Multiple viral infections in a group of intravenous drug users: hepatitis B virus exposure is the risk factor

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Objective Infection with hepatotropic viruses is associated with a variable degree of liver disease, and there is evidence that more severe lesions are related to the association with another viral infection. The aim of this investigation is to establish the relationship between different viral infections occurring in the same individual and the presence and progression of liver disease.

Design The study population comprises 754 intravenous (IV) drug abusers exposed to hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) or cytomegalovirus (CMV). All individuals were followed for an average of 2 years. Liver disease was assessed by liver function tests, 99m-technetium (^{99m}Tc) liver scintigraphy, and also by liver biopsy in a subset ($n = 136$) of patients. The different viral patterns and presence of disease were analysed by logistic regression, and the risk factors were calculated. Contingency tables of patients with single or associated infections were drawn up to evaluate progression of liver disease.

Results Association of HIV with at least one other viral infection was constant. Surface antigens of HBV (HBsAg) were always associated with HIV ($n = 19$); in this group, 18 patients had signs of liver disease. A past infection with HBV, as revealed by the presence of at least antibodies against the surface antigen (HBsAb) and antibodies against the core antigen of HBV (HBcAb), was detected in 463 patients (61.4%). The overall prevalence of HCV antibodies was 63.91% ($n = 482$). In 96.8% of the 406

patients tested, HCV-RNA was detected by reverse transcriptase polymerase chain reaction (RT-PCR). The majority of patients with high alanine transaminase (ALT) had anti-HBV antibodies in the presence of HCV (56.1%). At the end of follow-up, all of these patients showed signs of active liver disease, and scoring was significantly worse than in patients with either HBV or HCV alone. An infection/reactivation of CMV was found in patients previously exposed to HBV and with increased ALT values.

Conclusions Data emerging from this study reveal the association of HCV or CMV, or both, with a previous HBV infection, as demonstrated by HBsAb and HBcAb, and rapid progression of the disease in this group of patients. A previous HBV infection therefore appears to be an important risk factor for subsequent viral-related liver disease. *Eur J Gastroenterol Hepatol* **13**:1347–1354 © 2001 Lippincott Williams & Wilkins

Introduction

Co-infection with different hepatotropic viruses contributes to the development of more severe liver damage from both clinical [1–6] and histological points of view [7,8]. There is also evidence that active liver disease may be present in patients with previous hepatitis B virus (HBV) infection (only HBV antibodies) that is related to hepatitis C virus (HCV) infection [2,3]. Patients with HBV and HCV co-infection presented cirrhosis on liver biopsy more frequently than those with HBV infection alone, who instead developed hepatic fibrosis [3]. Furthermore, analysis of liver biopsies from HBV-positive patients with increased

serum alanine transaminase (ALT) levels revealed more severe lesions in those with cytomegalovirus (CMV) co-infection [8]. Again, it has been reported that HCV- and human immunodeficiency virus (HIV)-positive patients develop cirrhosis more frequently than HIV-negative subjects [5], and that in the former death is often due to liver failure [6]. Prospective assessment is necessary to confirm these data from cross-sectional studies.

The present investigation was carried out in an attempt to establish whether co-infection with one or more viruses represents a major risk factor for developing

chronic liver disease, each virus with its specific site and type of effect upon liver tissue. A group of intravenous (IV) drug abusers exposed to multiple viral infections [9] was followed for a mean period of 2 years in order to evaluate the progression of liver disease and to correlate severity with a given pattern of multiple viral infections.

Material and methods

A total of 754 patients (560 male, 194 female; age 35 ± 8 years, mean ± 1 SD) with a past history of IV drug abuse were enrolled over a 7-year period. In the first year, 57 individuals were enrolled in the study; thereafter, the number of patients enrolled each year ranged from 105 to 153. Patients were referred to our outpatient department by two different rescue centres on account of abnormal liver function tests. Patients attending these centres are treated initially with methadone, followed by consultation with psychologists, and are screened for the presence of infections and disease. All individuals entering the present study had completed at least 6 months' follow-up by the referring centres. Entry into the study was determined by the finding of impaired liver function tests by the attending physicians at the rescue centres: all participants were referred to the outpatient clinic of the gastrointestinal unit, where they were submitted to the entire diagnostic protocol (including liver scintigraphy and endoscopy due to oesophageal varices); they were hospitalized only for liver biopsy.

Exclusion criteria were presence of acute liver disease, clinically manifest HIV infection, and the presence of autoimmune hepatitis. Screening for autoimmune hepatitis included antinuclear (ANA), anti-smooth muscle (ASMA), anti-mitochondrial antibodies (AMA) and autoantibodies directed against liver and kidney membranes (LKM). The presence of autoantibodies was determined regularly during follow-up. Patients with a titre $\geqslant 1/80$ were not included in the study population, but were followed separately.

Alcohol consumption and the recent or present use of potentially hepatotoxic drugs were checked with the referring centres. We decided, arbitrarily, to allow up to 75 g of ethanol per day; abuse of a larger quantity was considered an exclusion criterion, and the report by relatives, partners or friends of occasional excess was a criterion for withdrawal from the study. Assessment of past ethanol consumption was carried out by means of a questionnaire. The form had to be filled in by all participants; we decided to exclude those individuals who failed to return the completed questionnaire after being asked for a second or third time. The use of 1 mg flunitrazepam and 2.5 mg lorazepam per day was allowed.

Every patient was tested for surface antigen of HBV (HBsAg), antibodies against the surface antigen (HBsAb), anti-hepatitis D virus (HDV), hepatitis D antigen (HDV-Ag), anti-hepatitis B core antigen (HBc) total antibodies and IgM, anti-HCV antibodies, anti-HIV antibodies, and anti-CMV antibodies (IgG and IgM; the transient presence of IgM and a high titre of IgG was considered to indicate a recent reactivation or re-infection). The status of HBV infection is considered active when either HBsAg or IgM antibodies against the core antigen of HBV (HBcAb) and HBV-DNA are present. A patient with HBsAb and HBcAb, referred to here as HBV antibody positive, is regarded as one who had been infected in the past but has no circulating virus at present.

HBsAg, anti-HBsAb and anti-HBcAb were screened by Elecsys® Immunoassay (Roche, Basel, Switzerland). HBV-DNA was detected by a competitive polymerase chain reaction (PCR) method (Cobas Amplicor HBV Monitor™, Roche, Basel, Switzerland). The presence of anti-HCV antibodies was revealed using the second-generation immune-enzymatic test by Ortho (Ortho Diagnostic System, Raritan, NJ, USA), and then confirmed by the second-generation immunoblot assay by Chiron (Chiron-RIBA, Emeryville, CA, USA), and later by the third-generation immune-enzymatic test (anti-HCV EIA Cobas® Core, Roche, Basel, Switzerland), in which serum specimens are incubated with recombinant antigens from the HCV core, NS3, NS4 and NS5 regions. Positive samples were confirmed by Inno-LIA™ HCV Ab III update immunoassay (Innogenetics, Ghent, Belgium).

Testing for the presence of HCV genome was performed on RNA ethanol extracted from 100 µl plasma, reverse transcribed and amplified by PCR (Cobas Amplicor HCV Monitor, Test v2.0). Primers were KY78 and KY80 to define a sequence of 244 nucleotides within the highly conserved 5′ untranslated region of HCV-RNA.

CMV IgG and IgM, HDV-Ab were detected by an indirect enzyme immunoassay method (EIA Well-Radim, Liege, Belgium). HDV antigens were revealed using a monoclonal anti-HDV antibody (EIA Well-Radim Liege, Belgium). The HIV antibody test used was the Access® Immunoassay System (Sanofi Diagnostics Pasteur, Marnes La Coquettes, France), which uses the p25 recombinant protein and peptides gp 41 and gp 36 as antigens for binding to the patient's antibodies.

The presence of liver damage was monitored monthly by serum ALT values, regardless of severity of the disease [10]. For the purposes of this study, the ALT determination indicating presence of disease was arbitrarily fixed at 1.5-fold above the upper range of normal.

Severity of disease was assessed by liver biopsy and technetium-99m (^{99m}Tc) liver/spleen scintigraphy [11,12]. After informed consent, liver biopsies were collected upon entry to the study in 23 patients, and at the end of follow-up (29.2 ± 3.7 months) in 19 patients. In those patients who refused biopsy, semi-quantitative assessment of liver damage was defined [11,12] as follows: (i) *mild*: presence of increased serum transaminases and normal uptake at liver/spleen scintigraphy; (ii) *moderate and chronic*: scintigraphy showing normal uptake of radionuclide by the right hepatic lobe, with increased uptake by the left lobe and none by the spleen; inflammatory activity was defined by ALT levels and liver histology, when available; (iii) *severe/cirrhosis*: liver scintigraphy revealing decreased and irregular uptake of ^{99m}Tc by the right hepatic lobe, with increased uptake by the left lobe and spleen; inflammatory activity was determined by transaminases. The presence of cirrhosis and activity were confirmed by histology, when available. Ultrasound (US) examination of the liver was carried out according to protocol only after the radionuclide scan [13], and in the presence of moderate/chronic or severe/cirrhosis stage of liver disease. Specific US patterns were, respectively, finely dyshomogeneous and grossly dyshomogeneous with irregular margins.

A total of 275 patients left this natural history study once a decision to treat the infection had been agreed upon between the patient, the doctors at the gastrointestinal unit, and the staff of social workers and psychologists at the rescue centre. The basal clinical observation before starting treatment coincides with the last point of this study. On this occasion, 136 participants agreed to undergo liver biopsy, and these data are included. In order to evaluate the reliability of the method used in the assessment of liver damage, an attempt was made to correlate the grading and staging obtained by ^{99m}Tc liver scan and transaminases with the liver pathology score [14].

Logistic regression was used to assess the risk factors associated with the different viral patterns present in all individuals and to calculate the odds ratio. The importance of the association of different viruses in order to produce a liver disease was compared with the presence of each virus, and the relative contingency tables were built up, one for each class of viral marker. Statistical analysis was carried out using a chi-squared test to establish the significance in the number of patients belonging to different groups in 2×2 contingency tables.

To assess the progression of disease, contingency tables were built up to compare the number of patients presenting a worse outcome at the end of follow-up (shift from one class of disease to another) with those in whom no change was observed, depending on different viral patterns. The sums of individuals with HBV or HCV infection alone were compared with those presenting a co-infection with different hepatotropic viruses, in particular HBV associated with HCV, and HBV with CMV.

Results
Mean follow-up was 29.2 ± 3.7 months. A total of 392/754 people entering the study had normal serum ALT levels, and 362/754 had persistently increased values (Table 1).

Viral pattern
The prevalence of antibodies against HIV was 6.9% (52/754), and infection was always associated with at least one of the other viruses investigated. Of those people with HIV infection, 36 (69.2%) presented co-infection with either HCV or HBV, or both, as well as raised ALT levels (Table 1). Regarding the association with HBV, increased ALT values were recorded in the presence of HBsAg as well as antibodies (HBsAb, HBcAb and antibodies against the 'e' antigen of HBV [HBeAb]).

The overall prevalence of HBV infection, including evidence of past infection (presence of at least HBsAb plus HBcAb) was 61.5% ($n = 463$; see Table 1). The overall prevalence of HCV was 63.9% ($n = 482$; see Table 1): 406 patients were tested for circulating viral genomes by PCR, and 393 (96.8%) were positive for HCV-RNA. Antibodies to CMV were sought in 322 patients; 84% showed signs of a recent infection/re-infection or reactivation. High titres of IgG, i.e. > 10-fold the upper limit of the normal range, were present in 247 patients, 94 of whom presented associated IgM at low titres, which remained constant or decreased slightly during the study. In 23 patients, high values of IgM (131 ± 42 mg/dl, mean ± SD) were observed; these decreased during the study, but were still positive in 19 patients at the end.

The majority of patients in our study population had multiple viral infections, and the relationship of the viral patterns to serum transaminases was analysed by logistic regression and contingency tables.

Positivity for HIV and HBsAg in the same patient (Table 1) multiplies the odds of having increased serum transaminases by 21.4 (95% CI 2.51 to 183.05). Sixteen patients also presented an association with HCV (HIV plus HBsAg plus HCV positivity), and in 13 cases HIV was associated with HCV alone (Table 1).

HCV antibody positivity increases the odds of presenting with raised ALT levels by 2.5 (95% CI 1.66 to 3.61). Of the 61 patients with only HCV, 57 had normal

Table 1 **Alanine aminotransferase (ALT) activity and exposure to human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and cytomegalovirus (CMV) in the entire study population.**

HBsAg	HBV-Ab	HCV	CMV	HIV	n
Increased ALT					
	+	+			136
	+		+		97
		+	+		12
	+	+	+		67
+		+		+	16
		+		+	13
+				+	2
	+			+	2
	+	+	+	+	3
	+				6
		+			4
			+		4
					362 total
Normal ALT					
	+	+			59
	+		+		58
		+	+		63
	+	+	+		44
+		+		+	1
		+		+	6
			+	+	11
	+				38
		+			57
			+		55
					392 total

Patients with a given viral pattern are stratified according to the level of serum transaminase $\geqslant$ 1.5 times the upper normal range. Exposure to HBV is differentiated as past infection (presence of antibodies against the surface antigen [HBsAb] and antibodies against the core antigen of HBV [HBcAb]) and present infection (all patients with the surface antigen of HBV [HBsAg]). Hepatitis C-positive patients include those with HCV antibodies only, and those with positive polymerase chain reaction (PCR) (96.8% of tested HCV antibody positive, $n = 406$).

transaminases (Table 1). Those patients with signs of exposure to an additional virus had, in general, abnormal ALT values. Of the 327 patients (43.4% of the entire study population) with HCV antibodies and evidence of a past HBV infection (revealed by at least HBsAb and HBcAb positivity), 196 had only HBV and HCV: 30.6% ($n = 60$) with normal ALT and 69.4% ($n = 136$) with raised transaminases. In this same subgroup, 131/327 patients also carried other viruses (Table 1). The number of people with high ALT in the group with associated infections is significantly higher than in the group with only one infection (OR 4.66, $P < 0.001$).

The presence of a past HBV infection multiplies the odds for the presence of increased ALT levels by 17.1 (95% CI 10.22 to 28.66), and the associations with HCV, CMV or both are the most frequent variables (Table 1). On the other hand, the presence of HBV antibodies alone with no other evidence of present or past infections is associated with normal transaminases ($n = 38$ v. 6 with raised ALT).

An association of exposure to HBV and CMV was found in 156/322 individuals also investigated for CMV:

62.2% ($n = 97$) had raised ALT levels and 37.8% ($n = 59$) had normal values. A significantly larger number of people with high ALT levels was found in the group with associated infections than in the group with only one infection (OR 2.26, $P < 0.01$). The prevalence of an association between HBV, HCV and CMV was 35.4% (114/322 tested for CMV): 61.4% ($n = 70$) of patients had raised ALT levels (3 of whom carried HBsAg and HIV), and 38.6% ($n = 44$) had normal transaminases. A significantly larger number of patients with high ALT levels was observed in the group with associated infections than in the group with only one infection (OR 4.32, $P < 0.001$).

HCV and CMV were found to be associated in 75 patients: 16.0% ($n = 12$) had raised ALT levels and 84.0% had normal ALT levels ($n = 63$). The number of patients with high ALT levels in this particular group with associated infections is significantly lower than in the group with only one infection (OR 2.45, $P < 0.05$). Isolated positivity to anti-CMV antibodies (no serological marker of any other virus detectable) was not related to hepatic lesions. In fact, a higher prevalence of CMV antibody positivity was found in individuals with normal ALT (patients CMV positive with high

ALT: $n = 4$; patients CMV positive with normal ALT: $n = 55$; OR 0.091).

Liver disease

The presence and severity of liver disease were assessed according to ALT values and results of liver ^{99m}Tc scintigraphy and US performed in 639 patients upon entry to the study and repeated after 28.6 ± 3.3 months' follow-up in 356 patients (55.7%). The non-invasive staging and grading of liver disease using this procedure was correlated to that obtained by liver biopsy in a subgroup of patients who gave consent to enter a treatment trial at the end of the present observation (Table 2). In 29 patients, the histological score was between 3 and 6 for inflammatory activity and 1–2 for stage: 23 of these had mild, and six moderate, chronic active liver disease. A total of 68 patients had a histological score between 7 and 12 for inflammatory activity and 3-4 for stage: four had mild, and 55 moderate, chronic active liver disease, while in nine patients the ^{99m}Tc scan showed severe disease with spleen uptake greater than 25% on the postero-anterior film. Thirty-nine patients had a histological score between 13 and 18 for inflammatory activity and 5–6 for stage: two of these had chronic active liver disease but not a frankly cirrhotic liver, which was present in 37 scinti-scan. Thus, overall agreement of the non-invasive methodology with liver biopsy was

around 85%. This increases to 95% when uptake of the radionuclide by both spleen and background is particularly high in the more severe cases, but it drops to 81% in less severe cases and to 79% in mild disease (Table 2).

Liver scintigraphy and ALT, as well as other liver function tests, were normal in 106 (16.6%) patients, although in 48 there was evidence of multiple viral infections, including 35 with HIV infection.

Of the patients who entered the trial, 533 had evidence of associated liver disease (Table 3). The percentage of patients with moderate chronic active disease was higher, although not statistically significant, in the subgroups with a previous contact with HBV infection and either HCV or CMV co-infection, and in the groups with associated HIV infection (Table 3). A total of 23 patients were HBsAg positive (3.1% of the whole study population), and 16/23 (73.9%) showed evidence of associated liver disease (Table 3); the disease was mild in six cases, chronic active in two, and severe/cirrhosis in eight.

Progression of disease was evaluated as a shift from one class of liver disease to another in those patients who were investigated fully upon entry and at the end of follow-up ($n = 354$; see Table 4). Spontaneous

Table 2   Correlation between non-invasive assessment of severity of liver disease and activity grading and staging obtained by liver pathology.

	Mild liver disease		Chronic active liver disease		Severe liver disease/cirrhosis	
	Activity	Stage	Activity	Stage	Activity	Stage
1		13 *3*		1		
2		10 *5*		5		
3	9 *2*	3	36 *3*			
4	7 *6*	1		19 *5*		9
5	5		2	2		18 *2*
6	3		6			12 *5*
7	4		7 *2*			
8	1		17 *3*			
9			13 *2*			
10			6 *1*		2	
11			4		4	
12			8		3	
13					11	
14					4	
15					7 *4*	
16					4 *2*	
17					3 *1*	
18					3	

Liver biopsy was performed in 23 patients on entry to the study, and repeated at the end in only 19 patients. Since this pathology score was limited, it has not been used for assessing progression of liver disease. Results in these patients (shown in *italics*) were considered together with those from a subgroup who had initially denied consent to biopsy, and therefore underwent full non-invasive testing, and later consented in order to enter a therapeutic trial at the end of this observation period ($n = 136$). Three degrees of disease were assessed non-invasively: mild, chronic active, and severe liver disease or cirrhosis according to liver function tests and ^{99m}Tc liver scans. In each cluster, activity grading and disease staging are given as resulting from pathological examination. The correlation of activity grading with non-invasive assessment is very high ($r = 0.869$, $P < 0.001$). The correlation of disease staging with non-invasive assessment appears to be even higher ($r = 0.892$, $P < 0.001$).

Table 3   Severity of liver disease related to different pattern of viral infection in 639 patients who underwent full assessment of liver disease by laboratory biochemical and virological tests, ultrasound examination and ^{99m}Tc liver scan.

	Mild liver disease	Chronic active liver disease	Severe liver disease/cirrhosis
HBV past infection ($n = 67/533$, 12.6%)	48 (71.6%)	18 (26.9%)	1 (1.5%)
HCV infection ($n = 74/533$, 13.9%)	53 (71.6%)	21 (28.4%)	
HIV + HBsAg alone ($n = 1/533$, 0.2%)			1
HIV + HCV antibodies ($n = 6/533$, 1.1%)	4 (66.7%)	2 (33.3%)	
HBV past infection + HCV ($n = 358/533$, 67.2%)	221 (61.7%)	134 (37.4%)	3 (8.4%)
HBV past infection + CMV ($n = 11/533$, 2.1%)	7 (63.6%)	3 (27.3%)	1 (9.1%)
HBsAg or HBV past infection + HCV + HIV ($n = 15/533$, 2.8%)	6 (40.0%)	1 (6.7%)	8 (53.3%)
HBsAg + HCV + CMV + HIV ($n = 1/533$, 0.2%)		1	

CMV, cytomegalovirus; HCV, hepatitis C virus.
No sign of disease was detected in 106 patients, and this synopsis refers to the remaining 533. Thirty-six patients were referred to our institution with human immunodeficiency virus (HIV) infection and raised transaminases, constantly carrying some other viral infection. Of these, 23 were associated with surface antigens of hepatitis B virus (HBV) (HBsAg), and in 17 of these, there were signs of liver disease (lines 3, 7 and 8). Patients indicated as having a past HBV infection are those with antibodies against the surface antigen (HBsAb) and antibodies against the core antigen of HBV (HBcAb).

Table 4   Progression of liver disease related to different patterns of viral infection in a group of 356 patients investigated at the beginning and end of follow-up at 29.2 ± 3.7 months.

		Mild liver disease	Chronic active liver disease	Severe liver disease/cirrhosis
HBV past infection	Upon entry	45 (76.3%)	13 (22.0%)	1 (1.7%)
	End of follow-up	44 (74.6%)	14 (23.7%)	1 (1.7%)
HCV infection	Upon entry	51 (72.8%)	19 (27.2%)	
	End of follow-up	49 (70.0%)	21 (30.0%)	
HIV + HCV antibodies	Upon entry	4 (66.7%)	2 (33.3%)	
	End of follow-up	2 (33.3%)	3 (50.0%)	1 (16.7%)
HBV past infection + HCV	Upon entry	123 (61.8%)	76 (38.2%)	
	End of follow-up	87 (43.7%)	99 (49.7%)	13 (7.5%)
HBV past infection + CMV	Upon entry	7 (63.6%)	3 (27.3%)	1 (9.1%)
	End of follow-up	2 (18.2%)	7 (63.6%)	2 (18.2%)
HBV + HCV + HIV	Upon entry	6 (66.7%)	1 (11.1%)	2 (22.2%)
	End of follow-up	1 (11.1%)	1 (11.1%)	7 (77.7%)

Clinical, biochemical and scintigraphic data were used to assess the severity of liver disease. The numbers and percentages of patients, with respect to the number of patients at entrance, for a given stage of liver disease, are given. The patient with both human immunodeficiency virus (HIV) antibodies and surface antigen of hepatitis B virus (HBV) (HBsAg) (Table 1) is still seriously ill, and is currently under treatment with protease inhibitors. The patient with HBV, hepatitis C virus (HCV), cytomegalovirus (CMV) and HIV infections shifted from the second stage of the disease to severely active cirrhosis. Patients indicated as having had a previous HBV infection are those with antibodies against the surface antigen (HBsAb) and antibodies against the core antigen of HBV (HBcAb).

improvement did not occur in any of the patients under study. Those with disease related to multiple infections had a more severe outcome at the end of follow-up.

HCV infection and an HBV seroconversion at entry were observed in 199 patients (Table 4): 123 (61.8%) patients were estimated to have mild liver disease and 76 (38.2%) to have chronic active disease. Upon completion of the study, 36 (29.3%) patients had a more severe stage of liver damage, from mild to chronic active disease ($n = 34$) and to cirrhosis ($n = 2$). In the group with chronic active disease, 11/76 (14.5%) developed cirrhosis. A contingency table of the sum of patients with HBV or HCV infection ($n = 129$) and worsening of disease ($n = 3$) and those with associated infection ($n = 199$) and progression ($n = 47$) were of very strong, statistical significance (OR 0.088).

Ten patients had both HBV seroconversion and CMV IgM/IgG high titre at entry to the study (Table 4): seven patients had mild liver disease and three had chronic active disease. At the end of the study, five patients from the former group had chronic active disease and one patient, who already had chronic active liver disease at entrance, developed cirrhosis.

A contingency table of data from patients with HBV and CMV infection ($n = 11$) and worsening of the disease ($n = 6$) and those with exposure only to HBV ($n = 59$) and progression of disease ($n = 1$) showed a high statistical significance (OR 0.022).

Likewise, in five of six patients with HCV or HBV infection, or both, seroconversion associated with HIV infection upon entry and mild liver disease shifted to the chronic active ($n = 1$) or to the cirrhotic stage ($n = 4$). No changes were observed in the other groups.

Discussion

Data emerging from the present investigation revealed the prevalence of viral infections in a group of Italian IV drug abusers, and stress the increased risk of developing more severe liver disease in the presence of multiple viral infections. The prevalence of infection for a single virus is similar to that reported by others, with an overall exposure to HIV, HCV and HBV of 6.9, 57.9 and 67.7%, respectively [15]. All HBsAg-positive patients were also HIV positive, thus suggesting that the presence of HIV prevents recovery from HBV infection, with a tendency towards chronic hepatitis and cirrhosis [16].

Considering the type of patients studied, we encountered great difficulty in evaluating liver disease. The initial protocol included liver biopsy, which could be performed only after informed consent was given in a very small proportion of cases (3.1%).

The reliability of serum transaminase levels as an index for inflammatory activity/necrosis and that of ^{99m}Tc liver/spleen scintigraphy for the stage of the architectural derangement of the liver are generally accepted [11,12]. Another issue that was very difficult to assess was past alcohol consumption and abuse. Those patients who returned the questionnaire declared an average consumption within the limits accepted in this trial; psychologists from the rescue centres were asked about each patient, and agreed with what had been stated. The patients who did not return the questionnaire were not included in the study.

The main finding of interest emerging from this series is that the association of HBV and CMV as well as HCV and HBV leads to more severe liver disease with a more rapid course.

In the present series, attention was also focused on patients who had a previous HBV infection (with circulating HBsAb and HBcAb, while the HBeAg system was negative in 2.6% of patients, $n = 14$) and raised serum ALT levels. Our data indicate the occurrence of liver disease is more frequent when antibodies against HCV or CMV, or both, are present. Raised serum transaminases are more common in patients with antibodies against HBV plus HCV, or HBV plus CMV, than in the presence of HBV antibodies alone. The viral patterns at greater risk of developing liver disease are the association of HBV antibodies and HCV infection ($n = 136/362$), past exposure to HBV and evidence

of recurrence of CMV infection ($n = 97/362$), and association of all three infections ($n = 67/362$; see Table 1).

Individuals with either HBV or HCV remained in the same class of liver disease throughout the study (Table 4). Of the 199 patients with signs of HBV and HCV association who entered the study, 123 had mild liver disease. In 36 of these, deterioration was observed. In the group with chronic active liver disease ($n = 76/199$), deterioration was also present. At the end of the study, 13 patients with associated HBV and HCV infections had developed severe liver disease and cirrhosis.

Several studies have indicated the simultaneous presence of different viruses as the aetiology of more severe liver disease [1,2,4,7]. The difference in the present group is the absence of HBc-IgM. This finding is consistent with the absence of circulating HBV, whereas the HCV antibodies (with 96.8% circulating HCV-RNA) and CMV detectable IgM and IgG at high titres suggest the presence of HCV and re-infection/reactivation of CMV. The presence of HCV by itself does not explain why this category of patients (HBV plus HCV antibodies) has a more severe disease. The hepatic damage is also more severe than in the presence of HCV alone. A possible explanation could be that previous HBV infection has led to a certain degree of damage, and the new HCV infection simply causes progression of the disease [17,18]. This interpretation is not, however, completely convincing, as the number of patients with associated infections shifting towards a more advanced stage of liver disease is higher (Table 3) than in patients with a single infection. Alternatively, an initial insult to the liver, or alert to the immune system from HBV infection, may enhance the liver damage from a second infection.

There are several potential mechanisms. Recent studies have shown that a polyclonal cytotoxic T-lymphocyte response to HBV can persist for decades after recovery from acute infection due to the presence of low amounts of HBV [19]. A subsequent infection (as in HCV or CMV co-infection) [17] might reactivate the immune response against HBV.

We would also speculate that once HBV infection has regressed (or when there is at least a lack of detectable circulating HBV-DNA), an integrated HBV genome can still codify for proteins more or less expressed on the cell membrane with major histocompatibility antigens [20,21]. A new viral infection, which would increase the number of leucocytes in the area, may produce discontinuity in the sinusoidal wall, thus allowing contact with the hepatocytes and, in turn, development of chronic active disease. Another recent study reveals that a possible mechanism for more serious

disease in the HBV/HCV association could be halted by replication of HBV with ongoing expression of HBV products in the liver, keeping CD4 activated and/or inducing apoptosis [22].

The presence of a single CMV infection does not appear to be related to the development of chronic liver disease, as revealed by its higher prevalence in patients with normal ALT levels in this and other studies [18]. However, in combination with a hepato-tropic virus, CMV increases the possibility of liver damage, especially in immunocompromised patients, although the pathogenic mechanisms involved remain to be evaluated. CMV infection seems to produce histological lesions located predominantly in the lobule [8,23–25], similar to those reported for HCV. The presence of 'lobular hepatitis' and sinusoidal damage could increase the response to other pathogens, perhaps through nonspecific antigen recruitment of cells or activating antigen-specific immunity.

Again, recent studies have indicated that many hepato-toxic agents (e.g. alcohol, endotoxins) induce sinusoidal defenestration and capillarization and may, through various pathways, effect fibrogenesis and cirrhosis [26]. Histological studies have shown that hepatic sinusoidal infection by HIV can induce the release of virions at basal membrane level, allowing contact with parenchymal cells and cytolysis [27].

In conclusion, the present data confirm the development of more severe liver disease as a result of HBV/HCV co-infection and we hypothesize a complex 'co-operation', in which a past HBV infection, as revealed by HBV seroconversion, and other viruses, such as HIV and CMV, play specific roles.

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