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Histology and virus expression in the liver: a prognostic puzzle in chronic hepatitis B

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Summary. Forty chronic untreated paediatric carriers of hepatitis B virus (HBV) infection, with no other causes of liver disease, were biopsied on presentation, when the disease was in the active viral replication phase. After a period ranging from 1 to 13 years, all patients underwent a control biopsy. At the time of the last biopsy, 31 of the patients were anti-HBe positive, whereas 9 persisted in the active replication phase. In this latter phase, necrotic and inflammatory lesions and the presence of nuclear HBcAg were found significantly more frequently than when replication had terminated. The necrotic and inflammatory lesions detected in the first biopsy of patients who subsequently underwent anti-HBe seroconversion were significantly more severe than in patients failing to reach seroconversion. All patients who maintained viral replication showed generalized nuclear reactivity for HBcAg on presentation; such reactivity was also found in 16 of 31 (52%) patients who reached anti-HBeAg seroconversion. All these cases had piecemeal necrosis (PMN) in the biopsy. PMN may therefore be considered as a positive prognostic factor in that it identifies those patients who may seroconvert with significant remission of liver disease

Key words: Chronic hepatitis B virus – Piecemeal necrosis – Immunohistochemistry – Liver

Introduction

Two main biological phases have been identified in hepatitis B virus (HBV) chronic infection (Bortolotti et al. 1983; Realdi et al. 1980). The first is associated with the presence of hepatitis B e antigen (HBeAg) and HBV-DNA in serum, which are considered to be expressions of active viral replication (Fattovich et al. 1986; Liaw et al. 1987). In the majority of patients, this phase is associated with inflammation. Piecemeal necrosis (PMN) has been considered to be the most relevant histological marker of this phase because of its potential significance in the evolution to cirrhosis (Burrel et al. 1988; Desmet et al. 1985; Scheuer 1977; Van Stapel et al. 1983). The second phase, which occurs later in the natural history of HBV infection, is characterized by termination of viral replication and appearance of antibody to HBeAg (anti-HBe) in serum; this phase is almost invariably associated with biochemical and histological remission of liver disease (Liaw et al. 1987; Rugge et al. 1986).

Several epidemiological, biological and clinical factors have been proposed as prognostically important in the evaluation of chronic liver disease due to HBV (Bianchi et al. 1987; Bortolotti et al. 1987, 1988, 1990; Liaw et al. 1987; Villari et al. 1991). Unfavourable prognostic significance has been attributed to focal expression of hepatitis B core antigen (HBcAg) in the liver, particularly when associated with PMN (Bianchi et al. 1987; Chu and Liaw 1987; Gerber and Thung 1987).

The aim of this study was to evaluate the prognostic significance of the histology and expression of viral antigens in the liver, in relation to seroconversion from HBeAg to anti-HBe, in a well-defined group of patients who were followed for several years during the normal course of their liver disease. For this purpose we have selected a group of untreated paediatric patients in whom hepatotoxic factors such as alcohol, drugs, environmental toxins and infection by other hepatotropic viruses were excluded.

Patients and methods

The study involved 40 untreated patients with chronic HBV infection [18 females and 22 males, with serum hepatitis B surface antigen (HBsAg) positive for at least 6 months. None of the patients

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Table 1. Demographic and clinical data at presentation (t_0); the 40 patients were divided into two groups (A, B) according to the presence (A1) or the absence (B1) of seroconversion to anti-HBe at the end of follow-up (t_1)

	Group A ($n=31$)	Group A1 ($n=31$)
	follow-up 5 years (range 1–13)	
Age (mean, years)	6.84 (range 1–13)	12.16 (range 3–17)
Sex (M/F)	18/13	
Serum HBV-DNA	31/31	0/31
Serum HBeAg	31/31	0/31
Alt (IU/l)	195±70	44±35
	Group B ($n=9$)	Group B1 ($n=9$)
	follow-up 4 years (range 1–8)	
Age (mean, years)	8.78 (range 4–14)	12.34 (range 7–20)
Sex (M/F)	4/5	
Serum HBV-DNA	9/9	9/9
Serum HBeAg	9/9	9/9
Alt (IU/l)	170±65	165±101

had cirrhosis at first observation and all of them were sero-negative for antibodies to hepatitis delta virus (HDV), hepatitis C virus (HCV) and human immunodefficiency virus (HIV) (Table 1).

At the time of diagnosis (t_0, time of first biopsy), all patients had abnormal transaminase levels and were in the phase of active viral replication (HBsAg+; HBeAg+; serum HBV-DNA+ = R+ phase). After a follow-up period ranging from 1 to 13 years, all patients underwent a control liver biopsy (t_1, time of last biopsy). At this time, 9 patients were found to persist in the active viral replication phase (t_1 = R+), whereas 31 patients were anti-HBe positive and HBV-DNA negative, indicating a previous seroconversion from HBeAg+ to anti-HBe+ (t_1 = R−). The control liver biopsy was taken in all patients at least 6 months after the demonstration of anti-HBe in serum.

The liver biopsies were performed using a Menghini needle. The biopsy fragments (at least 1 cm long) were fixed in 5% buffered formalin and embedded in paraffin. Sections of 5 μm were stained with haematoxylin and eosin, Gomori's method for reticulin and periodic acid-Schiff (PAS), before and after digestion with diastase. Liver biopsies were separately evaluated blindly by two of the authors (M.R. and M.G.); where opinions differed, the specimens were reconsidered jointly.

The following elementary lesions were coded using a semiquantitative method in a scale from 0 to 3 (from absent to severe): portal hepatitis (PH); portal-periportal fibrosis (PPF); PMN; hepatocyte degeneration (HD), i.e. ballooning, acidophilic condensation, Councilman bodies; intralobular lympho-histiocytic infiltration (ILI); focal necrosis (FN); confluent bridging necrosis (BN).

The histological diagnoses were based on the criteria proposed by Bianchi et al. (1977).

HBsAg, HBcAg, HBeAg, anti-HBe, anti-HDV and anti-HIV were investigated using radioimmunoassay kits or enzyme-linked immunoadsorbent assays (Abbott Laboratories, Chicago, Ill.) and using the ELISA method for anti-HCV (Ortho Laboratories Raritan, New Jersey). Serum HBV-DNA was investigated using the molecular hybridization technique suggested by Scotto (1983).

Testing for HBV antigens in the liver tissue was done immunohistochemically using Dakopatts polyclonal antibodies in the avidin biotin complex method.

Following Gudat et al. (1975) the expression of HBsAg and HBcAg in the hepatocytes was identified as follows: HBsAg in the cytoplasm (HBs-C) and membrane (HBs-M), HBcAg in the nucleus (HBc-N) and cytoplasm (HBc-C). Reactivity for the two antigens was also distinguished as focal (F) where reactivity was observed in less than 60% of liver cells and generalized (G), when reactivity was observed in more than 60% of liver cells.

The statistical significance of results was evaluated using the chi-square test.

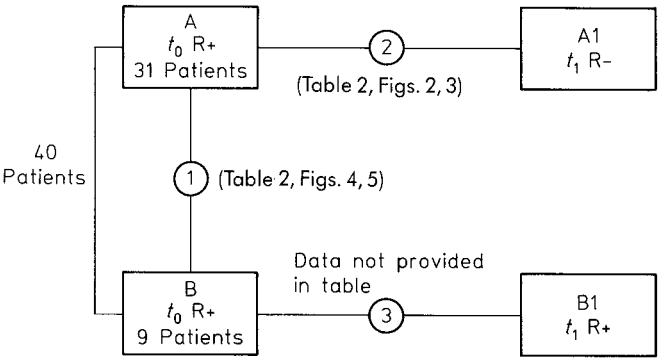

Fig. 1. Schematic representation of comparison made in the text. Patients were divided into four groups according to the outcome of the disease (A = $t0$ R+; A1 = $t1$ R−; B = $t0$ R+; B1 = $t1$ R+)

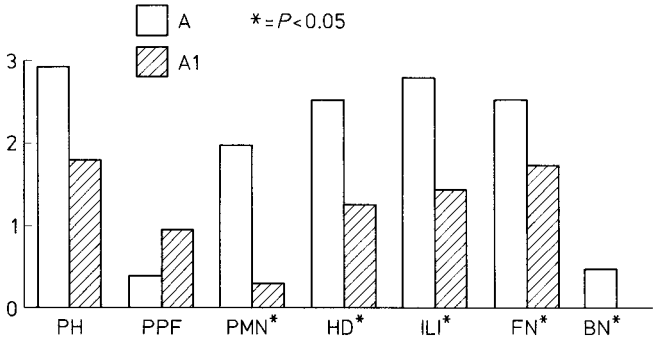

Fig. 2. Elementary cytohistological lesions: comparing A vs A1. *PH*, Portal hepatitis; *PPF*, portal-periportal fibrosis; *PMN*, piecemeal necrosis; *HD*, hepatocytic degeneration; *ILI*, intralobular lymphohistiocytic infiltration; *FN*, focal necrosis; *BN*, bridging necrosis

Results

Figure 1 illustrates the scheme for comparative evaluation of histological changes and viral antigen expression in the samples observed, distinguishing the R+ and R− phases at t_0 and t_1. At t_0, all patients were divided into two groups (A and B) according to the presence or absence of seroconversion to anti-HBe at t_1.

Figure 2 illustrates the mean value of representation of histological lesions investigated in biopsies taken during viral replication (t_0 = R+) and after seroconversion (t_1 = R−) (comparison 2: A vs A1). The presence of PMN, FN, BN, HD and ILI was found significantly associated with R+ phase ($P<0.005$). The same histological lesions showed in biopsies from patients failing to reach anti-HBe seroconversion showed no significant differences (comparison 3: B vs B1; data not provided in table form).

Table 2. Histological diagnoses from first and last biopsies in all patients considered (groups as indicated in Figure 1)

Histological diagnosis

First biopsy			Last biopsy		
A	HC = 1 (3%) CPH = 10 (32%) CAH = 20 (65%)	$P < 0.0001$ Comparison 2	A1	HC = 15 (48%) CPH = 13 (42%) CAH = 3 (10%)	
$P < 0.01$ Comparison 1					
B	HC = 1 (11%) CPH = 7 (63%) CAH = 1 (11%)	P = NS Comparison 3	B1	HC = 1 (11%) CPH = 5 (55%) CAH = 3 (33%)	

Table 3. Sensitivity (Sens.), specificity (Spec.), predictive positive value (PPV) and predictive negative value (NPV) of piecemeal necrosis (PMN), bridging necrosis (BN) and focal necrosis (FN) as indicators of subsequent seroconverion to anti-HBeAg

	PMN	BN	FN
Sens.	64.5	16.1	83.9
Spec.	88.9	100.0	22.2
PPV	95.2	100.0	18.8
NPV	42.1	25.7	28.6

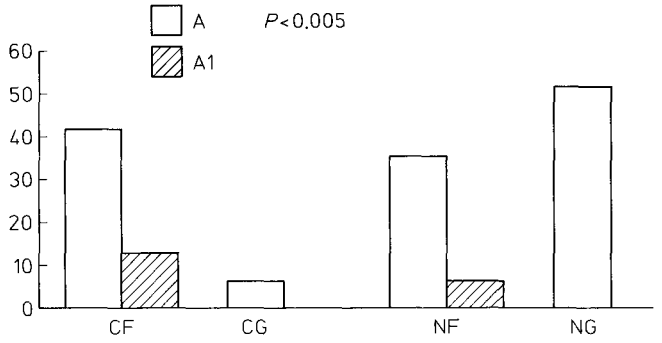

Fig. 3. Comparison of tissue reactivity for HBcAg in groups A and A1. *C*, cytoplasmic; *N*, nuclear; *f*, focal; *G*, generalized

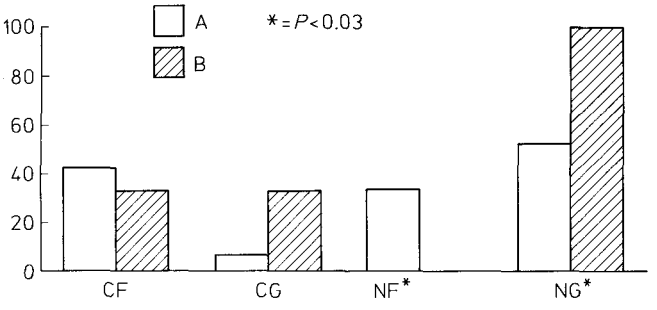

Fig. 4. Comparison of tissue reactivity for HBcAg in groups A and B. *C*, Cytoplasmic; *N*, nuclear; *F*, focal; *G*, generalized

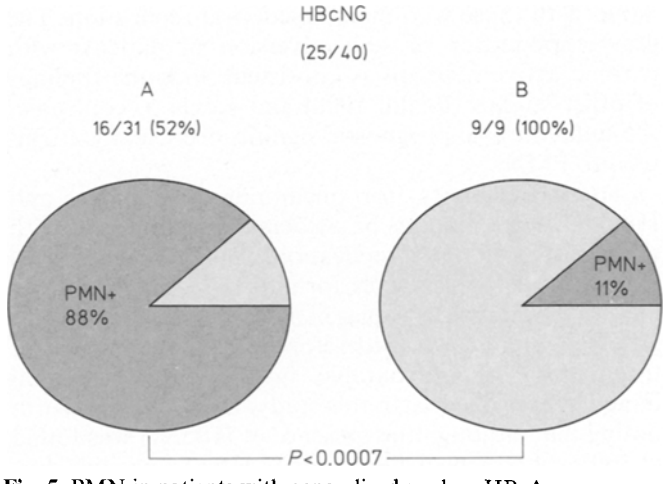

Fig. 5. PMN in patients with generalized nuclear HBcAg: comparing groups A vs B

Table 2 summarizes the first and last histological diagnoses in all 40 patients. The different distribution of the patients' diagnoses in the viral replication phase and after seroconversion proved significant ($P<0.0001$).

During active viral replication, the necrotic and inflammatory lesions in patients who subsequently seroconverted (group A) were found more severe than in patients who retained viral replication (group B). BN was observed only in group A patients and PMN was found in 65% of group A cases versus 11% of group B patients ($P<0.005$).

The sensitivity, specificity, positive and negative predictive values for necrotic and inflammatory lesions found in the first biopsy, as indicators of subsequent seroconversion to anti-HBe, are illustrated in Table 3.

Immunocytochemically demonstrable reactivity for HBsAg in a generalized cytoplasmic form (HBsCG) was found significantly more often after seroconversion (comparison 2: A vs A1; $P<0.05$). Membranous generalized (HBsMG) reactivity was significantly more frequent in the first biopsy of patients failing to seroconvert (comparison 1: A vs B; $P<0.02$).

For HBcAg liver cell reactivity proved to be significantly associated with the R+ phase (comparison 2: A vs A1 $P<0.0001$, see Fig. 3). Nuclear generalized reactivity for HBcAg (HBcNG) was found to be significantly associated with the first biopsy taken from patients with persistence of HBeAg (comparison 1: A vs B; $P<0.0001$ – see Fig. 4). Although generalized nuclear HBcAg was found in the first biopsy in all patients persisting in viral replication (group B), it was also detected in 52% of those who subsequently seroconverted to anti-HBe (group A). PMN was significantly more frequent, however, in the latter group than in the former (88% vs 11; $P<0.0007$; Fig. 5).

Discussion

The prognosis of chronic hepatitis B in the absence of superinfection by other viruses or of hepatotoxic factors

has been related to virological and histological features of liver disease. Viral replication is generally associated with active liver disease and PMN has been considered a predictive marker of evolution to cirrhosis (Liaw et al. 1988; Realdi et al. 1980; So et al. 1986). Termination of viral replication is associated with improvement or disappearance of both intralobular and periportal necrotic and inflammatory lesions (Burrel et al. 1988; Fattovich et al. 1986; Ramalho et al. 1988; Rugge et al. 1986).

The expression of HBV antigens in the liver has been correlated both with the virological phase of the infection and with necrotic and inflammatory lesions (Bianchi et al. 1987). In particular, active liver disease has been correlated with focal expression of HBcAg while inactive hepatitis has been related to HBsCG or HbcNG associated with HBsMG (Bianchi et al. 1987; Gudat et al. 1975). The results of our study confirm the association between the HBV replication phase and necrotic and inflammatory lesions. Both these features showed a significant reduction after seroconversion to anti-HBe. The R+ phase was also associated both with focal and generalized reactivity for HBcAg in the liver. As suggested in previous publications (Bortolotti et al. 1985, 1986; So et al. 1986), the seroconversion phase was characterized by a significant reduction of liver cell reactivity for HBcAg followed by a significant increase in cytoplasmic expression of HBsAg. At presentation, patients who subsequently seroconverted had more severe necrotic and inflammatory lesions including PMN when compared with those who maintained viral replication. The greater frequency of seroconversion in patients with chronic active hepatitis is consistent with the findings of other studies (Realdi 1990) and seems to contradict the unfavourable prognostic significance usually attributed to PMN.

Tissue reactivity for membranous HBsAg and HBcNG was found to be associated significantly with the persistence of viral replication. This finding is consistent with the low tendency for anti-HBe seroconversion observed by Sanchez-Tapias et al. (1988) in patients with HBcNG reactivity. It is therefore reasonable to consider this pattern as unfavourable, in relation to remission of active liver disease. In this study, however, it is worth noting that although the presence of HBcNG was found in 100% of patients who remained HBeAg positive during follow-up, this pattern was also found in 52% of those who subsequently seroconverted. In these latter cases, the presence of widespread HBcAg in the liver was associated with PMN in 88%, while in those cases who retained viral replication, PMN was only found in 11% of patients.

Thus the presence of HBcNG indicates a biologically heterogeneous disease in which the PMN distinguishes the cases of "permissive" infection (when PMN is absent) (Bianchi et al. 1987) from those which are likely to seroconvert (when PMN is present). Two complementary results therefore lead PMN to be considered as a favourable histological marker in chronic HBV infection: first, a higher percentage of seroconversion was found in patients with PMN than in patients with inactive disease (65% vs 11%); second, among cases with a virological pattern suggesting a persistent viral replication, PMN identified those who subsequently seroconverted.

Thus, in the model of chronic HBV infection considered here PMN seems to share the paradoxical prognostic import attributed by Liaw et al. (1988) to BN. Both types of necrosis have been suggested to be implicated in the morphogenesis of cirrhosis (Combes 1986; Rugge and Pollice 1990), but should nonetheless be considered as predictive of seroconversion and remission of necrotic and inflammatory activity. Our results suggest a re-appraisal of the evaluation of the histopathology of chronic hepatitis, bearing in mind the significance traditionally attributed to PMN as a histological marker of potential cirrhotic evolution (Baptista et al. 1988; Bianchi et al. 1977, 1987; Desmet 1985; Popper 1983; Scheuer 1977).

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