Original Paper

Clinical Significance of Elevated Alpha-Fetoprotein (AFP) in Chronic Hepatitis C without Hepatocellular Carcinoma

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ABSTRACT

Background/Aims: Alpha-fetoprotein is often measured in subjects with chronic hepatitis C for diagnosing hepatocellular carcinoma. However, its prevalence and clinical significance remain inconclusive in subjects without hepatocellular carcinoma. The study was to assess the clinical, virologic, and histopathological significance of elevated AFP in chronic hepatitis C without the presence of hepatocellular carcinoma.

Methodology: The retrospective study enrolled 102 consecutive subjects with a histological diagnosis of chronic hepatitis C. None had evidence of hepatocellular carcinoma by image study at enrollment and for at least 6 months' follow-up. The correlation between serum alpha-fetoprotein level and clinical, virologic, or histopathological records was reviewed.

Results: The prevalence of elevated serum alpha-fetoprotein (≥13.6ug/L) was 28.4% (29/102) in this study. Hepatic steatosis (≥5% hepatocytes), hepatic fibrosis (≥ stage II), uric acid ≥6.3mg/dL, asparate aminotransferase ≥40 IU/L, albumin <3.5g/dL, and fasting plasma glucose <126mg/dL were significantly associated with elevated AFP in multivariate analysis. However, neither hepatitis C virus genotype Ib infection nor viral load ≥$1x10^6$ copies/ml was related to elevated AFP. A serum alpha-fetoprotein level of 15.6ug/L was 34.3% sensitive and 83.6% specific for hepatic steatosis, was 28.2% sensitive and 95.8% specific for ≥ stage II hepatic fibrosis in Chronic hepatitis C.

Conclusions: Elevated alpha-fetoprotein is independently associated with hepatic steatosis (≥5% hepatocytes), ≥stage II hepatic fibrosis, increased level of uric acid (≥6.3mg/dL) or asparate aminotransferase (≥40 IU/L), and decreased level of albumin (<3.5g/dL) or fasting plasma glucose (<126mg/dL). Viral factors, including hepatitis C virus genotype 1b infection and viral load, are not related to elevated alpha-fetoprotein in hepatitis C virus-infected subjects.

KEY WORDS:
Alpha-fetoprotein; Chronic hepatitis C; Hepatic steatosis; Hepatic fibrosis

ABBREVIATIONS:
Alpha-Fetoprotein (AFP); Hepatocellular Carcinoma (HCC); Alanine Aminotransferase (ALT); Chronic hepatitis C (CHC)

INTRODUCTION

Alpha-fetoprotein (AFP) is a glycoprotein secreted from the yolk-sac and fetal liver in early embryonic life. However, AFP is then derived mainly from the liver in adulthood and is normally absent or undetectable in healthy adults. AFP has been widely used as a tumor marker for hepatocellular carcinoma (HCC) and it is also related to benign chronic liver disease or liver regeneration after hepatocyte destruction caused by viral hepatitis. Elevated AFP has been found to be associated with acute exacerbation with elevation of serum alanine aminotransferase (ALT) level and the presence of bridging necrosis in chronic hepatitis B (1,2). Besides, elevated AFP is also related to the development of germ cell tumors after birth or neural tube defects in fetus. Although AFP has long been recognized as a tumor marker, it has also long been recognized to be an imperfect marker due to its elevation in nonmalignant conditions.

Chronic hepatitis C (CHC) has been shown to be associated with the development of liver cirrhosis and HCC, and AFP has been recommended as a screening marker for HCC in subjects with CHC (3,4). The prevalence of elevated AFP shows racial variation in CHC, and it is reported to be 10-43% in Western countries and 29% in Taiwan (3,5,6,7). The pathogenesis and clinical significance of elevated AFP in CHC without HCC remain uncertain. Elevation of AFP is found to be associated with liver cirrhosis or fibrosis in Western countries and Taiwan (7,8,9). Some investigations also suggest that elevated AFP is also associated with increased ALT level in Western countries (9,10). Elevated AFP is ever found to be related to hepatitis C virus (HCV) genotype Ib infection in one investigation conducted in Taiwan, but not in Western countries (7,9). However, the shortcomings of these studies are relatively small number of enrolled patients and analysis of limited variables.

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This retrospective study was to determine the prevalence of elevated AFP in naïve HCV infected subjects without the development of HCC. This study also tried to evaluate the relationship between elevated AFP and clinical, histopathological or virological characteristics, including HCV viral load and HCV genotype. A receiver operating characteristic (ROC) curve was graphed to determine an appropriate level of AFP in predicting hepatic steatosis (≥5% hepatocytes) or ≥ stage II hepatic fibrosis that will give optimal sensitivity and specificity.

METHODOLOGY

Patients

Between October 2003 and November 2005, 102 consecutive subjects seeking interferon therapy with repetitive positive anti-HCV antibody over 6 months' follow-up, positive HCV ribonucleic acid (RNA) test within 3 weeks prior to liver biopsy, and histological diagnosis of CHC were retrospectively enrolled in this study **(Table 1)**. The subjects with either one of the following criteria would be excluded from the study: i) had a history of daily alcohol consumption ≥30g within 6 months prior to liver biopsy; ii) positive serology for hepatitis B surface antigen (HBsAg); iii) presence of other causes of chronic liver disease; iv) exposure to hepatotoxic medication within 6 months prior to liver biopsy; or v) history of prior anti-HCV treatment. There was coincidentally no history of intravenous drug abuse in the enrolled subjects. No patients had evidence of HCC by ultrasound (US) or other image studies at enrollment and for at least 6 months' follow-up.

TABLE 1 Baseline Demographic Data of Enrolled HCV-Infected Patients

Variables	Case (n)	Mean ± SD	Median (Range)
Age (yrs)	102	53.4±11.3	53.0 (26-76)
Gender			
Male / Female	69 / 33		
BMI (kg/m²)	101	25.1±3.0	25.0 (19.8-33.4)
Hepatic steatosis			
0 / 1 / 2 / 3	67 / 28/ 7 / 0		
Inflammation-necrosis grade			
0 / 1 / 2 / 3 / 4	0 / 7 / 72 / 23 / 0		
Fibrosis stage			
0 / 1 / 2 / 3	0 / 24 / 58 / 20		
HCV genotype			
1b	38		
Non-1b	30		
Hemoglobin (g/dL)	102	13.9±2.1	14.0 (6.2-17.8)
Platelets (x10⁴/uL)	102	14.7±6.2	14.2 (2.1-35.0)
ALT (IU/L)	102	131.9±121.6	96.0 (16-773)
AST (IU/L)	102	90.5±71.3	73.0 (24-443)
Total bilirubin (mg/dL)	102	0.94±0.46	0.80 (0.2-2.7)
FPG (mg/dL)	99	111.8±36.2	101.0 (75-296)
Albumin (g/L)	100	4.23±0.49	4.30 (1.9-5.2)
Uric acid (mg/dL)	99	6.5±1.7	6.60 (3.0-11.5)
AFP (ug/L)	102	28.28±81.43	7.95 (1.44-643.41)
ALK-P (U/L)	86	224.1±122.0	183.5 (81-910)

Histology

All subjects underwent a percutaneous liver biopsy to stage CHC as part of anti-HCV pretreatment assessment. Liver histology was reviewed by one pathologist who was blind to the clinical and laboratory findings of the patients. Results of histopathology were reported using a modified histological activity index scoring system, according to the classification of Knodell and Scheuer (11,12). Steatosis was graded as follows: 0, <5% steatosis; 1, 5-30% of hepatocytes with steatosis; 2, 30-70% of hepatocytes affected; 3, >70% of hepatocytes affected. Inflammation-necrosis grade was as follows: 0, no or minimal inflammation; 1, portal inflammation or lobular inflammation with no necrosis; 2, mild piecemeal necrosis or focal hepatocellular necrosis; 3, moderate piecemeal necrosis or severe focal cell damage; 4, severe piecemeal necrosis or bridging necrosis. Fibrosis was staged as follows: 0, no fibrosis; 1, enlarged fibrotic portal traces; 2, periportal or portal to portal septa; 3, bridging fibrosis with architectural distortion, no obvious cirrhosis; 4, cirrhosis.

Laboratory and Virologic Analysis

All subjects were screened for the presence of anti-HCV by a third-generation microparticle enzyme immunoassay (MEIA) (Abbott Laboratories, Wiesbaden-Delkenheim, Germany) and the presence of HBsAg by a chemiluminescent microparticle immunoassay (CMIA) (Abbott Laboratories, Wiesbaden-Delkenheim, Germany) according to the manufacturer's instructions. Routine laboratory tests included complete blood counts, fasting plasma glucose (FPG), albumin, total bilirubin (Bil-T), ALT, aspartate aminotransferase, (AST), uric acid, alkaline phosphatase (ALK-P), and AFP. The normal reference value of serum AFP was <13.6ug/L in healthy subjects. Routine laboratory test, body mass index (BMI), serum HCV measurements, and HCV genotyping were collected within one week prior to liver biopsy. Quantification of serum HCV RNA was performed according to the manufacturer's instructions (US patent no. 6913887, Qgene Biotechnology Corp., Kaohsiung, Taiwan). A 244-bp fragment of the HCV 5'-noncoding region (NCR) was amplified by the primer KY78 (5'-GAA AGC GTC TAG CCA TGG CGC-3') and KY80 (5'-TGA CGG ACT ATC CCA CGA ACG-3'.

The amplification was performed in a 50-ml reaction mixture containing 1X green polymerase chain reaction (PCR) master mix (SYBR®, Applied Biosystems, Foster City, CA, USA), 0.5μM of primers, and 5μl of c-deoxyribonucleic acid (cDNA). HCV genotyping was performed according to Simmonds' classification (13). Sequence alignment was performed by EMBOSS (European Molecular Biology Open Software Suite). PCR products were withdrawn and purified by PCR purification Kit (QIAquick®, Qiagen Inc., Hilden, Germany). PCR products were sequenced with reverse primer of HCV 5' NCR by DNA sequencer (ABI Prism 3100 Sequencer, Applied

Biosystems). The cost of checking HCV quantification and genotype should be paid by the patients rather than be covered by the government insurance system, therefore genotype was not checked in 34 subjects.

Statistical Analysis

The descriptive statistics were expressed with mean ± standard deviation and mean with range for continuous variables and proportion for categorical variables. The χ^2 test or Fisher's exact test, or Student's t test was used for comparison of categorical variables. Both univariate and multivariate analyses were used to determine the association between risk factors or clinical presentation of CHC and elevated AFP. Variables showing relevant association, with $p \leq 0.25$, on univariate analysis were included in the logistic regression model and a level of significance of 0.05 was adopted. A receiver operating characteristic (ROC) curve was graphed to determine an appropriate level of AFP in predicting hepatic steatosis or hepatic fibrosis ≥ II that gives optimal sensitivity and specificity. The statistical analysis was processed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 2 shows the results of univariate analysis of elevated AFP (≥13.6ug/L) and associated presentation in HCV-infected patients. Elevated AFP level was significantly associated with BMI ≥25kg/m², fibrosis stage ≥2, albumin <3.5g/dL, ALT ≥40 IU/L, and ALK-P ≥126U/L in univariate analysis ($p<0.05$). However, there was a trend that elevated AFP level was associated with age ≥50 years, liver inflammation-necrosis grade >2, hepatic steatosis (≥5% hepatocytes), HCV load <1x10⁶ copies/ml, platelet count <10 x 10⁴/uL, uric acid ≥6.3mg/dL, AST ≥40 IU/L, and FPG <126mg/dL ($p \leq 0.25$).

Table 3 shows the results of multivariate analysis of elevated AFP (≥13.6ug/L) and associated presentation in HCV-infected patients. Hepatic steatosis (≥5% hepatocytes), hepatic fibrosis (≥stage II), uric acid ≥6.3mg/dL, AST ≥40 IU/L, albumin <3.5g/dL, and FPG <126mg/dL were significantly associated with elevated AFP after adjusting for age, HCV viral load, hepatic steatosis, ≥ stage II hepatic fibrosis, hepatic inflammation-necrosis grade, body mass index (BMI), platelet counts, serum total bilirubin (BIL-T), alkaline phosphatase (ALK-P), albumin, uric acid, fasting plasma glucose (FPG) level, alanine aminotransferase (ALT), and asparate aminotransferase (AST) remained independently associated with elevated AFP in these subjects with CHC.

Based on ROC curve **(Figure 1)**, AFP level of 15.6ug/L is as high as 83.6% specific, but only 34.3% sensitive with positive predictive value of 63% in predicting hepatic steatosis (≥5% hepatocytes). Based on ROC curve **(Figure 2)**, AFP level of 15.6ug/L is as high as 95.8% specific, but only 28.2% sensitive with positive predictive value of 71% in predicting ≥ stage II hepatic fibrosis.

TABLE 2 Univariate Analysis of Elevated AFP (≥13.6Ug/L) and Associated Presentation in HCV-Infected Patients

Variables	AFP ≥13.6ug/L (%)	OR (95% CI)	*p*-value
Gender	102		
Male	19/69 (27.5%)	1.000	
Female	10/33 (30.3%)	1.144 (0.460-2.846)	0.772
Age	102		
<50 years	7/34 (20.6%)	1.000	
≥ 50 years	22/68 (32.4%)	1.845 (0.696-4.887)	0.214
BMI (kg/m²)	101		
<25	10/52 (19.2%)	1.000	
≥ 25	18/49 (36.7%)	2.439 (0.990-6.008)	0.050
Inflammation-necrosis grade	102		
1-2	20/79 (25.3%)	1.000	
3	9/23 (39.1%)	1.896 (0.712-5.048)	0.196
Fibrosis stage	102		
1	1/24 (4.2%)	1.000	
2-3	28/78 (35.9%)	12.880 (1.650-100.539)	0.003
HCV genotype	68		
1b	12/38 (31.6%)	1.000	
Non-1b	10/30 (33.3%)	1.083 (0.390-3.010)	0.878
HCV load	102		
<1x10⁶ copies/mL	24/73 (32.9%)	1.000	
≥1x10⁶ copies/mL	5/29 (17.2%)	0.425 (0.144-1.253)	0.114
Hemoglobin	102		
<10g/dL	2/7 (28.6%)	1.000	
≥10g/dL	27/95 (28.4%)	0.993 (0.181-5.430)	0.993
Platelets	102		
<10x10⁴/uL	10/25 (40.0%)	1.000	
≥10x10⁴/uL	19/77 (24.7%)	0.490 (0.189-1.275)	0.140
Albumin	100		
<3.5g/dL	4/5 (80.0%)	1.000	
≥3.5g/dL	25/95 (26.3%)	0.089 (0.010-0.837)	0.024
Uric acid	99		
<6.3mg/dL	11/48 (22.9%)	1.000	
≥6.3mg/dL	17/51 (33.3%)	1.682 (0.691-4.095)	0.250
ALT	102		
<40 IU/L	1/17 (5.9%)	1.000	
≥40 IU/L	28/85 (32.9%)	7.860 (0.991-62.305)	0.036
AST	102		
<40 IU/L	2/19 (10.5%)	1.000	
≥40 IU/L	27/83 (32.5%)	4.098 (0.883-19.029)	0.055
Total bilirubin	102		
<1.2mg/dL	20/81 (24.7%)	1.000	
≥1.2mg/dL	9/21 (42.9%)	2.288 (0.841-6.224)	0.100
ALK-P	86		
<126U/L	20/77 (26.0%)	1.000	
≥126U/L	7/9 (77.8%)	9.975 (1.912-52.042)	0.004
FPG	99		
<126mg/dL	26/79 (32.9%)	1.000	
≥126mg/dL	3/20 (15.0%)	0.360 (0.097-1.339)	0.116
Steatosis	102		
0	15/67 (22.4%)	1.000	
≥1	14/35 (40.0%)	2.311 (0.952-5.613)	0.061

DISCUSSION

The reported prevalence of elevated AFP in subjects with CHC varies widely because the sampled population in the literature differs greatly in different stages of CHC, such as HCC and compensated liver cirrhosis (3,7,8). Some studies even suggest that the prevalence of elevated AFP may be affected by ethnicity or genetic factor (5,9). The prevalence of ele-

TABLE 3 Multivariate Analysis of Elevated AFP (≥13.6 Ug/L) and Associated Presentation in HCV-Infected Patients

Variables	OR	(95% CI)	*p*-value
Steatosis	4.263	1.302-13.963	0.017
Stage 2-3	13.796	1.513-125.753	0.020
HCV load ≥1x10^6 copies/mL	0.273	0.070-1.069	0.062
Albumin ≥3.5g/dL	0.024	0.001-0.891	0.043
FPG ≥126mg/dL	0.161	0.29-0.900	0.038
Uric acid ≥6.3mg/dL	3.622	1.102-11.904	0.034
AST ≥40 IU/L	7.497	1.153-48.753	0.035

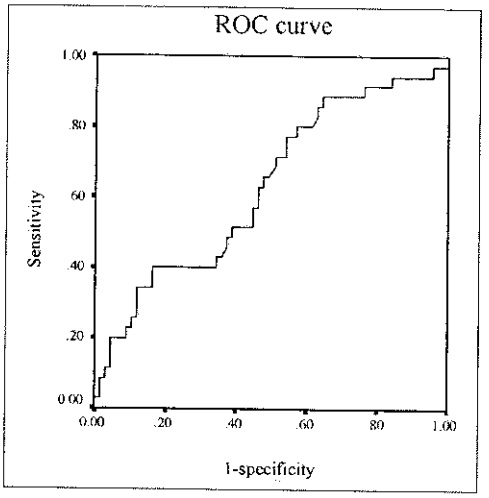

FIGURE 1
Receiving operating characteristic (ROC) curve was plotted to determine AFP level in predicting hepatic steatosis. AFP level of 15.6ug/L (arrow) is 34.3% sensitive and 83.6% specific for hepatic steatosis, with 63% positive predictive values.

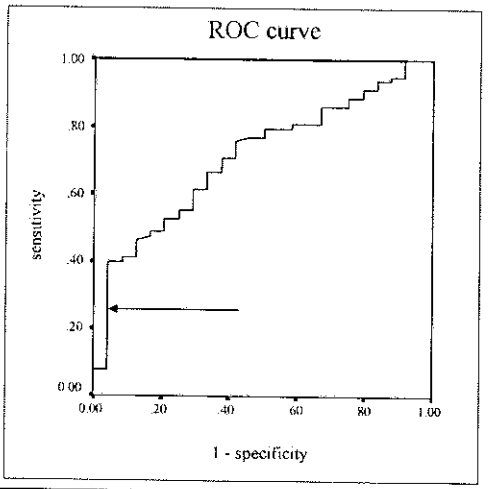

FIGURE 2
Receiving operating characteristic (ROC) curve was plotted to determine AFP level in predicting hepatic fibrosis. AFP level of 15.6ug/L (arrow) is 28.2% sensitive and 95.8% specific for ≥ stage II hepatic fibrosis, with 71% positive predictive values.

vated serum AFP is 28.4%, which is comparable to that (29%) of previous report reviewed by Chu *et al.* in Taiwan (7). However, in contrast to the study including 8.7% of subjects with cirrhosis reviewed by Chu *et al.*, the present study does not enroll any subjects with cirrhosis or HCC. Anyway, the present study is not a community-based study and it needs more studies to define the true prevalence of elevated AFP in HCV-infected subjects of Taiwan.

Although Nguyen *et al.* report that AFP greater than 100ng/mL is 97.3% specific for HCV-related cirrhosis and AFP greater than 200ng/mL is 100% specific for HCV-related HCC (6), it should be noted that all of the 3 subjects with AFP greater than 300ng/L had hepatocellular damage in this study because their ALT were all greater than 120 IU/L. The histological specimens showed stage III hepatic fibrosis and Grade III inflammation-necrosis in one subject, and stage II hepatic fibrosis and Grade II inflammation-necrosis were found in the other 2 subjects.

Consistent with the reports conducted in Taiwan and Western countries, the present study supports that hepatic fibrosis rather than hepatic necrosis-inflammation is closely associated with elevated AFP in subjects with CHC (7-9). Bayati *et al.* demonstrates that elevated AFP is found in 47% of HCV-infected subjects with cirrhosis and in 13% of HCV-infected subjects without cirrhosis (8). Hu *et al.* showed that elevated AFP is independently associated with stage III/IV hepatic fibrosis and the prevalence of elevated AFP is 15.3%, 24.5%, and 42% in HCV-infected subjects with stage 0-II, III, and IV, respectively (9). Chu *et al.* demonstrated that elevated AFP is associated with stage III/VI hepatic fibrosis in HCV-infected Chinese patients (7). The present study extends previous findings to suggest that not only stage IV or III hepatic fibrosis, but also stage II hepatic fibrosis is independently associated with elevated AFP in HCV-infected Chinese subjected. The prevalence of elevated AFP is 4.1% (1/24), 34.7% (20/58), and 40.0% (8/20) in HCV-infected Chinese with stage I, II, and III, respectively. In the present study, ROC curve was employed to assess the utilization of serum AFP level in predicting stage II/III hepatic fibrosis. The study shows that AFP level of 15.6ug/L is 95.8% specific with positive predictive value of 71% in predicting ≥ stage II hepatic fibrosis, although it is not sensitive (28.2%). In brief, elevated AFP provides a clinical clue for diagnosing stage II/III hepatic fibrosis in HCV-infected Chinese.

An AFP level of 15.6ug/L is 83.6% specific with positive predictive value of 63% in predicting hepatic steatosis (≥5% hepatocytes), and the sensitivity is only 34.3%. The possible reason for this observation that elevated AFP is related to hepatic steatosis is never mentioned in the literature, and the relationship between hepatic steatosis and elevated AFP deserves more investigation because the grade of steatosis commonly observed in this study is only grade 1.

Consistent with the literature conducted by Hu *et al.*, the present study conducted in Taiwan shows that viral factors, including HCV viral load and genotype 1b infection, are not associated with elevated AFP in HCV-infected subjects (9). Moreover, HCV viral load and genotypes show no significant correlation with hepatic necroinflammation and hepatic fibrosis in another literature conducted in Taiwan (14). However, Chu *et al.* in Taiwan suppose that HCV genotype 1b infection may lead to elevated AFP caused by more severe hepatic necroinflammation and fibrosis (7).

The AFP and albumin genes are suggested to be arranged in tandem by a similar structure and be derived from a common ancestral gene, and the switching action of AFP enhancer from AFP promot-

er to albumin promoter may lead to a reciprocal expression between AFP and albumin (15). The HCV-infected subjects with elevated AFP may thus be related to decreased albumin gene transcription and decreased albumin level.

The mechanism of the close association between decreased FPG (<126mg/dL) and elevated AFP in HCV-infected subjects is attributed to the inhibition of glucose production caused by the defect in the gap junctions and actions of sympathetic nerves in regenerating liver (16). Endo *et al.* also propose that increased hepatocyte proliferation may result in reduced gluconeogenesis caused by the immature function of regenerating hepatocytes (17).

Consistent with the report of Hu *et al.*, the present study shows that AST rather than ALT is an independent predictor of elevated AFP in HCV-infected subjects (9). In contrast to ALT that is exclusively located in the cytoplasm, AST is mainly located in mitochondria of hepatocytes and AST level will exceed the ALT level when AST is released from the mitochondrial AST compartments as a consequence of more severe hepatocellular damage. Moreover, the plasma clearance of AST is modulated by sinusoidal liver cells, which are damaged in the development of fibrosis or cirrhosis (18).

Increased serum level of uric acid (≥6.3mg/dL) is shown to be significantly related to elevated AFP in the present study. However, the relationship between uric acid and AFP is never mentioned in the literature. George *et al.* shows that elevated uric acid levels in serum and urine, without alteration of blood urea and creatinine levels, are observed in experimentally induced liver damage. They conclude that the increase of these metabolic parameters is compatible with the deterioration of liver functions during the pathogenesis of hepatic fibrosis (19). Usami *et al.* also suppose that the catabolism of purine nucleotides will increase in regenerating liver by demonstrating that decreased liver adenosine triphosphate and increased plasma xanthine and hypoxanthine can be observed in the rats undergoing partial hepatectomy (20).

In conclusion, this study found that a serum AFP level of 15.6ug/L is 28.2% sensitive and 95.8% specific for the diagnosis of ≥ stage II hepatic fibrosis in CHC without HCC. The present study demonstrates that viral factors, including HCV genotype 1b infection and viral load, are not related to elevated AFP in HCV-infected subjects. Elevated serum AFP is independently associated with hepatic steatosis (≥5% hepatocytes), ≥ stage II hepatic fibrosis, increased level of uric acid (≥6.3mg/dL) or AST (≥40 IU/L), and decreased level of albumin (<3.5g/dL) or FPG (<126mg/dL).

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