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Wit, F.W.N.M.; Weverling, G.J.; Weel, J.F.L.; Jurriaans, S.; Lange, J.M.A.

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Incidence of and Risk Factors for Severe Hepatotoxicity Associated with Antiretroviral Combination Therapy

Ferdinand W. N. M. Wit,[1,2,5] Gerrit Jan Weverling,[1,3] Jan Weel,[4] Suzanne Jurriaans,[2] and Joep M. A. Lange[1,2,5]

[1]National AIDS Therapy Evaluation Center, Departments of [2]Human Retrovirology, [3]Clinical Epidemiology and Biostatistics, and [4]Medical Microbiology, and [5]Division of Infectious Diseases, Tropical Medicine, and AIDS, Department of Internal Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

This retrospective cohort study investigated whether particular antiretroviral agents are associated with a higher risk for developing grade 4 liver enzyme elevations (LEEs) in patients with human immunodeficiency virus (HIV) type 1 infection who are starting to receive highly active antiretroviral therapy (HAART). Grade 4 LEE was defined as aminotransferase levels >10 times the upper limit of normal and >200 U above baseline levels. A multivariate Cox model was used to identify risk factors. The incidence of LEE was 6.3%. No patients died of LEE consequences. Risk factors were higher baseline alanine aminotransferase levels, chronic hepatitis B or C virus infection, antiretroviral therapy–naive patients undergoing their first HAART regimen, recent start of a regimen of nevirapine or high-dose ritonavir, and female sex. In hepatitis B virus (HBV)–coinfected patients, discontinuing lamivudine (3TC) use was a risk factor. In 97% of cases, ⩾1 risk factor was present. In HBV-coinfected patients using 3TC, continued use of 3TC should be considered, even if 3TC-resistant HIV strains develop.

Antiretroviral therapy is often complicated by the occurrence of drug-related toxicities [1]. Liver enzyme elevations (LEEs) are frequently associated with use of potent antiretroviral combination therapy [2–7]. LEEs associated with the use of nucleoside-analogue reverse-transcriptase inhibitors (NRTIs) might be caused by damaging mitochondrial DNA [8]. Nonnucleoside reverse–transcriptase inhibitor (NNRTI)–associated LEE is sometimes part of a hypersensitivity reaction [9, 10]. The mechanisms by which protease inhibitors contribute to the etiology of LEEs are unknown. It has been postulated that antiretroviral combination therapy–associated LEE in the setting of chronic viral hepatitis is part of an immune-restoration disease [3, 11].

The most well-established risk factors for LEE are chronic hepatitis B and C infections [2–7]. In addition, several other parameters (e.g., a greater increase in CD4 cell count after the start of antiretroviral therapy [5] and higher baseline levels of alanine aminotransferase [ALT] [2, 3]) are risk factors for LEE. Virtually every licensed antiretroviral drug has been associated with LEE [9, 10, 12–21]; however, most of this information is from case series or reports of randomized trials that compared a limited number of antiretroviral regimens.

Of several cohort studies [3–7] that compared the hepatotoxic effects of the licensed antiretroviral drugs in clinical practice, only one found an independent relationship between the occurrence of LEE and the use of a specific antiretroviral drug (i.e., ritonavir) [5]. These cohort studies only investigated possible associations between LEEs and the initial antiretroviral combination regimens. Because up to 53% of patients modify their initial antiretroviral combination regimen within the first year [22], discarding information about subsequent regimens will reduce the power to adequately detect differences in hepatotoxicity between antiretroviral agents. Most of the aforementioned cohort studies included mild cases (grade 2 or 3) in their definitions of LEE. Because most mild cases of LEE resolve whether antiretroviral therapy is continued or not [2, 3], they are of limited clinical relevance. Furthermore, as new antiretroviral agents become available, there is a continued need for studies that compare the hepatotoxicity of antiretroviral agents, especially since several reports have linked the use of antiretroviral agents to life-threatening hepatotoxicity [9, 23–27].

We previously investigated the incidence and outcome of, and risk factors for, grade 3+4 hepatotoxicity of antiretroviral combination therapy in our human immunodeficiency virus (HIV) type 1–infected outpatient population [3]. Since then, several new antiretroviral agents have been licensed for the treatment of HIV-1–infected persons in The Netherlands (e.g., nelfinavir and nevirapine). Here we report the results of a repeated analysis of an updated and extended data set, in which, while controlling for other established and potential risk factors, we investigated whether the use of any licensed antiretroviral drug is associated with a higher risk for developing grade 4 LEE.

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Reprints or correspondence: Dr. Ferdinand W. N. M. Wit, National AIDS Therapy Evaluation Center, Academic Medical Center, Rm. T0-120, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands (f.w.wit@amc.uva.nl).

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Patients and Methods

Patient population. This study included HIV-1–infected adults from the HIV outpatient clinic of the Academic Medical Center, University of Amsterdam, who initiated potent antiretroviral combination therapy for the first time between 1 July 1996 and 1 January 2000. Patients could be completely antiretroviral therapy naive or could have been pretreated with NRTIs. Potent antiretroviral combination therapy was defined as therapy containing ⩾3 antiretroviral agents. Patients participating in clinical trials were also included in this study. Patients routinely visited the HIV outpatient clinic every 12 weeks. After the start of therapy and following any change in regimen, patients were monitored more frequently (once every 4 weeks) for a 12-week period.

As described elsewhere [22], data collected from patients' medical records were date of birth, sex, risk factor(s) for HIV infection, use of antiretroviral and prophylactic drugs, and the occurrence of HIV-1–related events, as classified according to the 1993 Centers for Disease Control and Prevention (CDC) guidelines [28]. Toxicity data were recorded only if toxicity led to modification of the antiretroviral regimens. Information on patient adherence to the regimens was not collected. Data were collected on standardized case record forms. Source document and data entry verification were performed in a randomly selected group of patients who comprised ~25% of the total population. The quality of the data was satisfactory for the purpose of this study.

Laboratory data. Laboratory data, such as plasma HIV-1 RNA levels, CD4 cell counts, aspartate aminotransferase (AST) and ALT levels, and hepatitis B virus (HBV) and hepatitis C virus (HCV) serology, were retrieved electronically from the hospital information system. Baseline values for plasma HIV-1 RNA levels, CD4 cell counts, and AST and ALT levels were obtained within 26 weeks before the start of antiretroviral therapy (92.3% were obtained within 4 weeks). These measurements were routinely obtained every time a patient visited the outpatient clinic. Plasma HIV-1 RNA was determined by the following techniques: HIV-1 RNA QT assay (NASBA; Organon Teknika), HIV-1 QT assay (NucliSens; Organon Teknika), and HIV Monitor assay (Amplicor; Roche Diagnostic Systems). After August 1999, ultrasensitive HIV-1 RNA assays were used, either the NucliSens HIV-1 QT assay with 2 mL of plasma input or the Quantiplex bDNA 3.0 (Bayer). The patients' HBV and HCV serologic status was determined by immunoassay (AxSYM; Abbott GbmH Diagnostika).

Definitions of chronic viral hepatitis. As described elsewhere [3], patients were considered to have chronic HBV infection when HBV surface antigen (HBsAg) could be detected in plasma on 2 consecutive occasions ⩾6 months apart before the start of potent antiretroviral combination therapy [29]. As reported in an earlier study, HCV infection has a very high rate of persistence [30]; therefore, patients were considered to have a chronic HCV infection when antibodies against HCV (anti-HCV) were present. Patients without detectable HBsAg and anti-HCV were considered not to have chronic viral hepatitis.

Definitions of grade 4 and grade 3+4 LEEs. In accordance with AIDS Clinical Trials Group criteria [31], grade 4 LEE was defined as AST and/or ALT elevations ⩾10 times the upper limit of normal (ULN). The ULNs in our hospital are 37 and 47 U/L for ALT and AST, respectively. In addition, the absolute increase in aminotransferase levels needed to be >200 U/L greater than an individual's baseline value, to avoid misclassification of patients as a consequence of high baseline aminotransferase levels. Patients were regarded as having grade 4 LEE if they had aminotransferase elevation at least once, according to the above definition, after starting antiretroviral therapy. Grade 3+4 LEE was defined as AST and/or ALT elevations ⩾5 times the ULN, with an absolute increase in aminotransferase levels >100 U/L greater than an individual's baseline value. Elevations of γ–glutamyl transferase and alkaline phosphatase were not considered to be primary parameters of interest, since these do not reflect liver cell damage.

Clinical evaluation and outcome of grade 4 LEEs. To classify cases of grade 4 LEE as either symptomatic or asymptomatic and to assess outcomes of grade 4 LEEs, the medical records of patients with grade 4 LEEs were screened for evidence of clinical signs and symptoms during that episode. In addition, all available information on the etiology of the grade 4 LEE cases was recorded (e.g., acute hepatitis A virus [HAV] infection).

Statistical analysis. We considered the start of potent antiretroviral combination therapy to be the start of observation. Baseline characteristics of patients with and without grade 4 LEE were tabulated: age, sex, risk group for HIV-1 transmission, prior AIDS-defining illnesses, prior treatment with NRTIs, CD4 cell count, plasma HIV-1 RNA levels, AST and ALT levels, and chronic infection with HBV or HCV.

Group comparisons were made with the Wilcoxon rank sum test for continuous data and with the χ^2 statistic or Fisher's exact test for categorical data. The level of significance was set at 5% throughout the analyses. All reported P values are 2-sided. Data were analyzed with SAS software (version 8.0; SAS Institute).

To identify independent risk factors for the development of antiretroviral therapy–associated grade 4 LEE, a multivariate Cox proportional hazards model was constructed. Both fixed and time-dependent covariates were included in the model. The fixed covariates were the baseline parameters considered to be possible risk factors for LEE: hepatitis serologic status, AST and ALT values, age, sex, body weight, stage of HIV-1 disease (CDC classification), CD4 cell count, and plasma HIV-1 RNA levels. The time-dependent covariates were the use of specific antiretroviral drugs, plasma HIV-1 RNA levels, and (changes in) CD4 cell counts obtained after the start of antiretroviral therapy.

Because the risk for developing LEE is greatest during the first few months after starting antiretroviral therapy, the Cox model separated the first 12 weeks of therapy with a specific drug from the complete treatment episode with that specific drug. This was done by creating additional dummy variables that took the value 1 only during the first 12 weeks after the start of that specific antiretroviral drug. Furthermore, to investigate whether use of the first-line antiretroviral regimens is associated with an increased risk, 2 dummy variables were created (one for patients pretreated with nucleoside analogues and one for antiretroviral therapy–naive patients) that took on the value 1 as long as the initial potent antiretroviral combination regimen was not modified. Discontinuation of lamivudine (3TC) in patients with chronic HBV infection can cause an acute exacerbation of HBV replication, with clinical symptoms of hepatitis [32]. The Cox model accounted for this phenomenon by treating the first 16 weeks after discontinuing use of 3TC in patients with a chronic HBV infection as a "post-3TC" period.

A stepwise selection method was followed by using entry and removal criteria of 0.05 to identify the best-fitted multivariate model. The multivariate Cox proportional hazards analysis was repeated with grade 3+4 LEEs as the dependent variable to investigate whether the identified risk factors for grade 4 LEE would also apply for grade 3+4 LEE.

Results

Baseline characteristics. In total, 560 patients initiated potent antiretroviral combination therapy for the first time between 1 July 1996 and 1 January 2000. The median duration of follow-up was 3.0 years (interquartile range [IQR], 1.6–4.1 years). The baseline characteristics of these patients are listed in table 1.

Use of antiretroviral medication. Table 2 summarizes the use of specific antiretroviral drugs. Drugs used by <10% of all patients were zalcitabine, abacavir, saquinavir soft gel capsules, amprenavir, lopinavir, efavirenz, and hydrea. Therefore, our study had limited power to detect hepatotoxic effects of these drugs.

Incidence of grade 4 LEEs. Of the 560 patients, 44 (7.9%) developed grade 4 LEEs (figure 1). By screening the medical records of these patients, an obvious cause of the grade 4 LEE was identified for 9 patients. Four patients had an acute HAV infection, and 1 patient each had acute HCV infection, secondary syphilis, an episode of alcohol abuse (and was not using any antiretroviral drugs at that time), myositis, and rhabdomyolysis secondary to seizures. All 9 cases of grade 4 LEE resolved. These 9 cases were excluded from the analysis of outcome and risk factors for idiopathic grade 4 LEE, leaving 35 cases.

Outcome of grade 4 LEEs. Overall, 6 (17.1%) of 35 patients were symptomatic. The most frequently occurring symptoms were jaundice, dark urine, clay-colored stools, malaise, nausea, vomiting, and right upper quadrant discomfort. Two symptomatic patients had prolonged prothrombin time. Five of the 6 patients with symptomatic grade 4 LEE were coinfected with either HBV or HCV. In none of the cases was lactic acidosis diagnosed. In 1 patient, the grade 4 LEE coincided with a mild nevirapine-related rash. Both LEE and rash disappeared after discontinuing all antiretroviral therapy.

Twenty-three patients (66%) continued their antiretroviral regimen throughout the entire episode of grade 4 LEE. Twelve (34%) discontinued or interrupted therapy, some for reasons unrelated to the LEE. In all 35 patients, the grade 4 LEE resolved to grade 2 (2.5–5 times the ULN) or less. None of the patients died during the episode of grade 4 LEE. Two patients, one coinfected with HBV and the other with HCV, developed progressive liver disease after, respectively, 1 and 2 years (biopsy-proven cirrhosis with clinical symptoms of liver failure).

The median peak level of AST was 372 IU/L (IQR, 218–681), and that of ALT was 604 IU/L (IQR, 493–894). The median time to improvement of both AST and ALT levels to grade 2 or less was 8.9 weeks (IQR, 4.0–14.0). The patients who interrupted antiretroviral therapy had higher median peak levels of both AST (714 IU/L [IQR, 523–976] vs. 284 IU/L [IQR, 190–378]; $P = .0005$) and ALT (903 IU/L [IQR, 583–1435] vs. 533 IU/L [IQR, 476–651]; $P = .0174$) than patients who continued therapy. The median time to improvement of both AST and

Table 1. Baseline characteristics of the study population.

Characteristic	All patients ($n = 560$)	Patients with no grade 4 LEE ($n = 525$)	Patients with grade 4 LEE ($n = 35$)	P[a]
Age, median years (IQR)	37.8 (33.1–44.5)	38.1 (33.1–44.7)	36.2 (32.8–40.7)	.20
Sex, % women	21.8	20.8	37.1	.033
Risk group, %				
MSM	59.5	60.8	40.0	.020
Heterosexual	20.0	19.2	31.4	.081
Injection drug use	7.1	6.5	17.1	.031
Use of blood products	2.5	2.1	8.6	.051
Other	5.9	6.1	2.9	.71
Unknown	5.0	5.3	0.0	.25
AIDS, %	38.2	38.9	28.6	.28
Prior treatment with NRTIs, %	47.9	48.0	45.7	.86
CD4 cell count, median cells/mm^3 (IQR)	170 (50–320)	170 (50–320)	220 (90–370)	.24
HIV-1 RNA, median $\log_{10}$ (IQR)	4.6 (3.9–5.0)	4.6 (3.9–5.0)	4.5 (3.9–5.0)	.62
Liver enzymes, median IU/L (IQR)				
AST	31 (24–43)	31 (24–42)	43 (26–65)	.015
ALT	32 (21–52)	31 (21–50)	48 (23–77)	.021
Chronic viral hepatitis, %[b]				
HBV	8.8	6.5	42.9	<.0001
HCV	10.7	9.1	34.3	<.0001

NOTE. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; LEE, liver enzyme elevation; MSM, men who have sex with men; NRTI, nucleoside-analogue reverse-transcriptase inhibitor.

[a] Fisher's exact test or Wilcoxon rank sum test.

[b] Four patients had both chronic HBV and HCV infections.

Table 2. Use of antiretroviral drugs by study population.

Antiretroviral drug	No. (%) of patients ever treated	Cumulative no. of treatment episodes	Cumulative years of use
NRTIs			
Zidovudine	269 (48.0)	326	471
Didanosine	133 (23.8)	182	221
Zalcitabine	16 (2.9)	18	9
Stavudine	437 (78.0)	587	910
Lamivudine	483 (86.3)	624	1103
Abacavir	49 (8.8)	62	56
Protease inhibitors			
Indinavir	265 (47.3)	346	417
Saquinavir HGC	347 (62.0)	436	546
Saquinavir SGC	13 (2.3)	13	15
Ritonavir[a]	372 (66.4)	505	572
Nelfinavir	131 (23.4)	168	178
Amprenavir	5 (0.9)	5	3
Lopinavir	9 (1.6)	10	7
NNRTIs			
Nevirapine	205 (36.6)	241	344
Efavirenz	35 (6.3)	42	32
Hydroxyurea	40 (7.1)	49	56

NOTE. HGC, hard gel capsule; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside-analogue reverse-transcriptase inhibitor; SGC, soft gel capsule.

[a] All formulations of ritonavir (hard capsules, liquid, and soft elastic capsules).

ALT levels to grade 2 or less was not significantly different between patients who interrupted or continued antiretroviral therapy (data not shown). The median peak levels of AST and ALT and the time to improvement of both AST and ALT to grade 2 or less were not significantly different between patients with or without chronic viral hepatitis.

Risk factors for grade 4 LEE. As seen in table 1, there were differences in baseline characteristics between the patients who developed grade 4 LEE and those who did not. The 35 patients with idiopathic grade 4 LEE more often had chronic viral hepatitis, had higher baseline AST and ALT values, were more often infected via injection drug use or infected blood products, and were more often women.

Table 3 lists the risk factors significantly associated with grade 4 LEE in univariate and multivariate Cox proportional hazards analysis. The independent risk factors identified in the multivariate model were higher baseline ALT levels (hazard ratio [HR], 1.05/10 U increase), chronic HBV infection (HR, 9.2), chronic HCV infection (HR, 5.0), the use of first-line potent antiretroviral combination regimens in patients without prior NRTI treatment (HR, 2.8), recent start of nevirapine (HR, 9.6) or ritonavir (HR, 4.9), and female sex (HR, 2.8). Furthermore, among patients chronically coinfected with HBV, discontinuing the use of 3TC was associated with the development of grade 4 LEE (HR, 6.8).

There were several differences between the univariate and multivariate Cox models. Injection drug use was not a significant predictor in the multivariate model, because of the strong correlation between injection drug use and chronic HCV infections ($\kappa = 0.63$). Also, the recent start of saquinavir was not a significant predictor in the multivariate model, because saquinavir is often boosted with ritonavir (400 mg 2 times/day) in our patient population ($\kappa = 0.46$). The (long-term) use of ritonavir was not a significant predictor in the multivariate model, because most grade 4 LEEs associated with the use of ritonavir developed within the first 12 weeks of ritonavir use (12 of 19 events). The use of indinavir seemed to protect against grade 4 LEEs in the univariate but not the multivariate model, probably because indinavir-based regimens almost never contain nevirapine and/or high-dose ritonavir in our patient population. The use of first-line potent antiretroviral combination regimens in patients without prior NRTI treatment was not identified as a risk factor in the univariate analysis but did contribute significantly in the multivariate analysis and significantly improved the overall fit of the multivariate model, compared with the model without this covariate (difference in -2 log likelihood, 5.9; $\chi^2_{(1)}$; $P < .02$).

At the time of grade 4 LEE diagnosis, 15 (43%) of the 35 patients had a chronic HBV infection, 12 (34%) had a chronic HCV infection, 25 (71%) had a chronic HBC and/or HCV infection, 12 (34%) had recently started using ritonavir, 5 (14%) had recently started nevirapine, 13 (37%) were women, and 5 (14%) had recently discontinued the use of 3TC while a chronic HBV infection was present. Nineteen of the 35 patients had not been pretreated with an NRTI before they began their first potent antiretroviral combination regimen: 13 (68%) of these 19 were still using their first antiretroviral regimen at the time of the grade 4 LEE diagnosis. When the risk factors were combined, 34 (97%) of the 35 patients had ≥ 1 of these risk factors present at the time of the grade 4 LEE diagnosis. Table 4 lists the incidence rates per 100 person-years of follow-up for each binary covariate of the multivariate model and for patients having 0, 1, 2, 3, or 4 of these risk factors.

A high proportion (75%) of patients with grade 4 hepatotoxicity who were using ritonavir also had chronic viral hepatitis. Therefore, we investigated whether ritonavir would pref-

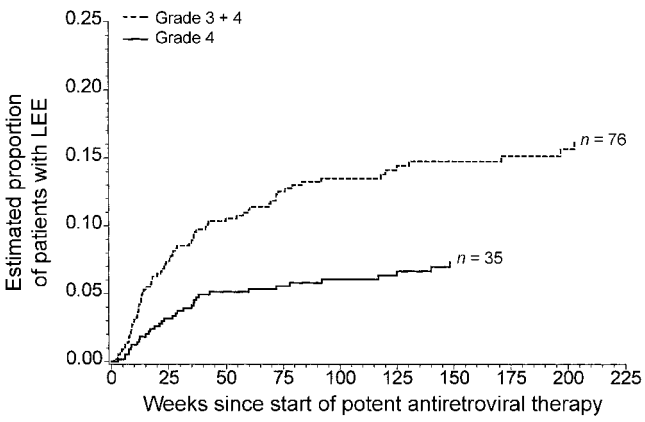

Figure 1. Kaplan-Meier estimate of time to development of grade 3+4 and grade 4 liver enzyme elevations (LEEs).

Table 3. Univariate and multivariate Cox proportional hazards analyses of risk factors for grade 4 and grade 3+4 liver enzyme elevations (LEEs).

	Univariate analysis			Multivariate analysis					
	Grade 4 LEEs			Grade 4 LEEs			Grade 3+4 LEEs		
Risk factor	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Female sex	2.3	1.2–4.6	.017	2.8	1.3–5.8	.007			
Injection drug use[a]	3.1	1.3–7.4	.013						
Baseline ALT level[b]	1.08	1.04–1.13	.0004	1.05	1.01–1.11	.030	1.03	0.99–1.07	.11
Chronic HCV infection	4.9	2.4–9.9	<.0001	5.0	2.3–10.7	<.0001	3.2	1.8–8.3	<.0001
Chronic HBV infection	9.6	4.9–18.8	<.0001	9.2	4.1–20.6	<.0001	4.6	2.6–8.3	<.0001
Recent discontinuation of 3TC[c]	25.9	9.6–70.2	<.0001	6.8	2.1–22.7	.0018	3.5	1.3–5.6	.012
Recent start of NVP[d]	5.3	2.0–14.2	.001	9.6	3.2–28.3	<.0001	2.8	1.08–7.2	.035
Recent start of RTV[d]	4.5	2.0–10.2	.0003	4.9	2.0–12.1	.0007			
Recent start of SQV[d]	3.3	1.3–8.3	.013						
Use of IDV[e]	0.38	0.15–0.97	.043						
Use of RTV[e]	2.0	1.03–3.9	.040				1.9	1.2–2.9	.0084
First-line antiretroviral therapy[f]				2.8	1.2– 6.4	.014			

NOTE. 3TC, lamivudine; ALT, alanine aminotransferase; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; HR, hazard ratio; IDV, indinavir; NVP, nevirapine; RTV, ritonavir; SQV, saquinavir.

[a] Injection drug use as risk factor for human immunodeficiency virus infection.

[b] Per 10 U/L increase.

[c] Sixteen-week period after discontinuation of 3TC in patients with chronic HBV infection.

[d] Twelve-week period after start of that particular drug.

[e] Whole treatment episode.

[f] Patients without prior nucleoside-analogue reverse-transcriptase treatment using first-line antiretroviral combination therapy vs. subsequent regimens.

erentially increase the risk for developing grade 4 hepatotoxicity in patients with chronic viral hepatitis. We fitted an interaction term between ritonavir and chronic viral hepatitis in the multivariate Cox model, but the fit of the model did not improve, indicating that the incidence of ritonavir-associated grade 4 LEE is not increased by the presence of chronic viral hepatitis. Only 1 of the 5 patients who developed grade 4 LEE shortly after starting nevirapine had a chronic viral hepatitis (HCV). Because of these relatively low numbers, there was insufficient statistical power to fit an interaction term between nevirapine and chronic viral hepatitis into the multivariate Cox model.

To investigate whether the use of low-dose ritonavir carries the same risk for developing grade 4 hepatotoxicity, we classified the use of ritonavir as either low dose ($\leqslant$200 mg twice daily) or high dose ($\geqslant$300 mg twice daily) and fitted these variables into the multivariate Cox model. The use of low-dose ritonavir was not associated with the development of grade 4 hepatotoxicity (no events occurred in the low-dose group). The HR of high-dose ritonavir was 5.8 (95% confidence interval [CI], 2.3–14.4; $P = .0002$).

The combined use of nevirapine with the NRTI stavudine (d4T) was implicated in the development of severe hepatotoxicity in one study [33]. To investigate a possible interaction between nevirapine and d4T in the development of LEE, we determined the incidence of grade 3 or 4 LEE during the first 6 months following the start of nevirapine. Data were right censored in case nevirapine was stopped within 6 months. Among the 205 patients who ever used nevirapine, nevirapine was combined with d4T in 104 patients and with zidovudine in 69; 32 patients did not use d4T or zidovudine. LEE occurred in 5 of 104 patients who used d4T, 2 of 69 patients using zidovudine, and 0 of 32 patients who used neither d4T nor zidovudine. By comparing the incidence of LEE in the d4T group with that in the zidovudine group, we found an odds ratio of 1.7 (95% CI, 0.3–9.0).

To investigate the dynamics of plasma HBV DNA concentrations in patients for whom the occurrence of grade 4 LEE was temporarily associated with the discontinuation of 3TC, we measured plasma HBV DNA concentrations in stored plasma samples (figure 2). Three of the 5 patients had low or intermediate plasma HBV DNA concentrations at the moment they stopped using 3TC, and HBV DNA levels increased strongly after the discontinuation of 3TC. The remaining 2 patients already had high concentrations of plasma HBV DNA when they stopped 3TC. In both of them, the HBV DNA declined to much lower concentrations after the grade 4 LEE improved. We randomly selected 5 HBsAg-positive control subjects who did not develop LEE shortly after stopping 3TC. Three of the 5 control subjects had undetectable plasma HBV DNA concentrations (<200 copies/mL) at the time they discontinued the use of 3TC, and 2 control subjects had high concentrations. The plasma HBV DNA concentrations remained stable in 4 of 5 control subjects. One of the control subjects with undetectable HBV DNA showed a strong increase

Table 4. Incidence rates of grade 4 liver enzyme elevations (LEEs) per 100 person-years of follow-up for selected risk factors.

Risk factor	No. of grade 4 LEEs	Total person-years of follow-up	Incidence rate/ 100 person-years of follow-up
Sex			
Male	22	1208	1.82
Female	13	276	4.71
Chronic HCV infection			
No	23	1362	1.69
Yes	12	122	9.84
Chronic HBV infection			
No	20	1379	1.45
Yes	15	105	14.3
Recent discontinuation of 3TC[a]			
No	10	99	10.1
Yes	5	6.1	81.6
Recent start of NVP[b]			
No	30	1435	2.09
Yes	5	49.1	10.2
Recent start of RTV[b]			
No	23	1378	1.67
Yes	12	106	11.3
First-line antiretroviral therapy[c]			
No	6	434	1.38
Yes	13	265	4.91
No. of risk factors present			
0	1	782	0.13
1	6	508	1.18
2	16	166	9.64
3	11	26.2	42.0
4	1	2.65	37.7
Total	35	1485	2.36

NOTE. 3TC, lamivudine; HBV, hepatitis B virus; HCV, hepatitis C virus; NVP, nevirapine; RTV, ritonavir.

[a] Sixteen-week period after discontinuation of 3TC in patients with chronic HBV infection.

[b] Twelve-week period after start of that particular drug.

[c] Patients without prior nucleoside-analogue reverse-transcriptase inhibitor treatment using first-line antiretroviral combination therapy vs. subsequent regimens.

in HBV DNA but did not develop LEE. None of the patients or control subjects had restarted 3TC at the time points used for this analysis.

LEEs of grade 3 or higher. We defined grade 3+4 LEE as cases in which AST and/or ALT values were ⩾5 times the ULN. Of the 560 patients, 95 (17%) developed grade 3+4 LEE. The medical records of these patients showed an obvious cause of the grade 3 LEE for 17 patients. Nine of the 17 also met the criteria for grade 4 LEE and were described above. Two patients were treated with intravenous chemotherapy for pulmonary or visceral Kaposi sarcoma, 2 developed toxic hepatitis after the start of combination therapy for *Mycobacterium avium* infection, 1 was treated with interleukin-2 and the murine monoclonal antibody OKT3, 1 had an itraconazole intoxication, 1 had severe myopathy, and 1 had pancreatitis. These 17 patients were excluded from the analysis, leaving 76 patients (14%; figure 1).

We repeated the multivariate Cox analysis by using the occurrence of grade 3+4 LEE as a dependent variable. The results were different from the analysis of grade 4 LEE alone (table 3). Chronic viral hepatitis, the discontinuation of 3TC in patients coinfected with HBV, and the recent start of nevirapine were again found to be risk factors for grade 3+4 LEE. The covariate "use of ritonavir" better fitted the data than the "recent start of ritonavir" covariate. Female sex was only weakly associated with the occurrence of grade 3+4 LEE (HR, 1.6; 95% CI, 0.91–2.7; $P = .11$. Antiretroviral therapy–naive patients did not have a higher risk for developing grade 3+4 LEE when they were receiving their first antiretroviral combination regimen. No additional risk factors were identified.

Discussion

In this patient population, we observed an incidence of grade 4 hepatotoxicity of 7.9%. Forty-four events occurred during 1548 patient-years of follow-up. A high proportion (20%) of these events had clearly identifiable causes other than antiretroviral drug–related toxicity, of which acute HAV infection was the most common. Therefore, the incidence of "idiopathic" grade 4 hepatotoxicity, possibly related to the use of antiretroviral drugs, was 6.3%. Most of these events were not accom-

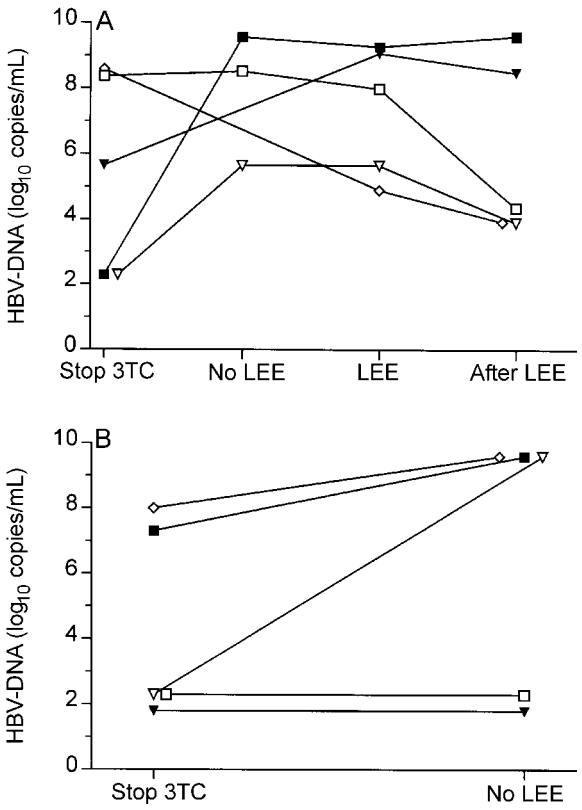

Figure 2. Changes in plasma hepatitis B virus (HBV) DNA concentrations in patients with chronic HBV infections who develop *(top)* or do not develop *(bottom)* grade 4 liver enzyme elevations (LEEs) after discontinuation of lamivudine (3TC). "Stop 3TC," time point just before discontinuation of 3TC; "No LEE," no LEEs present; "After LEE," LEEs have improved to grade 2 or lower.

panied by clinical symptoms. No patient died as a direct result of the grade 4 hepatotoxicity. Only 2 patients, one coinfected with HBV and the other with HCV, developed progressive liver disease after 1 and 2 years, respectively. For two-thirds of the patients, the antiretroviral regimen was not changed during the episode of grade 4 LEE. The grade 4 LEE improved in all patients, regardless of whether patients continued or stopped/modified their antiretroviral regimen. Risk factors for developing grade 4 LEE were higher baseline ALT levels, chronic viral hepatitis, use of their first potent antiretroviral combination regimen by antiretroviral therapy-naive patients, recent start of nevirapine or high-dose ritonavir, and female sex. In patients coinfected with HBV, discontinuing the use of 3TC was also a risk factor. In 34 (97%) of 35 patients, at least 1 of these risk factors was present at the time the grade 4 LEE was diagnosed.

Our finding that the recent introduction of ritonavir or nevirapine into an antiretroviral regimen is a risk factor for grade 4 LEE confirms several reports [5, 9, 34, 35]. Even though the HRs are quite high (ritonavir, 4.9; nevirapine, 9.6), the crude absolute risks, unadjusted for other risk factors, are low (ritonavir, 3.2%; 95% CI, 1.7%–5.6%; nevirapine, 2.4%; 95% CI, 0.8%–5.6%). The grade 4 LEE associated with the use of ritonavir seems to be the result of a direct toxic effect of the drug, because there was no interaction between the use of ritonavir and the presence of chronic viral hepatitis, and the use of low-dose ritonavir was not associated with the occurrence of grade 4 LEE. All grade 4 LEE associated with the use of nevirapine improved, even though 4 of these 5 patients did not interrupt the use of nevirapine. Just 1 patient had a concurrent rash. This makes the hypothesis that nevirapine-associated grade 4 LEEs are part of a hypersensitivity reaction less plausible, arguing more in favor of the hypothesis that these LEEs are caused by a direct toxic effect of the nevirapine itself [36]. Because few patients were using other NNRTIs (e.g., efavirenz), we could not investigate whether this is an effect specific to nevirapine or a class effect [37]. We found no evidence for the hypothesis that combining nevirapine with d4T results in a higher incidence of LEE than the combination of nevirapine with zidovudine, but our statistical power to detect a difference was low [33].

Patients chronically infected with HBV who discontinue the use of 3TC are prone to developing grade 4 LEE (incidence, 22%; 95% CI, 7%–44%), and the withdrawal of 3TC can result in increased HBV replication [32]. Furthermore, the use of potent antiretroviral combination therapy may result in an improved cell-mediated immunity to HBV antigens. Together, increased HBV replication and improved immunity may result in immune-mediated hepatocellular damage. The observed dynamics of the plasma HBV DNA concentrations after the discontinuation of 3TC in this subset of patients suggest an etiologic role of HBV in the development of their grade 4 LEEs.

We did not find an association between the occurrence of grade 4 LEE and antiretroviral agents other than ritonavir, nevirapine, and 3TC. However, because zalcitabine, abacavir, saquinavir, amprenavir, lopinavir, efavirenz, and hydrea were used by <10% of all patients, our results should not be interpreted as evidence of absence of hepatotoxic effects of these agents.

The use of first-line potent antiretroviral combination regimens, compared with subsequent regimens, was associated with a higher risk for developing grade 4 LEE in antiretroviral therapy–naive patients but not in patients with prior exposure to NRTIs. It is hypothesized that at least some grade 4 LEEs result from immune reconstitution [5, 38]. If this hypothesis is true, a possible explanation for the higher risk in antiretroviral therapy–naive patients might be that these patients experience the strongest immune reconstitution. In the pretreated patients, the prior use of NRTIs may have already resulted in some increases in CD4 cells, making the immune reconstitution following the start of first-line potent antiretroviral combination regimens less pronounced. After 12 weeks of follow-up, the median increase in CD4 cell counts was higher in the therapy-naive group than in the pretreated group: 130 (IQR, 50–210) versus 70 CD4 cells (IQR, 20–140), respectively; $P < .0001$. Neither absolute CD4 cell counts nor changes in CD4 cell counts were associated with the occurrence of grade 4 LEE in the Cox analysis, but these crude measures might not accurately reflect changes in specific immune function. Baseline plasma HIV-1 RNA levels or changes in these levels after the start of therapy were also not significant risk factors. Another possible explanation for the higher risk in antiretroviral therapy–naive patients might be a selection bias: Pretreated patients predisposed to LEE may have developed LEE previously while receiving NRTIs alone, and the offending agents may have been discontinued.

Women had a higher risk for developing grade 4 LEE than men (HR, 2.6). To our knowledge, this has been published before only once, in the FTC-302 study [33]. The reason why this association was not found in other cohort studies, including our previous analysis of a part of this data set [3], might be that all of these cohort studies included mild cases in their definitions of LEE. In accordance with this, the sex difference was much weaker in our current study when we considered grade 3+4 LEE . Why women have a higher incidence of grade 4 LEE than men is unclear. We did not find evidence that the effect of sex is caused by the lower body weight of women.

The risk profile observed for grade 4 LEE could not be completely reproduced when we combined grade 3 and 4 events. As discussed above, the effect of sex became less pronounced. The inclusion in the multivariate model of the covariate "use of ritonavir for the whole treatment period" produced a better fit to the data than the covariate "recent start of ritonavir use." The effect of using first-line antiretroviral therapy in therapy-naive patients was not associated with grade 3+4 LEE, suggesting that at least some of the grade 4 LEEs have an etiology different from that of grade 3 LEEs. Another explanation might

be that the significance of the "first-line" covariate in the grade 4 analysis was a chance result, but this issue can be solved only by validation of our results in other data sets.

We conclude that the use of potent antiretroviral combination therapy results in a high incidence of grade 4 LEE. Patients should be carefully evaluated for the presence of treatable causes of the LEE. Most cases are asymptomatic and resolve whether antiretroviral therapy is modified or not. Therefore, the occurrence of grade 4 LEE does not automatically necessitate the discontinuation of all or some antiretroviral drugs; instead, a more expectative approach might be warranted. However, in cases of grade 4 LEE associated with the recent start of nevirapine, we recommend a vigilant approach, because nevirapine has been linked to several fatal cases of hepatotoxicity [33]. Furthermore, hepatic steatosis with lactic acidosis should be ruled out as a possible cause for the grade 4 LEE. Unfavorable long-term outcome is associated with the presence of a chronic viral hepatitis. Patients coinfected with HBV who are using 3TC should consider continuing the use of 3TC, even if they develop 3TC-resistant HIV strains.

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