Med Microbiol Immunol (2011) 200:39–49
DOI 10.1007/s00430-010-0172-z

ORIGINAL INVESTIGATION

Prevalence and characteristics of hepatitis B and C virus infections in treatment-naïve HIV-infected patients

Stefan Reuter · Mark Oette · Frank Clemens Wilhelm · Bastian Beggel · Rolf Kaiser · Melanie Balduin · Finja Schweitzer · Jens Verheyen · Ortwin Adams · Thomas Lengauer · Gerd Fätkenheuer · Herbert Pfister · Dieter Häussinger

Received: 8 July 2010 / Published online: 19 September 2010

Abstract In HIV-infected treatment-naïve patients, we analyzed risk factors for either chronic hepatitis B (HBV) infection, occult HBV infection (OHBV) or a positive hepatitis C (HCV) serostatus. A total of 918 patients of the RESINA-cohort in Germany were included in this study. Before initiating antiretroviral therapy, clinical parameters were collected and blood samples were analyzed for antibodies against HIV, HBV and HCV, HBs antigen and viral nucleic acids for HIV and HBV. Present or past HBV infection (i.e. HBsAg and/or anti-HBc) was found in 43.4% of patients. HBsAg was detected in 4.5% (41/918) and HBV DNA in 6.1% (34/554), resulting in OHBV infection in 2.9% (16/554) of patients. OHBV infection could not be ruled out by the presence of anti-HBs (50.1%) or the absence of all HBV seromarkers (25%). A HCV-positive serostatus was associated with the IVDU transmission route, non-African ethnicity, elevated liver parameters (ASL or GGT) and low HIV viral load. Replicative HBV infection and HCV-positive serostatus both correlated with HIV resistance mutations ($P = 0.001$ and $P = 0.028$). HBV and HCV infection are frequent co-infections in HIV treatment-naive patients. These co-infections influence viral evolution, clinical parameters and serological markers. Consequently, HIV patients should routinely be tested for HBV and HCV infection before initiating HIV treatment. OHBV infection constituted almost half of all HBV infections with detectable HBV DNA. Due to a lack of risk factors indicating OHBV infection, HBV diagnosis should not only include serological markers but also the detection of HBV DNA.

Keywords HBV · HCV · Co-infection · Resistance mutations · Occult infection

S. Reuter and M. Oette contributed equally to this work.

This study is conducted for the RESINA study group.

S. Reuter (✉) · D. Häussinger
Clinic of Gastroenterology, Hepatology and Infectious Diseases, University Hospital, Moorenstr. 5, 40225 Duesseldorf, Germany
e-mail: Stefan.reuter@med.uni-duesseldorf.de

M. Oette
Clinic of General Medicine, Gastroenterology and Infectious Diseases, Augustinerinnen Hospital, Cologne, Germany

F. C. Wilhelm · R. Kaiser · M. Balduin · F. Schweitzer · J. Verheyen · H. Pfister
Institute of Virology, University of Cologne, Cologne, Germany

B. Beggel · T. Lengauer
Max Planck Institute for Informatics, Computational Biology and Applied Algorithmics, Saarbruecken, Germany

O. Adams
Institute of Virology, University Hospital of Düsseldorf, Düsseldorf, Germany

G. Fätkenheuer
Department of Internal Medicine I, University Hospital of Cologne, Cologne, Germany

Introduction

Liver disease has become a leading cause of disease progression, morbidity and mortality in HIV-infected patients. In this context, hepatitis B virus (HBV) or hepatitis C virus (HCV) are frequently causing liver disease, as these infectious agents share modes of transmission with HIV [1, 2]. Furthermore, HBV and HCV co-infections increase the risk of hepatotoxicity caused by HIV antiretroviral treatment regimens [3].

Prevalence of HBV co-infection and the presence of HBV serological markers depend on epidemiology and the

route of infection [4, 5]. Chronic HBV infection is defined by the persistence of HBsAg for more than 6 months and is usually accompanied by detectable HBV DNA. HBV infection in immunocompetent HIV-negative persons results rarely in chronification (<10%), while in HIV co-infection, chronification is more frequent and is associated with a rapid progression to cirrhosis [6, 7].

In some cases, HBV replication with detectable HBV DNA occurs in the absence of HBsAg, termed occult HBV (OHBV) infection. The underlying mechanisms of OHBV infection have not been elucidated yet [8–12]. Variation within prevalences of OHBV infection in HIV-infected individuals has been observed [4, 5, 13–15]. Some substances included in HIV-treatment regimens are also active against HBV; treatment should therefore be initiated concomitantly.

The goal of this study was to identify subgroups of patients with an increased risk for HBV chronification. The prevalence of OHBV infection in HIV-infected patients was determined and possible risk factors were analyzed.

The prevalence of HCV–HIV co-infected patients in western countries is 30% [3]. HCV disease course, increasing HCV viral load, severity of liver disease and progression to cirrhosis and liver failure is known to be influenced by HIV infection. It remains a matter of debate to what extent HCV co-infection influences the natural course of HIV infection [3, 16, 17]. The determination of the impact and frequency of HCV co-infection as well as possible risk factors is important for screening and appropriate treatment choices.

Few data are available on HBV or HCV co-infection in treatment-naïve HIV patients. To improve the understanding of the epidemiology and the possible risk factors of both infections, the prevalence and distribution of serological and molecular markers for HBV and HCV were analyzed in a large multicenter cohort of treatment-naive HIV patients (RESINA cohort) [18]. Furthermore, patterns of transmitted resistance in HIV and HBV infection were analyzed and correlated with clinical parameters.

Methods

Patients

RESINA is a large prospective and well-characterized cohort of treatment-naive HIV-infected patients in North Rhine-Westphalia, Germany ($n = 2{,}000$) [18]. At baseline, a HIV resistance analysis is performed. Out of the RESINA cohort, all treatment-naive HIV patients from the two largest recruiting centers (i.e. from the University Hospitals of Cologne and Düsseldorf) were included in this study ($n = 918$). Clinical and epidemiological parameters included age, gender, mode of transmission, ethnicity, and CDC stage. Basic laboratory values included lymphocyte typing, AST, ALT, GGT, AP, LDH, total bilirubin, and complete blood cell count.

Serological analysis

Commercial serological assays from ABBOTT (Architect and Axsym, ABBOTT Wiesbaden, Germany) were used for the determination of HBsAg ($n = 918$), anti-HBs ($n = 842$), anti-HBc ($n = 918$) and anti-HCV ($n = 918$).

Determination of HBV DNA

In 554 out of 918 samples, we performed a qualitative HBV DNA analysis by using an in-house PCR. This allowed us to sequence the samples directly in case of a positive PCR result. We used different primer sets for the amplification due to differences in the primer binding region of different HBV genotypes (P1 = tgctgctatgcctc atcttc, P2 = caragacaaaagaaaattgg, P3 = caaggtatgttgccc gtttgtcc, P4 = ggtawaaagggactcamgatg; primers P1/P2 and P2/P3 and P3/P4 or P1/P4 were combined). A PCR product was sequenced with the amplification primers. Sequence analysis was carried out with the DNA Lasergene SeqMan (GATC Biotech, Konstanz, Germany). We performed pools of 10 samples with 100 µl plasma from each sample and extracted nucleic acids from the complete volume of 1 ml for PCR. The detection limit of the PCR reaction was 150–200 copies/ml with an input volume of 100 µl. A result was called negative, when amplification with neither primer combination was successful.

Definitions

Chronic HBV infection was assumed in cases with detectable HBV DNA and/or HBsAg. In analyses referring to detectable HBV DNA, the term "replicative HBV infection" was used.

HBV genotypic resistance testing

Viral DNA was isolated from 200 µl patient serum using QIAamp DNA Blood Mini-kit (Qiagen, Hilden, Germany). Polymerase chain reaction was performed using HotStarTaq kit (Qiagen, Hilden, Germany) and 3 different primer pairs alternatively described by Allen et al. [19]. PCR products were purified using the QIAquick spin PCR purification kit (Qiagen, Hilden, Germany) and extended using the PCR primers Taq Dye Terminator Ready Reaction Mix, BigDye® version 2.0 (Applied Biosystems, Foster City, CA, USA). Extension products were purified using MultiScreen purification plates (Millipore, Bedford,

MA, USA) and Sephadex G-50 superfine (Amersham Biosciences, Upsala, Sweden) and were run on an ABI Prism 3130xl capillary sequencer. The sequences were edited using the DNASTAR Lasergene (GATC GmbH Konstanz, Germany). Sequences were screened for resistance mutations, HBV-genotype and possible escape mutants using Geno2pheno[HBV] and HIV-GRADE-HBV (www.genafor.org, www.HIV-GRADE.de).

HIV genotypic resistance testing

HIV viral load was determined in 912/918 samples. HIV resistance analysis was carried out as described previously [20]. Resistance testing was performed by population-based sequencing detecting mutations in the protease (PR) and reverse transcriptase (RT) of the HIV-1 *pol*-gene. Only major mutations were scored as resistance-associated according to the current Stanford HIV Drug Resistance Database (accessed November 6, 2009) (available at http://hivdb.standford.edu and according to www.HIV-GRADE.de).

Statistical methods

The statistical analysis was performed using the statistical environment R in version 2.8.1 (www.r-project.org). Wilcox Rank Sum Test was utilized for variable attributes (HIV viral load, CD4 abs./rel., GOT, GPT, GGT and age) to compare the median of independent test samples. Fisher's Exact Test was applied for categorical attributes. All results are expressed as absolute values and percentages. A 95% confidence interval was chosen to be statistically significant ($P = 0.05$). For the multivariate analysis, we utilized logistic regression and stepwise forward feature selection. The ROCR package determined the AUC (area under the receiver operating characteristic). The model selection was done with the help of the Akaike Information Criterion (AIC) together with tenfold cross validation to assess the quality of a model. To curb overtraining, the datasets were split into an 80% training set and a 20% test set. The feature and model selection was done on the training set, while the final performance evaluation in terms of AUC was done on the test set. Finally, to compute the impact of the risk factors, we used the complete datasets.

Results

Patient characteristics

In this cohort, the median age of the patients was 37 years (min: 17, max: 77), with a percentage of 75.2% (690/918) men. Ethnical origins were 77.7% (713) Caucasian, 17.1% (157) African black, 3.5% (32) Asian, 1.0% (9) Latin-American, and 0.8% (7) unknown. The most frequent mode of transmission was men who have sex with men (MSM) (47.1%, 432) followed by heterosexual transmission (23.1%, 212), origin from endemic country (17.0%, 156), intravenous drug abuse (IVDA) (7.1%, 65), blood transfusion (0.7%, 6), occupational exposure (0.2%, 2), perinatal transmission 0.1%, 1), and unknown exposure (4.8%, 44).

Seromarkers for HBV and HCV

In 57.5% (520/918) of the patients, no serological signs of HBV infection (anti-HBc and HBsAg neg) were detected, while 43.4% (398/918) of the patients showed indicative seromarkers of present or past HBV infection (i.e. HBsAg and/or anti-HBc). Isolated anti-HBs was detected in 11.5% (97/842) of the patients. These patients with isolated anti-HBs were regarded as immunized against HBV, although a detailed history of immunization was not available and seroconversion after HBV infection could not be ruled out. HBsAg was found in 4.5% (41/918), and anti-HBs was detected in 43.2% (364/842) of the patients. Anti-HBc was identified in 42.8% (393/918); of these, 21.8% (86/393) showed only isolated anti-HBc without any other marker, corresponding to 10.2% of all patients (86/842). Anti-HBc was detected in significantly higher frequencies in patients showing HBV DNA ($P < 0.001$) (Table 2). HBV DNA was detected in 6.14% (34/554) of the patients. Eighteen out of 34 patients with detectable HBV DNA levels were HBsAg positive. Out of the 34 patients with detectable HBV DNA, 16 patients were HBsAg negative, revealing a frequency of OHBV infection of 2.9% (16/554) (Table 1). Of the 16 identified OHBV patients (i.e. HBsAg negative but detectable HBV DNA), three were isolated anti-HBc positive, 2 were isolated anti-HBs positive and both antibodies were detected in 7 patients. In 4 cases, OHBV was associated with a completely negative HBV serology (HBV viral load was low in all 4 OHBV samples, as judged by the intensity of the bands in our in-house PCR and all samples were double checked for exclusion of contamination). Nevertheless, no significant correlation between OHBV infection and possible risk factors or serological markers could be identified.

Anti-HCV was found in 10.6% (97/918) of the patients, and in 8.3% (8/97) of these patients, HBsAg and/or HBV DNA were detected indicating an active HBV co-infection. Isolated anti-HBc was associated with a positive HCV status ($P = 0.0002$).

Table 1 Prevalence of occult HBV (OHBV) and HBsAg in 554 HIV-positive patients with analysis of HBV DNA

	HBV DNA negative	HBV DNA positive	Anti-HBC+ Anti-HBS+	Anti-HBC+ Anti-HBS−	Anti-HBC− Anti-HBS+	Anti-HBC− Anti-HBS−
Total (*n*)	520	34				
OHBV (*n*)	–	16	7	3	2	4
%		2.9 (16/554)	4.3 (7/164)	6.4 (3/47)	3.8 (2/53)	1.9 (4/213)
HBsAg pos. (*n*)		18		15		3
%		3.2 (18/554)		2.7 (15/554)		0.5 (3/554)
HBsAg pos. (*n*)	6			6		
%	1.1 (6/554)			1.1 (6/554)		
HBsAg pos. (*n*)	514		157	44	51	209
%	92.8 (514/554)		95.7 (157/164)	93.6 (44/47)	96.2 (51/53)	98.1 (209/213)

Prevalence, co-morbidities, and risk factors of replicative HBV in HIV

In a multivariate analysis using a logistic regression model, we analyzed factors associated with isolated anti-HBc-positive serostatus (including HIV viral load, CD4 count, mode of transmission, anti-HCV, AST, ALT, GGT, and ethnicity). Origin from an HIV endemic country increased the likelihood (odds) of isolated anti-HBc by a factor of 4.38. Additionally, a positive anti-HCV status increased the odds of isolated anti-HBc by a factor of 3.70. Table 3 summarizes the results of the obtained prediction model. Using the listed factors yielded an AUC of 0.71 and, therefore, showed intermediate prediction capacity.

The proportion of patients with elevated transaminases was significantly higher in replicative HBV (i.e. DNA-positive) infection when compared to patients without detectable HBV DNA (AST elevation $P < 0.001$, ALT $P = 0.003$). As shown in Table 2, a significant correlation was found between elevated AST and ALT levels and DNA-positive vs. DNA-negative patients ($P < 0.001$ and 0.005).

HCV-positive serostatus, identification of risk factors

In a multivariate analysis using a logistic regression model, we analyzed factors associated with HCV-positive serostatus (including HIV viral load, CD4 count, AST, ALT, GGT, age, mode of transmission and ethnicity). IVDU had the greatest impact on a positive HCV serostatus, increasing the odds by a factor of 191.78. Ninety-two percent (61/66) of the patients with a history of IVDU were HCV positive. Additionally, elevated values for either AST or GGT, non-African ethnicity and low HIV viral load were independent and significant predictors for positive HCV serology. The combination of these five factors is highly discriminative and supports a logistic regression model with an AUC of 0.90 evaluated. Table 4 lists the results of the final regression model.

HBV resistance analysis

We could sequence 30 of the HBV DNA–positive samples and found the following genotypes: 18× A, 7× D, 3× G, 1× C, 1× E. Overall, 19 patients had important HBV mutations and these were all HBV treatment-naïve (Table 5). Mutations conferring drug resistance were found in 5 cases (underlined). HBV drug resistance associated mutations were found in 4 patients with OHBV (4 of 13 = 30.8%) whereas only one of the 17 HBsAg positive patients (5.9%) showed such a mutation ($P = 0.138$). Three of the 4 OHBV cases with drug resistance mutations were negative for all HBV serological markers.

HIV resistance analysis

HIV-associated resistance mutations were found in 8.3% (76/912) of therapy-naïve patients. 46% (35/76) of the patients showed NRTI mutations (M41L, A62V, D67N, T69D, L74V, M184V, L210W, and K219E/Q), 47% (36/76) NNRTI mutations (L100I, K101E, K103N, V106A, V108I, V179D/E, Y181C, G190A, and P225H) and 18% (14/76) PI mutations (L33F, M46I/L, V82A, I84V, and L90M). There was no association between transmitted HIV and HBV resistance associated mutations in co-infected patients. However, the presence of HIV resistance associated mutations was significantly correlated with parameters of active HBV infection: HBsAg ($P = 0.042$), HBV DNA ($P = 0.001$), OHBV infection ($P = 0.019$) and with a positive HCV serostatus ($P = 0.028$).

Discussion

The high discrepancy of findings among studies demands the regional surveillance of HBV infection and the

Table 2 Prevalence, co-morbidities, and univariate analysis of risk factors for replicative HBV in HIV

	HBV DNA pos. $n = 34$	HBV DNA neg. $n = 520$	P value
Median age (range 17–77)	36	37.5	0.472
Male gender %	82.4 (28)	72.5 (377)	0.316
HIV transmission %			
MSM	58.8 (20)	43.8 (228)	0.213
Heterosexual	20.6 (7)	23.1 (120)	0.685
Endemic country	14.7 (5)	19.6 (102)	0.511
IVDA	5.9 (2)	6.5 (34)	1.000
Other/Unknown		6.9 (36)	
Ethnical origin %			
Caucasian	85.3 (29)	75.4 (392)	0.295
African/black	11.8 (4)	19.2 (100)	0.367
Asian	2.9 (1)	3.5 (18)	1.000
Other/Unknown		1.9 (10)	
HIV viral load (copies/ml)			
Median (range 50–500,000)	39.069	52.350	0.934
<10,000	18.1% (6)	23.8% (123)	0.532
10,000–100,000	45.5% (15)	39.7% (205)	0.583
>100,000	36.4% (12)	36.6% (189)	1.000
CD4 count/mm^3			
Median (range 0–1,023)	196	214	0.613
<200	50.0% (17)	45.8% (238)	0.723
200–500	35.3% (12)	44.6% (232)	0.373
>500	14.7% (5)	9.6% (50)	0.368
CD4 count (rel.)			
Median (range 1–56)	16	15	0.742
<10%	30.3% (10)	29.7% (154)	1.000
10–20%	39.4% (13)	42.1% (218)	0.856
>20%	30.3% (10)	28.2% (146)	0.842
CDC-state			
A	47.1% (16)	49.0% (247)	0.861
B	11.8% (4)	21.8% (110)	0.197
C	41.2% (14)	29.2% (147)	0.174
Liver parameters			
Median AST (U/l) (range 9–690)	42	32	**<0.001**
Median ALT (U/l) (range 6–805)	40	29	**0.003**
Median GGT (U/l) (range 5–1,532)	43	36	0.087
AST > ULN	66.7% (22)	35.2% (181)	**<0.001**
ALT > ULN	51.5% (17)	27.7% (141)	**0.005**
GGT > ULN	43.8% (14)	32.7% (167)	0.246
HBV serology			
HBsAg	52.9% (18/34)	1.2% (6/520)	**<0.0001**
Isolated Anti-HBs	3.2% (1/31)	10.9% (51/467)	0.234
Isolated Anti-HBc	9.7% (3/31)	9.4% (44/467)	1.000
anti-HBc/HBs neg	12.9% (4/31)	44.8% (209/467)	**<0.001**
anti-HBc/HBs pos	22.6% (7/31)	33.6% (157/467)	0.241
anti-HBc pos total	73.5% (25/34)	41.0% (213/520)	**<0.001**
HCV serology pos %	12.1 (4/33)	9.1 (47/516)	0.534

Bold values are statistically significant ($P < 0.01$)

Table 3 Identification of risk factors for isolated anti-HBc, results of the regression model

Predictor	Odds ratio	95% Confidence interval	*P* value
Origin from endemic country	4.38	(2.44, 7.89)	<0.0001
GGT > ULN	1.62	(0.95, 2.76)	0.068
Anti-HCV positive	3.70	(1.85, 7.39)	0.0002

ULN upper limit of normal

Table 4 Identification of risk factors for a positive HCV serostatus, results of the regression model

Predictor	Odds ratio	95% Confidence interval	*P* value
IVDU	191.78	(70.60, 520.98)	<0.0001
HIV viral load $< 4 \times 10^5$	8.02	(1.52, 42.35)	0.012
AST > ULN	2.47	(1.16, 5.26)	0.016
GGT > ULN	2.43	(1.13, 5.20)	0.020
Non-African ethnicity	2.68	(2.26, 3.18)	0.008

79/918 observations had to be removed due to missing values

IVDU i.v. drug use, *ULN* upper limit of normal

identification of risk factors for chronification. Our study contributes to the understanding of the epidemiology of HIV-Hepatitis B and C co-infection in Germany.

Prevalence, co-morbidities and risk factors of replicative HBV in HIV

There is evidence that HIV may influence the natural course of HBV infection. HIV positive patients have higher rates of HBV chronification and lower rates of seroconversion to anti-HBs [21, 22]. In the here analyzed cohort of therapy naïve HIV patients, 6.1% also had a replicative HBV infection. Other studies had revealed a worldwide prevalence of chronic HBV in HIV-infected patients of approximately 10% [22]. In analogy to other studies, the proportion of patients with elevated transaminases in our cohort was significantly higher in replicative HBV infection [21, 23].

In our cohort, isolated anti-HBc was found in 10.2% of the patients. Of the 16 OHBV patients, three isolates were anti-HBc positive. Sucupira et al. found isolated anti-HBc in 14% of all OHBV cases [24]. In general, OHBV infection is found in low frequencies in patients with isolated anti-HBc [13, 25]. The presence of anti-HBc alone is more frequent in HIV infected women than men [26], a finding that was confirmed in the present analysis.

It was postulated that the detection of anti-HBc only is a strong indicator for replicative HBV infection [4, 27]. In this study, no difference was found between isolated anti-HBc positive patients and those with other HBV markers.

Table 5 HBV associated mutations found in HIV co-infected patients

No.	Genotype	HBV-polymerase mutations	HBsAg mutations
1	A	L140I	**N131K**
2	A	N122D, G152GR, K168KR, S219A	L95S, **K160EK**, V190A, S193L, I195IT, S210R, F220C
3	A	N122H, M129L, V163I, R217L	**S167***, S207N, V209L
4	A	R138K, S219A	**G130N**, S210R
5	A	R138KR, S219A	**G130DGNS**, S210R
6	A	N131DN, I187L, V191IV, L217R, L229M	**Q101H, K122KR, A159V**, S167L, V168AV, **W182*W**, P217LP, F220L
7	A	T128AT, S219A	S210R
8	C	R153QR, S223A, I224V	**G145GR**, S210N
9	D	F122L, Q130P, N139S	T125M, **T131A**
10	D	F122V, H126R, L145M	T118V, A128V
11	D	H126R	T118A
12	D	H126R, Y135H, V173L, L180M, M204V, Q215S	L97P, T118A, **G130E, E164G**, I195M, S204N, S207R
13	D	L115M, H126R	T118V, A128V, I208IT
14	D	S119PST, F122L, Q130P, L132M, I169T, M171V, A211G	**L109LQ**, T125M, F161L, P203A
15	D	Y135S	L84HL, **T127P**, Y200CY, Y206F, S207N
16	E	V191IV	S167LS, **W182*W**, L226*L
17	G	H100HQ, V103IV, D118DN	I92IN
18	G	–	A166V, S174N, P214LP
19	G	V142T, L180M, M204V, L229F	**M133I, Y134H**, I195M, C221F

The underlined HBV-polymerase mutations are related to drug selection. HBs-mutations in bold represent escape mutants

* Stop codon

Due to the discrepancy between studies, the role of isolated anti-HBc as a marker for chronic HBV infection in HIV treatment-naive patients remains uncertain. Future studies should consider the fact that HBV viremia may be transient in isolated anti-HBc positive patients and may be undetected in single point analyses [4]. Furthermore, in recent infections the frequency of anti-HBc may be lower and could hereby bias the numbers found in different studies.

OHBV, prevalence and risk factors

Occult infection with HBV is a type of HBV infection defined by the presence of HBV DNA in the serum or liver without detectable HBsAg. The diagnosis of OHBV infection in HIV-infected patients is of high importance due to the subsequent choice of dual active agents against both HBV and HIV. We observed a frequency of 2.9% of OHBV infection, and these patients represented almost half of all active HBV infections (i.e. DNA positive). Great variations in the prevalence of OHBV infections have been shown in several studies: with low levels of 0% in a Spanish cohort (85 patients) [5] up to 37% in a French study (30 patients) [28]. This range of prevalence of OHBV infections could be explained by the observation that viremia in OHBV infection is detectable in a short time frame only. Long-term follow-up with sequential analyses revealed higher numbers of OHBV infection [4, 27] (Table 6). Six studies focussing on OHBV infection in treatment-naive patients could not identify any risk factors for OHBV infection [27, 29–33]. In this study, elevated transaminases were associated with chronic HBV infection (HBsAg and HBV DNA positive), but not with OHBV infection. In analogy, other studies could also not detect elevated levels of transaminases in OHBV infection [14, 15, 33–35], while ALT and AST were higher in the HBsAg positive patients [14].

Here, the serological markers of HBV infection in OHBV infection were diverse and no single parameter could be assigned as an indicator for OHBV infection. When comparing our data on OHBV infection with a study in the treatment naïve setting by Filippini and co-workers, we found isolated anti-HBc less frequently (36 vs. 19%), while more of our patients were either both anti-HBc and anti-HBs negative (9 vs. 25%) or both anti-HBc and anti-HBs positive (21 vs. 44%) [27]. This comparison demonstrates the great variability of serological markers in OHBV infection. Furthermore, this study demonstrates that the detection of anti-HBs cannot exclude the possibility of coexisting OHBV infection. Therefore, due to a significant rate of OHBV infection in HIV positive patients and a lack of risk factors indicating OHBV infection, the determination of serological markers should routinely be accompanied by the analysis of HBV DNA.

HCV-positive serostatus, identification of risk factors

HCV co-infection was found in 10.6% of the patients. 8.3% of them were co-infected with HBV and HCV. Transmission by IVDU, elevated AST, elevated GGT, non-African ethnicity, and a low HIV viral load were independently associated with HCV positive serostatus. Furthermore, a high correlation between isolated anti-HBc and HCV-positive serostatus was detected, while isolated anti-HBs (indicating past HBV infection) was significantly less frequent in patients with HCV-positive serostatus. Nevertheless, HCV-positive serostatus did not increase the risk for HBV infection. These findings are supported by others [6, 36, 37], but it remains a matter of debate as to what extent HCV co-infection influences the natural course of HIV infection [3, 16, 17, 38]. In our cohort, HCV-positive serostatus was independently associated with a lower HIV viral load. In support of our findings, a slower disease progression was found in HCV co-infection in two other recent studies [39, 40]. In contrast, a better immune status and improved CD4 cell recovery was detected in HCV-negative patients in Switzerland [38]. Discrepant results have been published with regard to the association between OHBV infection and HCV infection. HCV may influence HBV-infection, leading to undetectable HBsAg and promoting OHBV infection [6, 34]. In contrast, Lo and co-workers found an association between the absence of chronic HCV and OHBV infection [15]. In this study, no correlation between HCV-status and OHBV infection could be identified.

HBV resistance analysis

HBV-polymerase mutations related to drug resistance were found in 5/30 cases (16.7%) (Table 5, underlined). Transmitted HBV drug resistance thus constitutes a relevant problem in patients with active HIV–HBV co-infection. Four out of five HBV-associated drug resistance mutations were found in patients with OHBV and in three cases no seromarkers were found, either pointing at a recent infection or indicating a possible influence of these mutations on the expression of HBV seromarkers.

HBV genotypes A and D were most prevalent, and a similar distribution of genotypes was found in a Spanish cohort [41]. HBV genotype A is known to prevail in central and northern Europe and North America, while HBV genotype D is spread worldwide with focuses on the Mediterranean region and the Middle East [42]. Higher response rates to interferon alpha were found for HBV genotype A when compared to genotype HBV-D [43].

Table 6 Current literature of occult HBV (OHBV) infection in HIV-infected patients in alphabetical order

Reference	*n*	Prevalence of OHBV	Risk factors for OHBV	Method of detection	HIV-treatment status
Araujo-NM 2008, Brazil [34]	43 (anti-HBc+)	6/43 (14%)	HCV seropositivity	Semi-nested and real-time PCR, detection limit 100 c/ml (both)	91% ART
Azadmanesh-K 2008, Iran [29]	22 (anti-HBc+)	3/22 (13.6%)	n.d.	Rotor Gene 3000, Corbett Research, Australia. Detection limit 112 c/ml	Naive (1 exeption)
Filippini-P 2006, Italy [27]	86 (serial follow-up over 6 months)	17/86 (19.8%)	n.d.	Perkin-Elmer 9700, USA, detection limit 100c/ml	Naïve
Gandhi, RT 2003, USA [25]	84	1/84 (1.2%)	n.d.	HBV TMA Assay, Gen-Probe, CA, detection limit 84 c/ml	Mixed
Hofer-M EJCMID 1998, Switzerland [4]	57 (anti-HBc+)	17/57 (29.8%) HBV DNA persistence 51/57 (89.5%) HBV DNA intermittently	None	Nested PCR, detection limit 100 c/ml	n.d.
Laguno-M 2008, Spain [44]	238 (HCV co-infection)	15/238 (6.3%)	None	TaqMan HBV Test, Roche Diagnostics, CA, detection limit 35 c/ml	Mixed
Lo Re III-V 2007, USA [15]	179 (anti-HBc+)	17/179 (10%)	Absence of chronic HCV HIV-RNA > 1,000 c/ml.	HBV TMA Assay, Gen-Probe, CA, detection limit 15 c/ml	Mixed
Morsica 2009 Italy [45]				Semi-nested PCR, detection limit 2.6 c/ml	
Nunez M 2002, Spain [5]	85	0/85 (0%)	n.d.	HBV Monitor, Roche Diagnostics, Netherlands, detection limit 200 c/ml	Mixed
Nebbia-G JMV 2007, Great Brittain [35]	343 (anti-HBc+)	48/343 (14%)	No anti-HBs Heterosexual transmission Afro-Caribbean descent	TaqMan HBV Test, Roche Diagnostics, UK, detection limit 35 c/ml	Mixed
Palacios 2008, Spain [46]				Cobas TaqMan, Roche Molecular Systems, USA, detection limit 112 c/ml	
Piroth L 2002, France [37]	37 (anti-HBc+)	13/37 (35%)	None	See below	Mixed
Piroth-L 2008, France [47]	368 (anti-HBc+)	5/368 (1.4%)	n.d.	Qual. PCR, INSERM U271, France, detection limit 16 c/ml	n.d.
Piroth-L 2008, France [47]	111 (HCV co-infection)	6/111 (5.4%)	anti-HBc+ status, low CD4 or CD8 count, no previous ART	Qual. PCR, INSERM U271, France, detection limit 16 c/ml	Mixed
Pogány-K 2005, Netherlands [30]	93 (anti-HBc+) (<24 Wo vor ART)	4/93 (4%)	n.d.	Cobas amplicor HBV Monitor 2.0, Roche Diagnostics, detection limit 200 c/ml	Naïve
Quarleri-J 2007, Argentina [48]	72 (anti-HBc+)	4/72 (5.6%)	n.d.	Method: PCR–RFLP, detection limit 200 c/ml	100% ART
Rai-RR 2007, India [31]	58	7/58 (12%)	n.d.	Full Article not available via PubMed	Naïve
Ramia-S 2008, Lebanon [49]	87	25/87 (28.7%)	n.d.	Not indicated	n.d.
Rodriguez-Torres 2007, Multinational [50]	866 HIV/HCV co-infection	0/866 (0%)	n.d.	Cobas amplicor HBV Monitor, Roche Diagnostics, detection limit 200 c/ml	Mixed
Santos-EA 2003, Brazil [23]	115 (anti-HBc+)	17/115 (14.8%)	n.d.	Detection limit 100 c/ml	Mixed
Sheng-WH 2007, Taiwan [32]	633	3/179 (7.3%) (isolated anti-HBc+) 3/65 (4.6%) (no serological markers)	n.d.	LighterCycler instrument, Roche Molecular Biochemicals, detection limit 1,000 c/ml	Naïve

Table 6 continued

Reference	*n*	Prevalence of OHBV	Risk factors for OHBV	Method of detection	HIV-treatment status
Shire-NJ 2007, USA [14]	909	12/909 (1.3%)	Lower HBV DNA Lower transaminases	Stratagene Mx3000P Real Time PCR (10-specimen sample pools)	Mixed
Shire-NJ 2004, USA [33]	38 (anti-HBc+)	4/38 (10.5%)	n.d.	Cobas amplicor HBV Monitor, Roche Diagnostics, detection limit 200 c/ml	Naive
Sucupira-MV 2006, Brazil [24]	32 (anti-HBc+)	6/32 (1.9%)	n.d.	7700 SDS System, Applied Bio Systems, detection limit 100 c/ml	Mixed
Taylor-L 2008, USA [51]	549 women	>3%	HCV positivity	Not indicated	n.d.
Torres-Barranda 2006, Mexico [52]	38	7/38 (18.4%)	none	Not indicated	n.d.
Tsui-JI 2007, USA [53]	400 women (anti-HBc+)	2%	CD4 < 200/µl	Cobas amplicor HBV Monitor, Roche Diagnostics, detection limit 200 c/ml	n.d.
Wagner-AA 2004, France [28]	30 (anti-HBc+)	11/30 (37%)	Absence of chronic HCV	Nested PCR, detection limit 350 c/ml	n.d.

OHBV infection was defined as detectable HBV DNA without HBsAg. Sensitivity of the assays used in the different studies varied, and results may therefore have been influenced by the testing method

n.d. not defined

HIV resistance analysis

HIV-associated resistance mutations were found in 8.3% of ART naïve patients with a predominance of NRTI (46%) and NNRTI (47%) mutations compared to PIs (18%). HIV resistance mutations were significantly correlated with all markers of active HBV infection (HBsAg ($P = 0.042$), HBV DNA ($P = 0.001$) and OHBV infection ($P = 0.019$)) as well as a positive HCV status. In HBV or HCV co-infected patients, all HIV mutations were NRTI or NNRTI associated.

There are some limitations to this study: the number of active HCV infections was not assessed as HCV RNA was not determined. Furthermore, a qualitative HBV DNA analysis was performed and no quantitative determination of HBV DNA content. Therefore, correlations between the amount of HBV particles and different forms of active infection (OHBV or active HBV infection) could not be performed. HBeAg and anti-HBe results are not available. As a future task follow-up analyses for patients with OHBV will be performed.

Conclusions

Both HBV and HCV infection are frequent in HIV-infected patients. Furthermore, the chronification of HBV occurs at a relatively high rate. It is therefore advisable to test all patients for possible co-infections before initiating HIV treatment. Elevated AST and ALT levels could here give hint for replicative HBV infection, whereas the detection of isolated anti-HBc might be an indicator for severe immune dysfunction.

In comparison with other study cohorts, the rate of HBV DNA–positive infections was relatively low, while OHBV constituted almost half of these HBV DNA–positive infections. Due to a lack of risk factors indicating OHBV, the determination of serological markers should routinely be accompanied by analysis of HBV DNA.

Risk factors found to be independently associated with HCV-positive serostatus included HIV transmission by IVDU, elevated values for AST or GGT, non-African ethnicity and a low HIV viral load.

HIV resistance mutations were significantly correlated with active HBV infection and with a positive HCV status.

Acknowledgments The RESINA study is supported by a grant from the German Ministry of Health and Social Security (grant no. AZ 319-4476-02/3). We are indebted to Professor W. H. Gerlich for fruitful discussion and critical reading of the manuscript. We would like to thank Eugen Schülter, Claudia Müller and Angelika Hergesell for valuable help in data acquisition.

References

1. Weber R, Sabin CA, Friis-Moller N et al (2006) Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. Arch Intern Med 166:1632–1641
2. Stürmer M, Doerr HW, Gürtler L (2009) Human immunodeficiency virus: 25 years of diagnostic and therapeutic strategies and their impact on hepatitis B and C virus. Med Microbiol Immunol 198(3):147–155
3. Rockstroh JK (2006) Influence of viral hepatitis on HIV infection. J Hepatol 44:S25–S27
4. Hofer M, Joller-Jemelka HI, Grob PJ et al (1998) 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 17:6–13
5. Nunez M, Rios P, Perez-Olmeda M et al (2002) Lack of 'occult' hepatitis B virus infection in HIV-infected patients. AIDS 16:2099–2101
6. Jilg W, Sieger E, Zachoval R et al (1995) Individuals with antibodies against hepatitis B core antigen as the only serological marker for hepatitis B infection: high percentage of carriers of hepatitis B and C virus. J Hepatol 23:14–20
7. Thio CL, Netski DM, Myung J et al (2004) Changes in hepatitis B virus DNA levels with acute HIV infection. Clin Infect Dis 38:1024–1029
8. Yamamoto K, Horikita M, Tsuda F et al (1994) Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. J Virol 68:2671–2676
9. Jeantet D, Chemin I, Mandrand B et al (2004) Cloning and expression of surface antigens from occult chronic hepatitis B virus infections and their recognition by commercial detection assays. J Med Virol 73:508–515
10. Vivekanandan P, Kannangai R, Ray SC et al (2008) Comprehensive genetic and epigenetic analysis of occult hepatitis B from liver tissue samples. Clin Infect Dis 46:1227–1236
11. Filippini P, Coppola N, Pisapia R et al (2007) Virological and clinical aspects of HBV-HCV coinfection in HIV positive patients. J Med Virol 79:1679–1685
12. Raimondo G, Pollicino T, Romano L et al. (2010) A 2010 update on occult hepatitis B infection. Pathol Biol (Paris)
13. Neau D, Winnock M, Jouvencel AC et al (2005) Occult hepatitis B virus infection in HIV-infected patients with isolated antibodies to hepatitis B core antigen: Aquitaine cohort, 2002–2003. Clin Infect Dis 40:750–753
14. Shire NJ, Rouster SD, Stanford SD et al (2007) The prevalence and significance of occult hepatitis B virus in a prospective cohort of HIV-infected patients. J Acquir Immune Defic Syndr 44:309–314
15. Lo RV III, Frank I, Gross R et al (2007) Prevalence, risk factors, and outcomes for occult hepatitis B virus infection among HIV-infected patients. J Acquir Immune Defic Syndr 44:315–320
16. Dorrucci M, Valdarchi C, Suligoi B et al (2004) The effect of hepatitis C on progression to AIDS before and after highly active antiretroviral therapy. AIDS 18:2313–2318
17. Klein MB, Lalonde RG, Suissa S (2003) The impact of hepatitis C virus coinfection on HIV progression before and after highly active antiretroviral therapy. J Acquir Immune Defic Syndr 33:365–372
18. Sagir A, Oette M, Kaiser R et al (2007) Trends of prevalence of primary HIV drug resistance in Germany. J Antimicrob Chemother 60:843–848
19. Allen MI, Deslauriers M, Andrews CW et al (1998) Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. Hepatology 27:1670–1677
20. Oette M, Kaiser R, Daumer M et al (2004) Primary drug-resistance in HIV-positive patients on initiation of first-line antiretroviral therapy in Germany. Eur J Med Res 9:273–278
21. Hadler SC, Judson FN, O'Malley PM et al (1991) Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. J Infect Dis 163:454–459
22. Puoti M, Airoldi M, Bruno R et al (2002) Hepatitis B virus coinfection in human immunodeficiency virus-infected subjects. AIDS Rev 4:27–35
23. Santos EA, Yoshida CF, Rolla VC et al (2003) Frequent occult hepatitis B virus infection in patients infected with human immunodeficiency virus type 1. Eur J Clin Microbiol Infect Dis 22:92–98
24. Sucupira MV, Mello FC, Santos EA et al (2006) Patterns of hepatitis B virus infection in Brazilian human immunodeficiency virus infected patients: high prevalence of occult infection and low frequency of lamivudine resistant mutations. Mem Inst Oswaldo Cruz 101:655–660
25. Gandhi RT, Wurcel A, McGovern B et al (2003) Low prevalence of ongoing hepatitis B viremia in HIV-positive individuals with isolated antibody to hepatitis B core antigen. J Acquir Immune Defic Syndr 34:439–441
26. Neau D, Winnock M, Galperine T et al (2004) Isolated antibodies against the core antigen of hepatitis B virus in HIV-infected patients. HIV Med 5:171–173
27. Filippini P, Coppola N, Pisapia R et al (2006) Impact of occult hepatitis B virus infection in HIV patients naive for antiretroviral therapy. AIDS 20:1253–1260
28. Wagner AA, Denis F, Weinbreck P et al (2004) Serological pattern 'anti-hepatitis B core alone' in HIV or hepatitis C virus-infected patients is not fully explained by hepatitis B surface antigen mutants. AIDS 18:569–571
29. Azadmanesh K, Mohraz M, Aghakhani A et al (2008) Occult hepatitis B virus infection in HIV-infected patients with isolated hepatitis B core antibody. Intervirology 51:270–274
30. Pogany K, Zaaijer HL, Prins JM et al (2005) Occult hepatitis B virus infection before and 1 year after start of HAART in HIV type 1-positive patients. AIDS Res Hum Retroviruses 21:922–926
31. Rai RR, Mathur A, Mathur D et al (2007) Prevalence of occult hepatitis B & C in HIV patients infected through sexual transmission. Trop Gastroenterol 28:19–23
32. Sheng WH, Kao JH, Chen PJ et al (2007) Evolution of hepatitis B serological markers in HIV-infected patients receiving highly active antiretroviral therapy. Clin Infect Dis 45:1221–1229
33. Shire NJ, Rouster SD, Rajicic N et al (2004) Occult hepatitis B in HIV-infected patients. J Acquir Immune Defic Syndr 36:869–875
34. Araujo NM, Branco-Vieira M, Silva AC et al (2008) Occult hepatitis B virus infection in HIV-infected patients: Evaluation of biochemical, virological and molecular parameters. Hepatol Res 38:1194–1203
35. Nebbia G, Garcia-Diaz A, Ayliffe U et al (2007) Predictors and kinetics of occult hepatitis B virus infection in HIV-infected persons. J Med Virol 79:1464–1471
36. French AL, Operskalski E, Peters M et al (2007) Isolated hepatitis B core antibody is associated with HIV and ongoing but not resolved hepatitis C virus infection in a cohort of US women. J Infect Dis 195:1437–1442
37. Piroth L, Binquet C, Vergne M et al (2002) The evolution of hepatitis B virus serological patterns and the clinical relevance of isolated antibodies to hepatitis B core antigen in HIV infected patients. J Hepatol 36:681–686
38. Greub G, Ledergerber B, Battegay M et al (2000) Clinical progression, survival, and immune recovery during antiretroviral

therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. Lancet 356:1800–1805
39. Morsica G, Bagaglio S, Ghezzi S et al (2007) Hepatitis C virus (HCV) coinfection in a cohort of HIV positive long-term non-progressors: possible protective effect of infecting HCV genotype on HIV disease progression. J Clin Virol 39:82–86
40. Zhang X, Xu J, Peng H et al (2008) HCV coinfection associated with slower disease progression in HIV-infected former plasma donors naive to ART. PLoS One 3:e3992
41. Ramos B, Nunez M, Martin-Carbonero L et al (2007) Hepatitis B virus genotypes and lamivudine resistance mutations in HIV/hepatitis B virus-coinfected patients. J Acquir Immune Defic Syndr 44:557–561
42. Lindh M, Andersson AS, Gusdal A (1997) Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus–large-scale analysis using a new genotyping method. J Infect Dis 175:1285–1293
43. Erhardt A, Blondin D, Hauck K et al (2005) Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. Gut 54:1009–1013
44. Laguno M, Larrousse M, Blanco JL et al (2008) Prevalence and clinical relevance of occult hepatitis B in the fibrosis progression and antiviral response to INF therapy in HIV-HCV-coinfected patients. AIDS Res Hum Retroviruses 24:547–553
45. Morsica G, Ancarani F, Bagaglio S et al (2009) Occult hepatitis B virus infection in a Cohort of HIV-positive patients: Correlation with hepatitis C virus coinfection, virological and immunological features. Infection 37:445–449
46. Palacios R, Mata R, Hidalgo A et al (2008) Very low prevalence and no clinical significance of occult hepatitis B in a cohort of HIV-infected patients with isolated anti-HBc seropositivity: the BHOI study. HIV Clin Trials 9:337–340
47. Piroth L, Lafon ME, Binquet C et al (2008) Occult hepatitis B in HIV-HCV coinfected patients. Scand J Infect Dis 40:835–839
48. Quarleri J, Moretti F, Bouzas MB et al (2007) Hepatitis B virus genotype distribution and its lamivudine-resistant mutants in HIV-coinfected patients with chronic and occult hepatitis B. AIDS Res Hum Retroviruses 23:525–531
49. Ramia S, Mokhbat J, Ramlawi F et al (2008) Occult hepatitis B virus infection in HIV-infected Lebanese patients with isolated antibodies to hepatitis B core antigen. Int J STD AIDS 19:197–199
50. Rodriguez-Torres M, Gonzalez-Garcia J, Brau N et al (2007) Occult hepatitis B virus infection in the setting of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) co-infection: clinically relevant or a diagnostic problem? J Med Virol 79:694–700
51. Taylor L, Gholam P, Delong A et al (2008) Occult hepatitis B virus (HBV) and hepatitis C virus (HCV) viremia in women with and at-risk for HIV/AIDS. In: 17th International AIDS conference. Mexico City, Mexico
52. Torres-Baranda R, Bastidas-Ramirez BE, Maldonado-Gonzalez M et al (2006) Occult hepatitis B in Mexican patients with HIV, an analysis using nested polymerase chain reaction. Ann Hepatol 5:34–40
53. Tsui JI, French AL, Seaberg EC et al (2007) Prevalence and long-term effects of occult hepatitis B virus infection in HIV-infected women. Clin Infect Dis 45:736–740