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Predictors of hepatitis B virus genotype and viraemia in HIV-infected patients with chronic hepatitis B in Europe

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Background: Both natural history and treatment outcome of hepatitis B virus (HBV) infection are influenced by genotypes and viral load. Information about factors determining HBV genotype distribution and viraemia in HIV/HBV-co-infected patients is scarce.

Methods: All HIV-positive patients living in Europe and Argentina recruited in EuroSIDA (1994–2006) were tested for serum HBV surface antigen (HBsAg). Chronic carriers were further characterized virologically at one central laboratory. Variables influencing HBV genotype distribution and viraemia were assessed using logistic regression.

Results: From 16505 HIV patients enrolled in EuroSIDA, 1179 (7.1%) were HBsAg positive, of whom 474 had specimens that allowed inclusion in the virological substudy. Overall 293 (62%) were treated with anti-HBV active antiretroviral drugs at the time of testing. Hepatitis delta virus superinfection was recognized in 14% and hepatitis C virus (HCV) antibodies in 27%. Serum HBV DNA was detectable in 315 (66.5%) and HBV genotyping gave results in 170 (35.9%) patients. HBV genotype distribution was as follows: A (72.9%), D (17.1%), G (1.8%), E (1.2%), F (1.2%) and C (0.6%); another 5.9% were co-infected with multiple HBV genotypes. In the multivariate analysis, the best predictor of HBV genotype A infection was risk exposure other than intravenous drug use, whereas predictors for detectable HBV viraemia were lower CD4 counts and lack of HCV antibodies.

Conclusion: A substantial proportion of HIV-positive patients with chronic hepatitis B show detectable HBV viraemia despite being treated with anti-HBV active antiretroviral drugs (mainly lamivudine). Low CD4 counts were associated with an independent higher risk of detectable HBV viraemia, which supports an earlier introduction of antiretroviral therapy, including anti-HBV drug(s) more potent than lamivudine.

Keywords: epidemiology, HBV genotypes, HBV viraemia

Introduction

Chronic hepatitis B virus (HBV) infection currently affects 5%–10% of individuals with HIV infection in developed countries, despite HBV vaccination being widely available and successful for preventing HBV infection.[1,2] Co-infection with HIV alters the natural history of chronic hepatitis B, with higher serum HBV DNA concentrations, lower liver enzyme levels and faster progression to liver cirrhosis, particularly in those with low CD4 counts.[2–5] Liver-related mortality has now become one of the leading causes of non-AIDS deaths among HIV-positive persons in developed regions, where antiretroviral therapy is widely used,[6] and chronic hepatitis B and/or C are the major contributors.

Only recently it has become apparent that serum HBV DNA level is one, if not the major, determinant of the speed of liver disease progression in patients with chronic hepatitis B.[7–9] On the basis of this observation, most current guidelines recommend considering HBV therapy in patients with serum HBV DNA > 2000 IU/mL.[10,11] Moreover, different HBV genotypes may

show distinct behaviour in terms of natural history[12,13] and response to therapy.[14,15] Information about HBV genotype distribution and viraemia in HIV/HBV-co-infected patients is scarce.[15] This knowledge is important since several new antiviral agents active against HBV have recently been approved, some of which are dually active against HBV and HIV.[2,15] The aim of this study was to describe the virological characteristics of HIV-infected patients with chronic hepatitis B in the EuroSIDA cohort.

Patients and methods

Study population

EuroSIDA is a prospective study of 16505 HIV-1-infected patients at 93 centres across Europe, Israel and Argentina; further details have been reported elsewhere.[16] Briefly, for each cohort the centres provide data on consecutive patients seen at the outpatient clinics beginning in May 1994 until a pre-defined number of patients enrol from each site. To date, eight cohorts of patients have been recruited. Data are collected prospectively at clinical sites and are extracted and sent to the coordinating centre at 6 monthly intervals. For cohorts I–III, eligible patients were those who had had a CD4 count <500 cells/mm^3 during the previous 4 months. The CD4 count restriction was removed for cohorts IV–VII. At recruitment, in addition to demographic and clinical information, a complete antiretroviral treatment history is obtained, together with the most recent CD4 count and plasma HIV RNA measurements. At each follow-up visit, details on all CD4 counts and plasma HIV RNA values measured since the last follow-up visit are extracted, as are the dates of starting and stopping each antiretroviral drug received and the use of drugs for prophylaxis against opportunistic infections. The dates of diagnosis of all AIDS-defining illnesses, non-AIDS-defining malignancies and other serious infections are also recorded. The present analysis includes follow-up to a median date of January 2008.

Information on serum HBV surface antigen (HBsAg) status in EuroSIDA has been collected since 1997; patients who died or were lost to follow-up before this date did not routinely have information on HBsAg collected. Centres which have determined HBsAg genotype or measured HBV DNA after 1997 report test results to the coordinating centre via the data collection form details at www.cphiv.dk. The EuroSIDA plasma repository was set up in 1997 and collects plasma samples from all HIV patients at 6 monthly intervals. Patients with unknown HBsAg status and with a stored plasma sample were identified in 2006 and HBsAg status determined. Patients who tested HBsAg postive were then tested for serum HBV DNA and genotyped in a central laboratory. In addition, patients with unknown HBV genotype, but reported to be HBsAg positive by the centres who had stored samples, were identified and tested for serum HBV DNA and genotyped. All patients gave written informed consent for participation in the study and approved testing of clinical samples. Further information is available at www.cphiv.dk.

Quantification of HBV viraemia and genotyping

Serum HBV DNA was measured in all HBsAg-positive samples using a bDNA assay (Versant HBV v3.0, Siemens, Berkeley, CA, USA), which is a signal amplification procedure with a linear dynamic range from 357 to 18×10^6 IU/mL.[17] HBV genotyping was performed using the LiPA HBV genotype v2.0 assay (Innogenetics, Ghent, Belgium), a commercial hybridization line probe assay that detects specific sequences in the S region of the HBV genome.[18]

Statistical analysis

Characteristics of patients were compared using χ^2 tests for categorical variables and non-parametric Wilcoxon or Kruskall–Wallis tests for continuous variables. Patients were categorized into three groups: HBV genotype A, non-A genotypes and patients without a recorded genotype. Logistic regression, using forward selection with entry criteria of $P<0.1$, was used to identify which factors were associated with genotype A versus non-genotype A (excluding patients for whom a genotype could not be determined), and for comparisons between subjects with undetectable serum HBV DNA and viraemic patients. Baseline was defined as the date of the serum sample. All data were analysed using SAS version 9.1 (Statistical Analysis Software, Cary, NC, USA).

Results

Of 16505 HIV patients enrolled in EuroSIDA, 1179 (7.1%) tested HBsAg positive. Of these, 477 patients had stored serum samples available (as described above) but three of these patients did not have sufficient material for virological analyses and were therefore excluded. HBsAg-positive patients excluded from this analysis were less likely to have been infected via homosexual exposure ($P=0.0038$), were less likely to be from Central or Northern Europe ($P<0.001$), were slightly younger ($P=0.012$) and were recruited into EuroSIDA more recently ($P<0.0001$).

The main characteristics of the 474 HIV/HBV study population are depicted in Table 1. Most individuals were male (84%), Caucasian (85%), and men who have sex with men (MSM) (51%). Median age was 38 years. Median CD4 count was 295 cells/mm^3. Overall, 91% were or had been exposed previously to antiretroviral therapy, of whom 293 (62%) included at least one anti-HBV active agent at the time of testing. Hepatitis delta virus (HDV) superinfection was recognized in 14% and hepatitis C virus (HCV) antibodies in 27% of patients.

Serum HBV DNA was detectable in 315 (66.5%) out of 474 HBsAg-positive patients. HBV genotype could be obtained for 170 (54.0%) HBV viraemic patients. Lack of HBV genotype results in the remaining HBsAg-positive specimens was mainly due to too low serum HBV DNA concentrations. HBV genotype distribution was as follows: A (124; 72.9%), D (29; 17.1%), G (3; 1.8%), E (2; 1.2%), F (2; 1.2%) and C (1; 0.6%); another 5.9% were co-infected with multiple HBV genotypes (5 A/D, 3 A/G, 1 A/D/G).

There were significant differences in the regional distribution of HBV genotypes ($P=0.0031$). As shown in Figure 1, HBV genotype A was the predominant variant in all regions except South Europe/Argentina, where non-A genotypes (mainly genotype D) were similarly prevalent. In Northern Europe, HBV genotype A was by far the most frequent HBV variant.

Of 124 patients infected with HBV genotype A, only 3 (2.4%) were female, compared with ~20% of those with non-A genotypes or unknown genotypes ($P<0.0001$). Similarly, only 12% of patients with HBV genotype A were infected through intravenous drug use compared with 54% of those with non-A genotypes and 26% of those with unknown HBV genotypes ($P<0.0001$). Patients with non-A genotypes were more likely to be co-infected with HCV (50%), compared with patients with unknown HBV genotypes (31%) or genotype A (10.5%) ($P<0.0001$). In addition, patients with unknown HBV genotypes had significantly higher CD4 counts at baseline (326 cells/mm^3)

Table 1. Main baseline characteristics of the HIV/HBV-co-infected study population

	n (%) or median (IQR)			
	All patients *n*=474 (100%)	Genotype A *n*=124 (23.4%)	Genotype non-A *n*=46 (8.7%)	*P* value
Gender				
male	400 (84.4)	121 (97.6)	37 (80.4)	<0.0001
female	74 (15.6)	3 (2.4)	9 (19.6)	
HIV risk category				
MSM	243 (51.3)	95 (76.6)	15 (32.6)	<0.0001
IDU	116 (24.5)	12 (9.7)	25 (54.3)	
heterosexual	71 (15.0)	7 (5.6)	4 (8.7)	
other	44 (9.3)	10 (8.1)	2 (4.4)	
Ethnic origin				
white	403 (85.0)	110 (88.7)	39 (84.8)	0.40
other	41 (15.0)	14 (11.3)	7 (15.2)	
HCV antibody				
negative	284 (59.9)	91 (73.4)	20 (43.5)	<0.0001
positive	127 (26.8)	11 (8.9)	22 (47.8)	
unknown	63 (13.3)	22 (17.7)	4 (8.7)	
HDV antibodies				
negative	386 (81.4)	103 (83.1)	27 (58.7)	<0.0001
positive	63 (13.3)	6 (4.8)	16 (34.8)	
unknown	25 (5.3)	15 (12.1)	3 (6.5)	
Prior AIDS	144 (30.4)	44 (35.5)	14 (20.4)	0.34
ARVs at/before baseline				
naive	44 (9.3)	11 (8.9)	4 (8.7)	0.81
ART	81 (17.1)	25 (20.2)	9 (19.6)	
cART	349 (73.6)	88 (71.0)	33 (71.7)	
Baseline date (month/year)	12/98 (5/97–1/02)	11/98 (3/97–7/00)	2/98 (5/97–1/02)	0.015
CD4 count (cells/mm^3)	295 (165–450)	237 (115–386)	244 (108–431)	0.0004
CD4 nadir (cells/mm^3)	140 (57–238)	101 (31–189)	173 (52–244)	0.0006
Plasma HIV RNA ($\log_{10}$ copies/mL)	2.70 (1.81–4.00)	3.00 (1.93–4.34)	2.70 (1.90–4.09)	0.15
Age (years)	37.8 (33.1–45.3)	38.2 (33.4–45.5)	35.3 (30.7–41.5)	0.10
Time since first HBsAg+ test (years)	1.0 (0.2–3.0)	1.3 (0.3–2.7)	1.8 (0.4–3.3)	0.18
Serum HBV DNA ($\log_{10}$ IU/mL)	2.8 (2.6–5.7)	7.3 (5.5–7.3)	6.4 (4.4–7.3)	<0.0001

MSM, men who have sex with men; IDU, intravenous drug user; ARVs, antiretrovirals; ART, antiretroviral therapy; cART, combination antiretroviral therapy.

compared with patients with genotype A (237 cells/mm^3) or non-A genotypes (244 cells/mm^3) (P=0.0004). Interestingly, those with non-A genotypes had the highest CD4 nadir prior to baseline (P=0.0006). Patients with HBV genotype A had significantly higher median serum HBV DNA levels (17 857 100 IU/mL) compared with non-A genotypes (2 512 130 IU/mL) or unknown genotypes (357 IU/mL) (P<0.0001).

In the multivariate analysis, the only factors associated with being infected with HBV genotype A versus all other genotypes (patients with unknown genotypes were excluded from the analysis) were HIV exposure category and region of Europe. Compared with intravenous drug users (IDUs), MSM had a 10-fold increased risk of infection with HBV genotype A (Figure 2). After adjustment, compared with patients from Southern Europe/Argentina, HIV/HBsAg-positive patients from northern Europe tended to be more frequently infected with HBV genotype A (P=0.054), but most probably this was a surrogate of more common homosexual behaviour there.

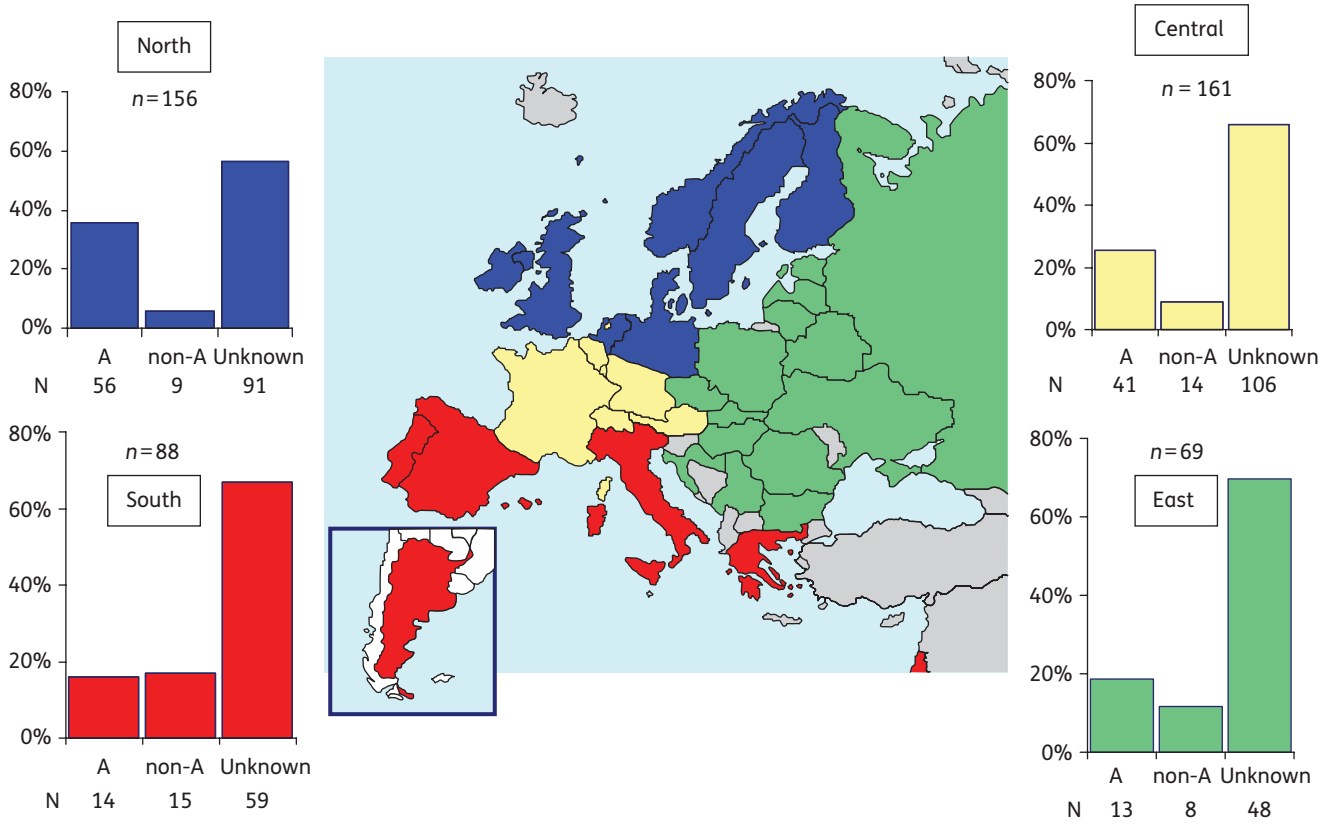

Figure 1. Regional distribution of hepatitis B virus genotypes. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

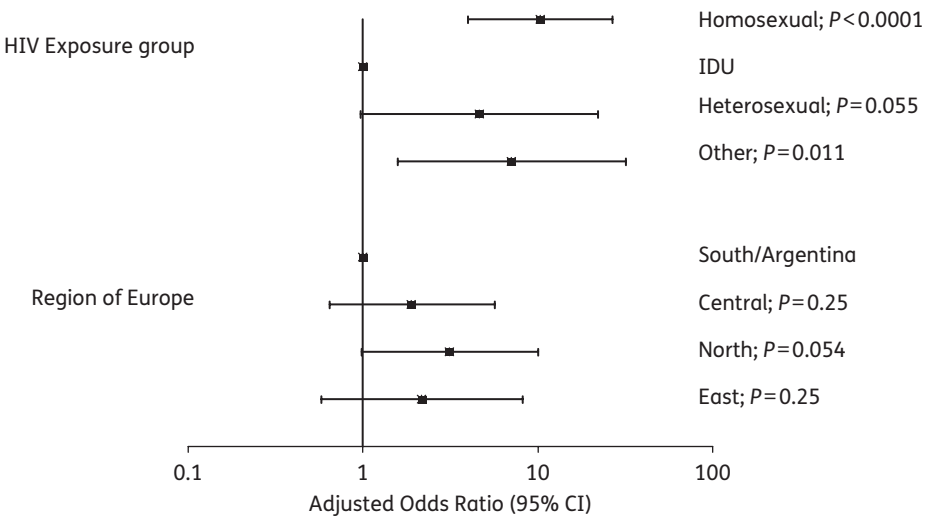

Figure 2. HBV genotype distribution (A versus non-A) according to risk group and region (adjusted odds).

Overall, 159 HBsAg-positive patients [33.5%, 95% confidence interval (CI) 29.3%–37.7%] had serum HBV DNA < 357 IU/mL. Conversely, 96 patients (20.3%) had serum HBV DNA > 10^7 IU/mL. It should be highlighted that HBV genotype distribution tended to differ comparing distinct HBV viral load strata; up to 75/124 (60.5%) of HBV genotype A showed serum HBV DNA > 10^7 IU/mL compared with 21/46 (45.7%) of non-A genotypes (P=0.083). Table 2 splits up the study population into different strata of HBV viral load taking into account exposure to distinct anti-HBV agents. Overall, serum HBV DNA levels > 2000 IU/mL, which

Table 2. Stratification of serum HBV-DNA levels according to antiviral exposure at the time of testing

Serum HBV DNA (IU/mL)	All patients $n=474$	No antiretroviral therapy or anti-HBV agents $n=78$	Antiretroviral therapy with lamivudine (no tenofovir)[a] $n=270$	Antiretroviral therapy with tenofovir (with or without lamivudine or emtricitabine) $n=22$	Antiretroviral therapy with no anti-HBV agents $n=103$
<357 (aviraemic)	159	21 (26.9%)	95 (35.2%)	10 (45.4%)	33 (32.0%)
357–2000	136	27 (34.6%)	77 (28.3%)	8 (36.4%)	24 (23.3%)
2001–10^7	83	11 (14.1%)	56 (20.6%)	4 (18.2%)	12 (11.6%)
>10^7	96	19 (24.4%)	43 (15.9%)	0 (0)	34 (33.1%)

[a]One subject was treated with emtricitabine as the only anti-HBV agent.

Table 3. Predictors of undetectable serum HBV DNA; univariate and multivariate analyses

	Univariate			Multivariate		
	OR	95% CI	*P* value	OR	95% CI	*P* value
HCV antibody status						
negative	1.00	—	—	1.00	—	—
positive	1.97	1.28–3.04	0.0021	1.73	1.07–2.80	0.025
unknown	1.05	0.58–1.90	0.88	1.05	0.57–1.93	0.87
Baseline CD4 count						
per 2-fold increase	1.23	1.05–1.45	0.013	1.24	1.05–1.46	0.013
HDV antibodies						
negative	1.00	—	—	—	—	—
positive	1.46	0.85–2.51	0.17	1.16	0.64–2.10	0.63
unknown	0.08	0.01–0.61	0.014	0.09	0.01–0.66	0.018
Time since first HBsAg+						
per year	1.07	0.99–1.15	0.089	1.06	0.98–1.14	0.14

have been associated with an increased risk of liver-related complications[7–9] and with indication for HBV therapy,[10,11] were recognized in 76 out of 181 patients (42%) not receiving anti-HBV drugs. In the extreme, serum HBV DNA levels >10^7 IU/mL, which are associated with low response to either interferon or oral nucleoside analogues, were recognized in 53 (69.7%) patients. Of 293 patients who included anti-HBV agents as part of the antiretroviral regimen, only 105 (35.8%) had undetectable HBV viraemia, which was slightly higher in patients receiving tenofovir than lamivudine as the only anti-HBV agent (45.4% versus 35.2%; $P=0.3$).

Overall, 293 (61.8%) out of 474 HIV/HBV-co-infected patients were taking anti-HBV antiretroviral agents at the time of testing. They were receiving lamivudine ($n=281$; 59.3%), tenofovir ($n=22$; 4.6%) and/or emtricitabine ($n=4$; 0.8%). There were no statistically significant differences when comparing HBV genotype groups in the proportion of patients receiving any anti-HBV agent (data not shown). In addition, there were no differences in the proportion of patients who had ever started anti-HBV treatment when comparing those who were currently aviraemic (105/159; 66%) versus those who were viraemic (188/315; 59.7%) ($P=0.18$). In contrast, in the group of patients who had started anti-HBV therapy there was a higher proportion with serum HBV DNA <10^7 IU/mL (250/378; 66.1%) than with HBV DNA >10^7 IU/mL (43/96; 44.8%) ($P=0.0001$).

Table 3 records the results of the univariate and multivariate analysis of factors associated with being HBV aviraemic. After adjustment, patients with a higher CD4 count at baseline were more frequently aviraemic [odds ratio (OR) 1.24 per each 2-fold increase of CD4 values], as well as more frequently HCV antibody positive (OR 1.73).

Of 449 HIV/HBsAg-positive patients tested for anti-HDV antibodies, 63 were positive (14.0%; 95% CI 10.9%–17.1%). Among 109 patients with HBV genotype A, 6 (5.5%) were anti-HDV antibody positive, compared with 16/43 (37.2%) with non-A genotypes and 41/297 (13.8%) with unknown genotypes ($P<0.0001$). There were no significant differences in the proportions of patients who were anti-HDV antibody positive comparing those who were HBV DNA viraemic (36/291; 12.4%) and aviraemic (27/158; 17.1%) ($P=0.17$), or comparing patients with serum HBV DNA <10^7 IU/mL (57/368, 15.5%) or >10^7 IU/mL (6/81, 7.4%) ($P=0.058$). Finally, the median serum HBV DNA level was slightly lower in those who were anti-HDV antibody positive [2.6 $\log_{10}$ IU/mL, interquartile range (IQR)

2.6–3.0 $\log_{10}$ IU/mL] compared with those who were anti-HDV antibody negative (2.8 $\log_{10}$ IU/mL, IQR 2.6–5.7 $\log_{10}$ IU/mL) ($P=0.012$).

Discussion

Co-infection with HBV complicates the management of HIV-infected individuals.[2] On the one hand, liver-related events, including decompensated cirrhosis and hepatocellular carcinoma, appear more frequently in co-infected patients than in HBV-monoinfected individuals.[3,19] Suppression of HBV replication by treatment with anti-HBV agents represents the best way to prevent progression of liver disease in these patients.[10,11] On the other hand, the use of antiretroviral agents to treat HIV-associated immunodeficiency is challenged by an increased risk of hepatotoxicity in subjects with underlying chronic hepatitis B.[20–22] A further complication derives from the fact that some antiviral agents possess dual activity against both HIV and HBV, and therefore have to be doubly monitored, avoiding selection of drug resistance by either virus, as recently highlighted in Africa.[23,24]

In the EuroSIDA study, two-thirds of HIV-positive patients with chronic hepatitis B showed detectable HBV viraemia despite almost two-thirds of them being treated with antiretroviral therapy including active anti-HBV drugs. It should be noted, however, that the study was conducted retrospectively on specimens collected from 1996 until 2008, and lamivudine was by far the most frequently prescribed single anti-HBV agent as part of any antiretroviral regimen. It is well known that the risk of selection of lamivudine resistance in HBV is increased in HIV-co-infected individuals.[25] More recently tenofovir and the combination of tenofovir plus emtricitabine (Truvada®) have replaced lamivudine as the preferred choice in HIV/HBV-co-infected patients.[2] Tenofovir either alone or in combination ensures a more durable suppression of HBV replication, and preliminary evidence suggests that this effect may be associated with a halt and regression of HBV-related liver fibrosis in HIV/HBV-co-infected patients.[26,27] Although we could not record liver fibrosis staging in the EuroSIDA population, the recognition of detectable HBV viraemia in 66% of the co-infected population should reinforce the need to monitor and treat appropriately chronic hepatitis B in HIV-positive patients. It is worrisome that nearly one-third of the 91% of HIV/HBV-co-infected patients who received antiretroviral therapy in our study did not receive any anti-HBV agent. As pointed out by others,[1,3] chronic hepatitis B has unfortunately been neglected in the HIV-positive population for a long time. It should be noted, however, that the median date of testing in our study was 1998, before oral drugs other than lamivudine were available to treat hepatitis B and before release of recommendations about how to manage chronic hepatitis B in HIV-positive patients.

In the EuroSIDA study, low CD4 counts were independently associated with a higher risk of detectable HBV viraemia. Since persistent serum HBV DNA levels >2000 IU/mL are associated with an increased risk of liver damage,[7–9] complete HBV suppression should be the goal in patients with established liver disease as any HBV replication must be viewed as deleterious.[10,11] Complete suppression of HBV viraemia is also desirable from a viral resistance standpoint as the risk of resistance positively correlates with viral load, as recently pointed out in the GLOBE study.[28] In HIV-positive patients with chronic hepatitis B, the recognition that low CD4 counts are associated with HBV viraemia may further support recent guidelines that recommend considering an earlier introduction of antiretroviral therapy including anti-HBV agents in HIV/HBV-co-infected patients.[29–31] Moreover, potent anti-HBV drugs (avoiding lamivudine as single active agent) must be part of the chosen antiretroviral regimen.[24]

HBV genotype A was by far (73%) the most prevalent in HIV-positive patients with chronic hepatitis B in EuroSIDA. Only in Southern Europe and Argentina was HBV genotype D equally represented. There was a strong association between HBV genotype A infection and MSM, as previously shown by others.[32,33] In contrast, non-A genotypes were more often found in IDUs, and not surprisingly up to 50% of them were also HCV antibody positive. It is noteworthy that HBV genotype A patients tended to show significantly higher serum HBV DNA levels, regardless of anti-HBV drug exposure. This is important since HBV viraemia is associated with the risk of liver-related complications[7–9] and selection of HBV drug resistance.[34]

Chronic HDV was recognized in 14% of HIV-positive patients with chronic hepatitis B in EuroSIDA. This rate is lower than in other series which included more IDUs,[35] as more than half (51%) of HBsAg-positive patients in EuroSIDA were MSM, and IDUs only represented a quarter of patients. Delta hepatitis occurred more frequently in HBsAg-positive patients carrying non-A HBV genotypes than in those infected with genotype A (37% versus 6%, respectively), most probably because there was a strong segregation of HBV genotypes by risk group category. Serum HBV DNA viraemia was lower and more often undetectable in HBsAg-positive patients with delta hepatitis than in the rest, as expected given the inhibitory effect on HBV replication caused by HDV superinfection.[36] Given the dependence of HDV on HBV, and that delta hepatitis is the most aggressive form of chronic viral hepatitis,[36] it is of great interest to clarify to what extent potent anti-HBV oral drugs may be of benefit[37] in ameliorating the excessive liver damage caused by hepatitis delta in HIV/HBV-co-infected patients.[38,39]

Our study has several limitations. First, only a fraction of the whole 1179 HIV/HBV-co-infected population could be characterized virologically, due either to lack of stored sera ($n=705$; 60%) or to too low or undetectable serum HBV DNA in a subset of the rest (159; 34%), which precluded further genotyping. Moreover, HBV e antigen status was unknown, and this might have influenced viral load levels to some extent, regardless of the HBV genotype. Despite this, to our knowledge the current study is the largest virological characterization of HBV conducted so far in HIV-co-infected individuals. A second limitation of our study regards the period examined, being somewhat out of date, especially considering that the advent and widespread use of tenofovir has dramatically changed the landscape of HIV/HBV co-infection. Indeed, the results of our study reinforce the current recommendation to avoid lamivudine use as the only anti-HBV agent in HIV/HBV-co-infected patients.[2] The risk of virological failure is tremendous, along with the inherent selection of resistance and cross-resistance to other antivirals.[23,24]

In summary, a substantial proportion of HIV-positive patients with chronic hepatitis B in EuroSIDA showed detectable HBV viraemia until recently, despite many of them being treated with active anti-HBV antiretroviral drugs (mainly lamivudine). Low

CD4 counts are associated with an independent higher risk of detectable HBV viraemia, which further supports recent recommendations in favour of an earlier introduction of antiretroviral therapy, including anti-HBV drug(s) more potent than lamivudine alone, in HIV/HBV-co-infected patients.

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Transparency declarations

None to declare.

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