Impact of occult hepatitis B virus infection in HIV patients naive for antiretroviral therapy

Pietro Filippini[a], Nicola Coppola[a], Raffaella Pisapia[a], Carlo Scolastico[a,*], Cecilia Marrocco[a], Antonella Zaccariello[a], Cesare Nacca[b], Caterina Sagnelli[a], Giulio De Stefano[c], Teresa Ferraro[d], Carlo De Stefano[e] and Evangelista Sagnelli[a,b]

Objective: To study the impact of occult hepatitis B virus (HBV) infection in 115 consecutive anti-HIV-positive, hepatitis B surface antigen-negative patients, naive for antiretroviral treatment.

Methods: Of these 115, 86 patients were followed for at least 6 months (range 6–36) with serial determinations of HIV RNA and HBV DNA by polymerase chain reaction and other laboratory tests.

Results: Of the 86 patients having a follow-up, plasma HBV DNA was detected in 17 (19.8%), 13 on admission and four during follow-up. HBV DNA was more frequently found in patients with isolated anti-hepatitis B core (HBc; 35.5% of 31 cases) than in those lacking anti-HBc and anti-hepatitis B surface (8.8% of 41, $P < 0.005$), or showing both (21.4% of 14). Twenty-eight patients (32.5%) experienced a hepatic flare during the follow-up; this event was more frequent in the 17 HBV-DNA-positive patients than in the 69 negative (64.7% versus 24.6%, $P < 0.005$). Of the 13 HBV-DNA-positive patients on admission, 11 receiving HAART containing lamivudine became HBV-DNA negative, but two of these again became positive and experienced a hepatic flare during treatment and two both during and after lamivudine treatment. A hepatic flare also occurred under lamivudine treatment in two of the four patients in whom HBV DNA became detectable during follow-up. The role of immune reconstitution inflammatory syndrome and HAART in inducing a hepatic flare was found to be marginal in 49 patients with no HBV or hepatitis C virus marker.

Conclusion: The study suggests that HBV occult infection, relatively frequent in anti-HIV-positive patients, is associated with hepatic flares.

AIDS 2006, **20**:1253–1260

Keywords: HAART, hepatic flare in HIV infection, HIV infection, lamivudine treatment, occult hepatitis B virus infection

Introduction

Sensitive polymerase chain reaction (PCR) assays detect hepatitis B virus (HBV) DNA in plasma and liver tissue of both hepatitis B surface antigen (HBsAg)-positive and HBsAg-negative individuals [1–3]. 'Occult' or 'silent' HBV infection are the terms currently used to identify the detection of HBV DNA in HBsAg-negative patients

From the [a]Department of Public Medicine, Section of Infectious Diseases, Second University of Naples, Naples, Italy, the [b]Division of Infectious Diseases, Azienda Ospedaliera San Sebastiano, Caserta, Italy, the [c]Division of Infectious Diseases, Azienda Ospedaliera Matera, Matera, Italy, the [d]Division of Infectious Diseases, Azienda Ospedaliera Catanzaro, Catanzaro, and the [e]Division of Infectious Diseases, Azienda Ospedaliera Potenza, Potenza, Italy.
*In memory of Carlo Scolastico who passed away in August 2005.
Correspondence to Evangelista Sagnelli, Department of Public Health, Section of Infectious Diseases, Second University of Naples, c/o Ospedale Gesù e Maria, Via D. Cotugno 1, 80135 Naples, Italy.
Tel: +39 081 5666271; fax: +39 081 5666206; E-mail: evangelista.sagnelli@unina2.it
Received: 20 November 2005; revised: 9 February 2006; accepted: 13 February 2006.

ISSN 0269-9370 © 2006 Lippincott Williams & Wilkins

[4–6]. Mason *et al*. [7] demonstrated that HBV replication may persist for years in the livers of HBsAg-negative patients after they have recovered from acute HBV infection, because they found both HBV transcription and an intact direct repeat region of HBV DNA in the livers of HBsAg-negative patients 3–67 months after acute hepatitis B infection.

Several studies have given evidence of the presence of low-level HBV replication in patients with chronic hepatitis C virus (HCV) with or without antibodies to the HBV antigens [1,2,4–6]. Patients with chronic HCV and occult HBV infection more frequently have a severe liver disease [8], lack of response to interferon treatment [9,10], and a higher risk of developing hepatocellular carcinoma [11,12] than those with chronic HCV alone.

Studies on the prevalence of occult HBV infection in anti-HIV-positive patients have been performed, but conflicting data are reported [13,14].

Several reports have described a reactivation of HBV replication in HBsAg/anti-HIV-negative individuals receiving prednisone or chemotherapy, followed by a hepatic flare after the discontinuation of treatment that is at times severe or even fatal [15–17]. It is very difficult in anti-HIV-positive patients with occult HBV infection to attribute a hepatic flare to changes in HBV replication as a result of the development of HBV mutants under lamivudine or tenofovir treatment or after the discontinuation of these drugs [18,19], because HAART [20–22] and immune reconstitution inflammatory syndrome (IRIS) [23,24] may both cause hepatic flares. The identification of the cause of a hepatic flare in these patients is, however, of clinical relevance because clinical and therapeutic decisions should be based on the pathogenesis of the flare.

Therefore, we studied occult HBV infection in a multicentre prospective study on 115 consecutive anti-HIV-positive, HBsAg-negative patients, naive for HIV treatment at the beginning of the observation. The patients were enrolled in five wards of infectious diseases in southern Italy and were followed for at least 6 months (median 18, range 6–36) to monitor for changes in HBV replication and hepatic flares.

Patients and methods

Five wards of infectious diseases in southern Italy, two in the Campania region (one in Naples and one in Caserta), two in Basilicata (Potenza and Matera), and one in Calabria (Catanzaro), participated in the study. These five centres have cooperated in several clinical investigations using the same laboratory methods and the same clinical approach. The study was planned as an observational prospective study by all the investigators during a preliminary consensus meeting.

Enrolment started in February 2001 and ended one year later; 115 consecutive anti-HIV-positive, HBsAg-negative patients naive for antiretroviral therapy were included.

Throughout the observation, the anti-HIV-positive patients (HIV subjects) were left untreated or received treatment according to the current international guidelines [25,26].

For all patients a no-end follow-up was planned. A total of 86 patients were observed monthly as outpatients in one of the five participating clinical centres for at least 6 months (median 18, range 6–36), whereas 29 were lost to follow-up because of lack of compliance. No significant difference was observed between the 86 patients having a follow-up and the 29 who were lost to follow-up with regard to demographic, clinical and laboratory characteristics observed on admission. Of the 86 patients with follow-up, 21 did not receive antiretroviral treatment, 38 received protease inhibitor-based antiretroviral therapy, 14 received non-nucleoside reverse transcriptase inhibitor-based treatment, 11 received both protease inhibitors and non-nucleoside reverse transcriptase inhibitors, and the remaining two received a three nucleoside reverse transcriptase inhibitor regimen. None of the 86 patients with a follow-up was treated with tenofovir or emtricitabine. No patient received chemotherapy during the observation period.

For each patient serum and plasma samples were obtained at the time of the first observation and then every 3 months during the follow-up. The samples were stored at $-80^{\circ}C$ and not thawed until used for this investigation. The patient's informed consent was always obtained.

The stage of HIV infection and related diseases was defined according to the Centers for Disease Control classification published in 1993 [27].

Patients were considered as having a 'hepatic flare' when they showed an increase in the serum alanine aminotransferase value of at least three times the previous value. Risk factors for a hepatic flare were recorded throughout the follow-up: the presence of plasma HBV DNA, alcohol intake, intravenous drug abuse, HAART (ritonavir or nevirapine, in particular), and lamivudine treatment or its discontinuation.

Patients were considered as having IRIS if the Shelburne criteria were fulfilled [23,24]: an antiretroviral therapy-induced increase in the CD4 cell counts or a decrease in the HIV-RNA plasma level in patients with AIDS showing symptoms consistent with an infectious/inflammatory condition appearing while on ART, which

may not be explained by an expected course of a previously recognized infection, or by the side-effects of treatment.

HBV viraemia was determined in all the HBsAg-negative plasma samples by a qualitative PCR, extensively described in previous papers [2,8,9]. Briefly, viral DNA was extracted from 200 μl of each plasma specimen using microspin columns (QIAamp blood kit, Qiagen GmbH, Hilden, Germany) and then amplified using primers located in the core region of HBV (upstream primer 5′–TTGCCTTCTGACTTCTTTCC–3′, nucleotides 1955–1974, and downstream primer 5′–TCTGCG AGGCGAGGGAGTTCT–3′, nucleotides 2401-2381). PCR was performed in a programmable thermal cycler (Perkin Elmer 9700; Perkin Elmer, USA) with the following three-step cycling profile: 93° for 30 s, 48° for 30 s, 68° for 60 s, for a total of 35 cycles. The amplified product was detected by hybridization with a specific probe in the core region (GEN-ETI-K HBV core; DiaSorin Biomedica Saluggia, VC, Italy). Sample preparation and DNA extraction were carried out in a different room from the one in which the amplified samples were handled, and a filter pipette was used for all steps. Positive (plasma sample of HBeAg-positive patients) and negative (DNA/RNA-free water) were included in all runs of extraction and amplification; moreover, a negative control was included after every 10 samples to minimize possible contamination. To evaluate the sensitivity of our PCR, serial dilutions of a control specimen with a known HBV viral load (100 000 copies/ml) were made until a dilution of 10 copies/ml was obtained. The lowest limit of detection by this method is 100 copies/ml.

In HBV-DNA-positive plasma samples the viral load was determined by quantitative PCR using the HBV Amplicor Monitor (Roche Molecular Systems, Branchburg, New Jersey, USA); the lowest limit of HBV-DNA detection of this method is 600 copies/ml.

HBsAg-negative subjects were considered as carrying occult HBV infection if HBV DNA was detected in the plasma first by the qualitative PCR assay and then by the quantitative PCR; in the patients who were HBV-DNA positive by the qualitative PCR and negative by the less sensitive quantitative assay, a qualitative PCR was repeated and the previous positive results were always confirmed.

In plasma samples obtained from patients who became HBV-DNA positive at the time a hepatic flare occurred under lamivudine treatment, mutations in the HBV-DNA polymerase gene (YMDD motif) were determined by PCR and direct sequencing using forward primer 5′–GCCCGTTTGTCCTCTACT–3′ (nucleotides 466–483) and reverse primer CAAAACAAAAAGATGGG GATA (nucleotides 843–863) (accession no. AB205190) [28]. Briefly, PCR products were purified using a QIA quick kit (Qiagen) and sequenced using an autosequencer (CEQ 8000 autosequencer; Beckman Coulter, Fullerton, California, USA).

Antibodies to HIV-1 and HIV-2 were determined by commercial enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, Illinois, USA and DiaSorin Biomedica), and the reactivity of samples was confirmed by a Western blot analysis (Genelabs Diagnostics, Science Park Drive, Singapore), which identifies both HIV-1 and HIV-2 strains.

HBV serum markers were detected using commercial immunoenzymatic assays [Abbott Laboratories for HBsAg, anti-hepatitis B surface (HBs) and anti-hepatitis B core (HBc), and DiaSorin Biomedica for hepatitis B e antigen and anti-hepatitis B e). The anti-HCV antibodies were determined using a third-generation commercial immunoenzymatic assay (Ortho Diagnostic Systems, Neckargemund, Germany). Antibodies to the hepatitis A virus (total and IgM) were determined using a commercial immunoenzymatic assay (Abbott Laboratories).

The lymphocyte subsets (CD4, CD8) were assessed by flow cytofluorimetry using monoclonal antibodies and fluorescence-activated cell sorter scan (Becton Dickinson, Mountain View, USA). Liver function tests were performed by routine methods every month.

The mean values were compared using the Student's *t*-test, the differences in the proportions were assessed by the χ^2 test with the Yates correction or by Fisher's exact test. A P value less than 0.05 was considered to be statistically significant.

Results

Of the 115 anti-HIV-positive subjects, 57 were anti-HBs negative/anti-HBc negative (group A), 16 were anti-HBs positive/anti-HBc positive (group B), and 42 were anti-HBs negative/anti-HBc positive (group C). Table 1 shows the demographic, clinical and laboratory data recorded on admission for the 115 patients, according to the presence or absence of antibodies to HBV. No significant difference was observed in these three groups of patients with regard to age, sex, presumed median duration of HIV infection, risk factors for the acquisition of both HIV and HBV infections, CD4 cell count, alanine aminotransferase and clinical stage of HIV infection (Table 1). Patients in group A (28.1% of 57 cases) less frequently than those in group C (50% of 42, $P < 0.05$) or B (43.7% of 16) were anti-HCV positive.

Plasma HBV DNA was detected in 18 patients at the time of the first observation and in another four during the

Table 1. Demographic, clinical and laboratory characteristics of 115 anti-HIV-positive/hepatitis B surface antigen-negative patients, according to the presence or absence of anti-hepatitis B surface or anti-hepatitis B core.

	Group A Anti-HBs neg/Anti-HBc neg	Group B Anti-HBs pos/Anti-HBc pos	Group C Anti-HBs neg/Anti-HBc pos
No. of cases	57	16	42
% men	63.2	62.5	69.0
Median age (range), years	34.5 (20–63)	34 (23–52)	38 (25–64)
% with risk factors for acquiring HIV or HBV infection			
IVDU	35.1	43.7	35.7
Male homosexuality	15.8	18.7	9.5
Heterosexuality[a]	36.8	18.7	26.2
Heterosexuality with HIV[a]	8.8	18.7	16.7
Blood transfusion	0	0	2.4
IVDU + male homosexuality	0	0	4.8
Unknown	3.5	0	4.8
Duration of HIV infection, years (mean ± SD)	4.9 ± 3.4	6.0 ± 4.5	6.6 ± 3.7
CD4 cell count, mean ± SD	365 ± 256	380 ± 237	333 ± 278
% of patients in the different CDC groups:			
A	66.7	73.3	53.7
B	11.2	20.0	26.9
C	22.3	6.6	19.5
% of anti-HCV-positive patients	28.1	43.7	50.0
% of patients:			
HBeAg positive	0	0	0
Anti-HBe positive	0	68.7	64.2
HBeAg/anti-HBe negative	100	31.3	35.8

CDC, Centers for Disease Control; HBV, hepatitis B virus; HBc, hepatitis B core; HBeAg, hepatitis B e antigen; HBs, hepatitis B surface; HCV, hepatitis C virus; IVDU, intravenous drug use.
[a]Heterosexuality = unsafe heterosexual exposure.

follow-up. Of these 22 HBV-DNA-positive patients by the qualitative assay, 10 had an undetectable viral load and 12 a low viral load (from 7.4×10^2 to 3.5×10^3 copies/ml).

Considering the 86 patients in the follow-up study, 17 were found to be HBV-DNA positive, 13 on admission and four in the subsequent observation: these HBV-DNA-positive patients were less frequent (three of 41, 8.8%) in the anti-HBs-negative/anti-HBc-negative group (group A[1]) than in the anti-HBs-positive/anti-HBc-positive group (group B[1]; three of 14, 21.4%) and in the anti-HBs-negative/anti-HBc-positive group (group C[1]; 11 of 31, 35.5%, $P < 0.005$).

The CD4 cell count (mean ± SD) was 380 ± 270 in HBV-DNA-positive patients and 290 ± 212 in HBV-DNA-negative subjects, a difference that was not significant to the statistical analysis. Moreover, HBV DNA was detected in 4.5% of the 22 patients with a CD4 cell count greater than 500 cells/μl and in 25% of the 64 subjects with a CD4 cell count lower than 500 cells/μl; this difference was also not statistically significant.

Of the 86 patients with a follow-up, 28 (32.5%) experienced a hepatic flare (Table 2); this occurred more frequently in the 17 patients with occult HBV infection than in the 69 HBV-DNA-negative patients (64.7 versus 24.6%; $P < 0.005$). No correlation was found between the HBV viral load and the occurrence of a hepatic flare in patients with a detectable HBV viral load.

In most patients with a flare more than one risk factor for its occurrence was recorded (Table 2); a multivariate analysis, however, was not performed because of the numerous risk factors and the relatively low number of patients. Of the 28 patients experiencing a hepatic flare during the follow-up, 18 were anti-HCV positive and 10 were anti-HCV negative; none of these 10 developed acute HCV during the observation.

Of the 13 patients who were HBV-DNA positive on admission, two did not receive HAART; one of these two experienced a hepatic flare after a 12-month observation (Fig. 1a). The other 11 patients were treated with HAART containing lamivudine and were HBV-DNA negative at the subsequent check points. Of these 11, four (Fig. 1b–e) became HBV-DNA positive under lamivudine treatment and experienced a hepatic flare; two of these four also experienced a hepatic flare after the discontinuation of the drug (Fig. 1d, e). Another two of these 11 patients (Fig. 1f, g) became HBV-DNA positive and experienced a hepatic flare, respectively, 4 and 8 months after lamivudine was discontinued because of lack of efficacy of the HAART regimen. The remaining five patients of the 11 treated remained HBV-DNA negative under HAART containing lamivudine and did not experience a hepatic flare.

Table 2. Risk factors for hepatic flare during HAART in 28 patients with a hepatic flare during follow-up.

Patient no.	HBV DNA positive	HF during lamivudine treatment	HF after the discontinuation of lamivudine	Treated with ritonavir or nevirapine or PI	IVDU or alcohol intake
1	Yes	No 3TC tx	No	Untreated	Yes
2	Yes	Yes	No	No	Yes
3	Yes	Yes	No	PI	Yes
4	Yes	Yes	Yes	PI	Yes
5	Yes	Yes	Yes	RTV, NVP	Yes
6	Yes	No	Yes	NVP	No
7	Yes	No	Yes	PI	No
8	Yes	Yes	No	No	Yes
9	Yes	Yes	No	PI	Yes
10	Yes	No 3TC tx	No	NVP, RTV, PI	No
11	Yes	No 3TC tx	No	No	Yes
12	No	Yes	No	PI	Yes
13	No	No	No	PI	No
14	No	No	No	PI, RTV	No
15	No	Yes	No	PI	No
16	No	Yes	No	PI	Yes
17	No	No 3TC tx	No	PI, RTV	No
18	No	Yes	No	NVP	Yes
19	No	Yes	No	PI, RTV	No
20	No	Yes	No	NVP	No
21	No	Yes	No	PI	Yes
22	No	Yes	No	PI	Yes
23	No	Yes	No	PI	No
24	No	Yes	No	PI	No
25	No	No 3TC tx	No	PI	No
26	No	No	No	Untreated	Yes
27	No	Yes	No	NVP	Yes
28	No	No	No	Untreated	No

HF, Hepatic flare; IVDU, intravenous drug use; No 3TC tx, no lamivudine treatment; NVP, nevirapine; PI, protease inhibitor; RTV, ritonavir.

Of the four patients who were HBV-DNA negative on admission and positive during the observation, two (Fig. 1h, i) had been receiving HAART including lamivudine for 12 and 16 months, respectively, when they became HBV-DNA positive and experienced a hepatic flare; the remaining two patients (Fig. 1j, k) were never treated with lamivudine, became HBV-DNA positive and experienced a hepatic flare after 18 and 9 months of observation, respectively.

All patients who became HBV-DNA positive by the qualitative assay during the follow-up showed an undetectable or very low HBV viral load (6.8×10^2 to 1.1×10^3 copies/ml). The YMDD mutant was never observed in the six patients who became HBV-DNA positive during lamivudine treatment; four of these six, however, showed an undetectable HBV viral load and two a very low viral load (6.8×10^2 and 8.1×10^2 copies/ml).

The role of HAART and IRIS in the development of flares was analysed in the 49 patients lacking the confounding effects of the hepatitis viruses (anti-HCV negative and HBsAg/HBV-DNA negative). A hepatic flare was found in none of the 13 patients who remained untreated during the follow-up, in 26.3% of the 19 receiving HAART and showing signs of IRIS, and in 11.8% of the 17 patients receiving HAART with no evidence of IRIS.

Discussion

Contrasting data are reported in the literature on the prevalence of occult HBV infection in anti-HIV-positive individuals: Nunez *et al.* [13] 0%, Neau *et al.* [29] 0.6%, Shire *et al.* [30] 10.5% and Hofer *et al.* [14] 89.5%. Differences in the patient selection (i.e. age, sex, risk factor for the acquisition of HBV infection, degree of immunodepression), in the percentage of patients receiving HAART containing lamivudine or tenofovir, and differences in the sensitivity of the PCR assays used might account for these discrepancies.

Using a sensitive PCR assay we found an occult HBV infection in approximately 20% of anti-HIV-positive/HBsAg-negative consecutive patients, naive for antiretroviral therapy on admission. Worthy of mention is the observation that four patients in this study were HBV-DNA negative on admission and HBV-DNA positive during the follow up, suggesting that fluctuations in HBV replication may occur, and that serial determinations of HBV DNA are required to identify occult HBV infection.

The presence of HBV antibodies in HBsAg-negative subjects is generally used in epidemiological studies as a marker of a previous HBV infection. However, the growing knowledge on the immune response to HBV

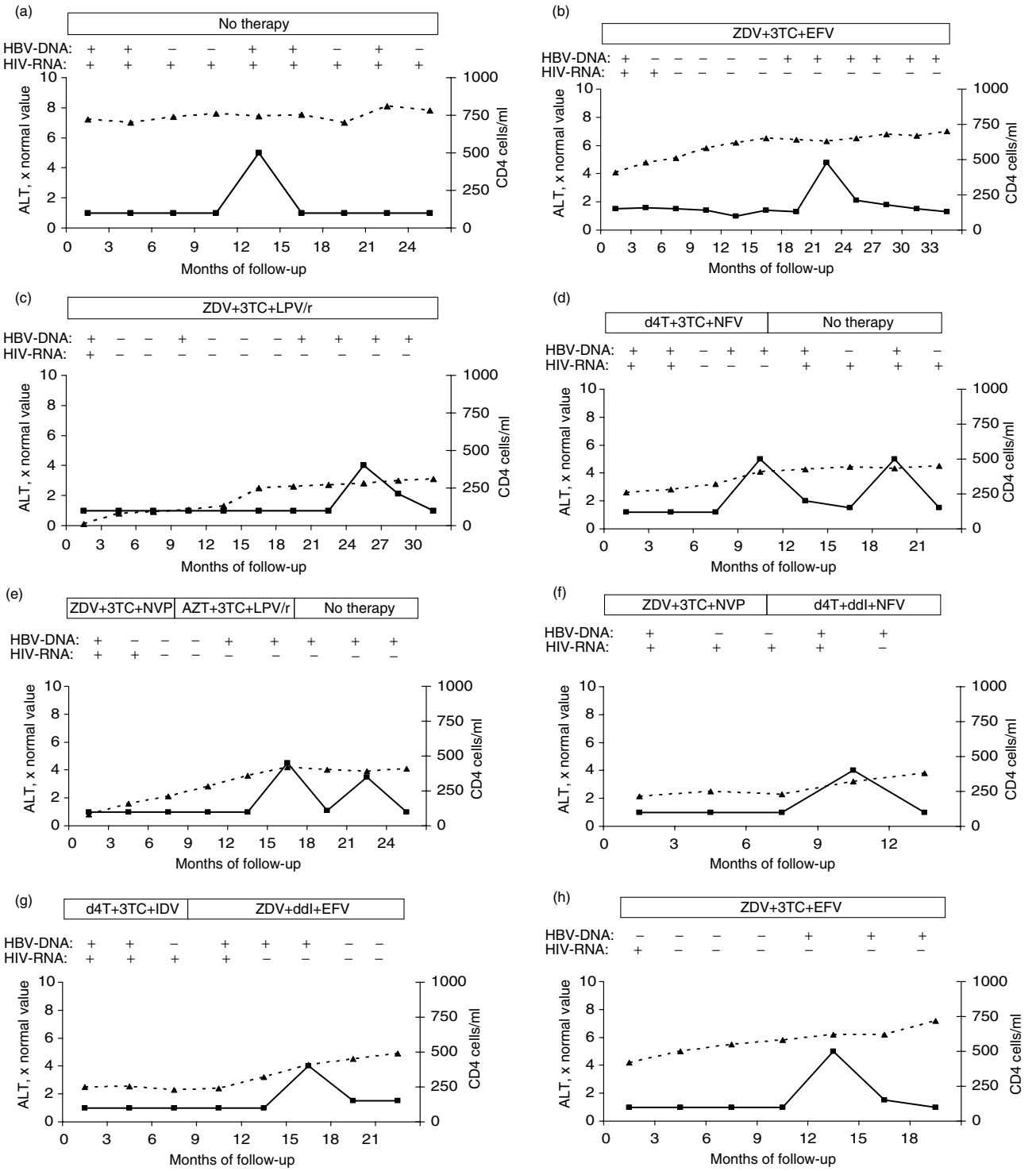

Fig. 1. Follow-up of the 11 patients with occult hepatitis B virus infection and occurrence of hepatic flare. —■— ALT levels; - - ▲ - - CD4 values. (a) Patient 1, male, 64 years, anti-hepatitis C virus (HCV) positive, history of alcohol intake; (b) Patient 2, male, 40 years, anti-HCV positive, history of intravenous drug use (IVDU); (c) Patient 3, male, 38 years, anti-HCV positive, history of IVDU; (d) Patient 4, female, 47 years, anti-HCV positive, history of IVDU; (e) Patient 5, male, 31 years, anti-HCV negative, history of alcohol intake; (f) Patient 6, male, 52 years, anti-HCV negative; (g) Patient 7, female, 45 years, anti-HCV negative; (h) Patient 8, male, 48 years, anti-HCV negative, history of alcohol intake; (i) Patient 9, male, 49 years, anti-HCV positive, history of IVDU; (j) Patient 10, male, 33 years, anti-HCV negative; (k) Patient 11, male, 44 years, anti-HCV positive, history of IVDU. ABC, Abacavir; ALT, alanine aminotransferase; ddI, didanosine; d4T, stavudine; EFV, efavirenz; HBV, hepatitis B virus; IDV, indinavir; LPV/r, lopinavir/ritonavir; NFV, nelfinavir; NVP, nevirapine; 3TC, lamivudine; ZDV, zidovudine.

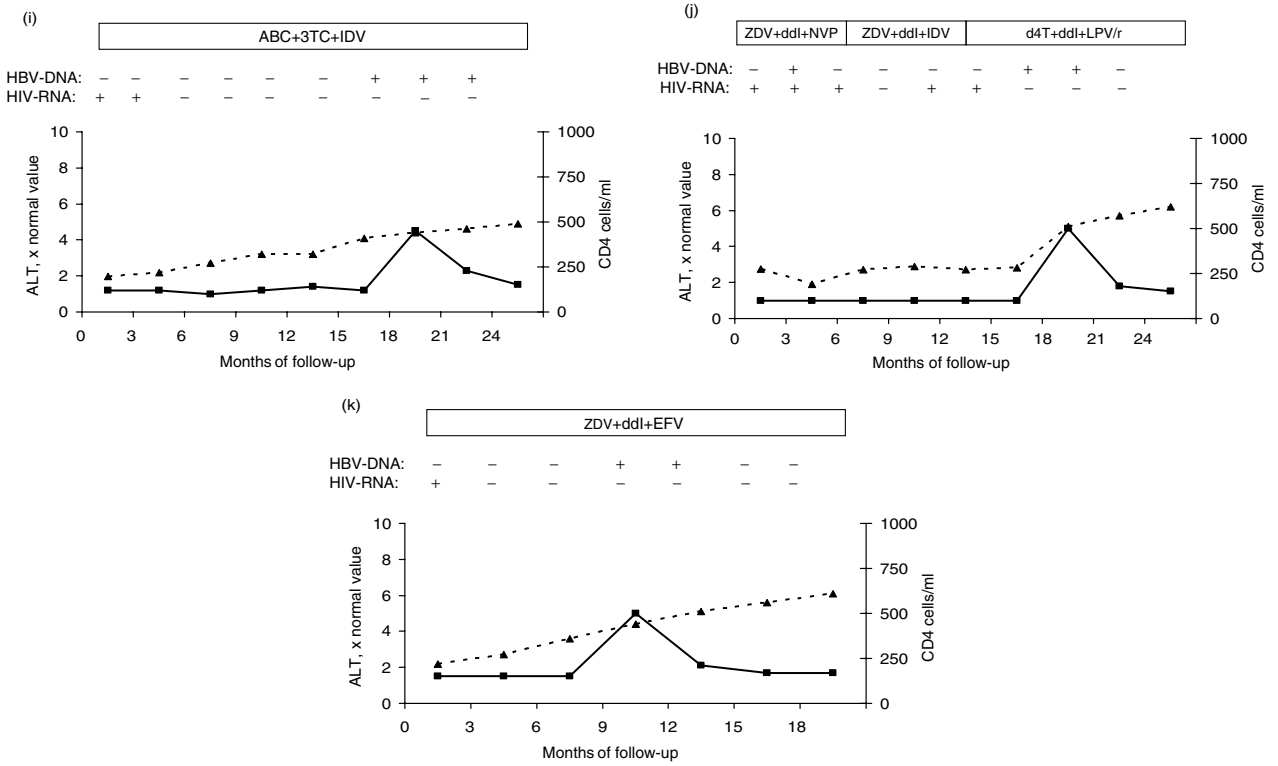

Fig. 1. (*Continued*).

seems to indicate that these antibodies are involved in the immune regulation exerted by the host on HBV replication [31]. Consistent with this are several studies on anti-HIV-negative/HCV-RNA-positive cases showing an association between occult HBV infection and the presence of the HBV antibody [1,2,4,32]. The present study, performed on anti-HIV-positive subjects, shows that approximately one third of cases with antibodies to HBV had detectable plasma HBV DNA, whereas the circulating HBV genome was rarely found in anti-HBs/anti-HBc-negative individuals, most of whom had possibly never become infected with HBV.

Lamivudine-based HAART is effective in suppressing HBV replication even in anti-HIV-positive patients with occult HBV infection, because most cases with occult HBV infection cleared HBV DNA during treatment. However, in approximately half of the lamivudine-treated patients occult HBV replication became detectable again after 12–40 months of lamivudine treatment, always associated with a hepatic flare. Although YMDD mutants were not detected in patients who became HBV-DNA positive during lamivudine treatment, the question of whether lamivudine may induce the selection of YMDD mutants also in occult HBV infection in anti-HIV-positive subjects still remains unanswered, because the HBV-DNA plasma levels in these cases were too low to allow detection. Similar to what is observed in HBsAg-positive subjects, both anti-HIV positive and negative [33–35], the discontinuation of lamivudine in HIV patients with occult HBV infection may be followed by reconversion to HBV-DNA positivity associated with a hepatic flare.

After the introduction of HAART, drug-induced hepatotoxicity has emerged as an important complication of antiretroviral treatment [20–22], the most frequent mechanisms of liver damage being a direct cytopathic effect of the drugs or mitochondrial toxicity and steatosis, or IRIS [23,24]. However, this study of hepatic flares in anti-HIV-positive subjects with no HBV or HCV serum marker shows that hepatic flares may be ascribed to the toxicity of HAART or to IRIS only in a small percentage of patients. Instead, a hepatic flare was found three times more frequently in patients with occult HBV infection than in those without.

In conclusion, HBV occult infection seems to be relatively frequent in anti-HIV-positive patients and may induce hepatic flares, even under lamivudine treatment or when this drug is discontinued.

Sponsorship: This study was performed with the support of Abbott. We thank Dr. Umberto Di Luzio Paparatti (Medical Director of Abbott Italy) for the invaluable support.

References

1. Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G. **Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease.** *N Engl J Med* 1999; **341**:22–26.

2. Sagnelli E, Coppola N, Scolastico C, Filippini P, Santantonio T, Stroffolini T, Piccinino F. **Virologic and clinical expressions of reciprocal inhibitory effect of hepatitis B, C, and delta viruses in patients with chronic hepatitis.** *Hepatology* 2000; **32**:1106–1110.
3. Koike K, Koboyashi M, Gondo M, Hayashi I, Osuga T, Takada S. **Hepatitis B virus DNA is frequently found in liver biopsy samples from hepatitis C virus-infected chronic hepatitis patients.** *J Med Virol* 1998; **54**:249–255.
4. Torbenson M, Thomas DL. **Occult hepatitis B.** *Lancet Infect Dis* 2002; **2**:479–486.
5. Raimondo G, Balsano C, Craxì A, Farinati F, Levrero M, Mondelli M, *et al*. **Occult hepatitis B virus infection.** *Digest Liver Dis* 2000; **32**:822–826.
6. Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. **Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely occult?** *Hepatology* 2001; **34**:194–203.
7. Mason AL, Xu L, Guo L, Kuhns M, Perrillo RP. **Molecular basis for persistent hepatitis B virus infection in the liver after clearance of serum hepatitis B surface antigen.** *Hepatology* 1998; **27**:1736–1742.
8. Sagnelli E, Coppola N, Scolastico C, Mogavero AR, Filippini P, Piccinino F. **HCV genotype and silent HBV coinfection: two main risk factors for a more severe liver disease.** *J Med Virol* 2001; **64**:350–355.
9. Sagnelli E, Coppola N, Scolastico C, Mogavero AR, Stanzione M, Filippini P, *et al*. **Isolated anti-HBc in chronic hepatitis C predicts a poor response to interferon treatment.** *J Med Virol* 2001; **65**:681–687.
10. Zignego AL, Fontana R, Puliti S, Barbagli S, Monti M, Careccia G, *et al*. **Relevance of inapparent coinfection by hepatitis B virus in alpha interferon-treated patients with hepatitis C virus chronic hepatitis.** *J Med Virol* 1997; **51**:313–318.
11. Urashima T, Saigo K, Kobayashi S, Imaseki H, Matsubara H, Koide Y, *et al*. **Identification of hepatitis B virus integration in hepatitis C virus-infected hepatocellular carcinoma tissues.** *J Hepatol* 1997; **26**:771–778.
12. Pollicino T, Squadrito G, Cerenzia G, Cacciola I, Raffa G, Craxì A, *et al*. **Hepatitis B virus mantains its prooncogenic properties in the case of occult HBV infection.** *Gastroenterology* 2004; **126**:102–110.
13. Nunez M, Rios P, Perez Olmeda M, Soriano V. **Lack of occult hepatitis B virus infection in HIV-infected patients.** *AIDS* 2002; **16**:2099–2101.
14. Hofer M, Joller Jemelka HI, Grob PJ, Luthy R, Opravil M. **Frequent chronic hepatitis B virus infection in HIV-infected patients positive for antibody to hepatitis B core antigen only. Swiss HIV Cohort Study.** *Eur J Clin Microbiol Infect Dis* 1998; **17**:6–13.
15. Lok A, Liang R, Chiu E, Wong K, Chan T, Todd D. **Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy. Report of a prospective study.** *Gastroenterology* 1991; **100**:182–188.
16. Roche B, Samuel D, Gigou M, Feray C, Virot V, Schmets L, *et al*. **De novo and apparent de novo hepatitis B virus infection after liver transplantation.** *J Hepatol* 1997; **26**:517–526.
17. Carpenter PA, Huang ML, Mc Donald GB. **Activation of occult hepatitis B from a seronegative patient after hematopoietic cell transplant: a cautionary tale.** *Blood* 2002; **99**:4245–4246.
18. Manegold C, Hannoun C, Wywiol A, Dietrich M, Polywka S, Chiwakata CB, Gunther S. **Reactivation of hepatitis B virus replication accompanied by acute hepatitis in patients receiving highly active antiretroviral therapy.** *Clin Infect Dis* 2001; **32**:144–148.
19. Altfeld M, Rockstroh JK, Addo M, Kupfer B, Pult I, Will H. **Reactivation of hepatitis B in a long term anti-HBs positive patient with AIDS following lamivudine withdrawal.** *J Hepatol* 1998; **29**:306–309.
20. Rodriguez-Rosado R, Garcia-Samaniego J, Soriano V. **Hepatotoxicity after introduction of highly active antiretroviral therapy.** *AIDS* 1998; **12**:1256.
21. Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. **Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection.** *JAMA* 2000; **283**:74–80.
22. Nunez M, Lana R, Mendoza JL, Martin-Carbonero L, Soriano V. **Risk factors for severe hepatic injury after introduction of highly active antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2001; **27**:426–431.
23. Shelburne SA, Hamill RJ, Rodriguez-Barradas MC, Greenberg SB, Atmar RL, Musher DM, *et al*. **Immune reconstitution inflammatory syndrome: emergence of a unique syndrome during highly active antiretroviral therapy.** *Medicine* 2002; **81**:213–227.
24. Shelburne SA, Visnegarwala F, Darcourt J, Graviss EA, Giordano TP, Clinton White A Jr, Hamill RJ. **Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy.** *AIDS* 2005; **19**:399–406.
25. Carpenter CC, Cooper DA, Fischl MA, Gatell JM, Gazzard BG, Hammer SM, *et al*. **Antiretroviral therapy in adults: updated recommendations of the International AIDS Society – USA Panel.** *JAMA* 2000; **28**:381–390.
26. Yeni PG, Hammer SM, Carpenter CC, Cooper DA, Fischl MA, Gatell JM, *et al*. **Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society – USA Panel.** *JAMA* 2002; **228**:222–235.
27. Centers for Disease Control. **Revised HIV classification system, AIDS definition**. *WV Med J* 1993; **89**:74.
28. Stuyver L, Locarnini SA, Lok A, Richman DD, Carman WF, Dienstag JL, Schinazi RF, and the HEP DART International Committee. **Nomenclature of antiviral-resistance human hepatitis B virus mutations in the polymerase region.** *Hepatology* 2001; **33**:751–757.
29. Neau D, Winnock M, Jouvencel AC, Faure M, Castera L, Legrand E, *et al*. **Occult hepatitis B virus infection in HIV-infected patients with isolated antibodies to hepatitis B core antigen: Aquitaine cohort, 2002–2003.** *Clin Infect Dis* 2005; **40**:750–753.
30. Shire NJ, Rouster S, Rajicic N, Sherman KE. **Occult hepatitis B in HIV infected patients.** *J Acquir Immune Defic Syndr* 2004; **36**:869–875.
31. Chisari FV, Ferrari C. **Hepatitis B virus immunopathogenesis.** *Annu Rev Immunol* 1995; **13**:29–60.
32. Marusawa H, Uemoto S, Hijikata M, Ueda Y, Tanaka K, Shismotohno K, Chiba T. **Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen.** *Hepatology* 2000; **31**:488–495.
33. Honkoop P, de Man RA, Niesters HG, Zondervan PE, Schalm SW. **Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy.** *Hepatology* 2000; **32**:635–639.
34. Barlow GD, France AJ. **Exacerbation of hepatitis B infection following cessation of lamivudine therapy.** *Int J STD AIDS* 2000; **11**:684.
35. Sellier P, Clevenbergh P, Mazeron MC, Cazals-Hatem D, Evans J, Cervoni J, *et al*. **Fatal interruption of a 3TC-containing regimen in a HIV-infected patient due to re-activation of chronic hepatitis B virus infection.** *Scand J Infect Dis* 2004; **36**:533–535.