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Primary Biliary Cirrhosis and Hepatitis C Virus Infection

Annarosa Floreani, M.D., Anna Baragiotta, M.D., Maria Grazia Leone, M.D., Vincenzo Baldo, M.D., and Remo Naccarato, M.D.

Department of Surgical and Gastroenterological Sciences, University of Padua, Padua, Italy; and Department of Hygiene and Public Health, Institute of Hygiene, University of Padua, Padua, Italy

OBJECTIVE: The aim of this study was to describe the clinical characteristics of hepatitis C virus (HCV)–infected patients with primary biliary cirrhosis (PBC) by comparison to patients with both antimitochondrial (AMA) positive and AMA negative PBC.

METHODS: All patients consecutively diagnosed as having PBC between 1973 and 1999 who had a regular follow-up of at least 2 yr were prospectively included in the study. The mean follow-up was 8.3 ± 5.7 yr. Survival was calculated according to Kaplan-Meier curves.

RESULTS: A total of 170 patients with PBC were considered. The syndrome with PBC and HCV infection (HCV-infected PBC patients) was recorded in 14 patients (13 women and one man), whereas 135 patients had AMA positive PBC and 18 had AMA negative PBC. Only three patients fulfilled the criteria for overlap syndrome involving PBC and autoimmune hepatitis. At presentation, the HCV-infected PBC group had significantly lower levels of ALP, γ-glutamyl transpeptidase, and IgM than the AMA positive or AMA negative PBC patients ($p < 0.01$). With regard to the autoantibody profile, there was a significant association with LKM and HCV-infected PBC patients (21.4%), whereas ANA was significantly higher in AMA negative PBC patients than in the other two groups (83% *vs* 21.4% in the HCV-infected PBC patients and 38.5% in the AMA positive PBC group). No differences were found regarding the association with autoimmune conditions. During follow-up, hepatocellular carcinoma (HCC) developed more frequently in the PBC/HCV overlap group (*i.e.*, three of 14 *vs* four of 135 patients with AMA positive PBC, $p < 0.05$). Survival curves were similar in HCV-infected PBC patients and AMA positive PBC, whereas the AMA negative group had a significantly slower decline (relative risk (RR) = 2.44, $p < 0.05$).

CONCLUSION: HCV-infected PBC patients are characterized by a biochemical profile with a modest rise in cholestatic enzymes but a high risk of developing HCC during follow-up. (Am J Gastroenterol 2003;98:2757−2762. © 2003 by Am. Coll. of Gastroenterology)

INTRODUCTION

Overlap syndromes are defined as conditions with the diagnostic features of more than one disease (1). In recent years, however, the term has been used to define the features of an autoimmune liver condition that is not entirely consistent with any of the established criteria for the diagnosis of autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), or primary sclerosing cholangitis (PSC) (1). True overlap syndrome involving PBC and AIH is a rare condition that has long been recognized. Czaja (2) reported that 15 of 199 patients with a primary diagnosis of AIH or PBC had features of both diseases, and Chazouilleres *et al.* (3) diagnosed an overlap syndrome in 12 of 130 PBC patients (9.2%). The overlap of AIH and PBC is very likely a form of PBC that develops a more hepatitis-like picture in genetically susceptible individuals (4). Autoimmune cholangitis used to be distinguished from PBC but is now considered a variant of the latter, the only principal discriminant from classical PBC being the lack of antimitochondrial antibodies (AMA).

To our knowledge, a syndrome involving PBC and hepatitis C virus (HCV) infection has not been described until now. The problem of an association between PBC and HCV was sporadically considered in the early 1990s, although only from the epidemiologic standpoint. In fact, the first question arose from the problem of false positive (anti-HCV+) results in the sera of patients with autoimmune conditions, because of high γ-globulin concentrations (5). Using immunoblotting confirmation tests, the prevalence of anti-HCV in PBC was reportedly one of 90 patients (6) and three of 55 (7). The clinical association between PBC and HCV is also important in terms of treatment, because interferon can exacerbate PBC (8). In the last 29 yr, we have had the opportunity to follow prospectively a large group of Italian PBC patients, some with associated HCV infection. The clinical characteristics of this latter group, compared with the patients with classical and AMA negative PBC, are reported here.

MATERIALS AND METHODS

Patients

The study considered all patients consecutively who were diagnosed with PBC between 1973 and 1999, with a pro-

spective follow-up of at least 2 yr. A total of 170 patients (159 women and 11 men) participated in the study. The diagnosis of AMA positive PBC was considered if all the following criteria were met: AMA present in a titer of at least 1:40; abnormal liver function tests, and diagnostic or compatible liver biopsy. The diagnosis of AMA negative PBC was considered when the following criteria were met: AMA negativity, antinuclear antibodies present in a titer of at least 1:160, abnormal liver function tests, diagnostic, or compatible liver histology. The diagnosis of PBC/AIH overlap syndrome was considered using the criteria described by Chazouillères *et al.* (3). In brief, patients were considered to have AIH features if two of the three following criteria were met: 1) alanine transaminase (ALT) levels at least 5 times the upper limit of normal (ULN) for the local laboratory; 2) serum IgG levels at least 2 times ULN and/or positivity for anti–smooth muscle antibodies; and 3) liver biopsy showing moderate or severe periportal or periseptal lobular inflammation. The diagnosis of PBC and HCV overlap syndrome was considered in the presence of the above-mentioned criteria fulfilling the diagnosis of PBC, plus the following: 1) confirmation of M2 in immunoblotting; and 2) HCV-RNA positivity in at least two serum samples at a 6-month interval.

D-Penicillamine (600 mg/day) was the only immunosuppressive drug taken by 60% of patients between 1979 and 1987. D-Penicillamine was discontinued in all subjects after 1987, and no patients enrolled thereafter were treated with this drug. The three patients with PBC and AIH overlap syndrome were treated with prednisolone (30 mg/day for the first 6 months, then a tailored maintenance dosage of 10–15 mg/day, or prednisolone associated with steroid-sparing azathioprine + ursodeoxycholic acid (15 mg/kg/day). Only two patients with autoimmune conditions associated with PBC (systemic lupus erythematosus and rheumatoid arthritis) were treated with prednisolone (7.5 mg/day) for 8 yr. At present, 98% of the patients are taking ursodeoxycholic acid (12–15 mg/day).

The revised scoring system for the diagnosis of AIH hepatitis was calculated according to the report of the International Autoimmune Hepatitis group (9)

The Mayo risk score, based on a combination of five variables (bilirubin, age, albumin, prothrombin time, and severity of edema) was calculated for each patient (10).

Evaluation of Liver Histology

Of 170 subjects, 162 underwent liver biopsy. In eight subjects (seven women and one man), liver biopsy was not performed; in particular, four patients refused this procedure, and in four patients biopsy was not proposed because of advanced age (>75 yr for all patients) at presentation.

Biopsy samples were examined in a blinded fashion using codes by a dedicated pathologist. The stains available included hematoxylin, stains for connective tissue, iron, and copper-associated protein. Histologic findings were classified according to Scheuer (11). The following histologic features were recorded: bile duct damage, granulomas, portal inflammation, interface hepatitis (piecemeal necrosis), ductular proliferation, lobular necrosis, and canalicular cholestasis.

Follow-Up

The patients were followed up regularly every 4–6 months by clinical and biochemical examination. Biochemical parameters included AST, ALP, immunoglobulins, bilirubin, red cell and white cell counts, prothrombin time, and α-fetoprotein. Liver ultrasound was performed every 12 months in precirrhotic patients and every 6 months in patients with histological stage IV PBC (cirrhosis).

The mean follow-up was 8.3 ± 5.7 yr

Laboratory Methods

Autoantibodies (AMA, ASMA, and ANA) were tested by the indirect immunofluorescence method using normal human gastric mucosa and normal rat kidney and liver. Sera were tested at an initial dilution of 1:5 and were considered to be positive when they produced a reaction at a dilution of $\geq$1:5.

Immunoblotting for M2 was performed using a commercial kit (Euroimmun, Luebeck, Germany). The test enables the identification of the following M2 antigens: 74 KDa = E_2 subunit of the pyruvate dehydrogenase complex (PDC); 55 KDa = protein X of the PDC; 51 KDa = E_2 subunit of the branched chain α-ketoacid dehydrogenase complex, E_2 subunit of the α-ketoglutarate dehydrogenase complex; 45 KDa = $E_1\alpha$-subunit of the PDC; 36 KDa = $E_1\beta$-subunit of the PDC. Samples were considered as M2 positive if at least one band at 74 KDa or 51 KDa stained intensely or if at least two bands at 55 KDa, 45 Kda, or at 36 KDa produced a strong signal.

Autoantibodies against soluble liver antigen/liver pancreas antigen were tested by ELISA (Euroimmun GmbH, Luebeck, Germany).

Testing for anti-HCV was done in duplicate by second-generation ELISA (Ortho Diagnostic System, Raritan, NJ) and confirmed by HCV-RNA detection using a commercially available qualitative polymerase chain reaction assay (Amplicor, Monitor assay; Roche Diagnostic Systems, Branchburg, NJ).

The other biochemical variables were assayed by standard methods

Statistical Analysis

Quantitative data are expressed as median and range. The *t* test was used for quantitative data, χ^2 for qualitative data, and Wilcoxon test for matched pairs. Survival was analyzed according to the Kaplan-Meier method and was assessed with the log-rank test. Statistical analysis was performed using SPSS software (SPSS, Chicago, IL).

Table 1. Clinical Features of Patients at Presentation

	HCV-Infected PBC (N = 14)	AMA + PBC (N = 135)	AMA − PBC (N = 18)
Male:female ratio	13:1	126:9	17:1
Age (yr, mean ± SD)	53.8 ± 6.7	51.4 ± 11.7	51.6 ± 10.5
Histologic Stage I–II	9 (64.2)	71 (52.3)	14 (77.8)
Stage III	3 (21.4)	39 (28.9)	3 (16.6)
Stage IV	2 (14.3)	17 (12.6)	
Not ascertained		8 (5.9)	1 (5.6)
Mayo score	4.1 ± 1.1	3.9 ± 0.9	3.7 ± 0.6
Bilirubin >2 mg/dl	1 (7.1)	8 (5.9)	1 (5.6)
ALP (median, range)	115.5 (60–757)*†	316.0 (41–2079)†	493.5 (88–1700)*
ALT (median, range)	75.5 (10–705)	68.0 (10–333)	64.5 (24–210)
GGT (median, range)	66.5 (26–719)*†	223.0 (25–1125)	221.5 (44–708)*†
Albumin (median, range)	4.2 (3.4–5.1)	4.2 (2.7–5.1)	4.4 (2.8–5.3)
Prothrombin time (s, median, range)	10 (6.5–10.7)	10.4 (7.2–13.5)	10.3 (8.5–11.5)
IgG (median, range)	1835.0 (934–2528)	1620.0 (682–4957)	1507.0 (819–2531)
IgA (median, range)	226.5 (143–700)	278.0 (94–718)	304.0 (100–716)
IgM (median, range)	255.4 (127–580)*†	465.0 (133–1598)†‡	411.0 (171–697)*‡
AIH score (median, range)	4.5 (0–16)*	4.0 (−5–14)‡	11.0 (−1–14)*‡
No. of subjects with decompensated cirrhosis (ascites, esophageal bleeding)		4 (2.9%)	

Normal limits: ALP = 53–141 U/L; GGT = 3–45 U/L; albumin = 3.5–5.5 g/dl; prothrombin time 10–12.5 s; IgG 700–1600 mg/dl; IgA 70–400 mg/dl; IgM 40–238 mg/dl.
* $p < 0.01$ HCV *vs* AMA negative.
† $p < 0.005$ HCV *vs* AMA positive.
‡ $p < 0.001$ AMA positive *vs* AMA negative.

RESULTS

In 135 patients a diagnosis of AMA positive PBC was recorded, whereas 18 patients had an AMA negative variant of PBC and 14 patients with AMA positive PBC were also HCV-RNA positive. This last group was considered as HCV-infected PBC patients. Three more patients fulfilled the diagnostic criteria for the PBC/AIH overlap syndrome. All patients were also tested for HBsAg, but none was found to be HBsAg positive. Clinical features of the patients are summarized in Table 1. HCV-infected PBC patients exhibited significantly lower serum levels of alkaline phosphatase, γ-glutamyl transpeptidase, and IgM than the AMA positive or AMA negative groups. In contrast, no significant changes were found regarding male-female ratio, age, histologic stage, Mayo score, or serum levels of bilirubin, ALT, IgG, and IgA. The AMA negative PBC group had a significantly higher AIH score than either HCV-infected or AMA positive PBC patients.

The clinical and virologic details of the HCV-infected PBC patients are summarized in Table 2. Of the patients, 13 were female and one was male. In the nine patients whose genotyping was available, genotype 1 was found in five patients, genotype 2 was found in three, and genotype was not determined in one. The source of infection was transfusion in one patient, whereas 13 patients had a history of injections using glass syringes with nondisposable needles. At presentation, nine patients had histologic features compatible with early disease (five had inflammatory bile duct

Table 2. Clinical Characteristics of HCV-Infected PBC Patients

Patient	Sex	Age (yr)	HCV-RNA	Genotype	PBC Stage	Source of Infection	Outcome
A.P.	F	53	+	2a/2c	I	Glass syringes	Alive
B.M.	F	54	+	1b	III	Glass syringes	Alive
C.C.	F	50	+	1b	IV	Glass syringes	Alive
C.C.	F	73	+	2a/2c	II	Glass syringes	Alive
D.B.R.	F	56	+	1b	I	Glass syringes	Alive
D.B.M.	F	54	+	N.A.	I	Glass syringes	Dead (liver failure)
P.E.	F	52	+	2c	III	Glass syringes	Alive
P.L.	F	44	+	N.A.	I	Glass syringes	Alive
S.G.	F	50	+	N.A.	I	Glass syringes	Dead (HCC)
S.F.	F	53	+	N.A.	III	Glass syringes	Alive
V.T.	F	57	+	N.A.	II	Transfusion	Dead (liver failure)
E.O.	M	55	+	N.D.	II	Glass syringes	Alive
G.W.	F	58	+	1b	II	Glass syringes	Dead (HCC)
N.M.	F	45	+	1b	IV	Glass syringes	Dead (HCC)

F = female; M = male; N.A. = not available; N.D. = not determined.

Table 3. Associated Autoimmune Conditions of Patients at Presentation

	HCV-Infected PBC (N = 14)	AMA + PBC (N = 135)	AMA − PBC (N = 18)
Sjögren's syndrome	7 (50%)	64 (47.4%)	6 (33.3%)
Raynaud's disease	6 (42.8%)	43 (31.8%)	6 (33.3%)
Autoimmune thyroiditis	3 (21.4%)	24 (17.7%)	6 (33.3%)
Scleroderma	1 (7.6%)	7 (5.2%)	4 (22.2%)
Rheumatoid arthritis		11 (8.1%)	1 (5.5%)
CREST		7 (5.1%)	1 (5.5%)
Psoriasis	2 (14.2%)	3 (2.2%)	1 (5.5%)
IDDM		2 (1.4%)	1 (5.5%)
SLE		1 (0.7%)	1 (5.5%)
Vitiligo	2 (14.2%)	1 (0.7%)	

CREST = Calcinosis, Raynaud's Esophagus, Sclerodactyly, Telangietasia; IDDM = insulin-dependent diabetes mellitus; SLE = systemic lupus erythematosus.

lesions, four of them with granulomatous bile duct lesions, regarded as Stage I disease; four patients had Stage II lesions, marked by aggregates of lymphoid cells and bile duct proliferation; a cellular infiltrate surrounding the damaged bile ducts contained lymphocytes, plasma cells, eosinophils, and histiocytes). Three patients had Stage III and two had Stage IV disease; all liver specimens were consistent with the diagnosis of PBC. None of the patients with hepatitis C received interferon therapy.

Table 3 summarizes the associated autoimmune conditions at presentation. There were no significant differences in the association of autoimmune conditions among the three patient groups. The most representative conditions were Sjögren's syndrome and Raynaud's disease.

As for the autoantibody profile, liver/kidney microsomal antibodies (LKM) type 1 were significantly associated with the HCV-infected PBC group (21.4%), whereas ANA positivity was significantly higher in the AMA negative PBC group than in the other two groups (83.3% *vs* 21.4% in the HCV-infected PBC group and 38.5% in the AMA positive group) (Table 4). In all affected patients, ANA revealed a speckled pattern. The AMA negative PBC group was also tested for soluble liver antigen/liver pancreas antigen by ELISA and immunoblotting, but none of the 18 patients proved positive.

Table 4. Autoantibody Profiles of Patients at Presentation

	HCV-Infected PBC (N = 14)	AMA + PBC (N = 135)	AMA − PBC (N = 18)
ANA+	3 (21.4%)	52 (38.5%)*	15 (83.3%)*
ASMA+		27 (20.0%)	2 (11.1%)
LKM+	3 (21.4%)†	1 (0.7%)†	
ANA+, ASMA+		15 (11.1%)	2 (11.1%)
Microsomal thyroid antibody	3 (21.4%)	24 (17.7%)	6 (33.3%)

* $p < 0.001$ AMA + PBC *vs* AMA − PBC and AMA + PBC *vs* HCV-infected PBC patients.

† $p < 0.005$ HCV-Infected PBC *vs* AMA + PBC patients.

Table 5. Major Events During Follow-Up

	HCV-Infected PBC (N = 14)	AMA + PBC (N = 135)	AMA − PBC (N = 18)
Follow-up, (yr ± SD)	11.1 ± 6.0	6.2 ± 4.9	8.3 ± 5.7
Bleeding		20 (14.8%)	1 (5.6%)
Ascites	5 (35.7%)	19 (14.1%)	2 (11.1%)
HCC	3 (21.4%)	4 (3.0%)	
Extrahepatic cancer		8 (5.9%)	
OLTx		7 (5.2%)	
Death*	5* (35.7%) (*5)	32* (23.7%) (*29)	1* (5.6%) (*1)

OLTx = orthotopic liver transplantation.

* Due to liver failure.

† HCV-infected PBC *vs* AMA + PBC, $p < 0.05$.

Table 5 summarizes the major events during follow-up. Hepatocellular carcinoma (HCC) developed more frequently in the HCV-infected PBC group (three of 14 *vs* four of 135 patients with AMA positive PBC, $p < 0.05$). Deaths were recorded in 35.7%, 23.7%, and 5.6% respectively. The majority of deaths were due to liver failure, except in three patients of the AMA positive group who died of myocardial infarction. Survival curves were similar in HCV-infected and AMA positive PBC, whereas the AMA negative group had a significantly slower decline in the curve (RR = 2.44, $p < 0.05$) (Fig. 1).

DISCUSSION

The results of our study demonstrate that PBC is a heterogeneous disease and that its natural history is influenced by several factors such as age and other risk factors. Among PBC patients, the group with HCV superinfection (designated as the HCV-infected PBC patient group) is very different at presentation and carries a high risk of developing HCC. There are two points to stress. First, all cases of HCV positivity were true infections (documented by PCR positivity), and all cases of PBC with HCV superinfection had true PBC (not a clinical picture of chronic cholestasis

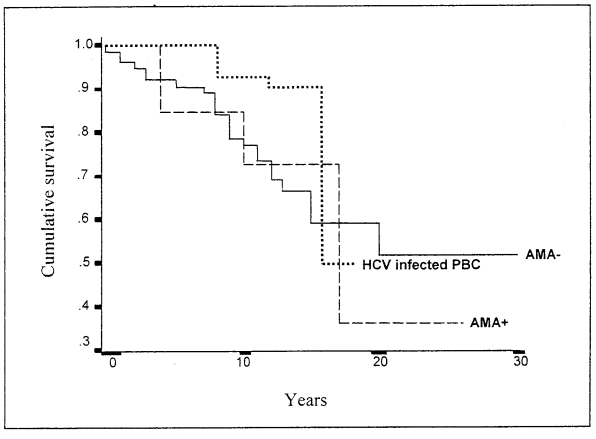

Figure 1. Kaplan-Meier survival curves.

associated with HCV). In fact, all HCV positive PBC patients were also tested for mitochondrial antigens, confirming the diagnosis of PBC. Second, HCV superinfection is not surprising in European countries, inasmuch as epidemiologic studies on the Italian general population show that HCV prevalence increases with age, reaching a peak of 30–35% among individuals >65 yr of age (12–14). This cohort effect is due to the HCV epidemic during and after World War II, when parenteral treatment with glass syringes was popular in Italy for administering antibiotics as well as long-term treatment with vitamins, tonics, and liver aids for various conditions (including infections, pneumonia, malnutrition) or for immune prophylaxis against tetanus. Injections were given by nonprofessional individuals at home or by health care professionals in hospitals. Another risk factor for HCV transmission during and after World War II was tuberculosis, for which prevention and care by the Italian Public Health Authorities were very well organized. Intramuscular injections with nondisposable syringes and a history of tuberculosis have been demonstrated as independent factors associated with community-acquired chronic HCV infection (15). These epidemiologic aspects explain the high rate of HCV in our PBC population. The problem is how HCV influences the presentation and natural history of PBC. Because HCV becomes chronic in >85% of infected patients, it is hardly surprising that all PBC patients superinfected with HCV are HCV-RNA. Genotyping was only possible in nine of 14 patients whose serum had been stored in the freezer. However, the 1b genotype was more common, as expected for infections acquired before the 1980s.

We chose not to treat these patients with interferon in light of the potential for interferon to exacerbate autoimmune conditions (16).

In this group of patients, liver histology showed the histologic characteristics of PBC; however, other specific features of HCV (*e.g.*, microvescicular steatosis) were not recognized. The biochemical profile did not differ significantly from those of the AMA positive and AMA negative PBC groups, suggesting that HCV attenuates the cholestatic enzymes. We have no explanation for how HCV can modify the liver enzymes in these patients. Certainly, none of the patients had ever taken interferon. However, the most important point is the significantly higher incidence of HCC developing during follow-up in this group of PBC patients by comparison with PBC patients without HCV infection. This confirms our previous report in a large group of PBC patients who were followed for a total of 1187 person-years (17). The present study has a longer follow-up, ranging from 2 to 27 yr. Other European and American studies have examined the risk of hepatic and extrahepatic malignancies in PBC (18–20) using a suitable method and with an adequate follow-up. Several differences attributable to local customs emerge from these studies (for example, the Newcastle group found HCC risk significantly associated with male gender and alcohol abuse).

Our results suggest the need to screen all PBC patients for anti-HCV and to monitor HCV-infected PBC patients closely with ultrasound and α-fetoprotein assay every 6 months. The actual survival was much the same in this group as in the AMA positive PBC group, whereas the AMA negative group showed a significantly slower decline in the curve. This observation needs to be confirmed in larger samples, however. The statistical significance in the survival curves is appreciable after a 15-yr follow-up, and all three groups in our series had much the same mean follow-up. PBC-related deaths were caused by liver failure, except three cases of myocardial infarction in the AMA positive group.

The HCV-infected PBC group did not differ significantly from the other groups. With respect to the autoantibody profile, LKM showed a significantly greater association with the HCV-infected PBC group than with the other two groups. As expected, ANA was positive in 90% of the AMA group. LKM type 1, the serologic marker of a subset of autoimmune hepatitis, is also found in a proportion of patients with HCV-related chronic liver disease, including patients with the following features: female sex, middle-age onset, low-to-moderate transaminase levels, and normal immunoglogulin serum levels (21, 22). In patients with anti-LKM type 1 and hepatitis C, the viral load observed has been lower than in cases of hepatitis C without autoantibodies (23), suggesting that autoimmune mechanisms may cooperate with viral infection in sustaining disease activity.

In conclusion, the HCV-infected PBC patients are a particular subgroup of PBC patients who are characterized by a biochemical profile with only moderately increased cholestatic enzymes but a high risk of developing HCC during follow-up. These clinical features demand careful follow-up, including ultrasound and an α-fetoprotein assay every 6 months.

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Reprint requests and correspondence: Prof. Annarosa Floreani, M.D., Div. Gastroenterologia, Via Giustiniani, 2, 35128 Padova, Italy.

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