Hepatic and Extrahepatic Malignancies in Primary Biliary Cirrhosis

ANNAROSA FLOREANI,[1] ANNA BARAGIOTTA,[1] VINCENZO BALDO,[2] TIZIANA MENEGON,[2] FABIO FARINATI,[1] AND REMO NACCARATO[1]

Hepatocellular carcinoma (HCC) is rarely reported as a complication of primary biliary cirrhosis (PBC). However, data suggest that patients with PBC have an increased incidence of breast cancer when compared with the general population. Our aim was to analyze the incidence of malignancies in a large series of PBC patients from North-East Italy; to compare findings with those obtained in the general population of the same geographical area, as derived from the general cancer registry; and to study any possible adjunctive risk factor for malignancy. The overall sample included 175 patients (9 males, 166 females). The mean age at presentation was 50.8 years (range 23-77); 17 patients had histological stage I, 45 had stage II, 76 had stage III, and 37 had stage IV. The prevalence of gynecological diseases obtained from the past history of the females included 19.9% miscarriages, 12% hysterectomies, and 2.4% curettages. The follow-up period was 1,187 person/years (average 6.8 yrs per person as a mean). The comparison of the incidence of malignancies between the study group and the general population was obtained by the proportional incidence ratio (PIR), which is the ratio between the cases observed and the expected number of cases in the study group. Logistic regression analysis was performed utilizing the risk factors significantly associated with cancer development in the univariate analysis. Extrahepatic malignancies developed in eight cases (4.5%) and HCC in a further four cases (2.3%), all associated with cirrhosis. Two of the four patients with HCC had a superinfection with hepatitis C virus (HCV). Breast cancer developed only in two patients. The PIR for HCC was 26.27 (95% CI 6.8-46.5), whereas the PIR for breast cancer was 0.43. The logistic regression analysis indicated that a history of cigarette smoking and HCV-RNA positivity were independent variables for the development of HCC. HCC has a relatively high prevalence in PBC and HCV superinfection may play an important part in favoring HCC. The incidence of breast cancer is not significantly higher in PBC than in the general population of the same area. (HEPATOLOGY 1999;29:1425-1428.)

Hepatocellular carcinoma (HCC) has been reported as being a rare complication of PBC.[1-3] In fact, HCC development is more frequent in cirrhosis associated with viral causes, idiopathic hemochromatosis, or alcohol abuse.[4-6] However, Melia et al. hypothesized that HCC may be no less frequent in PBC than in other types of cirrhosis.[7] Moreover, in a previous study, we found four cases of HCC in an overall sample of 178 PBC patients enrolled in two referral centers.[8] When, in a prospective cohort study, we compared the incidence of HCC in stage IV PBC with that of the tumor in female cirrhotic patients, matched for age, with disease of different etiology, we obtained a similar incidence.[9] On the other hand, data suggest that patients with PBC have an increased incidence of extrahepatic malignancies when compared with the general population.[10-12] In particular, Wolke et al[11] found that the incidence of breast cancer was 4.4 times the prevailing rate. The biological basis for an association between PBC and breast cancer is obscure, but endocrine dysfunction has been hypothesized.[12]

The aims of the present study were therefore (1) to evaluate the incidence of hepatic and extrahepatic malignancies in a large series of PBC from North-East Italy; (2) to compare the data obtained with findings observed in the general population of the same geographical area; and (3) to study any possible risk factor for malignancy.

PATIENTS AND METHODS

Study Design. The present study included all patients consecutively diagnosed as having PBC between 1973 and 1996, who were prospectively followed up for at least 12 months. The patients were regularly followed up every 4 to 6 months by clinical and biochemical examination. Biochemical parameters included transaminases (aspartate transaminase, alanine transaminase), alkaline phosphatase, immunoglobulins, bilirubin, red cell and white cell count, 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).

Patients. One hundred and seventy-five patients (9 males, 166 females) were included in the study. The diagnosis of PBC was obtained in each case from biochemical, immunological and histological findings. The mean age at presentation was 50.8 years (range, 23-77).

Histological findings were classified according to Scheuer.[13] Seventeen patients had histological stage I, 45 had stage II, 76 had stage III, and 37 had stage IV.

The overall prevalence of associated autoimmune conditions was 66.9%. Sjogren's syndrome was the most frequent association: it was present in 38.3% of patients. Raynaud's phenomenon was observed in 13.7% and thyroid diseases in 4.6% of cases. Crest syndrome was recorded in 2.9% and rheumatoid arthritis in 2.9% of cases. Lupus Erythematosus Systemic, scleroderma, lichen ruber planus, and vitiligo were recorded in less than 3% of cases.

The prevalence of gynecological diseases obtained from the past history of the females included 19.9% miscarriages, 12% hysterectomies for uterine fibromyomas, and 2.4% curettages.

Past oral contraceptive consumption was negligible in these patients (4.8%). D-Penicillamine (at a dosage of 600 mg/day) was the

Abbreviations: HCC, hepatocellular carcinoma; PBC, primary biliary cirrhosis; PIR, proportional incidence ratio; HCV, hepatitis C virus; CI, confidence interval; HBV, hepatitis B virus.

From the [1]Department of Surgical and Gastroenterological Sciences, [2]Institute of Hygiene, University of Padova, Padova, Italy.

Received October 28, 1998; accepted January 29, 1999.

This work was partially supported by a Ministerial grant (MURST 60%).

Address reprint requests to: Dr. Annarosa Floreani, Div. Gastroenterologia, Via Giustiniani, 2, 35128 Padova, Italy. E-mail: aflor@ux1.unipd.it; fax:+44-049-8760820.

0270-9139/99/2905-0012$3.00/0

only immunosuppressive drug taken by 60% of patients during the period of 1979 to 1987. D-Penicillamine was discontinued in every subject after 1987, and no patient enrolled after said year was treated with this drug. Only two patients with severe autoimmune conditions associated with PBC (Lupus Erythematosus Systemic and rheumatoid arthritis) were treated with Prednisolone (7.5 mg/day) for 5 years. At present, 90% of the patients are taking ursodeoxycholic acid 500 to 600 mg/day.

Follow-up. The patients were followed up for a total of 1,187 person/years. The follow-up is slighly shorter when only females are considered, with 1,123 person/years, 6.8 person/years as a mean.

Statistical Analysis. All data were collected in a questionnaire. A name-related and coded file with an appropriate computer program eliminated duplicate testing.

The comparison of the incidence of malignancies between the study group and the general population was done by using the proportional incidence ratio (PIR).[14] This calculation was extended to the resident PBC population in the Veneto region (North-East Italy), *i.e.*, an overall sample of 109 PBC subjects (103 female, 6 male). Because the number of males was too small, the six males were excluded from the analysis.

The PIR is the ratio between the observed cases and the expected cases and is expressed as a percentage according to the following formula:

$$PIR = (R/E) \times 100$$

where R = observed cases at the site of interest in the group under study; E = expected cases at the site of interest in the group under study.

The expected number of cases of a particular cancer was obtained by multiplying the total cancers in each group in the data set under study by the corresponding age and cause-specific proportions in the standard, according to the following formula:

$$E = \sum_{i=1}^{A} t_i \, (r_i + /t_i^*)$$

where r_i^* = number of cases of the cancer of interest in the age group i in the standard population; t_i^* = number of cases of cancer (all sites) in the age group i in the standard population; t_i = number of cases of cancer (all sites) in the age group i in the study group.

The 95% confidence interval (95% CI) was calculated on the assumption that the observed number of cases in each class followed a Poisson distribution.

Data from the Veneto Cancer Registry[15] on the development of malignancy were utilized to compare the incidence of neoplasms. The cancer registry reporting data for 1987 to 1989 was utilized because it is closest to the mid period of our study. The reference general population included all females resident in the Veneto in 1991.

Associations between the incidence of neoplasms and several variables (familial history of malignancies, autoimmune conditions, gynecological abnormalities, HCV infection, cigarette smoking, oral contraceptives, and alcohol consumption) were estimated by the χ^2 test (Mantel Hanszel and Fisher's exact test, as appropriate). A *P* value less than or equal to .05 was considered significant, and an odds ratio with a 95% confidence interval was calculated for each parameter. The adjusted odds ration for each of the variables significantly associated with tumor development in the univariate analysis was calculated by multiple logistic regression analysis. Analyses were performed with the EPI-Info 6.04 computer program supplied by the Centers for Disease Control of Atlanta (Atlanta, GA), using a Statistical Package for the Social Sciences (SPSS ver 7.5.2), 1997, Chicago, IL, SPSS Inc.

Laboratory Methods. Testing for anti-HCV was performed in duplicate by second-generation enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Raritan, NJ) and confirmed by HCV-RNA detection using commercially available quantitative polymerase chain reaction assay (Amplicor Monitor assay; Roche Diagnostic Systems, Branchburg, NJ).

TABLE 1. Incidence of Malignancies During Follow-up

	Number of Cases	(%)	Incidence Per 100,000 People Per Year
Hepatic malignancies			
HCC	4	2.3	337.0
Extrahepatic malignancies			
Breast cancer	2	1.1	168.5
Skin melanoma	2	1.1	168.5
Endometrium	1	0.6	84.2
Colorectum	1	0.6	84.2
Kidney	1	0.6	84.2
Non-HD lymphoma	1	0.6	84.2
Total	12	6.9	

The other biochemical variables were assayed by the standard methods.

RESULTS

During follow-up, 12 malignancies were observed (6.7%)(Table 1): 4 were HCC and 8 were extrahepatic tumors. HCC developed in four female patients with underlying stage IV PBC. The age of the patients was 60, 61, 66, and 44; the diagnosis of HCC was reached 4 to 13 years after the initial diagnosis of PBC. Among the four cases with HCC, two were HCV-RNA positive. Excluding the four patients with HCC, anti-HCV was tested in 118 patients, with an overall prevalence of 11.5%.

The extrahepatic malignancies included two breast cancers, two skin melanomas, one endometrial cancer, one colo-rectal cancer, one kidney cancer, and one non-Hodgkin's lymphoma. The crude incidence rates per 100,000 are reported in Table 1.

Table 2 shows the PIR for the tumors. The PIR for HCC was 26.27 (CI, 6.8-46.5) indicating a strong association of HCC with PBC. As far as extrahepatic malignancies are concerned, a PIR greater that 4.0, indicating an association between PBC and cancer, was observed for skin melanoma.

The PIR for breast cancer was 0.43, thus indicating that the incidence of breast cancer in PBC patients is strongly lower than in the general population.

No correlation was found between hepatic and extrahepatic malignancies and the following variables: familial history of malignancies, autoimmune conditions, previous gynecological abnormalities, and oral contraceptives (Table 3). The logistic regression analysis indicated that a history of smoking (more than 10 cigarettes per day) and HCV positivity are independent variables for the development of HCC.

TABLE 2. Proportional Incidence Ratio (PIR) Calculated in 103 PBC Women Residents in the Veneto Region

	Number of Cases	PIR
Hepatic		
HCC	3	26.27
Extrahepatic		
Breast cancer	2	0.43
Skin melanoma	2	6.68
Endometrium	1	0.9
Colorectum	1	1.4
Kidney	1	3.8
Non-HD lymphoma	1	2.8
Total	11	–

TABLE 3. Risk Factors Associated With HCC. Adjusted Odds Ratios Derived From Logistic Regression Analysis

	OR	*P* Value
Alcohol consumption	0.0	NS
Autoimmune conditions	—	NS
Familial history of malignancy	1.7	NS
Anti-HCV positivity	22.5	.02
Gynecological abnormalities	0.3	NS
Cigarette smoking	26.5	.02

DISCUSSION

The data in our study confirm that Italian patients with PBC have a higher incidence of HCC, but a relatively lower incidence of extrahepatic malignancies than American and/or Northern European patients.[10-12] We found that the development of HCC was restricted to patients with stage IV disease, in whom the incidence was 4/37 (10.8%). This overall incidence confirms our previous findings (4/178 cases of HCC in two Italian series[8]), and is consistent with the results reported by Van Dam et al. in the Netherlands.[16] The overall incidence of HCC is similar to the one observed in a large series of PBC patients in Newcastle.[17] In the study from Newcastle, HCC was identified only in patients with advanced disease (with a 5.9% incidence among 273 patients with stage III/IV disease). Moreover, no comparison was made between the study group and the general population; however, the incidence of HCC was particularly high in male patients in the English study, whereas other potential confounding risk factors for HCC development, such as excessive alcohol consumption, hemochromatosis, HBV, and HCV superinfections, were excluded.

In our study group, HCC developed only in female patients, and logistic regression analysis showed a significant correlation with HCC and both smoking habits and HCV infection. As far as HCV infection is concerned, two considerations should be stressed. First of all, all HCV positivities were true infections, as documented by PCR positivity; on the other hand, all patients with PBC and HCV superinfection had a true PBC, and not a clinical picture of chronic cholestasis associated with HCV. In fact, all HCV–positive PBC patients were additionally tested for mitochondrial antigens (E2, OGDC in immunoblotting), confirming a definite diagnosis of PBC. Secondly, when we consider the prevalence of HCV in PBC patients, an 11.5 % rate was documented, which is similar to the one observed in the general population of Northern Italy.[18] In our general population, HCV prevalence increases with advancing age, reaching a peak of 25% among the age group over 55 years old (Chiaramonte, 1998, personal communication). We can assume that HCV infection in the liver of PBC patients might contribute to the process of carcinogenesis accelerating the natural history of PBC through a direct mechanism[19] or by increasing hepatocellular proliferation rate.[20] This association with a viral factor might also be responsible for the modification observed in the male-to-female ratio. Indeed a higher incidence in males has been reported in two series in the UK,[7,17] in which HCV–positive patients were not included, a fact that is not confirmed in our study group. The other major factor involved in liver carcinogenesis, HBV, plays no part in our series, since all the patients were hepatitis B surface antigen and HBV-DNA negative.

Also, smoking habit was indicated as an independent risk factor for HCC development by the logistic regression analysis. Indeed, smoking has been considered one of the risk factors for HCC, even recently in hepatitis B surface antigen chronic carriers.[21] Overall, the relevance of smoking in the cause of HCC is still debatable,[22] even though it can be assumed that smoke-related carcinogens play a role in the multiphase, multifactorial process of liver carcinogenesis, particularly in subgroups such as PBC patients that lack a direct carcinogenic factor.

As far as breast cancer is concerned, this is not as frequent in our series as might be expected on the basis of previous reports.[11,12] The fact that PBC patients have no increased risk of breast cancer is also not confirmed by a study carried out in Sweden.[23] This is difficult to explain, but probably reflects a different epidemiology of breast cancer risk factors in the general population as well. For example, the mortality rate for breast cancer between 1983 and 1987 was 93.6 per 100,000 in the United States.[24] Over the same period, between 1984 and 1988 this rate was 34.6 per 100,000 in Italy.[25]

Sex hormone imbalance is not the only predisposing factor in the development of breast cancer. As previously reported,[26] we found a high incidence of miscarriages and curettages in the history of our patients, whereas the use of contraceptives was negligible.

Dietary factors (especially high fat intake) or prolonged use of oral contraceptives may account for the different epidemiology of breast cancer.[27] Another factor, not sufficiently studied, is vitamin A deficiency, which has been shown to induce metaplasia in epithelial cells in mice, along with an increased susceptibility to malignancy.[28] This may indeed be relevant with respect to PBC. For instance, Mills et al.,[10] in a personal series of 23 PBC patients without tumor, found that the mean serum vitamin A concentration was significantly lower than in 55 age- and sex-matched controls. However, we determined serum carotenoid levels (total carotenoids, retinol, α-tocopherol, α-carotene, β-carotene) in a sample of 74 PBC patients and found that the mean values of these anti-oxidants were not significantly different from those observed in a large sample of the general population from the same geographical area (unpublished data, 1998). The relationship between vitamin A deficiency and cancer risk in PBC is therefore questionable. Overall, our data do not support the need for a more aggressive breast cancer screening program in PBC patients than as recommended by the World Health Organization.

We also failed to find any risk factor for extrahepatic malignancies such as familial history of malignancy, smoking, previous history of gynecological disorders, oral contraceptives, alcohol, and association with autoimmune conditions.

In conclusion, this study confirms that patients with PBC are not at a lower risk for HCC than cirrhotics with disease of various cause, thus indicating that they should be submitted for a regular follow-up protocol just like any other patient with cirrhosis, suggesting that additional factors, such as HCV co-infection or smoking habit, have an additional role in cancer development.

REFERENCES

1. Johnson PJ, Krasner N, Portmann B, Eddleston ALWF, Williams R. Hepatocellular carcinoma in Great Britain. Gut 1978;19:1022-1026.

2. Davidson AR, Tomlinson S, Calne RY, Williams R. The variable course of primary hepatocellular carcinoma. Br J Surg 1974;61:349-352.
3. Dickinson ER, Fleming CR, Ludwig J. Primary biliary cirrhosis. In: Popper H, Schaffner F, eds. Progress in liver disease. Volume 6. New York: Grune & Stratton 1979;487-502.
4. Ikeda K, Saitoh S, Arase Y, Tsubota A, Chayama K, Kumada H, Kawanishi M. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. HEPATOLOGY 1993;18:47-53.
5. Brechot C, Jaffredo F, Lagorce D, Genken G, Meyer zum Buschenfelde K, Papakonstontinou A, Hadziyannis S, et al. Impact of HBV, HCV and GBV-C/HGV on hepatocellular carcinomas in Europe: results of a European concerted action. J Hepatol 1998;29:173-183.
6. Bradbear RA, Bain C, Siskind V, Schofield D, Webb S, Axelsen E, Halliday JW, et al. Cohort study of internal malignancy in genetic hemochromatosis and other chronic non-alcoholic liver disease. J Natl Cancer Inst 1985;75:81-84.
7. Melia WM, Johnson PJ, Neuberger J, Zaman S, Portmann BL, Williams R. Hepatocellular carcinoma in primary biliary cirrhosis: detection by alfa-fetoprotein estimation. Gastroenterology 1984;87:660-663.
8. Floreani A, Biagini MR, Chiaramonte M, Milani S, Surrenti C, Naccarato R. Incidence of hepatic and extra-hepatic malignancies in Primary Biliary cirrhosis. Ital J Gastroenterol 1993;25:473-476.
9. Farinati F, Floreani A, De Maria N, Fagiuoli F, Naccarato R, Chiaramonte M. Hepatocellular carcinoma in Primary Biliary Cirrhosis. J Hepatol 1994;21:315-316.
10. Mills PR, Boyle P, Quigley Emm, Birnieg G, Garrett F, Watkin G, Malssweet RNM. Primary Biliary Cirrhosis: an increased incidence of extrahepatic malignancies? J Clin Pathol 1982;35:541-543.
11. Wolke AM, Schaffner F, Kapelman B, Sacks HS. Malignancy in Primary Biliary Cirrhosis. High incidence of breast cancer in affected women. Am J Med 1984;76:1075-1078.
12. Goudie BM, Burt AD, Boyle P, Mc Farlane G, Birnie GG, Mills PR, Gillis CR, et al. Breast cancer in women with PBC. British Medical Journal 1985;291:1597-1598.
13. Scheuer PJ. Primary Biliary Cirrhosis. Proc R Soc Med 1967;60:1257-1260.
14. World Health Organization. Statistical methods for registries. In: Jensen OM, Parkin DM, MacLennan R, Muir CS, Skeet RG, eds. Cancer registration: principles and methods. Lyon, France: International Agency for Research on Cancer, 1991;126-158.
15. Venetian Tumour Registry. Simonato L, Guzzinati S, Stocco CF, Tognazzo S, Vettorazzi M, Zambon P, eds. Padua: CLEUP, 1997.
16. Van Dam GM, Gips CH. Primary biliary cirrhosis in the Netherlands. An analysis of associated diseases, cardiovascular risk, and malignancies on the basis of mortality figures. Scand J Gastroenterol 1997;32:77-83.
17. Jones DE, Metcalf JV, Collier JD, Bassendine MF, James OFW. Hepatocellular carcinoma in primary biliary cirrhosis and its impact on outcomes. HEPATOLOGY 1997;26:1138-1142.
18. Bellentani S, Tiribelli C, Saccoccio G, Sodde M, Fratti N, De Martin C, Cristianini G. Prevalence of chronic liver disease in the general population of Northern Italy. The Dionysos study. HEPATOLOGY 1994;20:1442-1449.
19. Ray RB, Lagging LM, Meyer K, Ray R. Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblasts to tumorigenic phenotype. J Virol 1996;70:4438-4443.
20. Farinati F, Cardin R, D'Errico A, De Maria N, Naccarato R, Cecchetto A, Grigioni W. Hepatocyte proliferative activity in chronic liver damage as assessed by the monoclonal antibody MIB1 Ki67 in archival material: the role of etiology, disease activity, iron and lipid peroxidation. HEPATOLOGY 1996;23:1468-1475.
21. Yu MW, Hsu FC, Sheen IS, Chu CM, Lin DY, Chen CJ, Liaw YF. Prospective study of hepatocellular carcinoma and liver cirrhosis in asymptomatic chronic hepatitis B virus carriers. Am J Epidemiol 1997;145:1039-1047.
22. Colombo M. Hepatocellular carcinoma. J Hepatol 1992;15:225-236.
23. Loof L, Adami H, Sparen P, Danielsson A, Eriksson LS, Hultcrantz R, Lindrgren S, et al. Cancer risk in Primary Biliary Cirrhosis: a population-based study from Sweden. HEPATOLOGY 1994;20:101-104.
24. Kelsey JL, Gammon MD. Epidemiology of breast cancer. Epidemiol Rev 1990;12:228-240.
25. Lega italiana per la lotta contro i tumori. Associazione italiana di epidemiologia. Cancer in Italy. Incidence data from cancer registries 1983-1987. In: Zanetti R, Crosignani P, eds. Cancer in Italy. Incidence data from cancer registries 1983-1987: Torino, 1992.
26. Stellon AJ, Williams R. Increased incidence of menstrual abnormalities and hysterectomy preceding primary biliary cirrhosis. Br Med J. 1986;293:297-298.
27. Zaridze DG. Time trends and international geographical variations related to diet and cancer. In: Recent progress in research on nutrition and cancer. Chichester, UK: Wiley-Liss, Inc., 1990,21-34.
28. Anonymous. Vitamin A and cancer. Lancet 1980;i:575-576.