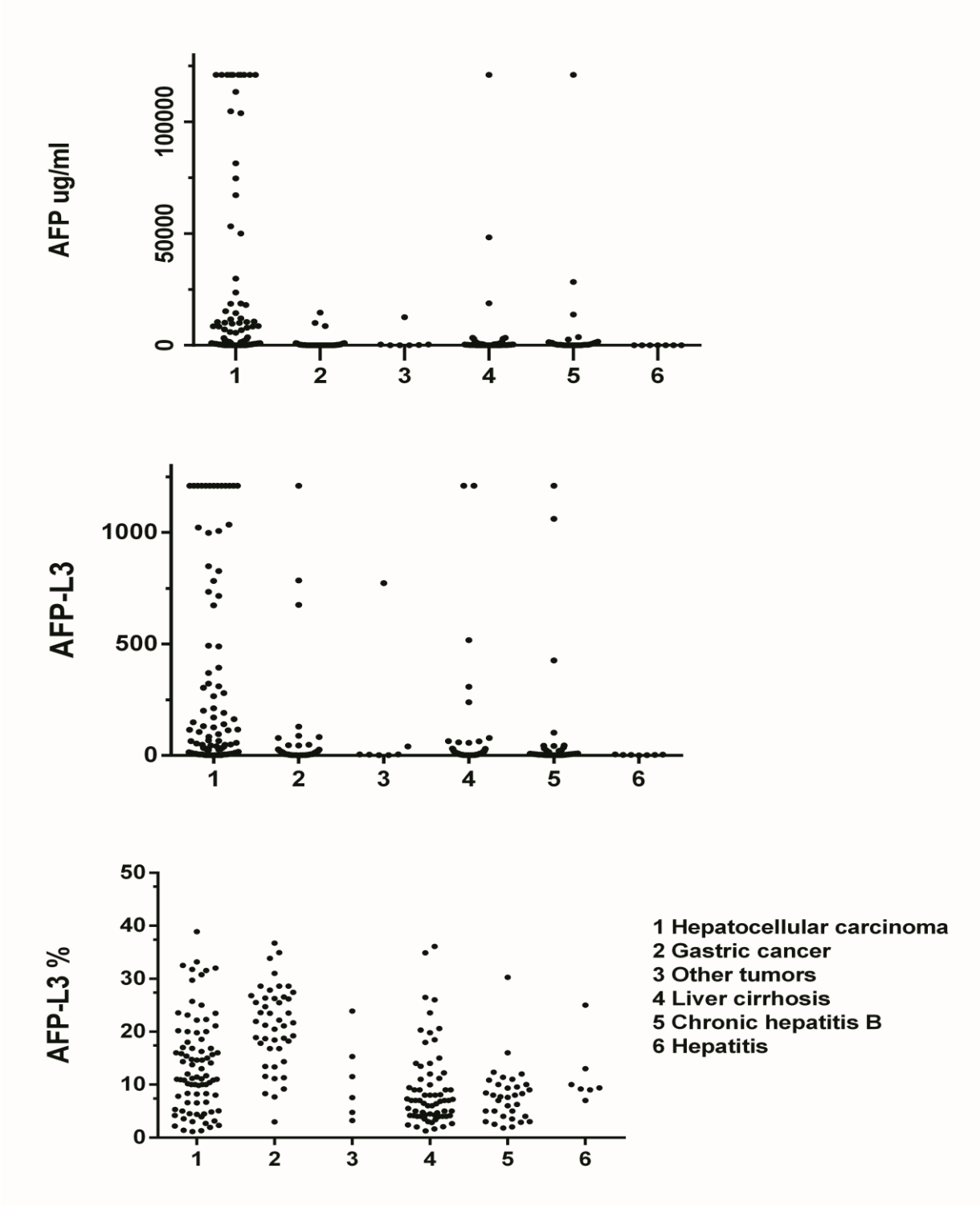

Figure 1 Comparison of AFP, AFP-L3, as well as AFP-L3% in the different groups of diseases as indicated.

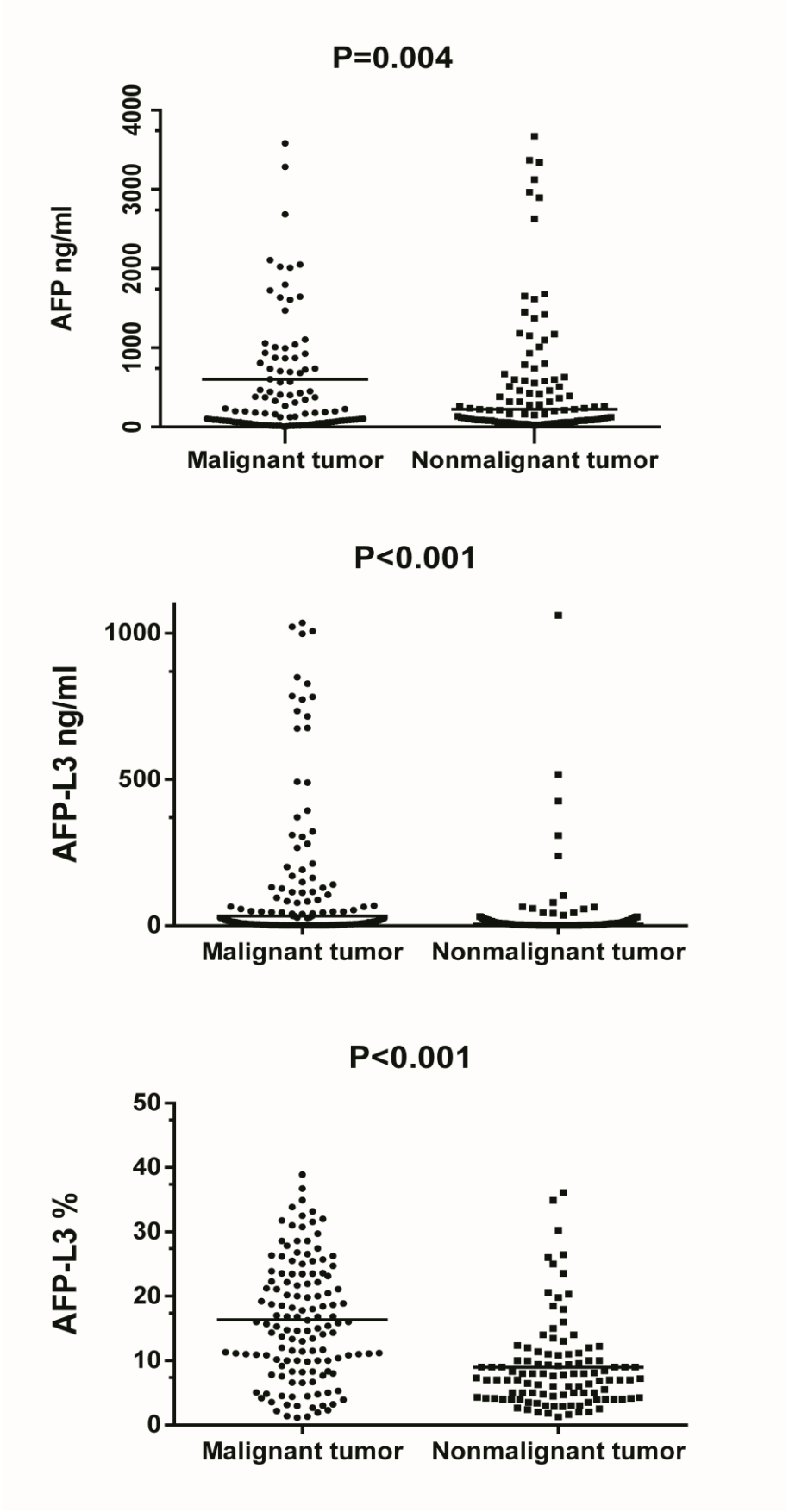

Figure 2 Comparison of AFP, AFP-L3, as well as AFP-L3% in malignant tumor and nonmalignant tumor groups.

Discussion

In clinic, the diagnosis of liver cancers primarily depends on the detection of serum AFP, imaging and pathological examinations. The AFP concentration over 400ng/ml was regarded to be ideal to diagnose HCC(5). However, during the routine clinical examinations, the serum AFP was usually found to be more than 400ng/ml in some patients who eventually were not clinically diagnosed as HCC, since cirrhosis, chronic hepatitis B, and even other types of tumor may also cause increased AFP. On the contrary, patients with mild or moderate AFP elevation had imaging or pathological evidence for tumors, thus were actually diagnosed to be HCC. The AFP-L3% is newly proposed to be a potential indicator for diagnosing malignant tumors, which increasingly reveals a higher diagnostic value in HCC than AFP(14, 15). The links of the pre-processed AFP-L3 to cancer etiology and progression have been investigated for a couple of years though, this field has not been availably and thoroughly explored to date(17).

It was believed that the AFP-L3 portion was much more sensitive to diagnose early-stage and/or small-sized HCCs when compared with AFP(7, 18, 19). And it was also reported to be reliably specific for HCC and was able to imply cancer features such as poor differentiation and/or cancerous aggression(20-23). We found that the low sensitivity (AFP-L3<0.605ng/ml) of the AFP-L3 to predict AFP-negative (AFP<7ng/ml) patients was consistent with the previous reports(24). The results suggest that AFP-L3 might show confined applications in terms of being an independent diagnostic biomarker for HCC, considering a remarkable fraction of patients with HCCs would be omitted by AFP.

In the present study, it is shown that HCC in patients with the level of serum AFP-L3 over 1ng/ml barely accounted for 38%, whereas the other non-neoplastic liver diseases accounted for nearly 40% in all the detected subjects. Our findings suggest that liver diseases should be first taken into account when AFP-L3 is larger than 1ng/ml. Once liver diseases are ruled out, it would then be put on high alert for tumors in other systems, especially for gastric cancers. Moreover, AFP and AFP-L3 were substantially greater in the sera of HCC patients than those with other tumors and non-neoplastic diseases. Therefore, as for patients who suffer from chronic hepatitis or cirrhosis whose AFP and AFP-L3 are mildly elevated in blood, regularly monitoring of AFP-L3 in blood is recommended; once a significant increase of the blood AFP-L3 occurs in a short period of time, liver carcinomatous changes should be under consideration. Some scholars thought that AFP-L3 measurement was allowed to predict the potential of biological malignancy of HCC prior to any therapy. To be noted, the AFP-L3 was also believed to contribute to the prediction of HCC relapse post curative therapy much earlier compared to the AFP detection(25-28).

Our data illustrate that the AFP-L3% in primary hepatic malignancy cohort was considerably higher compared with that in both chronic hepatitis B and cirrhosis counterparts. The increased AFP-L3% was highly associated with the degree of liver pathological changes of malignancy. Of note, we did observe several subjects in the cirrhosis and chronic hepatitis B groups with AFP of greater than 400ng/ml but with the AFP-L3% of no more than 10%. Although this portion of patients were not eventually diagnosed clinically with liver cancers, they should be highly recommended to continuously dynamic monitor of the AFP-L3%. If an evident elevation occurs in its serum level, the corresponding further detection should be done to early diagnose HCC and timely intervene. Finally, HCC should be considered within patients with chronic hepatitis B and/or cirrhosis, whose AFP-L3% index exceeds 10%. The AFP, AFP-L3 as well as AFP-L3% have

been observed to significantly increase in the sera of patients with HCC, cirrhosis and chronic hepatic diseases. Nevertheless, the levels of AFP and AFP-L3% were substantially greater in the malignant cohort compared to those in the nonmalignant counterpart. Previous research has indicated that high levels of AFP-L3% in blood predicted poor prognosis for patients with HCC(29).

In this study, a small number of nonmalignant tumor patients with the AFP-L3% over 10% could not be clinically diagnosed as malignant tumors, it is believed that such patients were likely to be at high risk of malignant tumors, and the instant changes of AFP-L3% and imaging examination should be monitored dynamically. Among the patients with malignant tumors and with the level of AFP-L3 over 1ng/ml, HCC accounted for 60%, and gastric cancer for nearly 40%. The increase of AFP-L3 in patients with gastric cancer was not as great as that in those with HCC, nonetheless, the AFP-L3% was similar in all these subjects. The AFP-L3% of these patients can be used as the diagnosis, prognosis and postoperative evaluation index for gastric cancer. The reason why these many patients with gastric cancer express AFP-L3 is unclear. We hypothesize that one of the gastric malignancies would have liver metastasis, but with the lesions being small or inconspicuous, and not clinically diagnosed. Furthermore, this part of gastric malignant tumors might be able to produce and secrete additional AFP-L3. These assumptions, of course, remain to be validated. Previous research reported that serum AFP-L3 was able to significantly increase in patients who suffered severe liver damages(30). In that study, 4 cases of hepatitis E had the AFP levels between 40ng/ml and 100ng/ml, among which 3 cases had AFP-L3% positively detected. Such a high positive rate is surprising, given that hepatitis E could cause acute and severe hepatitis, and it was speculated that AFP-L3% could be used as prognostic index of hepatitis E, which requires large size of samples and continuous follow-up to draw definite conclusions.

Conclusions

AFP-L3 is likely to contribute to differential diagnosis of HCC as well as other hepatic diseases, AFP-L3% is a reliable indicator for diagnosing benign and malignant tumors.

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None.

Authors' contributions

QL and ZWJ participated in study designing and data collecting, QL drafted the manuscript. All authors edited the manuscript and approved the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of the First Affiliated Hospital of Anhui Medical University. Written consent was obtained from each patient or an appropriate substitute decision-maker.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

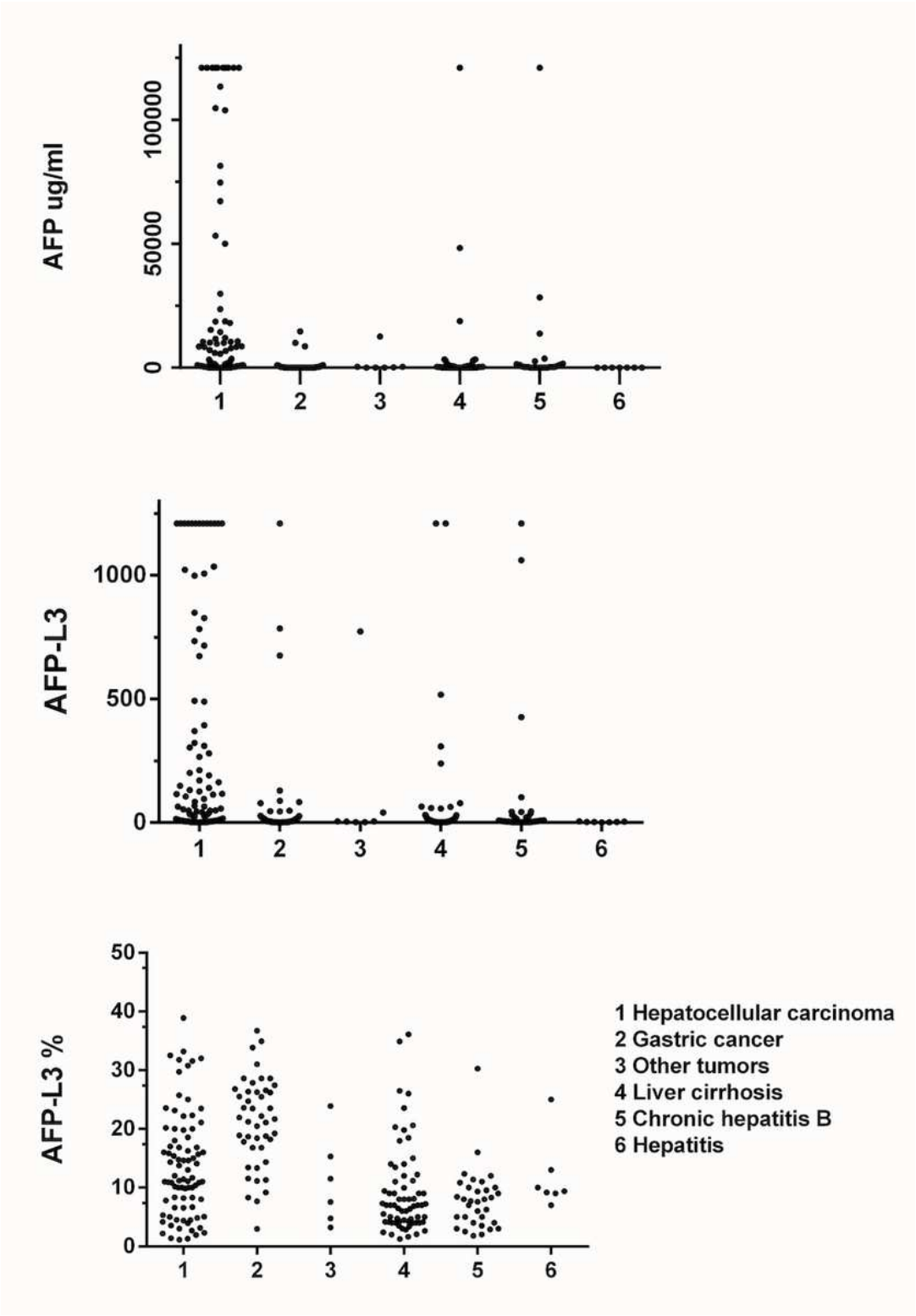

Figure 1

Comparison of AFP, AFP-L3, as well as AFP-L3% in the different groups of diseases as indicated.

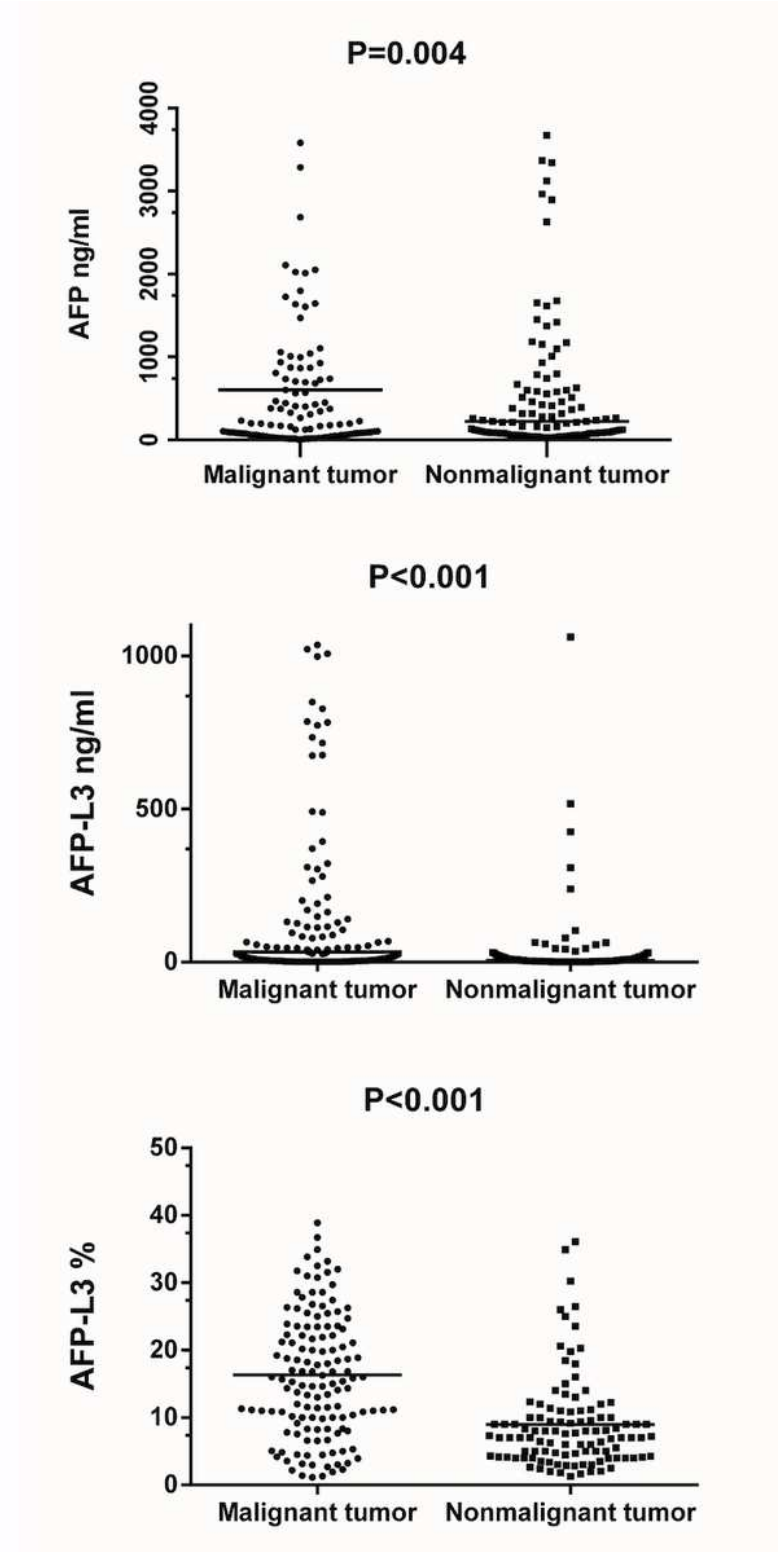

Figure 2

Comparison of AFP, AFP-L3, as well as AFP-L3% in malignant tumor and nonmalignant tumor groups.