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Anticardiolipin Antibodies in Patients With Liver Disease

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OBJECTIVE: Our aim was to test the hypothesis that anticardiolipin antibodies (aCL) may cause an antiphospholipid syndrome and thrombotic events in patients with liver disease.

METHODS: aCL were measured in 116 healthy controls and 372 patients with liver disease of different stage and etiology: 136 cases secondary to hepatitis C virus (HCV) infection, 139 due to hepatitis B virus (HBV) infection, 69 with alcoholic liver damage, and 28 cryptogenic in origin. Prior thrombotic events were recorded. The results were related to age, gender, stage, severity, and etiology of the liver disease, as well as to the occurrence of organ- and nonorgan-specific autoantibodies.

RESULTS: aCL were positive in 4.4% of controls and in 18.8% of patients ($p < 0.0002$). Patients with aCL were more frequently men with an advanced cirrhosis and simultaneous occurrence of anti–smooth-muscle antibodies (ASMA) in serum ($p < 0.0006$); their liver damage was often secondary to HBV (37.3%) or alcohol abuse (18.5%). At conditional logistic regression analysis, only the presence of ASMA (odds ratio [OR] = 3.02, 95% confidence interval [CI] 1.7–5.5, $p = 0.0003$), HBV (OR = 3.4, 95% CI 1.6–7.2, $p = 0.0013$), or alcoholic liver disease (OR = 5.3, 95% CI 2.3–12.2, $p = 0.0001$) were independently associated with aCL. Thrombosis was encountered in 24 patients (6.4%). At conditional logistic regression analysis, thrombosis was significantly associated with advanced age (OR = 1.07, 95% CI 1.0–1.1, $p = 0.0094$), development of hepatocellular carcinoma (OR = 17.8, 95% CI 1.6–196.0, $p = 0.01$), HBV etiology (OR = 6.3, 95% CI, 1.6–24.6, $p = 0.0076$), or cryptogenic liver disease (OR = 54.8, 95% CI 5–599.9, $p = 0.001$). Of the five patients with newly documented portal thrombosis during the follow-up, only one tested positive for aCL.

CONCLUSIONS: In patients with nonautoimmune liver disease, aCL production is an epiphenomenon of the liver damage and is not associated with thrombotic complications. These data do not support the hypothesis that HCV is a cause of the antiphospholipid syndrome. (Am J Gastroenterol 1999;94:2983–2987. © 1999 by Am. Coll. of Gastroenterology)

INTRODUCTION

Portal vein thrombosis is an important complication of cirrhosis. Its prevalence appears low (0.6%) in compensated stages but is as high as 25% in decompensated liver cirrhosis, usually associated with hepatocellular carcinoma (HCC) (1, 2). Portal vein thrombosis may induce a further increase in portal pressure and precipitate variceal bleeding; it thus constitutes a further hazard for liver transplantation (3). The high prevalence rates of thrombosis together with the relevant clinical sequelae of its occurrence have prompted the search for factors that may predispose cirrhotic patients to this complication. Recently, attention has focused on the role of antiphospholipid antibodies, in particular anticardiolipin antibodies (aCL). aCL are characteristically found in patients with systemic lupus erythematosus (SLE) or related autoimmune diseases (4) and, in this setting, their expression is significantly associated with thrombotic complications. On the other hand, more than one-half of patients with non-SLE autoimmune disorders are positive for lupus anticoagulant antibodies or aCL and, in this setting, their expression is not related to thrombotic disease (4).

A variety of infective agents have been implicated in non-SLE disorders leading to aCL production (5). Viral infections are the main causes of chronic liver disease. aCL have been reported in patients with liver damage secondary to hepatitis C virus (HCV) infection (13, 14), where they have been involved in the development of thrombosis, thrombocytopenia, and hepatic vein occlusion (6–12). Further, HCV has been suggested to cause the antiphospholipid syndrome (14).

From published data, it is unclear whether, in liver disorders, aCL production is uniquely associated with HCV infection or may be encountered in nonautoimmune, viral, and nonviral liver diseases; is related to severity and stage of the liver damage; is simply an epiphenomenon of the underlying liver damage and is accompanied in a nonspecific manner by other autoantibodies; or is of prognostic rele-

vance in identifying patients at risk for thrombotic events. This study was undertaken to ascertain the clinical relevance of aCL in patients with nonautoimmune liver disease of different stage and etiology.

MATERIALS AND METHODS

Patients

From June 1996 to January 1997 all in- and outpatients with chronic liver disease observed at the Division of Gastroenterology of our hospital were consecutively included to reach a comparable number in each etiological group. Patients with double etiology (*i.e.*, alcohol + virus, or double viral infection), autoimmune chronic hepatitis, or primary biliary cirrhosis were excluded. In total, 372 patients with different stage and severity of liver disease were included. One hundred-sixteen normal volunteers recruited from the medical staff served as controls; all tested negative for hepatitis B virus (HBV) and HCV markers, autoantibodies, and history of thrombotic events. Alanine transaminase (ALT) levels were normal in all of them.

In the 372 patients, liver disease was related to HBV in 139 cases, to HCV in 136 cases, to alcohol abuse (>80 g/daily for >5 yr) in 69 cases; in the remaining 28 cases it was cryptogenic. One hundred and thirty-one (96.3%) of the anti-HCV–positive and 115 (83.1%) of the hepatitis B surface antigen (HBsAg)-positive patients exhibited viral replication when tested by polymerase chain reaction (PCR). Among the 139 HBsAg-positive patients, 93 were anti-HBeAg positive (67.6%). Genotype 1b was found in 79 of 136 (58%) HCV-infected patients. None of the patients with a diagnosis of chronic HCV or HBV hepatitis was taking interferon therapy at the time of this study. Liver biopsy was performed in all patients with chronic hepatitis; in cirrhotics, it was performed only when signs of portal hypertension were not apparent. Ultrasound-guided fine liver biopsy was done in all patients with a nodular lesion (15). The presence of portal hypertension was defined as a diameter of portal vein >13 mm or maximum spleen axis >13 cm at ultrasound evaluation. Portal thrombosis was diagnosed by Doppler ultrasonography and enhanced computed tomography.

Thrombocytopenia was defined as a platelet count <140,000/μl. Prior episodes of thrombosis, myocardial infarction, or ischemic stroke were recorded.

Follow-Up Evaluation

After inclusion into the study, patients were invited to ambulatory visits at 6-month intervals for a mean of 12 months. Only 50 agreed to this program. At each follow-up visit patients were interviewed about the occurrence of thrombotic events; in particular, as most thrombotic events turned out to occur within the portal tract, color Doppler evaluation of the portal system was performed after 12 months in all cases. Moreover, in the 78 patients with HCC, enrolled in a therapeutic protocol for transarterial chemoembolization or percutaneous ethanol injections, patency of portal tract was also controlled by arteriography.

Methods

Standard laboratory chemistries were performed using well-validated procedures. Presence of antinuclear antibodies (ANA), antimitochondrial antibodies (AMA), anti–smooth-muscle antibodies (ASMA), and anti–liver-kidney microsomal antibodies (anti-LKM) were detected on serial dilutions of sera by indirect immunofluorescence, using commercially prepared slides (Incstar Corporation, Stillwater, MN). Titers >1:40 were considered positive. aCL of IgG isotype were evaluated in serum using a quantitative solid-phase enzyme-linked immunosorbent assay (IgG Selisa, Byk Gulden, Milano, Italy). In this assay a human serum was used to dilute samples using calibration in GPL units (γ phospholipid unit; 1 unit = 1 mg/ml of an affinity-purified standard IgG sample). The titer of each sample was calculated according to the manufacturer's recommendations; the intra- and interassay coefficients of variation (CV) were 4.1% and 12.1%, respectively. As the distribution of the normal range is logarithmic, a log transformation of the aCL values was used. The upper cut-off value of the test was chosen as the geometric mean plus 2SD of the normal values obtained in controls included in this study.

Values >9.1 GPL units were considered positive. The prevalence of aCL was determined at recruitment both in patients and controls; a second evaluation after 6 or 12 months was possible in 50 patients.

Anti-HCV antibodies were detected in serum using an enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Raritan, NJ). HCV RNA was determined after reverse transcription by nested PCR with primers for 5'UTR (16). The HCV genotype was assessed in all HCV RNA-positive patients by solid-phase hybridization (LIPA, Ghent, Belgium). HBsAg, anti-HBsAg, anti-HBeAg, HBeAg, and anti-HBcAg were evaluated by commercial assays. HBV DNA was evaluated by liquid-phase hybridization or nested PCR (17).

Statistical Analysis

Statistical analysis was performed using the SSPS version 6.0 for MacIntosh. For comparison of clinical data between aCL-positive and aCL-negative patients, with and without thrombosis, χ^2 and Mann-Whitney tests were used for discrete and continuous variables, respectively. Adjusted odds ratios (OR) were derived from a conditional logistic regression analysis.

RESULTS

The mean age of the 372 patients with liver disease was 51.5 ($\pm$14.6) yr, with a male predominance of 4:1. The mean age of the 116 controls was 54.4 yr ($\pm$13.2) , with a male:female ratio of 4:1. Prevalence of aCL was 4.4 % (5/116) in the control population and 18.8% (70/372) in patients with

Table 1. aCL Prevalence in 372 Patients Divided According to Stage, Severity, and Etiology of Liver Disease, N (%)*

	Total	HCV	HBV	Alcoholic	Cryptogenic
aCL-positive patients	70/372 (19)	11/136 (8)	31/139 (22)	23/69 (33)	5/28 (18)
Hepatitis (fatty liver + CPH + CAH)	22/185 (12)	3/73 (4)	15/69 (22)	1/20 (5)	3/23 (13)
Cirrhosis	31/109 (28)	2/31 (6)	11/36 (31)	16/37 (43)	2/5 (40)
HCC	17/78 (22)	6/32 (19)	5/34 (15)	6/12 (50)	0
Child-Pugh class					
A	19/103 (18)	5/45 (11)	4/28 (14)	8/27 (30)	2/3 (67)
B	22/63 (35)	2/14 (14)	7/27 (26)	13/20 (65)	0/2
C	7/21 (33)	1/4 (25)	5/15 (33)	1/2 (50)	0
Thrombocytopenia ($\times 10^3$)					
<50	7/29 (24)	0/5	6/21 (28)	1/2 (50)	0/1
50–140	33/113 (29)	6/40 (15)	12/37 (32)	14/35 (40)	1/1 (100)
>140	30/230 (13)	5/91 (5)	13/81 (16)	8/32 (25)	4/26 (15)
Thrombotic events	7/24 (29.1)	1/4 (25)	3/13 (23.0)	2/5 (40.0)	1/2 (50)

* Significant differences are reported in the text (*Results*).

aCL = anticardiolipin antibodies; HCV = hepatitis C virus; HBV = hepatitis B virus; HCC = hepatocellular carcinoma; CPH = chronic persistent hepatitis; CAH = chronic active hepatitis.

chronic liver disease ($p < 0.0002$). In both patients and controls, aCL increased with age: they were detected in 15.9% (15/94) of patients <40 yr, in 18.6% (46/247) of cases with age ranging from 41 to 70 yr, and in 29% (9/31) of those >70 yr ($p = 0.27$); this trend was not statistically significant. aCL were more frequently positive in men (80%) than in women, in patients or in controls.

When patients were stratified according to the stage, severity, and etiology of the liver disease (Table 1), aCL prevalence rates were directly related to the stage of the disease: they were found in 22 of 185 patients (11.8%) with chronic hepatitis and in 28.4% (31/109) of cirrhotics ($p = 0.0029$). The development of HCC was not associated *per se* with higher rates; aCL was found in 25.6% of cirrhotics with HCC (n = 78) and in 28.4% of cirrhotics without HCC (n = 109) ($p = 0.30$).

In patients with liver cirrhosis, regardless of the presence of HCC, liver failure (assessed by Child-Pugh score) appeared to influence the rate of aCL-positive patients: positive values were twice as higher in class B and C as in class A ($p = 0.05$) (Table 1). aCL positivity was not associated with thrombocytopenia ($p = 0.28$). In patients with liver damage secondary to HBV infection (n = 139), alcohol abuse (n = 69), or of cryptogenic origin (n = 28), aCL were found two to four times more frequently than in HCV-related liver disease (22%, 33%, and 18% *vs* 8%, respectively) ($p = 0.00023$) (Table 1). When etiological subgroups of patients were further stratified according to stage and severity of liver disease, this difference remained statistically significant.

In our series, the autoantibodies most frequently found were ASMA and aCL, present in 89 and 70 of 372 patients (23.9 and 18.8%, respectively). ASMA appeared equally distributed among the four etiological subgroups. Twenty-seven of 70 aCL-positive patients (38.5%) were also ASMA positive. When aCL titers were considered, titers >20 GPL/ml, a cut-off derived from the mean level plus 2 SD of controls after the exclusion of one outlier, were found exclusively in patients with non–HCV-related liver damage.

The clinical features of aCL-positive *versus* aCL-negative patients with chronic liver disease are reported in Table 2. Positive patients were more frequently men with advanced cirrhosis; they often had ASMA in serum and their liver damage was more often due to HBV infection or to alcohol abuse. Raised ALT levels, thrombocytopenia, and thrombotic events had no correlation with aCL levels. Conditional logistic regression analysis showed that the presence of aCL was significantly associated with ASMA (OR = 3.02, 95% confidence interval [CI] = 1.7–5.5, $p = 0.0003$), HBV (OR = 3.4, 95% CI 1.6–7.2, $p = 0.0013$), or alcoholic-

Table 2. Clinical Features of aCL-Positive (+ve) *vs* aCL-Negative (−ve) Patients With Chronic Liver Disease

	ACL+ve n (%)	ACL−ve n (%)	*p* Value
	70 (18.8)	302 (81.1)	
Mean age (yr)	53.4	55.9	0.5
Gender, M:F	4:1	2:1	0.013
Chronic hepatitis	22 (31.4)	163 (54.3)	
Cirrhosis	31 (44.2)	78 (25.8)	0.50
HCC	17 (24.2)	61 (20.1)	
Child-Pugh class			
A	19 (39.5)	83 (59.7)	
B	22 (45.8)	42 (30.2)	0.050
C	7 (14.5)	14 (10.0)	
Etiology			
HCV	11 (15.7)	125 (41.4)	
HBV	31 (44.3)	108 (35.8)	0.0023
Alcohol	23 (32.8)	46 (15.2)	
Cryptogenic	5 (7.1)	23 (10.6)	
Thrombocytopenia ($\times 10^3$)			
<50	7 (10.2)	22 (7.2)	0.28
50–140	33 (47.1)	80 (26.4)	
>140	30 (42.8)	20 (6.6)	
Thrombotic events	4 (5.7)	20 (6.6)	0.52
ALT (mean levels)	121.6	94.4	0.2
ANA, LKM, APCA+ve	6 (8.5)	15 (4.9)	0.00057
ASMA+ve (>1:80)	27 (38.5)	59 (19.5)	

HCC = hepatocellular carcinoma; HCV = hepatitis C virus; HBV = hepatitis B virus; ALT = alanine transaminase; ANA = antinuclear antibodies; LKM = liver-kidney microsomal antibodies; ASMA = anti–smooth-muscle antibodies; APCA = anti–parietal cell antibody.

Table 3. Clinical Features of Patients With Liver Disease Subdivided According to the Presence or Not of Venous Thrombosis

	With Thrombosis	Without Thrombosis	*p* Value
Number	348	24	
Mean age (yr)	50.6 ± 14.4	64.8 ± 10	<0.0001
Gender, M:F	2:1	5:1	NS
Chronic hepatitis	184	1	
Liver cirrhosis	102	7	<0.00001
HCC	62	16	
Child-Pugh class			
A	92	11	
B	53	10	0.0003
C	19	2	
HCV	133	3	
HBV	125	14	0.0074
Alcohol	64	5	
Cryptogenic	26	2	
ASMA	79	10	NS
Thrombocytopenia ($\times 10^3$)			
<50	25	4	
50–140	106	7	NS
>140	217	13	
Portal hypertension	88	15	0.0005

Abbreviations as in Table 2.

related liver disease (OR = 5.3, 95% CI 2.3–12.2, p = 0.0001). Age, gender, stage of liver disease (chronic hepatitis or cirrhosis), severity of cirrhosis (Child-Pugh class A *vs* B+C) and anti-HCV positivity did not enter the model.

Previous episodes of venous thrombosis had occurred in 24 (6.4%) of 372 patients. In 20 cases (83%) thrombosis was localized in the portal tract; the remaining four patients had suffered from myocardial infarction, ischemic stroke, deep venous thrombosis, and hepatic veno-occlusive thrombosis. Clinical features of patients with thrombosis are reported in Table 3. This complication occurred in 5.7% of the aCL-positive patients and in 6.6% of the 302 aCL-negative ones (p = 0.52). At univariate analysis, patients with venous thrombosis were significantly older than those without it, and were suffering from an advanced cirrhosis complicated by portal hypertension and usually associated with HCC. Etiology of liver disease, degree of thrombocytopenia, and occurrence of aCL or ASMA were not significantly related to a thrombotic event. Conditional logistic regression analysis showed that the occurrence of thrombosis was significantly associated with advanced age (OR = 1.07, 95% CI = 1.01–1.1, p = 0.0094), HCC (OR = 17.8, 95% CI 1.6–196.8, p = 0.001), HBV (OR = 6.3, 95% CI 1.6–24.6), or cryptogenic liver disease (OR = 54.8, 95% CI 5–599.9, p = 0.001). Gender, stage, etiology other than HBV, severity of cirrhosis, presence of aCL, and ASMA did not enter the model.

Follow-Up Data

Fifty of 372 (13.4%) patients were reevaluated for aCL at least 6 months after the first testing: 15 had HCV- or HBV-related cirrhosis and 35 hepatitis secondary to HCV, HBV, or alcohol. Only 50% of 30 patients positive for aCL at the initial testing remained positive; persistence of the reactivity was independent from the titer at the first testing. Four of 15 patients no longer reactive had spontaneous bacterial peritonitis at the time of the first test. Four of the remaining 20 (20%) aCL-negative patients at the initial screening were positive at the second test. During the follow-up, five patients developed portal venous thrombosis. All of them had an HCC associated with a liver cirrhosis, secondary to HCV and HBV infection in four and one cases, respectively. Only one of these five patients tested positive for aCL.

DISCUSSION

Antiphospholipid antibodies are a heterogeneous family of immunoglobulins directed against different protein-phospholipid complexes. Among them, anticardiolipin antibodies recognize the β2-glycoprotein I bound to anionic lipid surface. The clinical importance of antiphospholipid antibodies derives from their association with venous and arterial thrombosis, recurrent fetal losses, and thrombocytopenia, the so-called "antiphospholipid syndrome." Although different aCL isotypes have been investigated, from a clinical point of view the aCL IgG isotype appears to have a significant role. Recently, an association between thrombotic events and high aCL titers in patients with HCV-related liver diseases has been reported (13, 14).

Our survey in 372 patients with liver disease showed that at the univariate analysis aCL prevalence was positively related to the severity, stage, and etiology of the liver disease, as well as to the occurrence of non–organ-specific autoantibodies. At a multivariate analysis, however, only the presence of ASMA and the etiology were factors independently linked to the occurrence of aCL The finding that the occurrence of aCL was more frequent in HBV or alcoholic liver damage than in HCV infections disproves the claim that HCV is a cause of the antiphospholipid syndrome (13, 14).

The elevation of ASMA in patients with liver disease often represents a nonspecific epiphenomenon of liver cell necrosis. The fluctuation of the aCL levels is in keeping with the hypothesis of a nonspecific autoantibody production: when patients were tested again, aCL had disappeared in 50% independently of the initial titer and were newly found in 20% of those negative at the first screening. Low or moderate aCL reactivities are often transient, and this has been attributed to intercurrent infections (18, 19); overt infection, usually spontaneous bacterial peritonitis, was demonstrated in 25% of our patients with transient aCL reactivity.

The critical issue, raised by the finding of aCL, is whether these antibodies may be associated with and used for identifying patients with liver disease at risk for thrombotic complications. The results of this study provide a negative answer. At multivariate analysis the factors associated with

aCL occurrence (ASMA and non-HCV liver damage) were different from those linked to thrombotic complications (old age, HCC, HBV infection, or cryptogenic liver disease). Therefore, it appears that the two events (positivity of aCL and thrombosis) have different risk factors. This conclusion is supported also by the finding that throughout the 12 months of follow-up only one of five patients with newly documented thrombotic events was associated with aCL positivity.

Based on limited data, it has been hypothesized that HCV *per se*, rather than the associated antiphospholipid syndrome, plays a role in the thrombotic complications in cirrhosis (20). When comparing patients with HCV infection to those with HBV or alcoholism, higher F1 + F2 prothrombin fragment levels (regarded as a marker of thrombin generation) were measured in the former group. This contention is also disproved by our data, as thrombosis was found in HBV-, alcoholic-, and HCV-related liver damage. Our data fit well with the suggestion that in liver cirrhosis of any etiology there is an endothelial activation induced by endotoxemia that might lead to intravascular clotting activation and occurrence of thrombosis (21).

In conclusion, this study suggests that in patients with nonautoimmune liver diseases the production of aCL is a nonspecific epiphenomenon of the liver damage, and is not associated with thrombotic complications. This does not support the hypothesis that HCV causes the antiphospholipid syndrome.

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