

# Detection of autoantibodies in the serum of primary hepatocarcinoma patients

Fang Fang, Hua-Liang Wang, Ping Ye, Hai-Lin Deng, Gui-Ling Dong, Li-Ling Ma and Jian Wang

**Objective:** To study the significance of detecting autoantibodies in primary hepatocarcinoma (PHC) patients.

**Methods:** Autoantibodies were detected by indirect immunofluorescence assay. Antigens and antibodies of HBV were determined by enzyme immune assay. Antibody to HCV IgG was detected by enzyme-linked immunoabsorbent assay.

**Results:** The positive rate of autoantibody was 27.3% (38/139) in 139 PHC patients. The main type of autoantibodies in PHC was anti-nuclear antibody (36/38, 94.7%); others included anti-smooth muscle antibody (2/38, 5.3%), anti-mitochondria antibody (1/38, 2.6%), anti-midbody antibody (1/38, 2.6%), and anti-liver cell membrane antibody (2/38, 5.3%).

**Conclusions:** Detecting autoantibodies in PHC patients is of significance in studying the mechanism of autoimmune reaction and etiology in PHC. The diversity of autoantibodies might result from a wide variety of etiological factors involved in PHC development, and from a wide variety of overexpressed or mutated proteins involved in repeated cycles of necrosis and regeneration in hepatocarcinoma development.

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**Key words:** primary hepatocarcinoma; autoantibody; anti-nuclear antibody

## Introduction

Autoantibodies, the important diagnostic criterion for autoimmune diseases, could be found in several malignant tumors such as leukemia, breast cancer lung carcinoma, and nasopharyngeal carcinoma. Primary hepatocarcinoma (PHC) is one of them. There are few reports on detecting autoantibodies in PHC patients. In this research, we focused on the significance in detecting autoantibodies of PHC patients.

## Methods

## Patients

A total of 139 patients with PHC (123 were male and 16 female, aged 25-76) were studied. The diagnosis of PHC was based on ultrasonographic, CT, histological findings and serum alpha-fetoprotein (AFP) levels. As a control group, plasma of blood donors was collected from the Shanghai Blood Bank Center, Shanghai.

## Antigens and antibodies

Antigens and antibodies of HBV were determined by enzyme immune assay (Kehua Biotech Co, Ltd, Shanghai, China). Antibody to HCV IgG was detected by enzyme-linked immunoabsorbent assay (Fortune Long March-Trace Medical Science Co, Ltd, Shanghai, China).

## Autoantibodies

Autoantibodies were tested by indirect immunofluorescence using commercial Biochip slides (Euroimmun, Germany), kindly provided by Dr. Stoecker. Biochip slides were covered by human Hep-2 cell and frozen sections of different tissues including monkey liver, heart, rat liver, kidney and stomach. Serum and plasma were diluted by PBS-Tween. The assay was performed essentially according to the processing instruction. Autoantibodies were made visible with the LEICA DMLS fluorescence microscope.

## Statistical analysis

Pearson's  $\chi^2$  test was used for comparison.

*From the Department of Clinical Laboratory, Shanghai Eastern Hepatobiliary Hospital, Shanghai 200418, China (Fang F, Wang HL, Ye P, Deng HL, Dong GL, Ma LL and Wang J)*

*Correspondence: FangFang MD (Tel: 86-021-25070849; Fax: 86-021-25070850)*

**Table 1.** The detecting results of autoantibodies patterns and antigens or antibodies of HBV in 139 PHC patients

Antigens and antibodies patterns of HBV	No. of patients	Total positive	Autoantibody patterns									
			H	G	G + N	N	G + ASMA	AMA	G + N + LMA	H + M	LMA	
All negative	17	4(10.5%)	1	2	1	0	0	0	0	0	0	0
HBsAg⊕anti-HBe⊕anti-HBe⊕	98	26(68.4%)	4	14	3	1	2	0	1	0	1	0
anti-HBe⊕anti-HBc⊕12	4	(10.5%)	1	2	0	0	0	0	0	0	0	1
anti-HBs⊕anti-HBe⊕	4	2(5.3%)	0	1	0	0	0	0	1	0	0	0
anti-HBe⊕												
anti-HBs⊕	3	1(2.6%)	1	0	0	0	0	0	0	0	0	0
HBsAg ⊕ HBeAg ⊕ anti-HBe⊕anti-HBc⊕	2	0(0.0%)	0	0	0	0	0	0	0	0	0	0
HBsAg ⊕ HBeAg ⊕ anti-HBc⊕	1	0(0.0%)	0	0	0	0	0	0	0	0	0	0
HBsAg⊕anti-HBc⊕	1	0(0.0%)	0	0	0	0	0	0	0	0	0	0
Total	139	38(100.0%)	7	19	5	1	2	1	1	1	1	1

⊕: positive; H: ANA homogeneous; G: ANA speckled; N: nucleolar; ASMA: anti-smooth muscle antibody; AMA: anti-mitochondria antibody; M: anti-midbody antibody; LMA: anti-liver cell membrane antibody.

## Results

38 (38/139, 27.3%) patients were autoantibodies positive at a 1:100 or higher dilution. The results of autoantibody patterns and HBV antigens or antibodies are listed in Table 1.

All patients were negative for anti-HCV-IgG. Normal (<40U/L) and abnormal ALT results were observed in 35 (25.2%) and 104 (74.8%) patients, respectively. All of the blood donors were negative for antigens or antibodies of HBV and HCV, and all of ALT were normal.

In 50 blood donors, 2 (4%) with positive autoantibodies showed a significant difference from PHC patients ( $P < 0.01$ ). Moreover, 26 of the 38 autoantibody positive patients showed abnormal ALT; but 81 of 101 autoantibody negative patients showed abnormal ALT ( $P > 0.05$ ).

## Discussion

Autoantibodies were found in 38 of 139 (27.3%) PHC patients. 34 had a history of HBV infection. It is shown that a significant difference between chronic HBV infection (9.5%) and PHC patients. In the 38 autoantibody positive PHC patients, 4 (10.5%) had no evidence of HBV infection. It is suggested that autoantibody detection in PHC patients is of significance in studying the occurrence

mechanism of autoimmune reaction and etiological factors of PHC.

Many patterns of autoantibody exist in PHC. Anti-nuclear antibody (ANA) is the most frequent one with homogeneous, nuclear speckled and nucleolar distribution. In our study, 36 of the 38 (94.7%) autoantibody positive patients were ANA positive, suggesting that ANA is the major pattern in PHC. Other patterns were also observed: anti-smooth muscle antibody (ASMA), anti-mitochondria antibody, anti-midbody antibody and anti-liver cell membrane antibody, which could occur simultaneously with ANA in the same patient.

The diversity types of autoantibody might resulted from a wide variety of etiological factors for PHC development such as necrosis, viruses, alcohol and mold, and from a wide variety of overexpressed or mutated proteins involved in repeated cycles of necrosis and regeneration in hepatocarcinoma development.<sup>[1]</sup>

## Reference

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