Prevention and Treatment of Hepatitis B Virus Infection in HIV-infected Patients

T.E.M.S. de Vries-Sluijs

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Prevention and Treatment of Hepatitis B Virus Infection in HIV-Infected Patients

Preventie en behandeling van hepatitis B virus infectie
in HIV geïnfecteerde patiënten

Proefschrift

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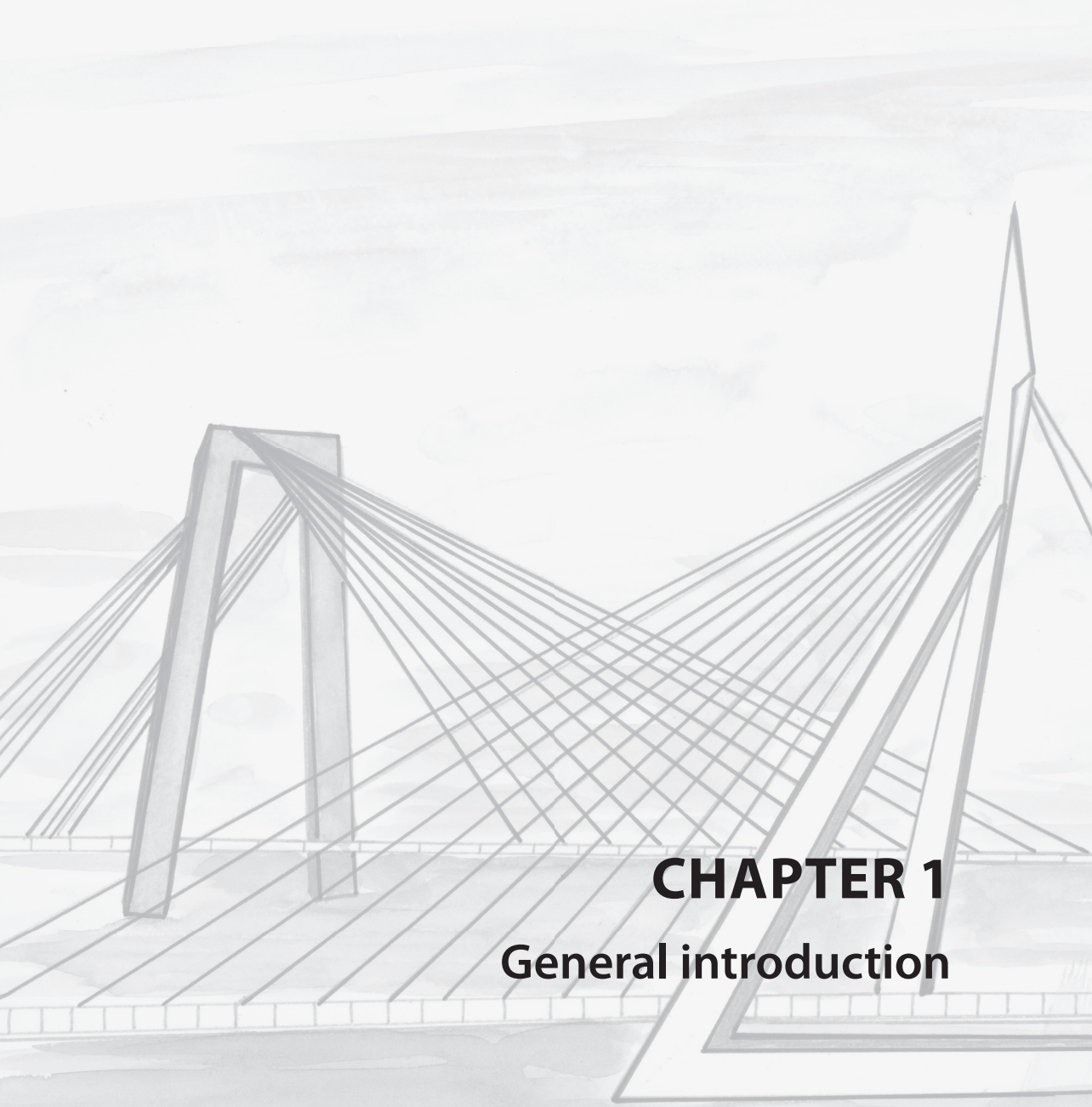
"A person is a person through other persons"
Desmond Tutu

Contents



CHAPTER 1

General introduction

General introduction

Hepatitis B Virus (HBV) infection in Human Immunodeficiency Virus (HIV) infected patients is an increasing problem. Both viruses are blood-borne pathogens transmitted through similar routes. With the development of highly active antiretroviral therapy (HAART) and consequently with an improvement in survival rates, liver-related disease has become a leading cause of mortality in HIV-infected patients and liver-related death has become the most frequent cause of non-AIDS related death [1-2].

HIV-infection

Infection with HIV and its end stage, acquired immunodeficiency syndrome (AIDS), is the major public health challenge of modern times, with over 25 million persons already deceased and worldwide an estimated 33 million people living with HIV/AIDS, the majority of whom are without access to therapy [3]. HIV is a member of the retrovirus family and can only replicate inside human cells. The first step in the infection of the cell by HIV is its binding to the target cell receptor, $CD4^+$ T-cell. After binding to a cell, HIV enters the target cell by a fusion process. At this stage, the virus loses its envelope-coat and RNA is released into the cytoplasm. Retroviruses use as a replication strategy the transcription of viral RNA into linear double-stranded DNA. The characteristic enzyme used for this process is called reverse transcriptase. The viral DNA is transported to the nucleus, where it is spliced into the host genome by the HIV enzyme integrase. After converting to messenger RNA (mRNA), the mRNA is transported outside the nucleus. Among these produced strands of mRNA are complete copies of HIV genetic material. This forms new viral particles together with HIV proteins and enzymes and are then released from the cell. The protease enzyme plays an important role at this stage of the HIV life cycle by cutting long protein strands into smaller parts, which are used to form mature viral particles.

During acute infection, viral replication occurs at an extremely rapid rate, producing the highest level of circulating virus observed at any time during infection [4]. According to the Centers of Disease Control and Prevention (CDC) classification HIV infection is divided into the following stages: viral transmission, primary HIV infection or acute HIV infection, seroconversion, clinical latent period, early symptomatic HIV infection, AIDS and advanced HIV infection characterized by a $CD4^+$-cell count < 50 cells/mm^3.

Antiretroviral drugs were introduced in 1987. Development of antiviral therapy has concentrated on inhibition of viral specific enzymes. The first enzyme targeted was HIV reverse transcriptase and both nucleoside analogs and non-nucleoside compounds have been found to block the enzyme. In 1987 the first nucleoside reverse transcriptase inhibitor (NRTI), zidovudine (AZT) was approved by the FDA and introduced as treatment for HIV. This was followed by duo therapy with 2 NRTI's for example Combivir, consisting of AZT and lamivudine (LAM). The second enzyme to be targeted was the HIV-specific protease. Protease inhibitors

(PI) obstruct the cleavage of the HIV polyprotein and prohibit the virus to assembly. After the introduction of PI, HAART was established as triple therapy including 2 NRTI's and 1 non-nucleoside reverse transcriptase inhibitor (NNRTI) or PI. After introduction of HAART, mortality and morbidity due to HIV decreased substantially and the reported decline proved to be consistent in developed countries [5] [6]. Research for new treatment modalities resulted in the finding of other drug classes, fusion inhibitors, integrase inhibitors and CCR5-receptor blockers.

Fusion inhibitors interact with components of the HIV envelope and prevent fusion of the virus with the cell membrane of the host. The integrase enzyme is essential for viral replication. Integrase inserts viral DNA into the cellular genome. Integrase inhibitors disrupt the viral life cycle and disturb viral replication. Final class of antiretroviral drugs is the CCR5-receptor blocker. CCR5 antagonists apply their antiretroviral activity against HIV by blocking entry of CCR5-tropic viruses into the $CD4^+$ T-cell.

HBV-infection

HBV infection is a global public health problem. It is estimated that there are more than 350 million HBV carriers in the world [7] of whom approximately 1 million die annually from HBV-related liver disease. Blumberg et al. discovered in the blood of an Australian aboriginal a previously unknown antigen (Australia antigen) and after several years this was found to be related to the parentally transmitted type B hepatitis [8]. HBV is one of the smallest human viruses known and belongs to the hepadnaviridae family. The life cycle of HBV is complex and is believed to occur preferentially in the hepatocyte. The HBV genome is a circular partially double-stranded DNA. After entry into the hepatocyte, the HBV-DNA is transported to the nucleus and converted to covalently closed circular DNA (cccDNA), which serves as the template for transcription of mRNA. The mRNA is transported to the cytoplasm where it codes for production of viral proteins. Out of these viral proteins and viral DNA in the cytoplasm of the hepatocyte new HBV-particles are assembled, that subsequently leave the cell into the circulation.

During the acute phase in adults the clinical manifestations range from subclinical or anicteric hepatitis (approximately 70%) to icteric hepatitis in 30% of the cases. During the chronic phase, manifestations range from an inactive carrier state to chronic hepatitis, cirrhosis and hepatocellular carcinoma. Acute infection will resolve spontaneously in 90% of the immunocompetent adults. The risk of a chronic HBV infection is highly dependent on the age at infection and the immune status of the patient. The infection rate among infants born to hepatitis B e antigen (HBeAg)-positive mothers is as high as 90% [9]. The initial phase in perinatally acquired HBV infection is characterized by high levels of HBV replication but no evidence of active liver disease is presented. The lack of liver disease despite high levels of HBV replication is believed to be due to immune tolerance to HBV. This immune tolerance

phase usually lasts 10 to 30 years. Transition to the immune clearance phase occurs during the second and third decade of life.

Two major types of antiviral drugs are being used for the treatment of chronic HBV mono-infection: drugs that modulate the HBV-specific immune response and drugs that directly interfere with virus replication (NRTI). Immunomodulatory drugs are interferon (IFN) and pegylated interferon (PEG-IFN). IFN was licensed for treatment in the early 1990s and in 2001 PEG-IFN was available, which resulted in simplification of the treatment regimen. In 1995 NRTI's were introduced, at first LAM, followed by adefovir (ADV) in 2002, entecavir (ETV) in 2005 and tenofovir (TDF) in 2008.

HIV/HBV co-infection

Among the estimated 33 million persons infected with HIV worldwide, an estimated 2-4 million are chronically infected with HBV [10]. Several factors influence co-infection, including geographic differences in the prevalence of chronic infection by age, the transmission route and the prevalence of persons at high risk for infection. For example, in sub-Saharan Africa high prevalence of chronic HBV is found among the adolescent and adult population at risk for sexually-acquired HIV, because of high HBV transmission perinatal and in early childhood. In the Western world low prevalence of chronic HBV is found because acute infections are acquired by adults who are less likely to develop chronic HBV infection. In contrast, in men having sex with men (MSM) the prevalence of chronic HBV infection is high, resulting in an estimated prevalence of HIV/HBV co-infection of 6-14% [11-15]. In the Netherlands the overall hepatitis B surface antigen (HBsAg) prevalence is estimated to be 0.3-0.5% [16] and in MSM between 1.9-6.3% [17-19]. In 2009 the prevalence of HBV among HIV-infected patients was estimated to be 8% in the Netherlands [20].

HIV infection is associated with a reduced clearance of HBsAg and HBeAg and a higher level of HBV replication [21-23]. In HIV/HBV co-infected patients the course of chronic liver disease is accelerated and patients show an up to 14-fold greater liver-related mortality, due to liver cirrhosis and hepatocellular carcinoma than patients infected with HBV alone [15, 21-22, 24-25].

The most prescribed NRTI combination from 1995-2001 was Combivir. This combination (including LAM) was very effective for HIV, but less for HBV treatment. Prolonged LAM-therapy, suppressing both HIV and HBV replication, has been identified as the major risk for the development of HBV resistance. Mutations typically occur in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of the catalytic domain of the polymerase gene of the hepatitis B virus. HIV/HBV co-infected individuals develop this YMDD mutation at a rate of 20% annually, with rates of 90% after 4 years of treatment. These rates are higher compared to resistance rates in mono-infected HBV patients [26-27]. In 2001 TDF, an acyclic nucleotide analogue reverse transcriptase inhibitor was licensed for treatment of HIV infection and in 2004 Combivir was largely replaced by Truvada, consisting of emtricitabine and TDF. The zid-

ovudine in Combivir had several side effects, such as anemia and lipodystrophy and a short half-life, requiring a twice daily dosing regimen. Both components of Truvada have a long half-life, which allows a once daily dosing schedule. In HIV/HBV co-infected patients the most important feature of TDF is activity in *in vitro* and *in vivo* studies against both wild type and LAM resistant HBV [28-29]. Moreover, TDF has a good resistance profile, and no convincing proof of HBV-resistant mutants to TDF has been presented so far [30].

As stated before liver complications and liver-related mortality due to chronic HBV in HIV-infected patients are high. Furthermore, among several HIV-infected risk groups the prevalence of acute and chronic HBV is high. Primary prevention of hepatitis B can be pursued by vaccination and is of great importance in HIV-infected individuals. However, compared to immunocompetent individuals a large proportion of HIV-infected patients (40-76% versus <10%) fail to respond to standard dose HBV vaccination schedules [31-33].

The aims of this thesis are:

Prevention of HBV infection in HIV-infected patients:

To compare the feasibility, compliance and effectiveness of an accelerated hepatitis B vaccination schedule compared to the standard vaccination regimen in a heterogeneous HIV-infected population.

To assess influenza immunization and hepatitis B vaccination responses in a cohort of HIV-infected patients.

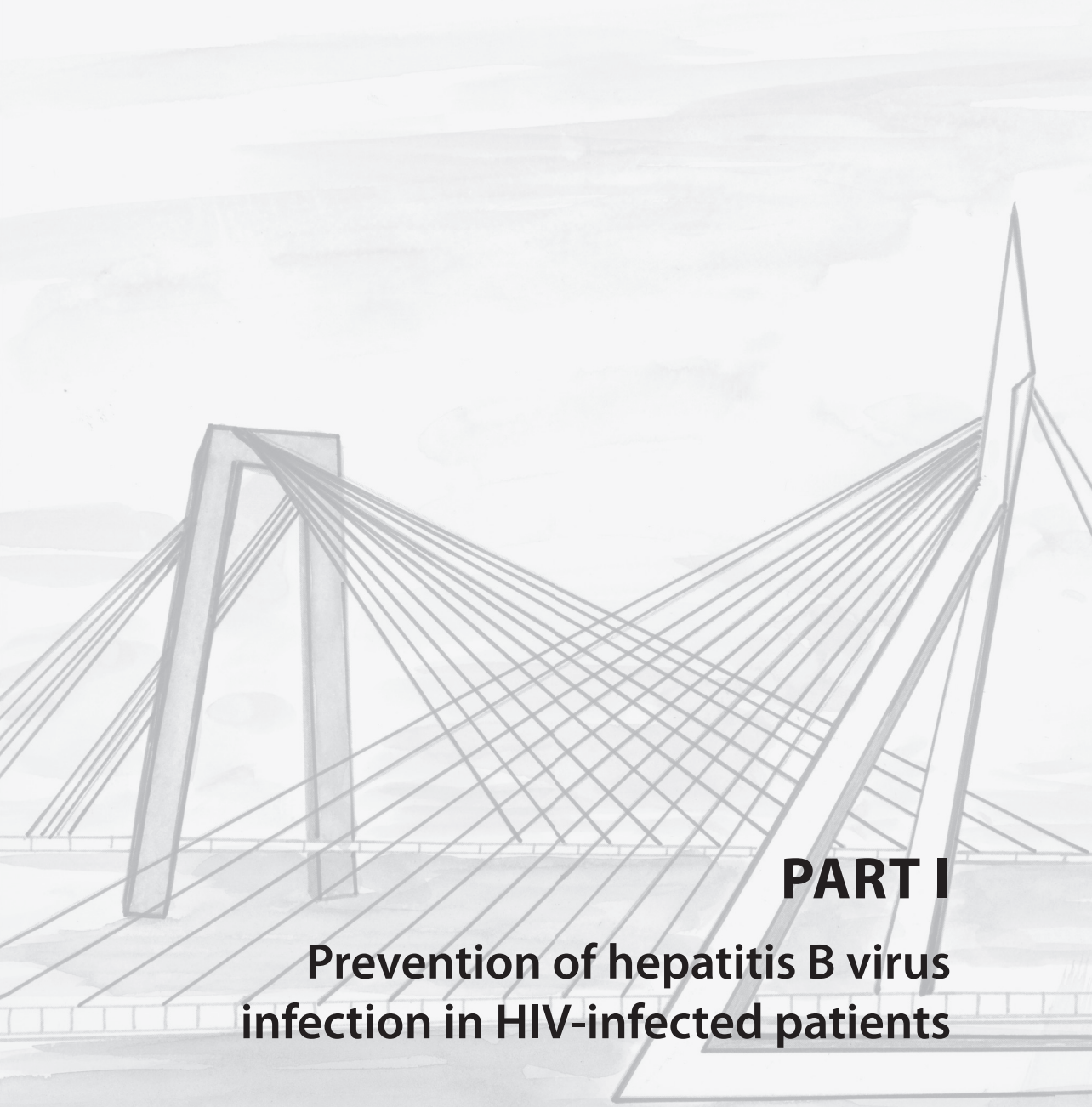
Treatment of HBV infection in HIV-infected patients:

To investigate viral kinetics, long-term efficacy and safety of tenofovir based treatment as part of antiretroviral therapy in a large cohort of co-infected patients.

References

1. Bica I, McGovern B, Dhar R, et al. Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. Clin Infect Dis 2001;32:492-7
2. Weber R, Sabin CA, Friis-Moller N, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. Arch Intern Med 2006;166:1632-41
3. http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009_en.pdf.
4. Kahn JO, Walker BD. Acute human immunodeficiency virus type 1 infection. N Engl J Med 1998;339:33-9
5. Mocroft A, Ledergerber B, Katlama C, et al. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. Lancet 2003;362:22-9
6. Sterne JA, Hernan MA, Ledergerber B, et al. Long-term effectiveness of potent antiretroviral therapy in preventing AIDS and death: a prospective cohort study. Lancet 2005;366:378-84
7. http://www.who.int/mediacentre/factsheets/fs204/en/index.html.
8. Blumberg BS, Alter HJ and Visnich S. A "New" Antigen in Leukemia Sera. JAMA 1965;191:541-6
9. Stevens CE, Beasley RP, Tsui J and Lee WC. Vertical transmission of hepatitis B antigen in Taiwan. N Engl J Med 1975;292:771-4
10. Alter MJ. Epidemiology of viral hepatitis and HIV co-infection. J Hepatol 2006;44:S6-9
11. Chun HM, Fieberg AM, Hullsiek KH, et al. Epidemiology of Hepatitis B virus infection in a US cohort of HIV-infected individuals during the past 20 years. Clin Infect Dis 2010;50:426-36
12. Denis F, Adjide CC, Rogez S, Delpeyroux C, Rogez JP and Weinbreck P. [Seroprevalence of HBV, HCV and HDV hepatitis markers in 500 patients infected with the human immunodeficiency virus] Seroprevalence des marqueurs des virus des hepatites B, C et D chez 500 patients infectes par le virus de l'immunodeficience humaine. Pathol Biol (Paris) 1997;45:701-8
13. Konopnicki D, Mocroft A, de Wit S, et al. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. AIDS 2005;19:593-601
14. Spradling PR, Richardson JT, Buchacz K, Moorman AC, Brooks JT and the HIVOSI. Prevalence of chronic hepatitis B virus infection among patients in the HIV Outpatient Study, 1996-2007. J Viral Hepat 2010
15. Thio CL, Seaberg EC, Skolasky R, Jr., et al. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). Lancet 2002;360:1921-6
16. Marschall T, Kretzschmar M, Mangen MJ and Schalm S. High impact of migration on the prevalence of chronic hepatitis B in the Netherlands. Eur J Gastroenterol Hepatol 2008;20:1214-25
17. Bleeker A, Coutinho RA, Bakker-Kok J, Tio D and de Koning GA. Prevalence of syphilis and hepatitis B among homosexual men in two saunas in Amsterdam. Br J Vener Dis 1981;57:196-9
18. Coutinho RA, Schut BJ, Albrecht-Van Lent NA, Reerink-Brongers EE and Jesdijk LS. Hepatitis B among homosexual men in The Netherlands. Sex Transm Dis 1981;8:333-5
19. http://rivm.openrepository.com/rivm/bitstream/10029/7356/1/441100024.pdf.
20. http://www.hiv-monitoring.nl/_site1134/images/091250-HIVM-2009-KL.pdf.
21. Colin JF, Cazals-Hatem D, Loriot MA, et al. Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. Hepatology 1999;29:1306-10
22. Gilson RJ, Hawkins AE, Beecham MR, et al. Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. Aids 1997;11:597-606

23. Hadler SC, Judson FN, O'Malley PM, et al. Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. J Infect Dis 1991;163:454-9
24. Sheng WH, Chen MY, Hsieh SM, et al. Impact of chronic hepatitis B virus (HBV) infection on outcomes of patients infected with HIV in an area where HBV infection is hyperendemic. Clin Infect Dis 2004;38:1471-7
25. Bonacini M, Louie S, Bzowej N and Wohl AR. Survival in patients with HIV infection and viral hepatitis B or C: a cohort study. AIDS 2004;18:2039-45
26. Leung NW, Lai CL, Chang TT, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001;33:1527-32
27. Wolters LM, Niesters HG, Hansen BE, et al. Development of hepatitis B virus resistance for lamivudine in chronic hepatitis B patients co-infected with the human immunodeficiency virus in a Dutch cohort. J Clin Virol 2002;24:173-81
28. Benhamou Y, Tubiana R and Thibault V. Tenofovir disoproxil fumarate in patients with HIV and lamivudine-resistant hepatitis B virus. N Engl J Med 2003;348:177-8
29. van Bommel F, Wunsche T, Schurmann D and Berg T. Tenofovir treatment in patients with lamivudine-resistant hepatitis B mutants strongly affects viral replication. Hepatology 2002;36:507-8
30. Snow A CB, Curtis M, Zhu Y, Heathcote EJ, Marcellin P, Borroto-Esoda K. Week 96 resistance surveillance for HBeAg-positive and negative subjects with chronic HBV infection randomized to receive tenofovir DF 300mg QD. Hepatology 2008;48:A977
31. Collier AC, Corey L, Murphy VL and Handsfield HH. Antibody to human immunodeficiency virus (HIV) and suboptimal response to hepatitis B vaccination. Ann Intern Med 1988;109:101-5
32. Cornejo-Juarez P, Volkow-Fernandez P, Escobedo-Lopez K, Vilar-Compte D, Ruiz-Palacios G and Soto-Ramirez LE. Randomized controlled trial of Hepatitis B virus vaccine in HIV-1-infected patients comparing two different doses. AIDS Res Ther 2006;3:9
33. Keet IP, van Doornum G, Safary A and Coutinho RA. Insufficient response to hepatitis B vaccination in HIV-positive homosexual men. Aids 1992;6:509-10



PART I

Prevention of hepatitis B virus infection in HIV-infected patients

CHAPTER 2

A randomized controlled study of accelerated versus standard hepatitis B vaccination in HIV positive patients

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Abstract

Background. In HIV-infected patients the immunogenicity of hepatitis B vaccines is impaired. The primary and secondary aims of our study were to investigate the effectiveness and compliance of an accelerated versus standard vaccination regimen in a HIV-infected population.

Methods. A non-inferiority trial with a response margin of 10% was designed. Included were patients ≥18 years old, with negative HBsAg and anti-HBc serology and not previously vaccinated against hepatitis B. Patients were stratified according to $CD4^+$-cell count: <200,200-500,>500. Participants received 10 μg HBvaxPRO® intramuscularly according to a 0-1-3 weeks schedule or the standard 0-4-24 weeks schedule. Anti-HBs levels were measured at week 28, considered protective ≥10IU/L.

Results. Modified intention to treat analysis in 761 patients was performed. Overall response difference was 50% (standard arm) versus 38.7% (accelerated arm) =11.3% (95%CI [4.3-18.3]), close to the response margin of 10%. However, the response difference in patients with a $CD4^+$-cell count > 500 cells/mm^3 was -1.8% (95%CI [-13.4-9.7]). Compliance was significantly superior with the accelerated schedule, 91.8% versus 82.7% ($p \leq 0.001$).

Conclusion. In HIV-infected patients compliance with an accelerated hepatitis B vaccination schedule is significantly better. The efficacy of an accelerated schedule proved to be non-inferior in patients with a $CD4^+$-cell count > 500 cells/mm^3.

Introduction

Safe and effective hepatitis B vaccines have been commercially available since 1982. Human immunodeficiency virus (HIV)-infected patients carry a high risk of contracting hepatitis B virus (HBV). The response to hepatitis B vaccines in HIV-infected patients is however impaired. Trials in HIV-infected patients in the pre- and post highly active antiretroviral therapy (HAART) era have yielded response rates between 17% and 72% [1-8]. Response rate depended on various factors including $CD4^+$-cell count, HIV viral load and dosing schedule.

Optimal compliance to the vaccination schedule is essential to achieve effective seroprotection against HBV. However, poor adherence to the standard immunization schedule (0, 1, 6 months) is a matter of concern [9-12]. In healthy volunteers accelerated hepatitis B vaccination has proven to be effective [13-14]. We tested the hypothesis that in unselected HIV-infected individuals an accelerated immunization schedule could have a positive impact on both the patient's compliance and outcome of vaccination.

The present study was designed to evaluate the protective efficacy by measuring the antibody response to hepatitis B vaccine administered according to an accelerated immunization schedule in comparison to a standard schedule.

Patients and Methods

Patients

We performed a large Dutch multi-centre, parallel group, open label, randomized non-inferiority study. Participants were randomized to either an accelerated schedule (t=0, 1 and 3 weeks) or the standard schedule (t=0, 4 and 24 weeks). The primary endpoint was response measured by anti-hepatitis B surface antigen (anti-HBs) titer with a response margin of 10% difference. The secondary endpoint was comparison of the compliance between the two study arms. We offered HBV vaccination to all HIV positive patients treated in 12 hospitals in the Netherlands specialized in HIV treatment (Erasmus MC, Rotterdam; Haga Hospital, The Hague; Medical Center Haaglanden, The Hague; University Medical Center Utrecht, Utrecht; OLVG, Amsterdam; Maasstad Hospital, Rotterdam; St. Elisabeth Hospital, Tilburg; LUMC, Leiden; AZM, Maastricht; Rijnstate Hospital, Arnhem; VUMC, Amsterdam; Radboud Hospital, Nijmegen). Patients were included if they were ≥ 18 years old, with negative hepatitis B surface antigen (HBsAg) and anti-hepatitis B core (anti-HBc) serology, without active opportunistic infection, not pregnant at time of inclusion, and had not been previously vaccinated against hepatitis B. A randomization sequence was generated at the Erasmus MC by an independent investigator. Patients were stratified according to centre and their $CD4^+$-cell count, assessed within the last 6 months, into three groups, < 200, 200-500, and > 500 cells/mm^3. Patients were randomized in various block sizes. At each centre sequentially numbered, opaque, sealed envelopes with

the randomization arm were stored securely. Enrollment and assignment of participants were done by the trial nurse at each site during the medical visit at the outpatient ward.

Each patient received a total of three dosages of 10 μg of HBvaxPro® (Aventis Pasteur MSD) intramuscularly in the deltoid region. Patients in the accelerated group received a reminder for anti-HBs testing in the month prior to week 28. In the standard group patients received a reminder for the last vaccination in the month prior to week 24. During this visit they were notified of the anti-HBs testing 4 weeks later.

When patients discontinued the vaccination schedule and response was not measured they were excluded from the modified intention to treat (MITT) and the per protocol (PP) analyses.

The study protocol was approved by the local Medical Ethical Committee of all participating hospitals and written informed consent was obtained from all subjects prior to study entry.

Assessments

Anti-HBs levels were measured on week 5 (initial response) and 28 (long-term response) in the accelerated schedule and on week 28 in the standard schedule. Quantitative anti-HBs testing were performed by AxSym Ausab (Abbott Diagnostic Division, Wiesbaden Germany) and the protective level of anti-HBs was defined as a titer ≥ 10 IU/L. At the time of vaccination we collected data on age, gender, transmission route of HIV infection, country of birth, weight, nadir $CD4^+$-cell count, $CD4^+$-cell count, plasma HIV-RNA level and use of antiretroviral therapy. Undetectable viral load was defined as an HIV-RNA less than 50 copies/ml.

Statistics

Based on previous studies we expected 50% protection against hepatitis B after initial vaccination in HIV positive patients [1-8]. Sample size was calculated as 400 patients in each study arm to have a power of 80%, considering the accelerated group to be clinically non-inferior to the standard group if the difference in response rate between the two groups was less than 10%.

The differences in response at week 28 between groups were calculated together with the 95% confidence interval. Subgroup analyses were pre-specified for the $CD4^+$-cell count stratification groups. The results are reported and interpreted according to the CONSORT statement on non-inferiority trials [15]. In addition, multivariate analysis of the treatment outcome was performed with logistic regression analysis. The data analysis was performed using SPSS for Windows, release 15 and SAS 9.2.

The analysis was performed in the MITT population and repeated in the PP population. In the MITT population patients with 3 vaccinations and an anti-HBs titer as endpoint beyond the stringent protocol time frame were included. The PP population included patients from the accelerated arm with the three vaccinations at the scheduled time points: vaccination 2 +/- four days, vaccination 3 +/- seven days, anti-HBs titer week 28 +/- 28 days. The standard arm included patients with 3 vaccinations at the three time points: vaccination 2 +/- 7 days, vaccination 3 +/- 28 days, anti-HBs titer week 28 +/- 28 days.

Results

Patients

Between March 2004 and October 2007, 841 patients were randomized for the study and allocated to intervention into one of the study arms. Thirty patients were excluded from participation due to reasons depicted in Figure 1. Of the 811 patients receiving allocated intervention, 50 patients did not complete the study for various reasons and were excluded from analysis (Figure 1). In the MITT, 761 patients were analyzed and data of 569 patients were available for the PP analysis.

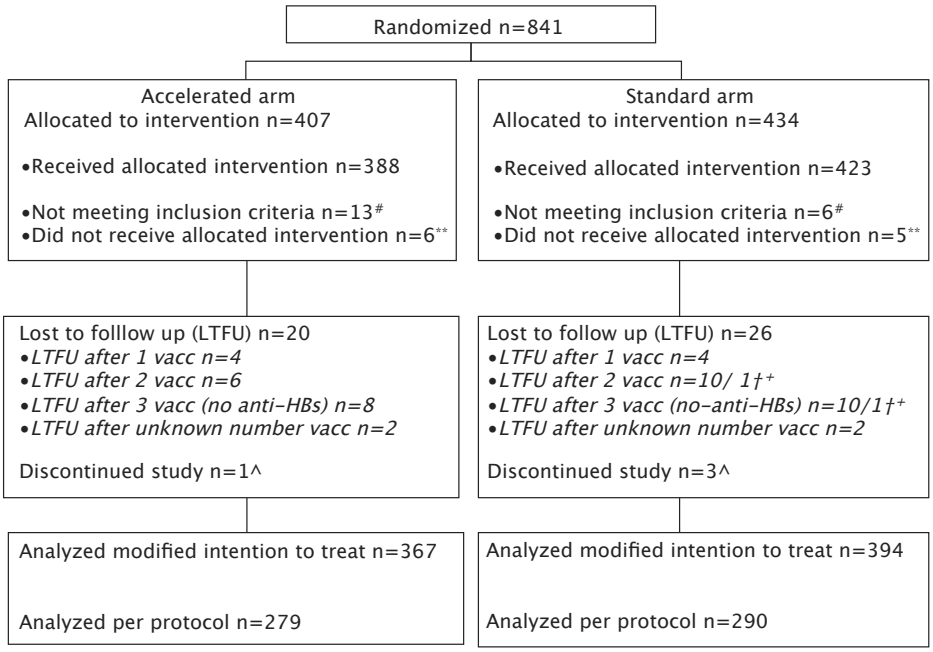

Figure 1. Flow chart patients included in study

#HBV serology of baseline sample performed at first vaccination proved positive (n = 13 in accelerated arm and n = 6 in standard arm)

**patients received no study medication (n = 4 in both study arms); patients withdrawal in n = 2 in accelerated arm en n = 1 (pregnant) in standard arm

+† = died

^ acute hepatitis B infection (n = 1 in accelerated arm and n = 2 in standard arm); allergic reaction (n = 1 in standard arm)

In the accelerated arm 407 patients were allocated to intervention and 388 patients received allocated intervention. In the standard arm 434 patients were allocated and 423 received the intervention. Patient characteristics in both groups were similar at baseline. Table 1 reports the distribution of age, gender, region of birth, body mass index, HIV risk,

start CD4$^+$-cell count, nadir CD4$^+$-cell count, HIV-RNA, usage and duration of HAART, Hepatitis A antibodies or Hepatitis C co-infection. The variation in body mass index was small and within the normal range.

Table 1. Baseline characteristics of study subjects with received allocated intervention

Variable	All N = 811	Accelerated schedule N = 388	Standard schedule N = 423
Age median (range)	40.0 yrs (19-77)	40.0 yrs (19-77)	40.0 yrs (19-73)
Male N (%)	547 (67.4)	252 (64.9)	295 (69.7)
Region[a] N (%)			
1	453 (55.9)	216 (55.7)	237 (56.0)
2	137 (16.9)	66 (17.0)	71 (16.8)
3	37 (4.6)	18 (4.6)	19 (4.5)
4	164 (20.2)	76 (19.6)	88 (20.8)
5	20 (2.5)	12 (3.1)	8 (1.9)
Body Mass Index N; median	725; 24.0	343; 23.8	382; 24.1
(25th-75th percentile)	(21.7-27.1)	(21.7-27.1)	(21.7-27.1)
HIV risk[b] N (%)			
1	322 (39.7)	157 (40.5)	165 (39.0)
2	447 (55.1)	212 (54.6)	235 (55.6)
3	12 (1.5)	6 (1.5)	6 (1.4)
4	9 (1.1)	4 (1.0)	5 (1.2)
5	2 (0.2)	0 (0.0)	2 (0.5)
7	19 (2.3)	9 (2.3)	10 (2.4)
Start CD4$^+$-cell count ; median	440	430	440
(25th-75th percentile)	(290-610)	(290-610)	(290-623)
Start CD4$^+$-cell count by category N (%)			
< 200 cells/mm^3	98 (12.1)	48 (12.4)	50 (11.8)
200-500 cells/mm^3	399 (49.2)	185 (47.7)	214 (50.6)
> 500 cells/mm^3	314 (38.7)	155 (39.9)	159 (37.6)
Nadir CD4$^+$-cell count N; median	807; 200	385; 190	422; 200
(25th-75th percentile)	(60-315)	(60-299)	(78-331)
HIV-RNA < 50 c/mL N (%)	475 / 811 (58.6)	231 / 388 (59.5)	244 / 423 (57.7)
On HAART N (%)	577 (71.1)	280 (72.2)	297 (70.2)
HAART duration N; median	577; 3.1 yrs	280; 3.3 yrs	297; 3.0 yrs
(25th-75th percentile)	(1.0-7.0 yrs)	(1.1-6.8 yrs)	(1.0-7.2 yrs)
HAV positive antibodies N (%)	379 (46.7)	177 (45.6)	202 (47.8)
HCV-RNA positive N (%)	17 (2.1)	9 (2.3)	8 (1.9)

[a]1=West and East Europe, USA
2=Sub-Sahara Africa
3=Mediterranean
4=South and Central America, Caribbean
5=Asian

[b]1=MSM (men having sex with men)
2=heterosexual
3=IV drugs
4=blood-blood contact
5=perinatal transmission
7=unknown

Treatment effect

Overall effect:

The overall response rate, defined as anti-HBs ≥ 10 IU/L, at week 28 in the standard arm and the accelerated arm was 50% and 38.7% respectively. The immunogenicity results of the two vaccination schedules according to MITT and PP population analysis are depicted in figure 2. The response difference in the overall MITT and PP analyses was 11.3% (95% CI [4.3-18.3]) and 12.3% (95% CI [4.2-20.4]) respectively. The 95% CI does not overlap 0; however the difference is small and compatible with the non-inferiority margin and therefore inferiority cannot

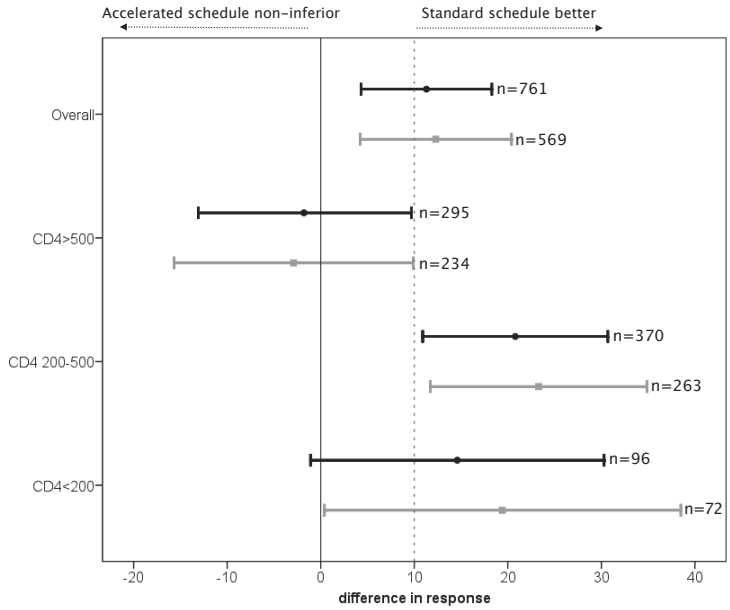

Figure 2. Results MITT and PP analysis according to $CD4^+$-cell count overall and by groups
Results MITT analysis shown in black
Results PP analysis shown in grey
SAS 9.2; results are reported and interpreted according to the CONSORT statement on non-inferiority trials

be concluded and the overall results were inconclusive. However, the treatment effect, i.e. sufficiently high levels of anti-HBs in the accelerated versus standard vaccination schedule, differed significantly in the $CD4^+$-cell count > 500 cells/mm^3 group from that in the group with $CD4^+$-cell count 200-500 cells/mm^3 (p=0.0034 in the MITT analysis and p=0.003 in the PP analysis) (Figure 2).

Effect by CD4⁺-cell count stratum:

The results showed that the response rates in the higher CD4⁺-cell count groups (i.e. 200-500 cells/mm³ and > 500 cells/mm³) in both schedules were 33.5% (accelerated) versus 54.3% (standard) and 53.4% (accelerated) versus 51.7% (standard), respectively. In comparison, in the low CD4⁺-cell count group these rates were 12.5% (accelerated) versus 27.1% (standard). The response differences in this non-inferiority trial showed that the accelerated schedule was non-inferior only in patients with CD4⁺-cell count > 500 cells/mm³ (-1.8%; 95% CI [-13.4 – 9.7]). In patients with CD4⁺-cell count 200-500 cells/mm³ the vaccination efficacy in the accelerated arm was inferior and in patients with CD4⁺-cell count < 200 cells/mm³ the result was inconclusive probably due to low patient numbers (Figure 2).

Effect by baseline characteristics:

The following variables were associated with an overall better response (independent of treatment arm): high CD4⁺-cell count, HAART use, female gender, undetectable HIV-RNA load ($p < 0.001$) and longer duration of HAART (≥4 years) ($p=0.03$). CD4⁺-cell count as a continuous variable showed a better response in favor of high CD4⁺-cell count, the odds ratio $(OR)_{standard}$

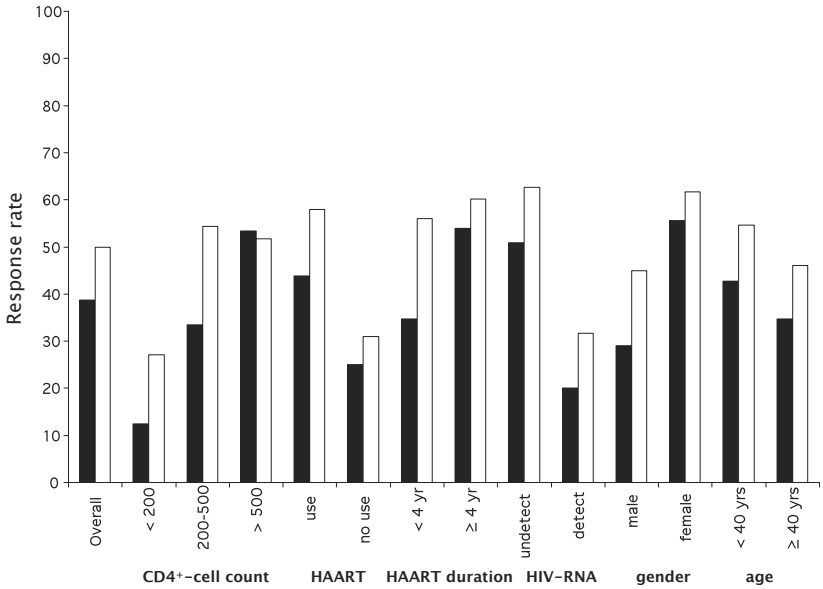

Figure 3. Vaccination results in all participants by clinical baseline characteristics.

Response accelerated arm shown in black

Response standard arm shown in white

Multivariate analysis of the treatment outcome was performed with logistic regression analysis. Significant effect differences were found for CD4⁺-cell count (> 500 versus 200-500 $p_{interaction}=0.0034$, > 500 versus < 200 $p_{interaction}=0.10$, < 200 versus 200-500 $p_{interaction}=0.51$). For all other clinical baseline characteristics no significant differences were found: HAART $p_{interaction}=0.30$, HAART duration $p_{interaction}=0.07$, HIV-RNA $p_{interaction}=0.99$, gender $p_{interaction}=0.19$, age $p_{interaction}=0.60$.

= 1.05 (95% CI [1.01-1.10]) (p=0.008) per increase of 50 CD4$^+$-cells; the $OR_{accelerated}$ = 1.13 (95% CI [1.08-1.18]) (p<0.001) per increase of 50 CD4$^+$-cells. The $p_{interaction}$ between the $OR_{standard}$ and $OR_{accelerated}$ = 0.03. Age as continuous variable also showed a better response in favor of younger age (p=0.008). After comparing patients younger than 40 years to those 40 years or older a similar pattern in overall response was seen (p=0.02). The effect of treatment by baseline characteristics on the vaccination response is shown in Figure 3.

In the CD4$^+$-cell count > 500 cells/mm^3 group, where non-inferiority was found, the effect

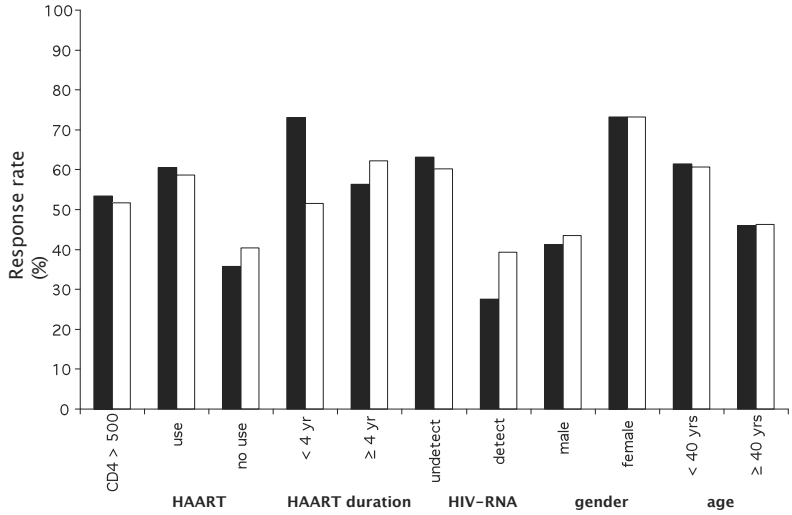

Figure 3a. Vaccination results in participants with a CD4$^+$-cell count >500 cells/mm^3 by clinical baseline characteristics.
Response accelerated arm shown in black
Response standard arm shown in white
Multivariate analysis of the treatment outcome was performed with logistic regression analysis.
No significant effect differences were found (HAART $p_{interaction}$=0.60, HAART duration $p_{interaction}$=0.09, HIV-RNA $p_{interaction}$=0.21, gender $p_{interaction}$=0.83, age $p_{interaction}$=0.94).

of HAART use, female gender, undetectable HIV-RNA load, younger age and longer duration of HAART remained significantly associated with an overall better response (Figure 3a). After correction for these factors in multivariable analysis the non-inferiority between the treatment arms in the CD4$^+$-cell count > 500 cells/mm^3 group was retained. The difference between accelerated and standard arm in this CD4$^+$-cell count group was 4.81% (95% CI [-05.6%-14.91%]); p=0.36 after correction for age, gender and HIV-RNA load. HAART use is highly correlated to HIV-RNA load and could therefore not be entered into the same model. Correcting for HAART use instead of HIV-RNA load gave similar results.

Five-week time point in accelerated schedule:

At week five samples from 332 of the 367 MITT patients in the accelerated arm were available for testing. In 53, 24 and 255 patients anti-HBs titers were ≥ 10 IU/l (positive responder), 3.0-9.9 IU/l (partial responder) and < 3.0 IU/l (non-responder) respectively. From the positive responders at week five, 47 (88.7%) patients subsequently had a protective titer at week 28. In the 255 non-responders at week 5, 189 (74.1%) patients were still non-responders at week 28. Nineteen out of twenty-four patients (79.2%) with a partial response at week 5 developed protective anti-HBs levels at week 28.

Compliance:

Compliance was defined as receiving three vaccinations according to the per protocol definition with or without a measured anti-HBs titer as end-point. The compliance with both vaccination schedules was significantly higher in the accelerated arm, 91.8% (n=356/388) versus 82.7% (n=350/423) in the standard arm (p≤ 0.001). Of the 105 non-compliant patients 10 patients stopped after the first vaccination; 16 patients stopped after two vaccinations and 79 persons received three vaccinations but not within the time interval definition of the per protocol analysis. Younger patients were significantly more non-compliant (p=0.006). All other baseline variables were not significantly different between the groups.

Adverse events:

No serious adverse events were observed. One patient was advised to discontinue the vaccination schedule because of an allergic reaction (urticaria and dyspnoe) possibly related to the vaccination, a known side effect in < 0.01% according to the manufacturer manual. Local reaction in the deltoid region was incidental present, but was not scored.

Discussion

To our knowledge this is the first large prospective randomized study on efficacy of different hepatitis B vaccination schedules in adult HIV positive patients. The results of our study show that the compliance with an accelerated schedule is significantly better to that with a standard schedule. Its efficacy is only non-inferior in patients with $CD4^+$-cell count > 500 cells/mm^3. This finding supports the use of an accelerated HBV vaccination schedule in HIV-infected patients with $CD4^+$-cell count > 500 cells/mm^3. The observation that response to different hepatitis B vaccination schedules varies by $CD4^+$-cell count is a unique finding.

According to the present Dutch national guidelines on the management of HIV infection HBV vaccination is offered according to a standard vaccination schedule (0, 1, 6 months) to all asymptomatic HIV-infected patients in several risk groups. This guideline includes all patients irrespective of the presence of several negative predictive factors of response, such

as low $CD4^+$-cell count or HIV-RNA load. The response rate to HBV vaccination in HIV-infected patients is known to be diminished [1-2]. However, the efficacy could be improved by proper timing of vaccine administration. In our study undetectable HIV-RNA was found to predict a better response irrespective of the vaccination schedule. This is in agreement with prior results showing that undetectable plasma HIV-RNA at first HBV vaccination was found to predict success (OR 3.47;95% CI, 1.5-7.6) in 194 HIV-infected patients [1]. Furthermore, an increased likelihood of developing a response was associated with a $CD4^+$-cell count ≥ 350 cells/mm^3 (p=0.008) in a previous study in 112 HIV infected patients [2]. This is in line with our own findings. A high $CD4^+$-cell count was associated with higher response rates.

Unlike most studies on HBV vaccination with a preponderance of males and MSM (men having sex with men), the majority of our study population consisted of heterosexuals and one third of the population were female. This allowed us to analyze the influence of gender and risk group. Female gender turned out to be a predictor of better response. This confirms earlier published results [16-17]. In contrast to previous published studies, membership of the MSM risk group was not a negative predictor for response. This could be explained by the composition of our population. Most study populations comprise a majority of males (MSM). Our population included 54% heterosexuals and 32 % women. Younger age was found to be associated with a better response (p=0.008), this was also documented in non HIV-infected patients. However, younger patients are less compliant. It has been suggested that humoral and cellular immune function may decrease over years and result in diminished vaccine effectiveness in older individuals [18-19].

Finally, usage of HAART was associated with development of a protective anti-HBs titer (p< 0.001). Moreover, longer duration of HAART showed a positive effect on response (p=0.03). This may be explained by restoration of cellular immunity induced by antiretroviral therapy, resulting in reducing polyclonal B cell activation [20]. Untreated HIV infection is characterized by an immunologic dysfunction and a reduced ability to respond appropriately to antigens. Ongoing HAART probably results in qualitative improvement of cellular immunity, next to the increase in T-cell count [21].

Despite identifying positive predictors of responding to vaccination, the overall response within both immunization schemes remains diminished. Poor results of HBV vaccination seen in different vaccination programs suggest the need for alternative strategies to prevent vaccination failure. Fonseca *et al.* studied the effect of double dosing HBV vaccination [2]. They found a statistically significant higher seroconversion rate associated with double dose compared with standard dose in 36/56 patients with $CD4^+$-cell count ≥ 350 cells/mm^3 (64.3% x 39.3%; p = 0.008). Rey *et al.* tested the hypothesis that doubling the number of hepatitis B vaccine injections might increase anti-HBs response rate. They assessed an increase in overall response from 55% after 3 vaccinations to 90% after 6 vaccinations (18/20 patients)

[7]. In the study of Sasaki *et al.* 80 patients received double dose of recombinant HBV vaccine and received either GM-CSF or placebo with the first vaccine dose. They found a significant increase in the seroconversion rate in the GM-CSF group. In our study we tested an accelerated schedule versus the standard schedule and found a better overall response in favor of the standard schedule, except for the higher $CD4^+$-cell count group where non-inferiority was found between the two treatment schemes with a better compliance in the accelerated schedule. The inferiority of the accelerated schedule in the lower $CD4^+$-cell count groups cannot be explained by a difference in HAART usage or the percentage of patients with undetectable HIV-RNA as they were equal in both groups. This is the first observation that response to different hepatitis B vaccination schedules varies by $CD4^+$-cell count. The underlying mechanism needs to be addressed in future studies. Perhaps the impaired immunity requires more time and longer intervals to benefit from repeated antigen stimulations.

Apart from the decreased response to HBV vaccination in HIV-infected patients, compliance to vaccination programs is poor irrespective of risk group. In our study overall compliance proved to be significantly better in the accelerated schedule ($p \leq 0.001$). Completing a vaccination schedule contributes to providing protective antibody levels in those individuals at high risk of exposure to HBV, due to sexual behavior or travelling to HBV endemic areas.

The strength of our study is the prospective randomized design and the large number of patients included. The population is heterogeneous reflecting day-to-day practice, and apart from a large number of MSM also comprises heterosexual patients and women. The high rate of HAART usage reflects a present-day HIV population and enables us to appreciate its value on the response to vaccination. The lowest $CD4^+$-cell count group represented only 12 % of the study population.

In conclusion, patients with $CD4^+$-cell count > 500 cells/mm^3 can be vaccinated against HBV according to an accelerated HBV schedule. As compliance is significantly better in the accelerated vaccination arm this schedule is preferable.

In all HIV-infected patients a better response rate is provided in patients on HAART with undetectable HIV-RNA load, longer duration of HAART use, female gender and younger age. Delaying hepatitis B vaccination in HIV-infected high-risk groups under all circumstances according to the above-mentioned predictors of success may not be warranted, but our findings suggest a more optimized and individualized timing can be applied.

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References

1. Overton ET, Sungkanuparph S, Powderly WG, Seyfried W, Groger RK and Aberg JA. Undetectable plasma HIV RNA load predicts success after hepatitis B vaccination in HIV-infected persons. Clin Infect Dis 2005;41:1045-8
2. Fonseca MO, Pang LW, de Paula Cavalheiro N, Barone AA and Heloisa Lopes M. Randomized trial of recombinant hepatitis B vaccine in HIV-infected adult patients comparing a standard dose to a double dose. Vaccine 2005;23:2902-8
3. Bruguera M, Cremades M, Salinas R, Costa J, Grau M and Sans J. Impaired response to recombinant hepatitis B vaccine in HIV-infected persons. J Clin Gastroenterol 1992;14:27-30
4. Carne CA, Weller IV, Waite J, et al. Impaired responsiveness of homosexual men with HIV antibodies to plasma derived hepatitis B vaccine. Br Med J (Clin Res Ed) 1987;294:866-8
5. Collier AC, Corey L, Murphy VL and Handsfield HH. Antibody to human immunodeficiency virus (HIV) and suboptimal response to hepatitis B vaccination. Ann Intern Med 1988;109:101-5
6. Keet IP, van Doornum G, Safary A and Coutinho RA. Insufficient response to hepatitis B vaccination in HIV-positive homosexual men. Aids 1992;6:509-10
7. Rey D, Krantz V, Partisani M, et al. Increasing the number of hepatitis B vaccine injections augments anti-HBs response rate in HIV-infected patients. Effects on HIV-1 viral load. Vaccine 2000;18:1161-5
8. Sasaki MG, Foccacia R and de Messias-Reason IJ. Efficacy of granulocyte-macrophage colony-stimulating factor (GM-CSF) as a vaccine adjuvant for hepatitis B virus in patients with HIV infection. Vaccine 2003;21:4545-9
9. Bailey CL, Smith V and Sands M. Hepatitis B vaccine: a seven-year study of adherence to the immunization guidelines and efficacy in HIV-1-positive adults. Int J Infect Dis 2008;12:e77-83
10. Nyamathi A, Liu Y, Marfisee M, et al. Effects of a nurse-managed program on hepatitis A and B vaccine completion among homeless adults. Nurs Res 2009;58:13-22
11. Panhotra BR, Saxena AK, Al-Hamrani HA and Al-Mulhim A. Compliance to hepatitis B vaccination and subsequent development of seroprotection among health care workers of a tertiary care center of Saudi Arabia. Am J Infect Control 2005;33:144-50
12. Suckling RM, Taegtmeyer M, Nguku PM, et al. Susceptibility of healthcare workers in Kenya to hepatitis B: new strategies for facilitating vaccination uptake. J Hosp Infect 2006;64:271-7
13. Bock HL, Loscher T, Scheiermann N, et al. Accelerated Schedule for Hepatitis B Immunization. J Travel Med 1995;2:213-217
14. Saltoglu N, Inal AS, Tasova Y and Kandemir O. Comparison of the accelerated and classic vaccination schedules against Hepatitis B: three-week Hepatitis B vaccination schedule provides immediate and protective immunity. Ann Clin Microbiol Antimicrob 2003;2:10
15. Piaggio G, Elbourne DR, Altman DG, Pocock SJ and Evans SJ. Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement. Jama 2006;295:1152-60
16. de Vries-Sluijs TE, Hansen BE, van Doornum GJ, et al. A prospective open study of the efficacy of high-dose recombinant hepatitis B rechallenge vaccination in HIV-infected patients. J Infect Dis 2008;197:292-4
17. Landrum ML, Huppler Hullsiek K, Ganesan A, et al. Hepatitis B vaccine responses in a large U.S. military cohort of HIV-infected individuals: another benefit of HAART in those with preserved CD4 count. Vaccine 2009;27:4731-8
18. Ginaldi L, De Martinis M, D'Ostilio A, et al. The immune system in the elderly: I. Specific humoral immunity. Immunol Res 1999;20:101-8

19. Wick G, Grubeck-Loebenstein B. The aging immune system: primary and secondary alterations of immune reactivity in the elderly. Exp Gerontol 1997;32:401-13
20. Malaspina A, Moir S, Kottilil S, et al. Deleterious effect of HIV-1 plasma viremia on B cell costimulatory function. J Immunol 2003;170:5965-72
21. Kroon FP, Rimmelzwaan GF, Roos MT, et al. Restored humoral immune response to influenza vaccination in HIV-infected adults treated with highly active antiretroviral therapy. AIDS 1998;12:F217-23

CHAPTER 3

A prospective open study of the efficacy of high-dose recombinant hepatitis B rechallenge vaccination in HIV- infected patients

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Abstract

Double-dose hepatitis B virus revaccination of human immunodeficiency virus (HIV)-infected patients proved to be effective in 50.7 % of 144 patients who had previously failed to respond to standard doses. In the multivariate analysis, female patients were found to have a significantly better response (p=0.03). The effect of age on the response depended on the viral load at time of revaccination. For patients with a detectable HIV-RNA load the effect of age was stronger (odds ratio (OR), 0.34 per 10 years older (95% confidence interval (CI), [0.16–0.72])); (p=0.005) than for patients with an undetectable HIV-RNA load (OR, 0.74 per 10 years older (95% CI, [0.50–1.09])); (p=0.12).

Introduction

Hepatitis B virus (HBV) infection in patients infected with HIV is an increasing problem in the western world. HIV and HBV have similar risk factors and routes of transmission. The prevalence of HIV/HBV co-infection among men having sex with men (MSM) is 6-10 % [1]. With the development of highly active antiretroviral therapy (HAART) and with better survival rates, liver disease has become a leading cause of mortality in patients with HIV infection [2]. Co-infection with HIV and HBV is associated with an 8-fold increase in mortality compared with HIV mono-infection and a 19-fold increase in mortality compared with HBV mono-infection [3]. In addition, HIV infection is associated with a reduced clearance of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) [4-5] and a higher level of HBV replication, increasing its potential for transmission. Progression to cirrhosis and flare-ups of hepatitis occur more often [6-7].

Prevention of HBV infection is of great importance for HIV-infected patients. However, compared with immunocompetent individuals, a large proportion of HIV-infected patients (40-76% versus < 10%) fail to respond to standard dose HBV vaccination [8-10]. Several groups have recently shown improved response rates when higher doses of HBV vaccine are used in the initial vaccination of HIV-infected patients. [9, 11-12].

HBV vaccination is offered to all asymptomatic HIV-infected individuals as recommended by the Dutch national guidelines. Approximately 50% of our HIV-infected cohort did not have an antibody response to the initial HBV vaccination with three dosages of 10 μg of HBvaxPRO®. In an attempt to achieve a higher response rate we prospectively revaccinated all non-responders (anti-hepatitis B surface antigen (anti-HBs) titers of 0 IU/L) three times at monthly intervals with a double dose of HBV vaccine.

Patients and Methods

The infectious diseases outpatient clinic of the Erasmus Medical Center offers HBV vaccination to all HIV-positive patients who are ≥ 18 years old, have negative HBsAg and anti-hepatitis B core (anti-HBc) serological results, do not have an active opportunistic infection, are not pregnant, and have not been vaccinated previously. The initial vaccination schedule consists of three dosages of 10 μg of HBvaxPRO®. Non-responders, defined as having an anti-HBs titer of 0 IU/L, were offered revaccination. A double dose (20 μg of HBvaxPRO®) was injected intramuscularly in the deltoid region at 0, 1 and 2 months, starting at a median of 5 weeks (25^{th}-75^{th} percentile 3-10 weeks) after completing the initial vaccination. One month after the last double dose, a blood sample was taken for quantitative anti-HBs testing (Axsym; Abbott). Age, gender, route of exposure for HIV infection, nadir $CD4^+$-cell count before and

$CD4^+$-cell count at the time of the initial vaccine administration, plasma HIV-RNA load, and antiretroviral therapy received at the time of revaccination were recorded.

HIV-RNA was quantitated using the Cobas Amplicor (Roche Molecular Systems, Penzberg, Germany). The detection limit was 50 copies/ml.

Univariate and multivariate logistic regression analysis were used to determine factors that predicted success for HBV revaccination. The data analysis was performed using SPSS for Windows, release 11.0.1.

In the multivariate logistic regression analysis age, gender, risk group, body mass index (BMI), $CD4^+$-cell count at the start of the initial vaccination schedule (<200, 200-500, ≥500 cells/mm^3), HAART (yes/no) and HIV-RNA load (detectable/undetectable), at the time of revaccination were included. The Medical Ethics Committee of the Erasmus Medical Center approved the study.

Results

One hundred forty-four HIV-infected patients who had an anti HBs titer of 0 IU/L after their initial HBV vaccination schedule were offered revaccination. The study population consisted of 108 males (75%) with a mean age 43.4 years (SD, 11.5 years). The mean BMI was 25.3 (SD, 4.6). HIV risk factors were male homosexual activity for 64 individuals (44.4%), heterosexual contacts for 72 individuals (50.0%), and miscellaneous for 8 patients (5.6%). At revaccination, 96 patients (66.7%) were receiving HAART, and 89 patients (61.8%) had a HIV-RNA load < 50 copies/ml. The median nadir $CD4^+$-cell count was 205 cells/mm^3 (25^{th}-75^{th} percentile, 90-330

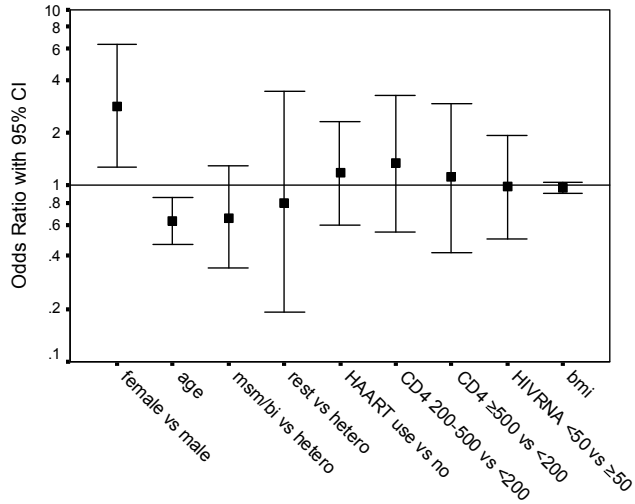


Figure 1: Results of univariate logistic regression analysis of factors predicting successful HBV vaccination (SPSS).

cells/mm^3). The median CD4$^+$-cell count at initial vaccination schedule was 360 cells/mm^3 (25th-75th percentile, 240-540 cells/mm^3); the distribution by CD4$^+$-cell count category of <200, 200–500 and ≥500 cells/mm^3 was, respectively, 26 (18.1%), 73 (50.7%), and 45 (31.3%). The CD4$^+$-cell count at the time of revaccination was known in 100 of 144 patients. They were similar to those at the time of the primary vaccination (340 cells/mm^3; 25th-75th percentile, 250-510 cells/mm^3). No side effects were reported during revaccination. Seventy-three of 144 patients developed anti-HBs titers ≥ 10 IU/L, giving a response rate of 50.7%. The median and mean time between anti-HBs titer determined in the initial schedule and the first vaccine dose in the revaccinating schedule was, respectively, 5 and 8 weeks (25th-75th percentile, 3-10 weeks). The median anti-HBs titer of the responder group was 107.9 IU/L (25th-75th percentile, 43.7-426 IU/L).

Univariate analysis showed that only female gender (OR, 2.8 female/male, [95%CI, 1.3-6.3]; p=0.009) and younger age (OR, 0.63 per 10 years older, [95%CI, 0.46-0.86]; p=0.002) were predictors of a successful response (Figure 1). Changes in HIV-RNA load or CD4$^+$-cell count between the initial and rechallenge vaccination schedule were not found to have an influence on outcome. However, we could not exclude an effect of changes in HIV-RNA load or CD4$^+$-cell count, given the limited numbers of patients available in this study.

In the multivariate analysis, female patients were found to have a significant better response (p=0.03), whereas the effect of age depended on the viral load at the time of revaccination (i.e., viral load is an effect modifier, p=0.05). For patients with a detectable HIV-RNA load (not

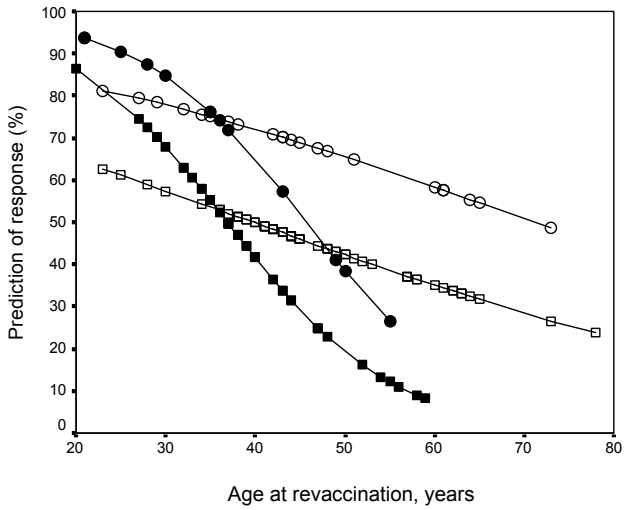


Figure 2: Multivariate logistic regression analysis (SPSS) of the relationship between age and the probability of success for HBV vaccination. Females have a significant better response rate than males. The effect of age depends on HIV-RNA load at revaccination. Shown are results for females with a detectable HIV-RNA load (●), females with an undetectable HIV-RNA load (○), males with a detectable HIV-RNA load (■), and males with an undetectable HIV-RNA load (□).

receiving HAART), the effect of age was stronger (OR, 0.34 per 10 years older, [95%CI, 0.16-0.72]; p=0.005) than it was for patients with an undetectable HIV-RNA load, all of whom were receiving HAART except for 2 HIV-2-infected patients (OR, 0.74 per 10 years older, [95%CI, 0.50–1.00]; p=0.12) (Figure 2). In conclusion, a response to revaccination with a double dose of HBV vaccine was more likely in patients < 40 years old irrespective of viral load (Figure 2). In patients ≥ 40 years old, a better response rate was observed in patients with an undetectable HIV-RNA load than in patients with a detectable HIV-RNA load. In the latter group, prediction of response decreased rapidly with age.

Discussion

To our knowledge, this is the first study describing the results of double-dose HBV rechallenge vaccination at monthly intervals in HIV-infected patients not responding to their initial vaccination. We revaccinated 144 patients who had failed to have an antibody response after standard vaccination and found a 50.7% response rate. Female gender was an independent predictor for an adequate response. This was not associated with a difference in BMI between men and women.

Our study shows that an undetectable HIV-RNA load is associated with a better response only in patients ≥ 40 years old. In patients < 40 years of age, the chance of a response is high, irrespective of HIV-RNA load. One possible explanation for this is a shorter duration of HIV infection and a limited depletion of specific memory T cells in the younger age group. The effect of undetectable HIV-RNA load on response in the older age group probably reflects long usage of HAART and partial recovery of immune system.

Overton et al. found that an undetectable HIV-RNA load was the only predictor of successful HBV vaccination in a multivariate analysis [13]. Because the outcome of their study group concerned the results of initial vaccination, this may not be in conflict with our results concerning revaccination of non-responders after initial vaccination. These patients probably are an immunological disadvantaged group.

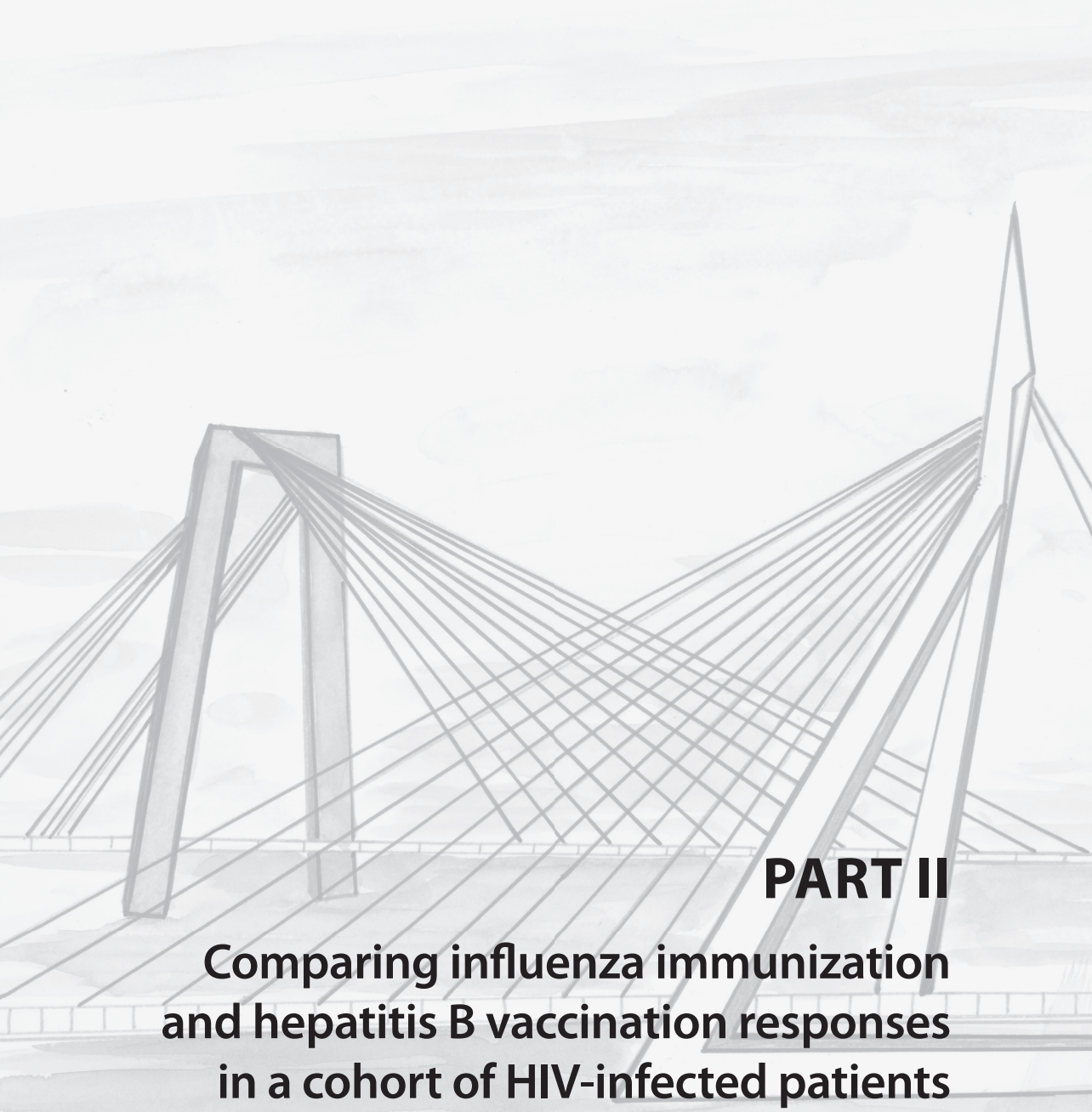
The optimal HBV vaccination schedule in HIV-infected individuals is still a matter of debate. Whether the results of revaccination in our study are due to the double dose or to the increasing number of monthly immunizations in initially non-responding individuals remains to be elucidated. Studies comparing standard and double doses in primary vaccination schedules are not conclusive. Cornejo-Juárez et al. compared 10 μg and 40 μg of HBV vaccine as the initial vaccination scheme in HIV-infected patients and found no significant difference [10]. Fonseca et al. enrolled HIV-infected patients in a primary vaccination serial study using a standard dose and a double-dose group. The double dose improved seroconversion only in those with $CD4^+$-cell counts ≥ 350 cells/mm^3 and low HIV viremia (p=0.008) [11]. In patients with predialysis chronic renal failure, McNulty et al. compared 20 μg and 40 μg doses as the

initial vaccination scheme and found 10 % more seroconversions in the higher dose group, but this difference was not statistically significant. A fourth dose increased the seroconversion rate in 4 of 31 non-responders [14]. The conflicting results may be due to small numbers of included individuals and different patient populations. According to Rey et al. rechallenging of non-responders to standard primary vaccination with HBV vaccination given at monthly intervals is very effective. In his study, 8 of 9 revaccinated patients responded [15]. Given that the response rate of the initial standard dose vaccination schedule at our institution is about 50 % and revaccinating contributed another 50 %, we achieved an overall response rate of 75 % among our HIV-infected patients.

In conclusion, double-dose HBV revaccination in HIV-infected non-responders produced a 50.7 % additional success rate, justifying post-vaccination anti-HBs screening. Future prospective studies are required to determine whether increasing the number of immunu-nizations is as effective as double-dose revaccination in non-responders to a standard initial vaccination schedule.

References

1. Nunez M, Soriano V. Management of patients co-infected with hepatitis B virus and HIV. Lancet Infect Dis 2005;5:374-82
2. Bica I, McGovern B, Dhar R, et al. Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. Clin Infect Dis 2001;32:492-7
3. Thio CL, Seaberg EC, Skolasky R, Jr., et al. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). Lancet 2002;360:1921-6
4. Bodsworth NJ, Cooper DA and Donovan B. The influence of human immunodeficiency virus type 1 infection on the development of the hepatitis B virus carrier state. J Infect Dis 1991;163:1138-40
5. Hadler SC, Judson FN, O'Malley PM, et al. Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. J Infect Dis 1991;163:454-9
6. Gilson RJ, Hawkins AE, Beecham MR, et al. Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. Aids 1997;11:597-606
7. Colin JF, Cazals-Hatem D, Loriot MA, et al. Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. Hepatology 1999;29:1306-10
8. Collier AC, Corey L, Murphy VL and Handsfield HH. Antibody to human immunodeficiency virus (HIV) and suboptimal response to hepatitis B vaccination. Ann Intern Med 1988;109:101-5
9. Keet IP, van Doornum G, Safary A and Coutinho RA. Insufficient response to hepatitis B vaccination in HIV-positive homosexual men. Aids 1992;6:509-10
10. Cornejo-Juarez P, Volkow-Fernandez P, Escobedo-Lopez K, Vilar-Compte D, Ruiz-Palacios G and Soto-Ramirez LE. Randomized controlled trial of Hepatitis B virus vaccine in HIV-1-infected patients comparing two different doses. AIDS Res Ther 2006;3:9
11. Fonseca MO, Pang LW, de Paula Cavalheiro N, Barone AA and Heloisa Lopes M. Randomized trial of recombinant hepatitis B vaccine in HIV-infected adult patients comparing a standard dose to a double dose. Vaccine 2005;23:2902-8
12. Scolfaro C, Fiammengo P, Balbo L, Madon E and Tovo PA. Hepatitis B vaccination in HIV-1-infected children: double efficacy doubling the paediatric dose. Aids 1996;10:1169-70
13. Overton ET, Sungkanuparph S, Powderly WG, Seyfried W, Groger RK and Aberg JA. Undetectable plasma HIV RNA load predicts success after hepatitis B vaccination in HIV-infected persons. Clin Infect Dis 2005;41:1045-8
14. McNulty CA, Bowen JK and Williams AJ. Hepatitis B vaccination in predialysis chronic renal failure patients a comparison of two vaccination schedules. Vaccine 2005;23:4142-7
15. Rey D, Krantz V, Partisani M, et al. Increasing the number of hepatitis B vaccine injections augments anti-HBs response rate in HIV-infected patients. Effects on HIV-1 viral load. Vaccine 2000;18:1161-5



PART II

Comparing influenza immunization and hepatitis B vaccination responses in a cohort of HIV-infected patients

CHAPTER 4

The response to Influenza compared to hepatitis B vaccination in HIV-infected patients

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Submitted for publication

Abstract

We studied the possible relationship between hepatitis B and influenza vaccination in HIV-infected patients. From a hepatitis B vaccination study in a HIV-infected cohort, we retrieved data on the history of influenza immunizations and stored serum samples were used to determine response to influenza vaccination. Outcome was defined as an influenza response rate, an influenza protection rate and an influenza geometric mean titer (GMT) of all 3 seasonal antigens. The outcome could be analyzed in 73 patients. In conclusion, a trend for higher GMT both in pre- and post- vaccination titers was observed in HBV vaccination responders compared to HBV non-responders.

Introduction

To reduce the risk of hepatitis B virus (HBV) infection it is currently recommended vaccinating all HIV-infected individuals against hepatitis B. Trials in HIV-infected patients in the pre- and post highly active antiretroviral therapy (HAART) era have yielded response rates between 17% and 72% respectively [1-8]. Response rates depend on various factors like $CD4^+$-cell count, HIV viral load and dosing schedule. A $CD4^+$-cell count ≥ 350 cells/mm^3 is associated with a higher likelihood of developing a protective immune response [4].

To reduce serious influenza-associated complications, influenza immunization is widely recommended for individuals with immune suppression. Like in HBV vaccination, HIV-infected patients approved to have an impaired response upon influenza immunization as compared to healthy controls [9-10].

Hepatitis B and influenza vaccines contain T-cell dependent antigens. The level of protection is associated with antibody levels. Failing to achieve an adequate vaccination response even in HIV-infected patients with a $CD4^+$-cell count > 500 cells/mm^3, suggests a persistent functional defect of the adaptive immune response.

To assess whether an impaired response to HBV and influenza immunization is related to the immune competence of the host or dependent on the vaccine used, we studied the possible relationship between both vaccinations in a group of HIV-infected patients.

Methods

Patients

From a cohort of 385 HIV-infected patients that participated in a HBV vaccination study (period 2004-2007) in our clinic, Erasmus MC, Rotterdam, the Netherlands, we gathered data on the history of influenza immunizations administered during the study period. We sent a questionnaire to the general practitioners of all patients. Data on age, gender, Body Mass Index (BMI), nadir $CD4^+$-cell counts, baseline $CD4^+$-cell counts, HAART usage, baseline HIV-RNA levels, number of influenza immunizations prior to HBV vaccination and response to HBV vaccination were available.

Vaccine and Antibody assays

Quantitative anti-hepatitis B surface antigen (anti-HBs) testing was performed by AxSym Ausab (Abbott Diagnostic Division, Wiesbaden Germany).

Available repository serum samples collected during the HBV vaccination study period and frozen at -20°C, were identified. We selected samples prior to and 2-3 months with a maximum of 6 months after influenza immunization. Inactivated influenza vaccines contain

the immunologically relevant envelope protein haemagglutinin (HA) of viral strains, as the presence of serum antibodies directed against HA is associated with clinical protection from disease. The standard assay to detect anti-HA antibodies is the haemagglutination inhibition (HI) test. Serial two-fold dilutions of serum were incubated with four haemagglutinating units of the respective vaccine strain and the reciprocal serum dilution that still inhibited the agglutination of turkey erythrocytes was determined as described previously [11]. Ferret sera raised against the test antigens were used as positive controls. All sera of each individual study subject were tested simultaneously. For statistical analysis a titer of 5 was arbitrarily assigned to sera with a titer < 10.

Serum samples were tested for antibodies against three influenza vaccine antigens corresponding to the vaccine composition of the season in which the vaccine was used. *Season 2003/2004*: H1N1 IVR-116 = A/New Caledonia/20/99, H3N2 ResVir-17 = A/Panama/2007/99, B/Shangdon/7/97; *season 2004/2005*: H1N1 IVR-116 = A/New Caledonia/20/99, H3N2 X-147 = A/Wyoming/3/03, B/Jiangsu/10/03; *season 2005/2006*: H1N1 IVR-116 = A/New Caledonia/20/99, H3N2 X-157 = A/New York/55/04, B/Jiangsu/10/03; *season 2006/2007*: H1N1 IVR-116 = A/New Caledonia/20/99, H3N2 IVR-142 = A/Hiroshima/52/05, B/Malaysia/2506/04.

When a patient was immunized more than once in the above mentioned seasons results of first influenza immunization were used for analysis. In the different seasons the A/H1N1 vaccine strain remained unchanged during the study period and therefore results were pooled. For influenza A/H3N2 and B viruses different vaccine strains were used in subsequent years, but since the HA antigens are variants of the same virus (sub)type and antibodies were detected against the homologous vaccine strains, results obtained with these different antigens were pooled as well to obtain more statistical power for analysis of the data.

Definitions and statistics

The following parameters for efficacy of vaccination were evaluated: seroconversion or response rate was defined as the percentage of patients with a fourfold titer increase and those with a titer of < 10 at baseline achieving a titer of ≥ 40; seroprotection or protection rate was defined as the percentage of patients with a HI titer ≥ 40, which is considered to be a clinically relevant titer, known to be associated with protection against severe influenza in healthy controls, after vaccination [11-12]. Besides response rate and protection rate, titers were transformed to a logarithmic scale and geometric means were used for further calculations. Geometric mean titers (GMT) are the strongest markers of the immunological capability of a group to respond to an antigen.

In the HBV vaccination study the protective level of anti-HBs was defined as a titer ≥ 10 IU/L. According to the response to HBV vaccination patients were grouped into HBV responders and HBV non-responders. The variables age, gender, BMI, nadir $CD4^+$-cell count, baseline $CD4^+$-cell counts, HAART use, baseline HIV-RNA levels and number of influenza vaccinations prior to the analyzed influenza immunization were studied. Continuous variables were

compared using the one-way ANOVA or Mann-Whitney U test; categorical variables were compared using the Chi-square test, where appropriate. GMT results were analyzed by one-way ANOVA. Calculations were performed using SPSS for Windows, version 17.0.

Results

We received information on influenza immunization for 302/385 (78.4%) participants of the HBV vaccination study. More than 50% of our HBV vaccinated cohort (n=175) were not immunized against influenza. One or more influenza immunizations were administered to 127 (42.1%) patients. The response to influenza immunization could be analyzed in 73 patients. The remaining 54 patients were not available for analysis due to various reasons: in 36 cases serum monsters were not available within the above-mentioned defined period, in 10 cases samples were retrospectively retrieved not according to the definition, 8 patients' samples had an insufficient volume.

Baseline characteristics of the HBV vaccine non-responders and responders are shown in Table 1. The median nadir $CD4^+$-cell count was < 200 cells/mm^3 in both groups and was significantly lower in the HBV responder group (p=0.04). The median $CD4^+$-cell count at 1st

Table 1. Baseline characteristics in HBV non-responders and HBV responders

	HBV non-responders N = 41	HBV responders N = 32
Age in yrs, median (25th-75th percentile)	42 (37.5-53.5)	43 (38.3-55)
Male (%)	29 (70.7)	15 (46.9)
BMI, median (25th-75th percentile)	24.5 (22.5-27.9)	24.6 (22.5-27.3)
Nadir $CD4^+$-cell count cells/mm^3, median (25th-75th percentile)	180 (70-280)	70 (10-175)
$CD4^+$-cell count cells/mm^3 at 1st HBV vaccination, median (25th-75th percentile)	500 (300-665)	385 (290-630)
$CD4^+$-cell count cells/mm^3 at infl immunization, median (25th-75th percentile)	480 (300-645)	425 (293-678)
HAART use, N (%)	33 (80.5)	29 (90.6)
Undetectable HIV-RNA at 1st HBV vaccination, N (%)	30 (73.2)	27 (84.4)
Undetectable HIV-RNA at infl immunization, N (%)	32 (78)	29 (90.6)
No infl immunizations prior to analyzed infl immunization, median (25th-75th percentile)	1 (0-5)	2 (0-3.8)
Time between sample analysis and infl immunization in months, median (25th-75th percentile)	3.6 (2.2-5.0)	2.6 (2.0-4.4)

HBV vaccination and influenza immunization was > 385 cells/mm^3 in both groups. There was a high percentage of HAART use with a corresponding rate of undetectable HIV-RNA that did not differ between the groups.

No differences in the other variables were found between the groups, besides the male/female ratio. In the HBV responders group significantly less male were present (p=0.04).

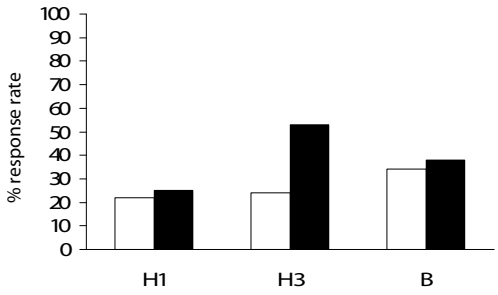


Figure 1a.
Percentage response rate in three influenza antigens (H1, H3 and B) in HBV non-responders and responders
White bar = HBV vaccine non-responders
Black bar = HBV vaccine responders

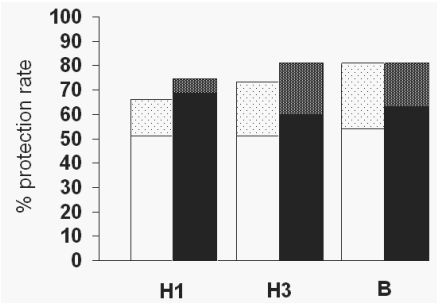


Figure 1b.
Percentage protection rate in pre- and post-influenza vaccination in three antigens (H1, H3 and B) in HBV vaccine non-responders and responders
White bar = pre-vaccination in HBV vaccine non-responders
White dotted bar = post-vaccination in HBV vaccine non-responders
Black bar = pre-vaccination in HBV vaccine responders
Black dotted bar = post-vaccination in HBV vaccine responder

The response rate, representing a fourfold titer increase after vaccination, was significantly higher in the HBV responder group only for A/H3N2 (p=0.01) (Figure 1a). The protection rate after vaccination was approximately 75 % and was not significantly different in HBV both groups for all three strains (Figure 1b). Pre-vaccination titers were already protective for a considerable percentage (50%) (Figure 1b). In Figure 2 the results are depicted for the mean GMT, with the 95% CI for the pre-vaccination titers and for the post-vaccination titers. For all three vaccine strains the pre-vaccination mean GMTs were higher in the HBV responders compared to the HBV non-responders although this difference was not statistically significant, p=0.09 for H1 antigen, p=0.7 for H3 antigen and p=0.3 for B antigen. Also the mean GMTs post-vaccination were higher in the HBV responders, again not statistically significant, p=0.3 for H1 antigen, p=0.3 for H3 antigen and p=0.2 for B antigen. The difference between HBV non-responders and HBV responders in post-vaccination titer, corrected for pre-vaccination titer, were again not statistically significant for all three strains, p=0.6 for H1 antigen, p=0.4 for H3 antigen and p=0.8 for B antigen.

After correction for male gender and nadir CD4$^+$-cell count in multivariate analysis the results for both HBV response groups remained comparable.

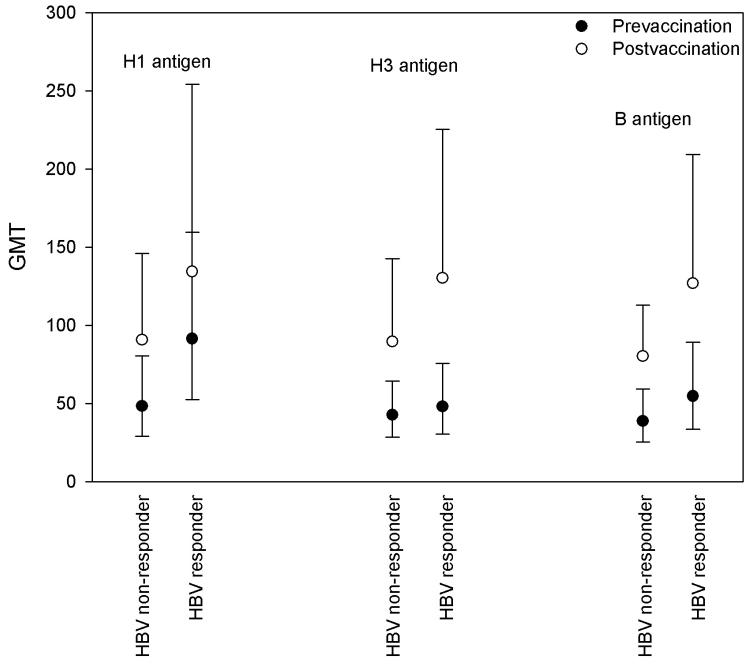


Figure 2. Mean GMT and 95% CI in pre- and post-influenza vaccination titers per antigen (H1, H3 and B) for HBV vaccine non-responders and responders
Black dots = pre-vaccination
Open dots = post-vaccination

Discussion

In a cohort of known HBV vaccine responders and non-responders we found merely a trend for higher post-vaccination titers (GMT) for all three influenza strains in the group of HBV responders.

The low response rate for all strains in both groups could be explained by high pre-vaccination titers, which can be caused by either natural exposure or previous vaccination. As the pre-vaccination protection rate in our study groups is approximately 55% the outcome of the response rate represents mostly a booster effect. Since the response rate is defined as a fourfold titer increase this may be difficult to achieve with high pre-vaccination titers.

The GMT was the only marker which showed a trend for higher immunological capability for response to all three strains in the HBV responder group compared to HBV non-responders.

Although most patients approved to have regained immune competence (as judged by a normal $CD4^+$-cell count and undetectable HIV-RNA load) at the time of vaccination, the immune restoration appears not to be complete. Peripheral blood $CD4^+$ T-cells, comprise only a minority of total T-cells during the chronic phase of the infection and are only a gross and aspecific measure for antigen specific T-cell dependent immunity. Mehandru et al. hypothesized that immune reconstitution in the peripheral blood does not coincide with immune reconstitution in the gastrointestinal mucosa [13]. Furthermore, in chronic HIV infection the architecture and function of the lymphoid tissue is disrupted by immune activation [14-15]. After vaccination the antigens are transported by antigen presenting cells to the lymph nodes where the dendritic cells present the antigen to the antigen-specific B-cells. The immune response upon vaccination should take place in these hyperplastic lymph nodes. This may partly explain the positive trend in HBV vaccine responders to achieve a higher GMT to influenza immunization irrespective of $CD4^+$-cell count and HIV-RNA.

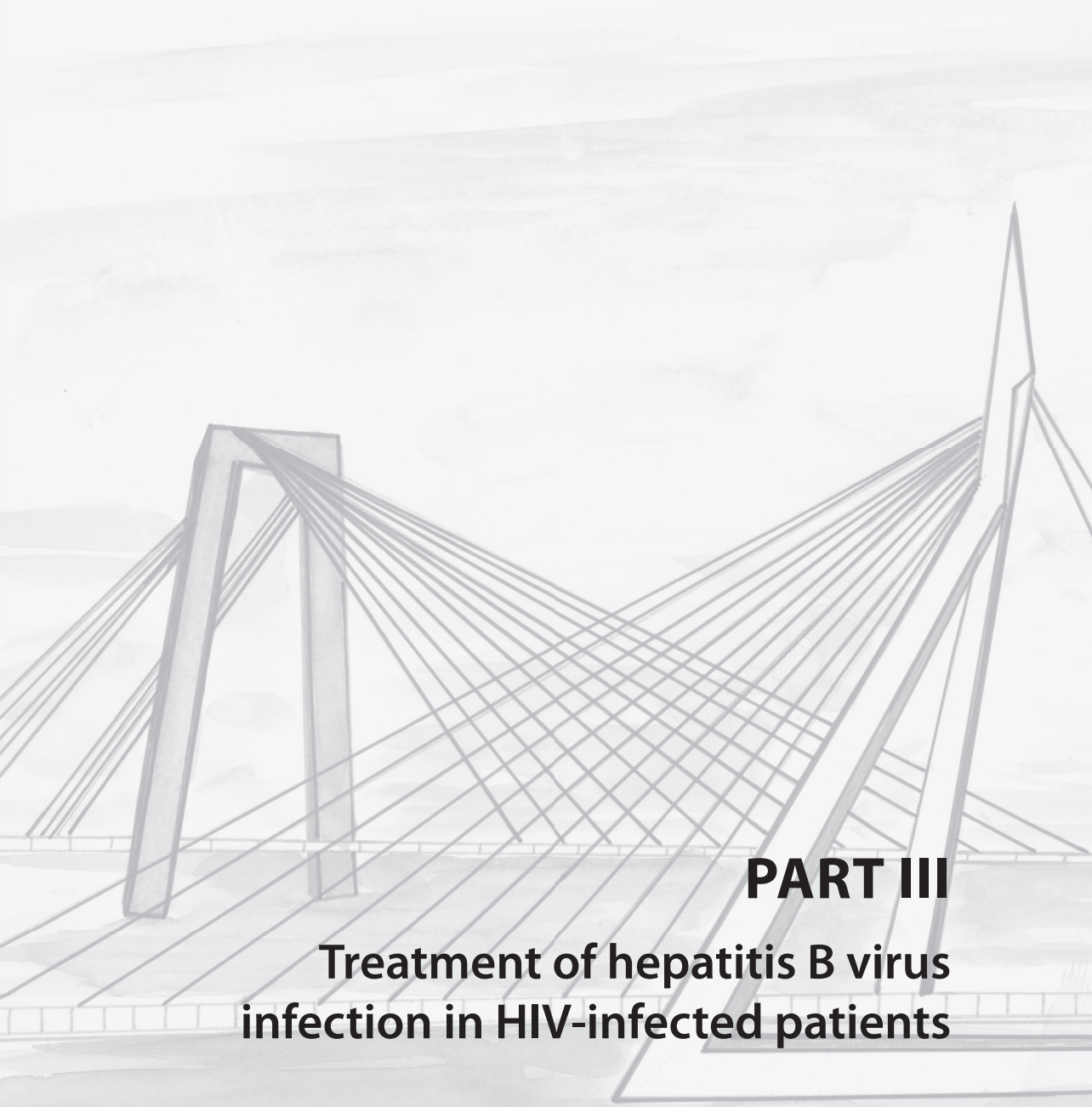
A limitation of our study is the retrospective design. We prospectively vaccinated HIV-infected patients in a HBV vaccination trial. All influenza data from these patients were retrospectively collected. After retrieving stored samples, the material was tested for antibodies against three influenza vaccine antigens corresponding to the vaccine composition of the season in which the vaccine was used. The number of patient samples that was available was limited. In the seasons in which our study was conducted (2004-2007) the A/H1N1 vaccine component remained unchanged and therefore the data of different seasons could be pooled. For A/H3N2 and influenza B the vaccine strains were updated during the subsequent influenza seasons, but we considered the antigens as variant of the same virus type and therefore also pooled these data. The protection rate pre-vaccination was relatively high.

Strength of our study is that we had the disposal of prospective data on HBV vaccination response in our HIV-infected population. Future research needs to address why some patients with normalized $CD4^+$-cell counts and undetectable HIV-RNA do not respond to influenza vaccination.

In conclusion, a trend for a higher GMT, both in pre- and post- influenza vaccination titers was observed in HBV vaccine responders compared to HBV non-responders. The differences in response to HBV and influenza vaccination in HIV-infected patients are probably related to defects in the host immune system, not represented by the $CD4^+$-cell count, and not to the vaccine itself. Underlying mechanisms tested by functional antigen specific T-cell assays may elucidate this defect.

References

1. Bruguera M, Cremades M, Salinas R, Costa J, Grau M and Sans J. Impaired response to recombinant hepatitis B vaccine in HIV-infected persons. J Clin Gastroenterol 1992;14:27-30
2. Carne CA, Weller IV, Waite J, et al. Impaired responsiveness of homosexual men with HIV antibodies to plasma derived hepatitis B vaccine. Br Med J (Clin Res Ed) 1987;294:866-8
3. Collier AC, Corey L, Murphy VL and Handsfield HH. Antibody to human immunodeficiency virus (HIV) and suboptimal response to hepatitis B vaccination. Ann Intern Med 1988;109:101-5
4. Fonseca MO, Pang LW, de Paula Cavalheiro N, Barone AA and Heloisa Lopes M. Randomized trial of recombinant hepatitis B vaccine in HIV-infected adult patients comparing a standard dose to a double dose. Vaccine 2005;23:2902-8
5. Keet IP, van Doornum G, Safary A and Coutinho RA. Insufficient response to hepatitis B vaccination in HIV-positive homosexual men. Aids 1992;6:509-10
6. Overton ET, Sungkanuparph S, Powderly WG, Seyfried W, Groger RK and Aberg JA. Undetectable plasma HIV RNA load predicts success after hepatitis B vaccination in HIV-infected persons. Clin Infect Dis 2005;41:1045-8
7. Rey D, Krantz V, Partisani M, et al. Increasing the number of hepatitis B vaccine injections augments anti-HBs response rate in HIV-infected patients. Effects on HIV-1 viral load. Vaccine 2000;18:1161-5
8. Sasaki MG, Foccacia R and de Messias-Reason IJ. Efficacy of granulocyte-macrophage colony-stimulating factor (GM-CSF) as a vaccine adjuvant for hepatitis B virus in patients with HIV infection. Vaccine 2003;21:4545-9
9. Amendola A, Boschini A, Colzani D, et al. Influenza vaccination of HIV-1-positive and HIV-1-negative former intravenous drug users. J Med Virol 2001;65:644-8
10. Zanetti AR, Amendola A, Besana S, Boschini A and Tanzi E. Safety and immunogenicity of influenza vaccination in individuals infected with HIV. Vaccine 2002;20 Suppl 5:B29-32
11. Gelinck LB, van der Bijl AE, Beyer WE, et al. The effect of anti-tumour necrosis factor alpha treatment on the antibody response to influenza vaccination. Ann Rheum Dis 2008;67:713-6
12. Beyer WE, Palache AM, Luchters G, Nauta J and Osterhaus AD. Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination? Virus Res 2004;103:125-32
13. Mehandru S, Poles MA, Tenner-Racz K, et al. Lack of mucosal immune reconstitution during prolonged treatment of acute and early HIV-1 infection. PLoS Med 2006;3:e484
14. Racz P, Tenner-Racz K, van Vloten F, et al. Lymphatic tissue changes in AIDS and other retrovirus infections: tools and insights. Lymphology 1990;23:85-91
15. Thacker TC, Zhou X, Estes JD, et al. Follicular dendritic cells and human immunodeficiency virus type 1 transcription in CD4+ T cells. J Virol 2009;83:150-8



PART III

Treatment of hepatitis B virus infection in HIV-infected patients

CHAPTER 5

Wild type and YMDD variant of hepatitis B virus: no difference in viral kinetics on lamivudine/ tenofovir therapy in HIV/HBV co-infected patients

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Abstract

Prolonged lamivudine therapy has been identified as the major risk for the development of resistance in HBV, with rates of 90% after 4 years of treatment. Tenofovir disoproxil fumarate showed activity against both wild type and lamivudine resistant HBV in HIV/HBV co-infected patients. In order to compare the efficacy of lamivudine/tenofovir treatment we investigated detailed HBV kinetics in 13 HIV/HBV co-infected patients with either wild type HBV or lamivudine resistant HBV.

The viral strains in both patient groups showed a biphasic viral decline pattern. Only in the first phase of viral decay, which reflects the clearance rate of the free virus from plasma, there was a statistically significant response in favor of the wild type group. After the first phase we observed a similar viral decline till 24 weeks of both groups. This is reassuring for many pretreated co-infected patients harbouring mutant viruses.

Introduction

Chronic hepatitis B virus (HBV) infection has become an important source of co morbidity in human immunodeficiency virus (HIV)-infected individuals. HIV-1 infection is associated with reduced frequency of spontaneous clearance of hepatitis B s antigen (HBsAg) and hepatitis B e antigen (HBeAg) and is associated with higher HBV-DNA levels, lower serum alanine amino-transferase (ALT) levels, and milder histological necro-inflammatory activity [1]. Despite this, progression to cirrhosis is more common [2].

Prolonged lamivudine therapy, suppressing both HIV and HBV replication, has been identified as the major risk for the development of HBV resistance. Mutations typically occur in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of the catalytic domain of the polymerase gene of HBV. HIV/HBV co-infected individuals develop resistance at a rate of 20% annually, with rates of 90% after 4 years of treatment. These rates are higher compared to resistance rates in HBV patients not infected with HIV [3-4]. Tenofovir disoproxil fumarate (TDF), an acyclic nucleotide analogue reverse transcriptase inhibitor, showed activity in *in vitro* and *in vivo* studies against both wild type and lamivudine resistant HBV in HIV/HBV co-infected patients [5-6]. Assessing the speed and variability in patterns of viral decay of both viral strains may be valuable in the design of future treatment strategies.

Therefore, we investigated HBV kinetics in 13 HIV/HBV co-infected patients treated with lamivudine/TDF combination therapy by using mathematical modeling [7-10]. We compared the efficacy of lamivudine/TDF for wild type infected patients versus patients harbouring an YMDD variant.

Patients and Methods

Patients

Thirteen HIV-1/HBV co-infected patients were included in this study after informed consent was obtained. Eight patients were pre-treated with highly active antiretroviral therapy (HAART) containing lamivudine for a median of 293 weeks (range 91-382 weeks) and had a mutation in the YMDD motif. Five patients were treatment naïve and had wild type virus. One patient was treated with lamivudine for a period of 178 weeks but stopped treatment 3 years prior to start of the study and no mutant virus was detectable. The HAART regimen of all 13 patients included lamivudine 300 mg daily and TDF 245 mg daily. All patients were male, suffered from HIV-1/HBV co-infection and were anti-HCV negative. All patients were treated with a combination of lamivudine and TDF and followed for a period of 24 weeks. Sequential sera, taken at day 1 (at t=0 and 8 h), days 2, 4, 7, 10, 14, 21, 28 and every 4 weeks thereafter until 24 weeks, were quantitatively assessed for HBV-DNA. The presence of YMDD mutants

was determined at t=0 and 24 weeks. Liver tests were performed on day 1 at t=0, days 7 and 28 and weeks 12 and 24.

Concurrent antiretroviral regimens in addition to TDF and lamivudine consisted of: (i) a non-nucleoside reverse transcriptase inhibitor (NNRTI) in eight patients (62%); (ii) a NNRTI and one nucleoside reverse transcriptase (NRTI) in three patients (23%); (iii) a boosted protease inhibitor (PI) in one patient (8%); (iv) a NRTI and an unboosted PI in one patient (8%).

Methods

HBV-DNA was isolated using the MagnaPure LC isolation station (Roche Applied Science, Penzberg, Germany) with a modified protocol HBV-02 in which the proteinase K digestion occurred first [11]. HBV-DNA serum levels were quantitatively assessed using the HBV-DNA TaqMan assay and calibrated using EUROHEP HBV-DNA standards [12]. The Taqman assay enabled accurate quantitative determination to levels of 1000 copies/ml [11].

At day 1 HBV polymerase mutant analysis was performed on HBV-DNA using a Line Probe assay (INNO-LiPA HBV DR; Innogenetics N.V., Gent, Belgium) [13]. Where the INNO-LiPA assay was indeterminate, sequence analysis was done. A selected genome region of the polymerase gene was amplified and sequenced with particular primers described earlier [14].

HIV-RNA was quantitatively assessed with the Cobas Amplicor (Roche Molecular Systems, Penzberg, Germany).

Mathematical modeling of viral decline was performed according to the model of Neumann as recently applied and described by van der Eijk *et al* [15].

Results

Thirteen patients participated in the study. Baseline characteristics are shown in Table 1. Nine patients were Caucasian, two patients were Black and two patients were Asian. In the YMDD variant group HIV-1 RNA was suppressed below detection level of 50 copies/ml. In the wild type group HIV-1 RNA varied from $1,3x10^4$ copies/ml to $> 10^5$ copies/ml.

There was no statistical significant difference in baseline HBV-DNA level between the wild type and YMDD variant group (p=0.28). The absolute viral decay of HBV-DNA in both groups was significant during the first four weeks of therapy. The median decline was 2.3 log (range 1.3-3.8) (p=0.02) in the YMDD variant group and 2.9 log (range 1.2-4.7) (p=0.04) in the wild type group. Between the two groups the difference was not significant (p=0.42).

Both the patient groups showed a very similar biphasic viral decline pattern of HBV-DNA in the first 28 days. Remarkably, during the first phase of viral decay, there was a faster decline of the wild type virus, (p=0.006) (Figure 1a), while during the second phase, until day 28, there was no statistically significant difference in viral decline between the two groups (p=0.98). The slope of the viral decay during the first phase was strongly correlated with baseline HBV-DNA level

Table 1. Baseline characteristics

patient	Age (yrs)	Duration of lamivudine (weeks)	HBV-DNA $\log_{10}$ copies/ml	HBeAg status	YMDD variant	CD4 start $x10^9$/l	ALT $^+$U/l
GROUP YMDD VARIANT							
1	36	313	6.78	Pos	YVDD/YIDD	0.3	46
2	46	326	8.96	Pos	YVDD	0.42	79
3	34	304	9.40	Pos	YVDD	0.47	235
4	49	183	9.45	Pos	YVDD/YIDD	0.46	80
5	53	382	8.68	Pos	YVDD	0.12	165
6	39	282	9.76	Pos	YVDD	0.37	98
7	36	166	6.48	Pos	YVDD	0.70	53
8	40	91	9.68	Pos	YVDD	0.48	46
Median			9.18			0.44	80
GROUP WILD TYPE							
9	37	178	9.92	Pos	-	0.32	402
10	55	-	10.32	Pos	-	0.28	114
11	41	-	9.36	Pos	-	0.2	363
12	36	-	8.11	Pos	-	0.1	1454
13	26	-	9.65	Pos	-	0.12	74
Median			9.65			0.20	363

$^+$ALT upper limit of normal = 40 IU/L

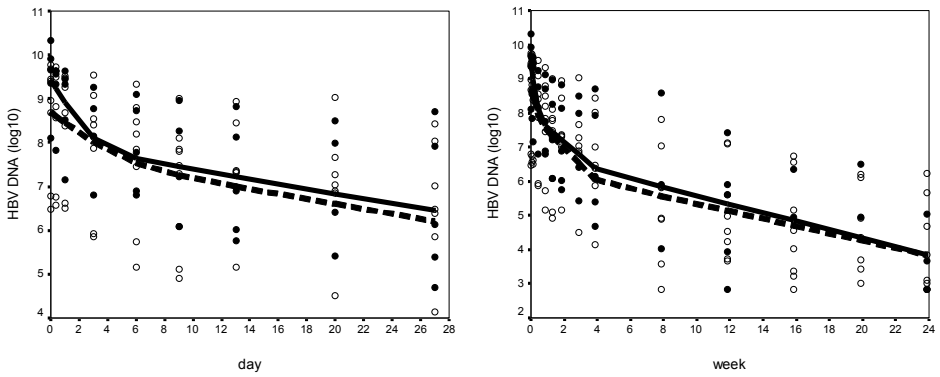


Figure 1 (a and b). Viral decline from baseline to week 24
Closed dots (•) represent observed HBV-DNA (copies/ml) data of the wild type virus group
Open dots (o) represent observed HBV-DNA (copies/ml) data of YMDD variant group
Solid line represents fitted HBV-DNA (copies/ml) data of the wild type virus group
Dotted line represents fitted HBV-DNA (copies/ml) data of YMDD variant group

within the wild type group (R=0.92), while for the YMDD variant group this correlation was weak (R=0.13). The median estimated time for first phase to become second phase was 3.7 days (range 2.6-7.6) and 2.8 days (range 1.9-4.8) in YMDD variant and wild type group respectively, (p=0.17).

From day 28 until week 24 a linear decline was observed (Figure 1b). Again there was no significant difference between the two groups (p=0.63). The median of the estimated effectiveness of TDF in blocking virus production in infected cells was 90% (range 82-99) for η = 0 and 85% (range 78-97) for η = 1 in the YMDD variant group and 96% (range 90-99) for η = 0 and 95% (range 89-99) for η = 1 in the wild type group. The median of the estimated death rate of virus producing infected cells was 14% (range 6-21) for η = 0 and 13% (range 5-20) for η = 1 in the YMDD variant group and 13% (range 3-21) for η = 0 and 12% (range 3-21) for η = 1 in the wild type group.

The median of the estimated half-life of free virus was 26.7 hours (range 26.2-27.0) and 13.7 hours (range 13.6-13.7) in the YMDD variant and wild type group respectively, (p=0.002). The median of the estimated half-life of infected hepatocytes was 4.8 days (range 3.4-10.8) and 5.4 days (range 3.2-20.5) in the YMDD variant and wild type group respectively, (p=0.72). Follow-up for 24 weeks was available for 12/13 patients. HBV-DNA became undetectable ($\leq 10^3$ copies/ml) in 5 patients and $\leq 10^5$ copies/ml in 10 patients. Moderate elevated baseline ALT (3 x upper limit of normal) was no predictor for early HBV decline in the first phase and up to 28 days (p=0.5). In the majority of patients (12/13) data were available on HBeAg and anti-HBe at start of treatment. At 12 weeks 3 out of 6 patients and at 24 weeks 6 out of 12 patients lost HBeAg. Seroconversion to anti-HBe occurred in three, two patients seroconverted to borderline anti-HBe and one patient had loss of HBeAg without seroconversion to anti-HBe. Two patients lost HBsAg. No side effects were reported and treatment was well tolerated and not interrupted.

YMDD sequencing was performed in 8 out of 13 patients at week 24. In three out of four patients with initially an YMDD variant, this variant persisted after week 24. In one patient sequencing could not be performed because of a negative HBV-PCR at week 24. At week 24 the wild type group showed no development of HBV mutations in two out of five patients, two patients had a negative HBV-PCR and could not be sequenced, one sample was missing.

Discussion

This study provides intensive viral kinetic data following lamivudine/TDF combination treatment of HIV/HBV co-infected patients with drug-resistant HBV mutants and wild type virus. The viral decay of HBV-DNA in both patients groups was overall the same.

Previous modeling studies in chronic infected HBV patients have demonstrated that a biphasic pattern of viral response occurs during the first 4 weeks of antiviral treatment with nucleoside analogues [10]. The decline of viral load during treatment with adefovir (ADV), an acyclic nucleoside reverse transcriptase inhibitor, also displayed a biphasic kinetic profile [9]. Efficacy of ADV in the treatment of wild type and YMDD variant hepatitis B virus is described and ADV is approved for the treatment of chronic HBV infection [16-17]. ADV as treatment

of HIV needs much higher doses than the approved dose used in HBV mono-infection and is associated with unacceptable risk for nephrotoxicity at these higher doses. TDF is capable of effectively blocking viral replication in patients with lamivudine-induced mutant viruses in HBV mono-infected patients as well as in HIV/HBV co-infected patients [15].

At this moment TDF seems a better treatment for HIV/HBV co-infected patients than lamivudine, although there is little known about selection of mutations against TDF during long term follow-up. From the limited data on TDF in HIV/HBV co-infection [18], there are no reports of resistance, as opposed to the annual 20% lamivudine resistance against HBV.

In the study reported here, the viral decay of HBV-DNA in both patients groups was overall the same. This could be explained by increased sensitivity of the YMDD variant to TDF [19-20]. In the recently published study of Lacombe *et al.* our results were confirmed in their long-term kinetics [21]. They concluded that YMDD mutations did not impact the long-term effect (517 days) of either TDF monotherapy or lamivudine/TDF combination therapy on HBV replication. In our study all patients received combination therapy, although in the YMDD mutant group TDF is probably the only effective drug in this combination. We cannot confirm nor contradict the results of the for mentioned study in which they conclude that the viral decline is influenced by initial HBV-DNA, as in our study no statistical difference between the two groups at baseline HBV-DNA existed. Only during the initial phase in our study, which lasted less than 7 days, we found a strong correlation with baseline HBV-DNA and viral decline in the wild type group.

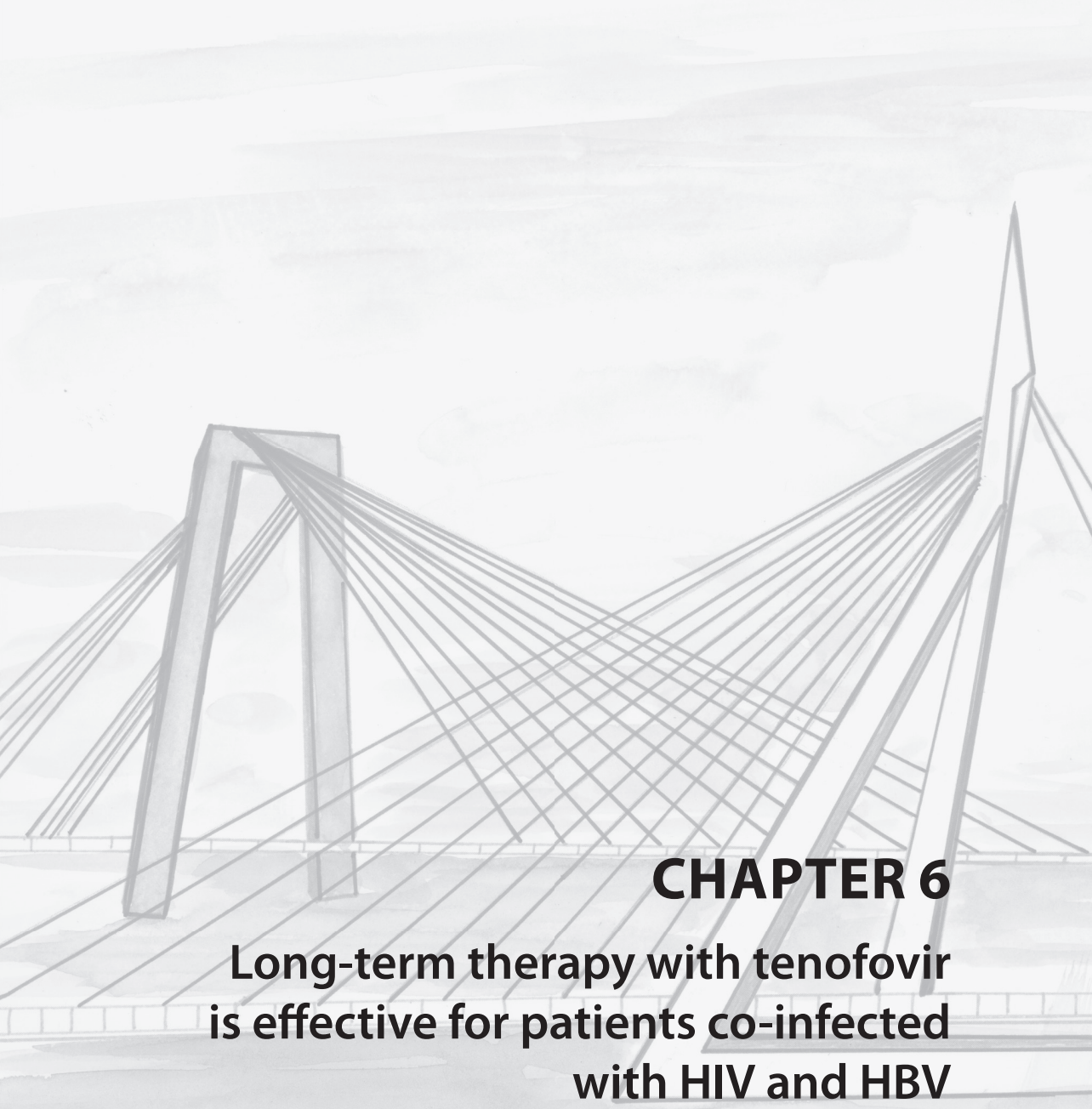
The second phase decline in HBV-DNA levels reflects the death rate of virus producing infected cells. The death of these cells requires a cellular immune response of the host. In HBV mono-infection a possible marker of the strength of host immune response is the level of ALT, which is an indicator of the level of cell death. The results of studies concerning the predictive value of pretreatment ALT levels among chronic HBV mono-infected and co-infected patients who were treated with antiviral therapy are in disagreement [8, 15, 21-23]. In our study baseline ALT in wild type was higher than in YMDD mutant without any correlation with HBV decline which is in agreement with van der Eijk *et al* [15]. This may be explained by HIV/HBV co-infection, resulting in less immunocompetence and therefore pretreatment ALT levels too low to produce a detectable association with the slope of viral decay. Also small numbers of patients and wide ranges may explain this phenomenon.

In conclusion, it is reassuring for many lamivudine pretreated co-infected patients harbouring YMDD variant virus that adding TDF to a HAART regimen showed a viral decline of HBV-DNA similar to wild type virus patients. Future studies with larger numbers of patients and longer periods of follow-up are relevant to document long-term outcome of lamivudine/TDF combination therapy in chronic HBV patients, with or without HIV co-infection.

References

1. Gilson RJ, Hawkins AE, Beecham MR, et al. Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. Aids 1997;11:597-606
2. Colin JF, Cazals-Hatem D, Loriot MA, et al. Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. Hepatology 1999;29:1306-10
3. Wolters LM, Niesters HG, Hansen BE, et al. Development of hepatitis B virus resistance for lamivudine in chronic hepatitis B patients co-infected with the human immunodeficiency virus in a Dutch cohort. J Clin Virol 2002;24:173-81
4. Leung NW, Lai CL, Chang TT, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001;33:1527-32
5. van Bommel F, Wunsche T, Schurmann D and Berg T. Tenofovir treatment in patients with lamivudine-resistant hepatitis B mutants strongly affects viral replication. Hepatology 2002;36:507-8
6. Benhamou Y, Tubiana R and Thibault V. Tenofovir disoproxil fumarate in patients with HIV and lamivudine-resistant hepatitis B virus. N Engl J Med 2003;348:177-8
7. Neumann AU, Lam NP, Dahari H, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science 1998;282:103-7
8. Nowak MA, Bonhoeffer S, Hill AM, Boehme R, Thomas HC and McDade H. Viral dynamics in hepatitis B virus infection. Proc Natl Acad Sci U S A 1996;93:4398-402
9. Tsiang M, Rooney JF, Toole JJ and Gibbs CS. Biphasic clearance kinetics of hepatitis B virus from patients during adefovir dipivoxil therapy. Hepatology 1999;29:1863-9
10. Wolters LM, Hansen BE, Niesters HG, Zeuzem S, Schalm SW and de Man RA. Viral dynamics in chronic hepatitis B patients during lamivudine therapy. Liver 2002;22:121-6
11. Pas SD, Fries E, De Man RA, Osterhaus AD and Niesters HG. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-901
12. Heermann KH, Gerlich WH, Chudy M, Schaefer S and Thomssen R. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. J Clin Microbiol 1999;37:68-73
13. Stuyver L, Van Geyt C, De Gendt S, et al. Line probe assay for monitoring drug resistance in hepatitis B virus-infected patients during antiviral therapy. J Clin Microbiol 2000;38:702-7
14. Osterhaus AD, Vos MC, Balk AH, et al. Transmission of hepatitis B virus among heart transplant recipients during endomyocardial biopsy procedures. J Heart Lung Transplant 1998;17:158-66
15. Eijk AA, Hansen BE, Niesters HG, et al. Viral dynamics during tenofovir therapy in patients infected with lamivudine-resistant hepatitis B virus mutants. J Viral Hepat 2005;12:364-72
16. Peters MG, Hann Hw H, Martin P, et al. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 2004;126:91-101
17. Perrillo R, Hann HW, Mutimer D, et al. Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. Gastroenterology 2004;126:81-90
18. van Bommel F, Wunsche T, Mauss S, et al. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology 2004;40:1421-5
19. Delaney WEt, Yang H, Miller MD, Gibbs CS and Xiong S. Combinations of adefovir with nucleoside analogs produce additive antiviral effects against hepatitis B virus in vitro. Antimicrob Agents Chemother 2004;48:3702-10

20. Ying C, De Clercq E, Nicholson W, Furman P and Neyts J. Inhibition of the replication of the DNA polymerase M550V mutation variant of human hepatitis B virus by adefovir, tenofovir, L-FMAU, DAPD, penciclovir and lobucavir. J Viral Hepat 2000;7:161-5
21. Lacombe K, Gozlan J, Boelle PY, et al. Long-term hepatitis B virus dynamics in HIV-hepatitis B virus-co-infected patients treated with tenofovir disoproxil fumarate. Aids 2005;19:907-15
22. Lewin SR, Ribeiro RM, Walters T, et al. Analysis of hepatitis B viral load decline under potent therapy: complex decay profiles observed. Hepatology 2001;34:1012-20
23. Wolters LM, Hansen BE, Niesters HG, et al. The influence of baseline characteristics on viral dynamic parameters in chronic hepatitis B patients treated with lamivudine. J Hepatol 2002;37:253-8



CHAPTER 6

Long-term therapy with tenofovir is effective for patients co-infected with HIV and HBV

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Abstract

Background and aims. We investigated the long-term efficacy and renal safety of tenofovir disoproxil fumarate (TDF), administered to patients co-infected with HIV and hepatitis B virus (HBV) as a part of antiretroviral therapy.

Methods. We performed a multicenter, prospective cohort study of 102 patients co-infected with HIV and HBV who were treated with TDF.

Results. At baseline, 80% of patients had a detectable viral load (HBV-DNA > 20 IU/mL). Among patients positive for hepatitis B e antigen (HBeAg, n=67), 92% had a VR (HBV-DNA < 20 IU/mL) after 5 years of treatment. There was no difference between patients with or without lamivudine-resistance at baseline (p=0.39). Loss rates of HBeAg and hepatitis B s antigen (HBsAg) were 46% and 12%, respectively. Among HBeAg-negative patients (n=15), 100% had a virologic response after 4 years of treatment and 2 (13%) lost HBsAg. Twenty subjects (20%, all HBeAg-negative) had undetectable HBV-DNA at baseline; during a median follow-up of 52 months (41–63 months), 19 (95%) maintained a virologic response and 2 (10%) lost HBsAg. Overall, one patient acquired a combination of resistance mutations for anti-HBV drugs and experienced a virologic breakthrough. Three (3%) patients discontinued TDF because of increased serum levels of creatinin. The estimated decrease in renal function after 5 years of TDF therapy was 9.8 mL/min/1.73m^2, which was most pronounced shortly after TDF therapy was initiated.

Conclusions. TDF, administered as part of antiretroviral therapy, is a potent anti-HBV agent with a good resistance profile throughout 5 years of therapy. Only small, non-progressive decreases in renal function were observed.

Introduction

Tenofovir disoproxil fumarate (TDF) was licensed for the treatment of human immunodeficiency virus (HIV) infection in 2001, and plays since then a pivotal role in HIV management. Currently, the combination of TDF and emtricitabine is the most widely prescribed nucleos(t)ide analogue reverse transcriptase inhibitor (NRTI) backbone in Europe. Because HIV and HBV share similar routes of transmission, prevalence of HBsAg-carriership is more than five-fold higher among HIV-infected patients compared to the general population [1-2]. Furthermore, HIV/HBV co-infected patients are at increased risk for development of cirrhosis and hepatocellular carcinoma, and have higher overall mortality rates compared to HIV mono-infected patients [3-6].

The efficacy of TDF in HBV therapy was first described in studies including mainly patients with HIV-1 co-infection [7-11]. Recent data showed the efficacy of TDF in the treatment of chronically HBV mono-infected patients as well [12]. TDF was superior to adefovir dipivoxil in both nucleos(t)ide-naïve HBeAg-positive and HBeAg-negative HBV patients, and appeared to be one of the most potent anti-HBV agents so far. Several reports showed that TDF was also effective in the nucleos(t)ide-experienced population, although conflicting results have been presented concerning patients with genotypic resistance to adefovir dipivoxil [13-16]. Moreover, TDF has a good resistance profile, and no convincing proof of HBV-resistant mutants to TDF has been presented so far [17]. Long-term therapy is indicated for all HIV/HBV co-infected and most HBV mono-infected patients treated with oral nucleos(t)ide analogues, as a sustained response after cessation of therapy is rare [18-19]. However, follow-up in studies investigating the efficacy of TDF in HIV/HBV co-infected and HBV mono-infected patients is limited to only two years. In addition, there are concerns about the risk of renal toxicity with TDF [20-25]. We investigated the long-term efficacy and renal safety of TDF administered as a part of antiretroviral therapy in a large cohort of HIV/HBV co-infected patients.

Materials and Methods

Study populations

Six Dutch centers specialized in HIV management participated in this multicenter cohort study. From 2001 to July 2006 all consecutive adult HIV-infected patients positive for hepatitis B surface antigen (HBsAg) for more than six months, and treated with TDF as a part of antiretroviral therapy for at least six months were included. Patients were excluded if they had hepatitis C or hepatitis *delta* co-infections, or received concomitant treatment with (pegylated) interferon during the on-treatment follow-up period. Patients were categorized to those with or without the presence of detectable HBV-DNA at baseline.

Follow-up of participants

Virologic, haematological and biochemical parameters were recorded at least at 6-month intervals in the first two years of follow-up and at yearly intervals thereafter. At every visit routine examination with measurement of serum alanine aminotransferase (ALT), creatinin, $CD4^+$-cell count, serum HIV-RNA, serum HBV-DNA, HBeAg, and anti-HBe took place. HBsAg status was measured in case of the combined presence of undetectable HBV-DNA and negative HBeAg. A mutation analysis was done (a) at baseline in all lamivudine (LAM)-experienced HBV patients, (b) in case of virologic breakthrough, defined as an increase in serum HBV-DNA level > 1 $\log_{10}$ (10-fold) above nadir on at least two occasions after initial virologic response, or (c) in case of serum HBV-DNA > 200 IU/mL at the end of follow-up. HBV genotype was determined at baseline. At baseline, the diagnosis of cirrhosis was based on the treating physician's judgment. Abdominal ultrasound was performed if there was clinical suspicion of progression to cirrhosis, development of decompensated liver disease or hepatocellular carcinoma.

Endpoints

The primary outcome was virologic response (VR), defined as serum HBV-DNA levels < 20 IU/mL during the on-treatment follow-up period. Secondary endpoints were HBsAg loss, HBeAg loss for HBeAg-positive patients, ALT normalization, and emergence of antiviral resistant mutations. Progression to cirrhosis was defined on clinical grounds, that is, albumin level < 3,5 g/dL, platelet count < 100,000 mm^3, clinical decompensation, and ultrasound demonstration of surface nodularity, splenomegaly, and > 15-mm portal vein diameter. Clinical decompensation was defined as development of ascites, encephalopathy, jaundice, or gastro-intestinal bleeding, defined to internationally agreed criteria [26]. Renal function was assessed by monitoring the estimated glomerular filtration rate (eGFR) in mL/min/1.73 m^2, which was calculated using the Modification in Diet in Renal Disease (MDRD) equation, based on the serum creatinin, age, sex and race.

Laboratory tests

ALT and creatinin levels were measured using automated techniques. Absolute numbers of CD4 T lymphocytes were assessed on whole blood by flowcytometry. HBsAg, HBeAg, and antibody against HBeAg (anti-HBe) were determined using commercially available enzyme immunoassays. HIV-RNA was quantitatively assessed with the Cobas Ampliprep/Cobas Amplicor version 1.5 (Lower limit of detection: 50 copies/mL; Roche Molecular Systems, Penzberg, Germany). HBV-DNA was quantified in serum as previously described [27-28]. The lower limit of this assay was recently determined at 20 IU/mL by probit analysis (M. Schutten, unpublished results). HBV genotype was determined by Sanger sequencing on a 752 basepair fragment in the S gene as previously described [29]. Antiviral resistance associated mutations were determined using the Inno-LIPA HBV DR v2 (Innogenetics NV, Zwijnaarde,

Belgium) for highly sensitive detection of mutant species and by Sanger sequencing of the HBV reverse transcriptase gene to detect mutations not present on the Inno-LIPA HBV DR v2 (rtT184, rtA194, rtS202, rtI233, rtM250).

Data analysis

Continuous variables are expressed as means ± standard deviation or median (interquartile range) where appropriate. Continuous variables were compared using the t-test or the Mann-Whitney test. Categorical variables were compared using the Chi-square or Fisher's exact test. Follow-up times were calculated from the date of TDF treatment initiation to the date of event or censorship. The cumulative probabilities of VR, HBeAg loss and HBsAg loss during treatment were calculated by the Kaplan-Meier method. Survival analysis with Cox regression model was used to analyze which baseline factors were associated with VR in patients with a detectable HBV-DNA at baseline (n=82). Changes in creatinin during treatment were analyzed with a repeated measurement model estimating an overall smooth quadratic decline while allowing for a random intercept and a decline per patient. Differences in decline between baseline characteristics like the use of ritonavir-boosted protease inhibitors were tested adding an interaction term with time in the model. All statistical tests are two-sided, and a p-value < 0.05 was considered to be statistically significant. SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) were used for all statistical analysis.

Results

Baseline characteristics of the study population are presented in Table 1. A total of 102 patients were included in this analysis. Ninety-two (90%) subjects were men and the mean age was 42±8.9 years. The treatment regimens that were used in addition to TDF were for most patients either a NRTI and a non-NRTI regimen (64%) or a NRTI and ritonavir boosted protease inhibitor regimen (24%). During the on-treatment follow-up all patients received concomitant treatment with either LAM or emtricitabine. Median follow-up of the whole study population was 55 (42-64) months.

Virologic response in patients with detectable HBV-DNA at baseline

Of 82 patients with detectable HBV-DNA at baseline, 67 (82%) subjects were HBeAg-positive at the initiation of TDF, and the mean HBV-DNA was 7.0±2.1 $\log_{10}$ IU/mL. Fifty (61%) patients were previously treated with LAM for a median duration of 42 (22-74) months. TDF was added to LAM therapy as a second anti-HBV drug in 45 (90%) of 50 patients and in 5 patients LAM was reintroduced in combination with TDF. In 33 (66%) subjects LAM-resistant mutations could be detected at the initiation of TDF. During a median follow-up of

Table 1. Baseline characteristics

	Detectable HBV-DNA	Undetectable HBV-DNA	P - value
	N=82	N=20	
Age (years)	42±8.7	43±10	0.68
Gender (male %)	77 (94%)	15 (75%)	0.02
Race			0.04
Caucasian	54 (66%)	8 (40%)	
Black	18 (22%)	10 (50%)	
Other	10 (12%)	2 (10%)	
BMI	23±5.2	25±3.4	0.31
ALT (xULN)	1.6 (1.0-2.7)	0.7 (0.4-1.0)	< 0.001
HBV-DNA (Log_{10}IU/ml)	7.0±2.1	UD*	< 0.001
HBeAg-positive	67 (82%)	0 (0%)	< 0.001
Genotype (N=81)			0.15
A	47 (62%)	5 (100%)	
other	29 (38%)	0 (0%)	
Presence cirrhosis	12 (15%)	2 (10%)	0.66
$CD4^+$-cell count	285 (120-473)	320 (155-460)	0.68
HIV-RNA (Log_{10} copies/mL)	3.1±1.6	2.0±1.3	0.002
Creatinin (mg/dL)	0.86±0.17	0.88±0.19	0.66
eGFR (mL/minute)	106±31	102±30	0.62
Treatment regimen			0.41
2 NRTI + 1 NNRTI	50 (61%)	15 (75%)	
2 NRTI + PI/r	20 (24%)	4 (20%)	
Other	12 (16%)	1 (5%)	
Concomitant anti-HBV therapy			0.26
Lamivudine	77 (94%)	20 (100%)	
Emtricitabine	5 (6%)	0 (0%)	
Previous anti-HBV therapy			
LAM-experienced	50 (61%)	18 (90%)	0.02
LAM-resistance at baseline	33 (40%)	0 (0%)	< 0.001
Duration of LAM therapy#	42 (22-74)	45 (24-64)	0.73

*Undetectable; # Months

56 (43-64) months, 72 (88%) patients achieved VR. For HBeAg-positive patients (n=67), the cumulative probability of achieving VR at 1, 2, 3, 4 and 5 years of treatment was 31%, 70%, 83%, 88%, and 92%, respectively (Figure 1). There was no significant difference between patients with or without LAM-resistance at baseline (p = 0.39) (Figure 2). In univariate analysis only HBeAg negativity at baseline demonstrated a trend towards a higher chance of achieving undetectable HBV-DNA (p = 0.09). HBeAg loss and HBsAg loss rates increased to 46% and 12% after 5 years of TDF therapy. For HBeAg-negative patients (n=15), the cumulative probability of achieving VR at 1, 2, 3 and 4 years of treatment was 47%, 85%, 85% and 100%, respectively (Figure 1). During follow-up 2 (13%) of 15 HBeAg-negative patients achieved HBsAg loss. Of 59 patients with elevated ALT levels at baseline, 46 (78%)

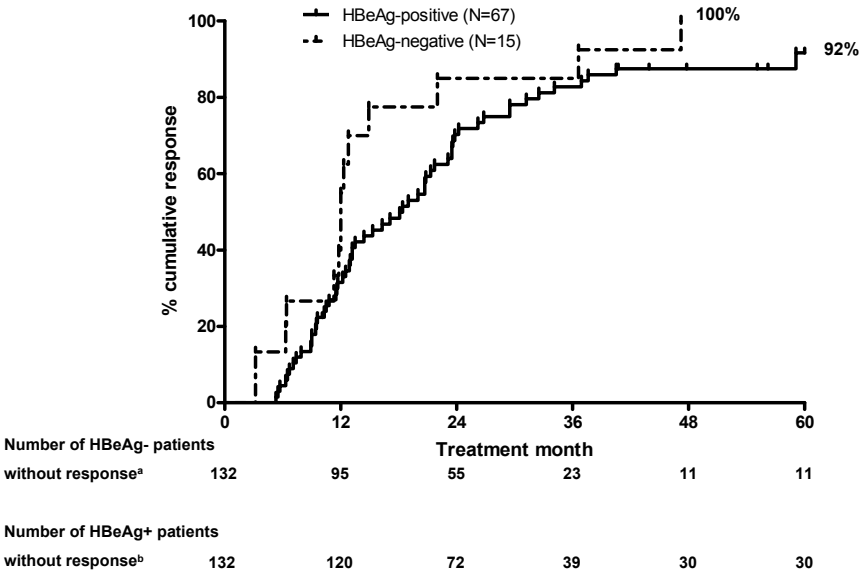

Figure 1 Kaplan-Meier curve for the cumulative probabilities of achieving VR, defined as HBV-DNA < 20 IU/mL, for HBeAg-positive (n = 67) and HBeAg-negative (n = 15) HIV/HBV with patients with detectable HBV-DNA at baseline (n = 82).

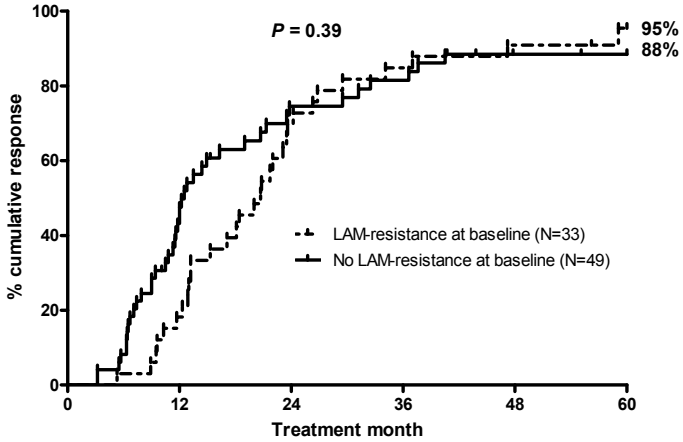

Figure 2 Kaplan-Meier curve for the cumulative probabilities of achieving VR, defined as HBV-DNA < 20 IU/mL, for HIV/HBV patients with detectable HBV-DNA at baseline (n = 82) with lamivudine-resistant (n = 33) or no lamivudine-resistant mutations (n = 49) at the initiation of TDF.

demonstrated ALT normalization at the end of follow-up. Three (4%) patients experienced a virologic breakthrough during the observation period. In two subjects no genotypic resistance could be detected; one patient demonstrated the combined presence of rtM204I, rtL80I, rtL180M, and rtA181V in the HBV polymerase gene (Figure 3B).

Virologic response in patients with undetectable HBV-DNA at baseline

Twenty patients (100% HBeAg-negative) had undetectable HBV-DNA at baseline: Two patients were treatment-naïve; 18 patients were pretreated with LAM for a median duration of 38 (24-64) months. In all patients TDF was added as a second anti-HBV drug per internal protocol. During a median follow-up of 52 (41-63) months 19 (95%) subjects maintained VR, and two (10%) patients showed HBsAg loss. One (5%) subject experienced a virologic breakthrough after which a hepatocellular carcinoma was diagnosed. No genotypic resistance could be detected.

HBV resistance surveillance

During a median follow-up of 55 (42-64) months nine of 67 (13%) HBeAg-positive and one of 15 (7%) HBeAg-negative patients with a detectable HBV-DNA at baseline did not achieve VR. Of these ten subjects, three experienced a virologic breakthrough as well. One of 20 patients with undetectable HBV-DNA at baseline demonstrated a virologic breakthrough. None of the subjects with a virologic breakthrough demonstrated LAM-resistant mutations at baseline. In two patients non-adherence was suspected, as a simultaneous rebound HIV-RNA was observed. A hepatocellular carcinoma was diagnosed in the other two patients, of whom one subject also demonstrated multiple anti-HBV drug-resistant mutations (rtM204I, rtL80I, rtL180M, and rtA181V). Of the patients with a detectable viral load at the end of follow-up without fulfilling the criteria of virologic breakthrough (n=7), four subjects showed LAM-resistance at baseline, and in one patient these substitutions persisted throughout the observation period. No therapy-resistant mutations were observed in the other patients at the end of follow-up.

Progression of hepatitis B and survival

Of the 14 cirrhotic patients at baseline, 3 developed de novo hepatocellular carcinoma after 10-32 months (Figure 3), and two subjects decompensated liver disease after 42 and 48 months of follow-up, respectively. In total, four patients died due to hepatocellular carcinoma progression (n=3) or complications related to end-stage liver disease (n=1). Of the 88 non-cirrhotic patients, none progressed clinically to cirrhosis or developed de novo hepatocellular carcinoma. Three patients died because of HBV-unrelated causes.

Entecavir as rescue therapy in patients with persisting HBV replication

In four patients who demonstrated persistent HBV replication during antiviral therapy, entecavir (ETV) (dosed at 1 mg once daily) was added to the treatment regimen as rescue therapy (Table 2). Patients were compliant with the treatment regimen, which is supported by the undetectable HIV-RNA levels in these four patients at the moment ETV was added. The addition of ETV resulted in undetectable HBV-DNA in all subjects after 3-15 months of follow-up; one patient also achieved HBeAg loss.

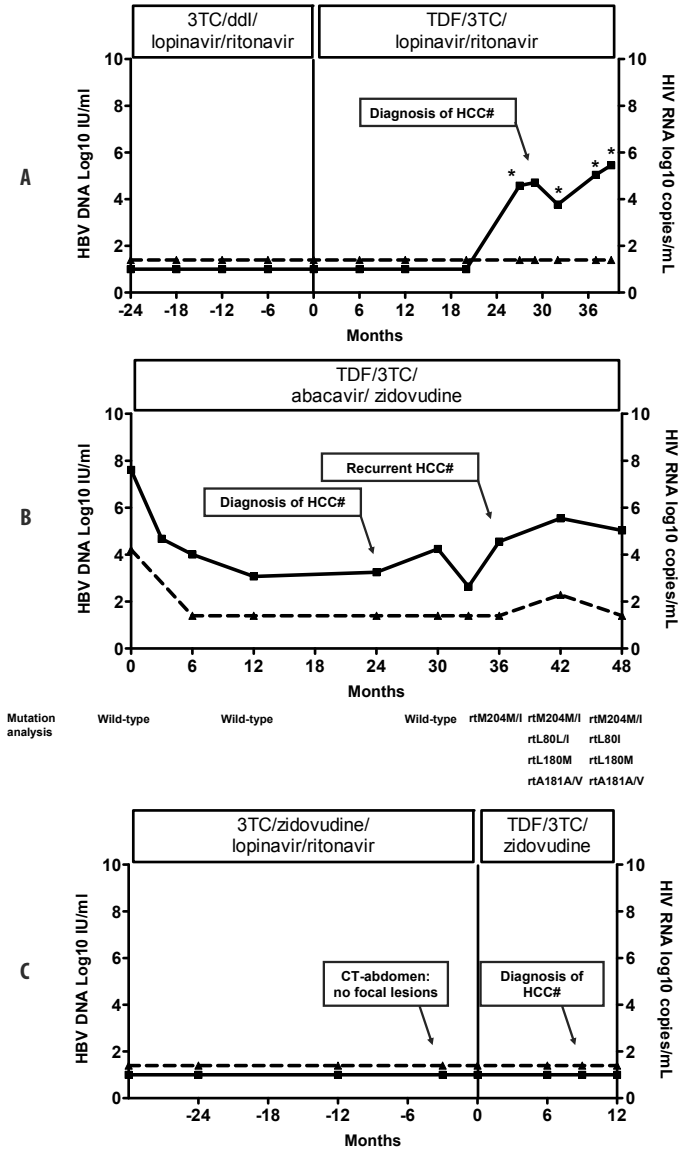

Figure 3 Clinical course of three patients who developed hepatocellular carcinoma throughout follow-up. Two patients demonstrated a virologic breakthrough as well.

■—— = HBV-DNA

▲----- = HIV-RNA

A. #: MRI abdomen demonstrated diffuse hepatocellular carcinoma in segment 4-8, with infiltration of the portal vein. In addition, there was a focal lesion in segment 2 with a diameter of 8mm, suspicious for hepatocellular carcinoma. *: Mutation analysis demonstrated wild-type hepatitis B virus

B. #: MRI abdomen demonstrated a focal lesion in segment 4a, suspicious for hepatocellular carcinoma with a diameter of 3.3 cm, for which he received treatment with radio-frequent ablation. After 36 months recurrent hepatocellular carcinoma was diagnosed.

C. #: MRI abdomen demonstrated a focal lesion in segment 8, suspicious for hepatocellular carcinoma, with a diameter of 13 cm. Two other focal lesions, suspicious for hepatocellular carcinoma were observed in segment 2 and 3, with a diameter of 1 cm

Table 2. Summary of patients with persistent HBV replication in whom entecavir was added as rescue therapy

	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	26	43	41	37
Gender	male	male	male	male
Previous therapy with LAM	no	yes	yes	no
At start of tenofovir				
HBeAg status	positive	positive	positive	positive
HBV-DNA (log_{10} IU/mL)	8.9	7.3	7.8	8.2
HIV-RNA (log_{10} copies/mL)	4.6	3.5	UD	3.5
HBV Genotype	A	A	A	A
Concomitant anti-HBV therapy	LAM	LAM	LAM	FTC
Virologic breakthrough	no	no	no	no
At time of initiation of entecavir				
Month of follow-up	42	48	48	15
HBV-DNA (log_{10} IU/ml)	5.3	3.3	4.2	4.2
HBeAg status	positive	positive	positive	positive
HIV-RNA (log_{10} copies/mL)	UD	UD	UD	UD
Mutation analysis	wild-type	wild-type	wild-type	wild-type
Non-compliance	no	no	no	no
Response to salvage therapy				
Salvage therapy	Addition of ETV	Addition of ETV	Addition of ETV	Addition of ETV
Follow-up (months)	15	27	15	12
HBV-DNA (log_{10} IU/mL) at last F/U	UD	UD	UD	UD
HBeAg status at last F/U	positive	negative	positive	positive

UD = Undetectable; F/U = Follow-up

HIV-RNA and CD4$^+$-cell count changes

At the initiation of TDF, the mean HIV-RNA was 2.9±1.6 log_{10} copies/mL, and 51 patients (50%) demonstrated serum HIV-RNA < 50 copies/mL. At the end of follow up a significantly increased proportion of patients (84%; $p < 0.001$) demonstrated undetectable HIV-RNA. The median CD4$^+$-cell count increased from 293 (138-470) cells/mm^3 at baseline to 455 (340-643) cells/mm^3 at the end of follow-up ($p < 0.001$).

Renal safety

Two patients (2%) experienced an increase in serum creatinin > 0.5 mg/dL after 5 (peak creatinin level: 1.5 mg/dL; eGFR: 54 mL/min) and 16 (peak creatinin level: 2.2 mg/dL; eGFR: 32 mL/min) months of follow-up, respectively. In both patients TDF was stopped, after which serum creatinin levels stabilized, but did not return to normal in both patients. In one of these patients this can also be explained by polyarteritis nodosa related to HBV with associated renal insufficiency. One additional subject TDF was discontinued after 45 months because of an increase in serum creatinin of 0.38 mg/dL from baseline. The mean eGFR at baseline was 105±30 mL/min/1.73m^2. The estimated decrease after five years of TDF therapy was 9.8 (95%CI: [5.4 – 14.2]) mL/min/1.73m^2. The major part of decline in renal function occurred

shortly after initiation of TDF therapy ($p = 0.02$), and was observed especially in those subjects with a baseline eGFR > 100 mL/min/1.73m^2 ($p < 0.001$). The use of ritonavir-boosted protease inhibitors was not related to decline in eGFR ($p = 0.60$).

Discussion

This is the first study to assess the long-term efficacy of TDF administered as part of antiretroviral therapy in a large cohort of HIV/HBV co-infected patients. Previous studies on the efficacy of TDF in both HIV/HBV co-infected and HBV-mono-infected patients were limited by a relatively short follow-up period for up to two years [7-8, 12, 14]. In our study, there is a median follow-up of almost five years, and, moreover, it presents the largest cohort of HIV/HBV co-infected patients treated with TDF so far. It is shown that after five years of follow-up, approximately 90% of patients achieved undetectable HBV-DNA (< 20 IU/mL), almost 50% of HBeAg-positive patients demonstrated HBeAg loss, and HBsAg loss was even observed in approximately 10% of subjects. There was no significant difference between patients with or without LAM-resistance at baseline. More importantly, only one patient demonstrated a combination of known anti-HBV drug-resistant mutations, and experienced a virologic breakthrough thereafter. In three patients TDF was discontinued because of increases of serum creatinin levels. The estimated decrease in renal function after at five years of TDF therapy was approximately 10 mL/min/1.73m2, and was most pronounced directly after initiation of TDF therapy.

The widespread use of highly active antiretroviral therapy (HAART) has significantly increased the life expectancy of HIV-infected patients, and liver disease has now emerged as a significant cause of non-AIDS-related death [3, 5]. A large prospective cohort study demonstrated active HBV infection to be strongly associated with liver-related mortality [3]. Current guidelines recommend, therefore, inclusion of HBV-active agents within the HAART regimen, and to initiate HAART early if an indication to treat HBV infection exists [30]. However, the benefits of long-term treatment may be negated by the development of anti-HBV drug resistance, which can lead to reversion of virologic and histological improvement. In two recently performed randomized clinical trials in HBV mono-infected patients, TDF resulted in HBV-DNA levels lower than 400 copies/mL in 76% and 93% of HBeAg-positive and HBeAg-negative patients, respectively [12]. Continued therapy produced additional viral suppression, HBeAg- and HBsAg-loss at week 72 and 96 [31-32]. Our study now shows TDF, combined with either LAM or emtricitabine, to be an effective anti-HBV agent through five years of therapy with 90% of HIV/HBV co-infected subjects achieving undetectable HBV-DNA.

In the phase III trials in HBV mono-infected patients no evidence of TDF-resistance was shown up to 72 weeks of treatment despite extensive resistance surveillance [17]. Until now TDF resistance has only been described in two HIV/HBV co-infected patients demonstrating

the A194T mutation in addition to LAM-resistance [33], yet the association between this mutation and TDF resistance was not confirmed in another study [34]. In our study, four subjects experienced a virologic breakthrough. In two patients this was explained by non-compliance and only one patient demonstrated a combination of LAM- and adefovir (ADV)-resistant mutations in the HBV polymerase gene. The rtA194T mutation was not observed. An interesting phenomenon was that two virologic breakthroughs occurred in association with the development of hepatocellular carcinoma. A satisfactory explanation for this relation could not be found. There are many reports which demonstrate an association between development of resistance and the risk of hepatocellular carcinoma, which is largely explained by the recurrence of viral replication; only one report noted that significantly more hepatocellular carcinomas were observed shortly after development of LAM resistance [35].

The recently published EASL guidelines on the management of hepatitis B state that "in patients receiving entecavir or tenofovir with a partial virologic response at week 48, some experts would suggest adding the other drug in order to prevent resistance in the long term"[36]. In agreement with the follow-up data of the two large phase III trials in HBV mono-infected patients [12], our study shows that most patients are still able to achieve undetectable HBV-DNA in the second year without changing the treatment regimen. Moreover, this is also the first report which demonstrates that adding ETV to existing TDF therapy is still effective after at least 15 months of treatment, and resulted in undetectable HBV-DNA in all patients. Our study, therefore, suggests that one can probably wait at least 24 months before adding ETV in patients who are viremic on a TDF-containing treatment regimen.

There have been concerns about the risk of renal toxicity with TDF due to an association between related compounds such as ADV and nephrotoxicity [37-38]. In our study, a small but significant increase in serum creatinin levels was observed after five years of treatment. Yet, only 3% of patients developed serum creatinin elevations which necessitated the discontinuation of TDF. Furthermore, serum creatinin elevations usually occurred early, which suggests that frequent monitoring of renal function is necessary shortly after initiation of TDF treatment, but that thereafter, monitoring can probably decreased [22]. Overall, this study supports the renal safety of TDF as a part of antiretroviral therapy through five years of treatment.

To date, no confirmed genotypic substitutions in the HBV polymerase gene associated with decreased sensitivity to TDF have been identified. Although direct sequencing does allow for all mutations to be identified, in vitro phenotypic confirmatory assays are mandatory to detect new substitutions. A limitation of our study is therefore, that we were only able to search for known anti-HBV drug-resistant mutations. In addition, no liver biopsies were available during follow-up in all our patients, and abdominal ultrasound was only performed if there was clinical suspicion of progression to cirrhosis, decompensated liver disease, or hepatocellular carcinoma. The frequency of progression of hepatitis B, and more specifically, the

development of cirrhosis and hepatocellular carcinoma, may therefore be underestimated in our study.

In conclusion, TDF administered as part of antiretroviral therapy, demonstrated to be a potent anti-HBV agent with a good resistance profile throughout five years of therapy. The antiviral efficacy of TDF was not influenced by presence of LAM resistance. Furthermore, this study supports the renal safety of TDF through five years of treatment, as only a small, non-progressive decline in renal function was observed. Nevertheless, close monitoring of renal function is still indicated. Adding ETV to the treatment regimen resulted in achievement of undetectable HBV-DNA in patients who demonstrate persistent HBV replication during a TDF-containing treatment regimen.

References

1. Bodsworth NJ, Cooper DA and Donovan B. The influence of human immunodeficiency virus type 1 infection on the development of the hepatitis B virus carrier state. J Infect Dis 1991;163:1138-40
2. Konopnicki D, Mocroft A, de Wit S, et al. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. Aids 2005;19:593-601
3. Weber R, Sabin CA, Friis-Moller N, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. Arch Intern Med 2006;166:1632-41
4. Hoffmann CJ, Seaberg EC, Young S, et al. Hepatitis B and long-term HIV outcomes in coinfected HAART recipients. Aids 2009;23:1881-9
5. Monforte A, Abrams D, Pradier C, et al. HIV-induced immunodeficiency and mortality from AIDS-defining and non-AIDS-defining malignancies. Aids 2008;22:2143-53
6. Nikolopoulos GK, Paraskevis D, Hatzitheodorou E, et al. Impact of hepatitis B virus infection on the progression of AIDS and mortality in HIV-infected individuals: a cohort study and meta-analysis. Clin Infect Dis 2009;48:1763-71
7. Benhamou Y, Fleury H, Trimoulet P, et al. Anti-hepatitis B virus efficacy of tenofovir disoproxil fumarate in HIV-infected patients. Hepatology 2006;43:548-55
8. Lacombe K, Gozlan J, Boelle PY, et al. Long-term hepatitis B virus dynamics in HIV-hepatitis B virus-co-infected patients treated with tenofovir disoproxil fumarate. Aids 2005;19:907-15
9. Matthews GV, Avihingsanon A, Lewin SR, et al. A randomized trial of combination hepatitis B therapy in HIV/HBV coinfected antiretroviral naive individuals in Thailand. Hepatology 2008;48:1062-9
10. Nelson M, Portsmouth S, Stebbing J, et al. An open-label study of tenofovir in HIV-1 and Hepatitis B virus co-infected individuals. Aids 2003;17:F7-10
11. van Bommel F, Wunsche T, Mauss S, et al. Comparison of adefovir and tenofovir in the treatment of lamivudine-resistant hepatitis B virus infection. Hepatology 2004;40:1421-5
12. Marcellin P, Heathcote EJ, Buti M, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med 2008;359:2442-55
13. Tan J, Degertekin B, Wong SN, Husain M, Oberhelman K and Lok AS. Tenofovir monotherapy is effective in hepatitis B patients with antiviral treatment failure to adefovir in the absence of adefovir-resistant mutations. J Hepatol 2008;48:391-8
14. van Bommel F, de Man RA, Wedemeyer H, et al. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. Hepatology;51:73-80
15. van Bommel F, Zollner B, Sarrazin C, et al. Tenofovir for patients with lamivudine-resistant hepatitis B virus (HBV) infection and high HBV DNA level during adefovir therapy. Hepatology 2006;44:318-25
16. Berg T, Moller B, Trinh H, et al. Tenofovir disoproxil fumarate (TDF) versus emtricitabine plus TDF for treatment of chronic hepatitis B (CHB) in subjects with persistent viral replication in receiving adefovir dipivoxil (ADV). J Hepatol 2008;48; suppl. 2: A76
17. Snow A, Chappell B, Curtis M, et al. Week 96 resistance surveillance for HBeAg-positive and negative subjects with chronic HBV infection randomized to receive tenofovir DF 300mg QD. Hepatology 2008;48; suppl. 1: A977
18. Dienstag JL, Cianciara J, Karayalcin S, et al. Durability of serologic response after lamivudine treatment of chronic hepatitis B. Hepatology 2003;37:748-55

19. Song BC, Suh DJ, Lee HC, Chung YH and Lee YS. Hepatitis B e antigen seroconversion after lamivudine therapy is not durable in patients with chronic hepatitis B in Korea. Hepatology 2000;32:803-6
20. Coca S, Perazella MA. Rapid communication: acute renal failure associated with tenofovir: evidence of drug-induced nephrotoxicity. Am J Med Sci 2002;324:342-4
21. Gallant JE, Moore RD. Renal function with use of a tenofovir-containing initial antiretroviral regimen. Aids 2009;23:1971-5
22. Gallant JE, Parish MA, Keruly JC and Moore RD. Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor treatment. Clin Infect Dis 2005;40:1194-8
23. Gallant JE, Winston JA, DeJesus E, et al. The 3-year renal safety of a tenofovir disoproxil fumarate vs. a thymidine analogue-containing regimen in antiretroviral-naive patients. Aids 2008;22:2155-63
24. Jones R, Stebbing J, Nelson M, et al. Renal dysfunction with tenofovir disoproxil fumarate-containing highly active antiretroviral therapy regimens is not observed more frequently: a cohort and case-control study. J Acquir Immune Defic Syndr 2004;37:1489-95
25. Nelson MR, Katlama C, Montaner JS, et al. The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. Aids 2007;21:1273-81
26. Lampertico P, Vigano M, Manenti E, Iavarone M, Sablon E and Colombo M. Low resistance to adefovir combined with lamivudine: a 3-year study of 145 lamivudine-resistant hepatitis B patients. Gastroenterology 2007;133:1445-51
27. Pas SD, Fries E, De Man RA, Osterhaus AD and Niesters HG. Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. J Clin Microbiol 2000;38:2897-901
28. Pas SD, Niesters HG. Detection of HBV DNA using real time analysis. J Clin Virol 2002;25:93-4
29. Pas SD, Tran N, de Man RA, Burghoorn-Maas C, Vernet G and Niesters HG. Comparison of reverse hybridization, microarray, and sequence analysis for genotyping hepatitis B virus. J Clin Microbiol 2008;46:1268-73
30. Hammer SM, Eron JJ, Jr., Reiss P, et al. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. Jama 2008;300:555-70
31. Heathcote EJ, Gane E, De Man R, et al. Two year tenofovir disoproxil fumarate (TDF) treatment and adefovir dipivoxil (ADV) switch data in HBeAg-positive patients with chronic hepatitis B (study 103), preliminary analysis. Hepatology 2008;48; suppl. 1: A158
32. Marcellin P, Buti M, Krastev Z, et al. Two year tenofovir disoproxil fumarate (TDF) treatment and adefovir dipivoxil (ADV) switch data in HBeAg-negative patients with chronic hepatitis B (study 102), preliminary analysis. Hepatology 2008;48; suppl. 1: A146
33. Sheldon J, Camino N, Rodes B, et al. Selection of hepatitis B virus polymerase mutations in HIV-coinfected patients treated with tenofovir. Antivir Ther 2005;10:727-3434. Delaney WEt, Ray AS, Yang H, et al. Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. Antimicrob Agents Chemother 2006;50:2471-7
35. Andreone P, Gramenzi A, Cursaro C, et al. High risk of hepatocellular carcinoma in anti-HBe positive liver cirrhosis patients developing lamivudine resistance. J Viral Hepat 2004;11:439-42
36. European Association For The Study Of The L. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol 2009;50:227-42

37. Cihlar T, Ho ES, Lin DC and Mulato AS. Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. Nucleosides Nucleotides Nucleic Acids 2001;20:641-8
38. Ha NB, Ha NB, Garcia RT, et al. Renal dysfunction in chronic hepatitis B patients treated with adefovir dipivoxil. Hepatology 2009;50:727-34

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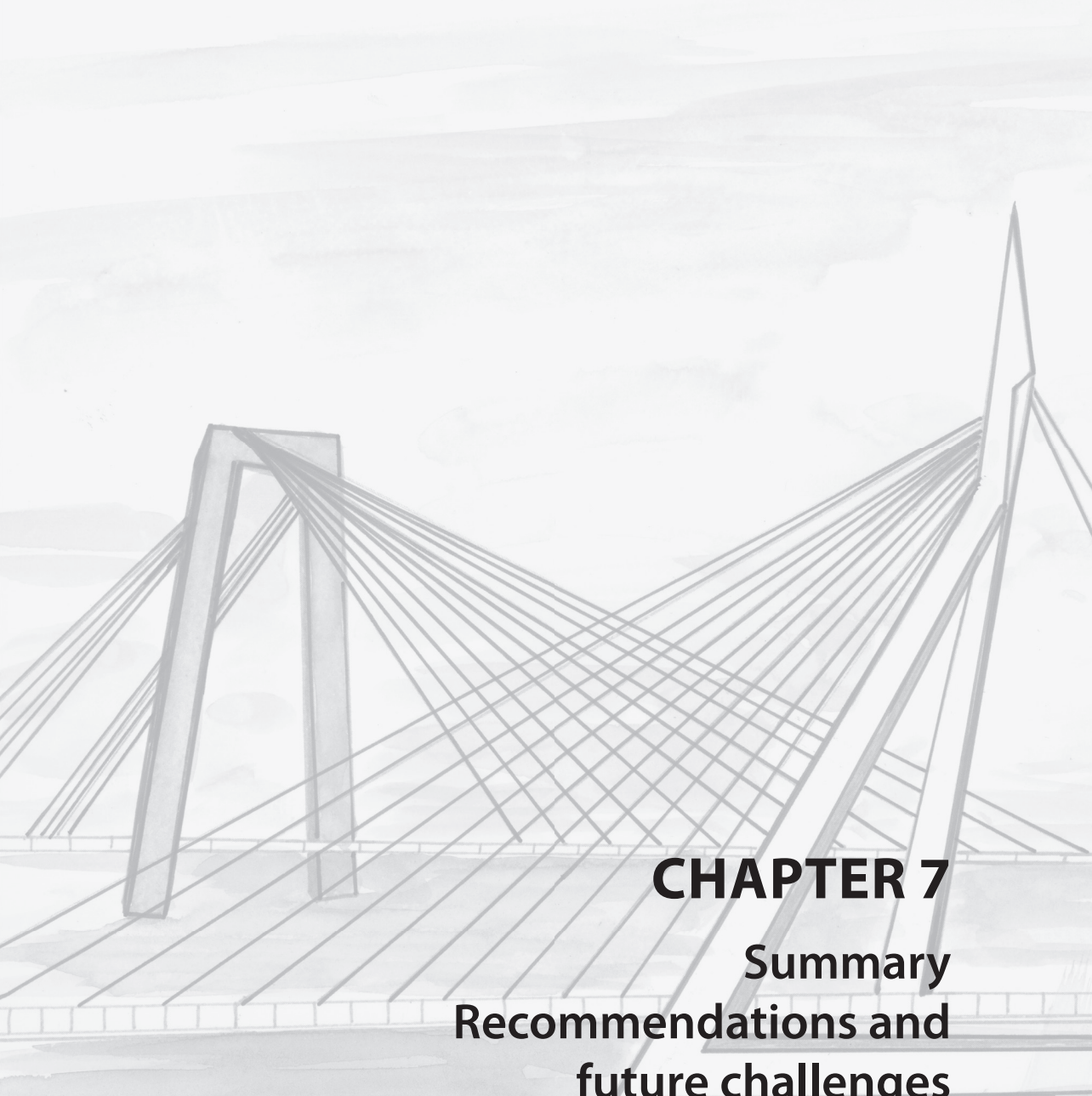


CHAPTER 7

Summary Recommendations and future challenges

Summary

The widespread use of HAART decreased the death rate by 80% among HIV-infected patients. Due to the increased survival liver diseases, such as chronic HBV infection have now emerged as a significant cause of non-AIDS-related death (15%). The objectives of this thesis are to describe different approaches to the prevention and treatment of HBV infection in HIV-infected individuals.

HBV vaccination in HIV-infected patients is a challenging opportunity for several reasons. First of all, the prevalence of HBV infection among men having sex with men (MSM) is high. This may lead to contracting the disease in unprotected MSM. Secondly, adherence to the standard hepatitis B vaccination schedule is a matter of concern and has proven to be difficult in daily practice both for doctors and patients. Thirdly, HIV-infected patients have an impaired response to HBV vaccination. In ***chapter 2*** we describe in a large prospective randomized, non-inferiority study the feasibility and effectiveness of an accelerated hepatitis B vaccination schedule (0, 1, 3 weeks) compared to the standard regimen (0, 4, 24 weeks). The results show that the compliance with an accelerated schedule is significantly better than that with a standard schedule, although its efficacy is only non-inferior in patients with a $CD4^+$-cell count > 500 cells/mm^3. In all HIV-infected patients a better response rate is provided in patients on HAART with undetectable HIV-RNA load, longer duration of HAART use, female gender and younger age.

We suggest to use these data by implementing an accelerated vaccination schedule in selected patients with a $CD4^+$-cell count > 500 cells/mm^3. As the number of vaccine non-response in this unselected population is high we recommend that HBV vaccination of HIV-infected patients should be followed by checking anti-HBs levels. Around 50 % of our HIV-infected patient cohort responded on initial HBV vaccination. In an attempt to achieve a higher response rate we prospectively revaccinated all non-responders (anti-HBs titer = 0 IU/L) three times at monthly intervals with a double dose HBV vaccine. In ***chapter 3*** we describe the outcome of this revaccination study. The results show that HBV double dose revaccination in HIV-infected non-responders produces a 51 % additional success rate. Our study shows that reponse to revaccination with a double dose of HBV vaccine is more likely in patients younger than 40 years of age, irrespective of viral load, while in patients older than 40 years an undetectable HIV-RNA load is associated with a better response rate. Eventually, we achieved an overall response rate to HBV vaccination of 75 % among HIV-infected patients.

In ***chapter 4*** we study the possible relationship between HBV and influenza vaccination in HIV-infected patients. A trend for higher geometric mean titers, both in pre- and post-influenza immunization was found in HBV vaccination responders compared to HBV non-responders. The differences in response to HBV and influenza vaccination in HIV-infected

patients are probably related to defects in the host immune system, not represented by the $CD4^+$-cell count, and not to the vaccine itself.

A HBV vaccination program targeting behavioral high risk groups such as MSM is in place nationwide since November 2002. Despite this program the HBV prevalence in HIV-infected patients in the Netherlands is 8%. HIV/HBV co-infected patients can be successfully treated with tenofovir/lamivudine as part of their HAART regimen. In ***chapter 5*** we describe the viral kinetics on tenofovir/lamivudine therapy in the first 24 weeks in co-infected patients with either wild type or lamivudine resistant HBV. We demonstrated that the viral response showed a biphasic pattern, with only during the first phase, which lasted less than 7 days, a statistical significant difference between wild type and mutant HBV-strains. After 24 weeks the viral decline in the YMDD variant group and the wild type group was similar. This was reassuring as many lamivudine pretreated co-infected patients harbor YMDD variant virus and this study showed that adding tenofovir to a HAART regimen resulted in a viral decline of HBV-DNA which is similar in both groups. These results raise the question whether the long-term outcome of antiviral therapy is as favorable as the short-term results. In ***chapter 6*** we describe the long-term efficacy of tenofovir administered as a part of antiretroviral therapy in a large cohort of HIV/HBV co-infected patients. It is shown that after five years of follow-up, approximately 90% of patients achieved undetectable HBV-DNA load. There was no significant difference between patients with or without lamivudine resistance at baseline. Furthermore, no confirmed genotypic substitutions in the HBV polymerase gene associated with decreased sensitivity to tenofovir have been identified in our cohort.

In conclusion: screening for HBV in HIV-infected patients is important. In patients with negative HBV serology prevention through individualized vaccination schedules with high compliance should be pursued. In HIV/HBV co-infected patients the HAART regime should include tenofovir/lamivudine (or emtricitabine) as the long-term efficacy, the resistance profile and the safety were excellent.

Recommendations and future challenges

The findings in ***chapter 2*** support the use of an accelerated HBV vaccination schedule in HIV-infected patients with a $CD4^+$-cell count > 500 cells/mm^3. Delaying HBV vaccination in HIV-infected high-risk groups under all circumstances according to the predictors of success (on HAART with undetectable HIV-RNA load and longer duration of HAART use) may not be warranted. Our findings suggest a more optimized and individualized timing can be applied increasing compliance and offering protection as fast as possible to those likely to respond to vaccination.

Landrum *et al.* assessed the risk of HBV infection, defined as HBsAg, anti-HBc or anti-HBs positive on at least two separate occasions, among vaccinated HIV-infected patients [1]. In

this observational cohort study 11.2% of non-responders to HBV vaccination developed a serological HBV infection compared with 5.1% in the responder group. In none of the responders with initial anti-HBs above 10 IU/L a chronic HBV infection developed as opposed to 35% in the non-responders. Furthermore a distinction was made between persistent and waning response after an initial positive response. The risk of HBV infection was not different between these groups suggesting that natural HBV infection will boost low titers of anti-HBs. It will be interesting to follow-up our large vaccination cohort with both HBV serology and anti-HBs levels over time. Several new research questions are worthwhile to be answered. For example: do hypo responders in our cohort (anti-HBs = 3-10 IU/L) have an equal risk of developing HBV infection compared to non-responders (anti-HBs = 0 IU/L)? Are responders independent of the height of their anti-HBs titer still at risk for developing signs and symptoms of chronic HBV infection? Is there a need for booster vaccination in HIV-infected patients after anti-HBs levels reached levels < 10 IU/L on the analogy of dialysis patients? Can successful implementation of HBV vaccination in this cohort influence the liver related mortality?

As described in ***chapter 3*** in our unselected cohort of HIV-infected patients we eventually achieved an overall response rate to HBV vaccination of 75 % after the combination of the initial and revaccination schedule. This finding justifies the policy to fulfill post-vaccination anti-HBs screening. In contrast, 25% of HIV-infected patients did not reach adequate anti-HBs levels and are still supposed to be at risk for contracting HBV infection. For these selected patients it will be interesting to study whether alternative vaccines with more powerful adjuvants, alternative vaccination schedules or vaccination routes are accessible to augment the response to HBV vaccination.

In ***chapter 4*** we conclude that the lack of response to HBV and influenza vaccination is probably related to the host immune system. Underlying mechanisms such as functional T cell assays may further elucidate this defect and will be useful to investigate in the future.

In ***chapter 6*** the long-term efficacy of tenofovir/lamivudine administered as part of a HAART regimen in HIV/HBV co-infected patients is documented. Since august 2008 tenofovir is also approved for treatment of chronic HBV mono-infection. Recent data have shown the efficacy of tenofovir in the treatment of this patient group [2]. In this study 76% of HBeAg-positive patients had an undetectable HBV-DNA load at week 48 and almost 50% achieved this level already at week 24. There was no difference observed in patients with or without lamivudine pre-treatment. An important different finding in treatment of HBV mono- and HIV/HBV co-infected patients is the time frame in which HBV-DNA load achieves undetectable levels. We showed that in 5 years time 90% of HIV/HBV co-infected patients had undetectable HBV-DNA loads and that a good resistance profile was demonstrated. According to the EASL guidelines adding entecavir in patients with a partial virological response at week 48 (detectable HBV-DNA) must be considered in order to prevent polymerase resistance in long term. This follows the paradigm of no replication equals no resistance. However this statement is based on

expert-opinion because long term studies in non-HIV-infected patients addressing this topic are lacking. In our study we successfully added entecavir to the treatment regimen in four patients with persistent detectable HBV-DNA load. However, our data suggest that one could wait 24 months (where the curve slope is steep) before adding entecavir. In fact, in our study entecavir was added in viremic patients at least 15 months later and still HBV-DNA levels became undetectable afterwards. Our data question the EASL recommendation with regard to the time point of adding a new drug. In fact, is their a need for adding a new drug at all?

We tried to identify the long-term detectable HIV/HBV co-infected patient. However, after prolonged follow-up and the introduction of a more sensitive quantitative HBV-DNA test in our laboratory no patients could be classified to fulfill the criteria of "long-term detectable" HBV-DNA. Based upon the above mentioned findings together with the finding that no tenofovir resistance was observed until now, it would be justified to follow a "wait and see" policy in HIV/HBV co-infected patients on a HAART regimen including tenofovir/lamivudine (or emtricitabine).

References

1. Landrum ML, Hullsiek KH, Ganesan A, et al. Hepatitis B vaccination and risk of hepatitis B infection in HIV-infected individuals. AIDS 2010;24:545-55
2. Marcellin P, Heathcote EJ, Buti M, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med 2008;359:2442-55

CHAPTER 8

Nederlandse samenvatting

Samenvatting

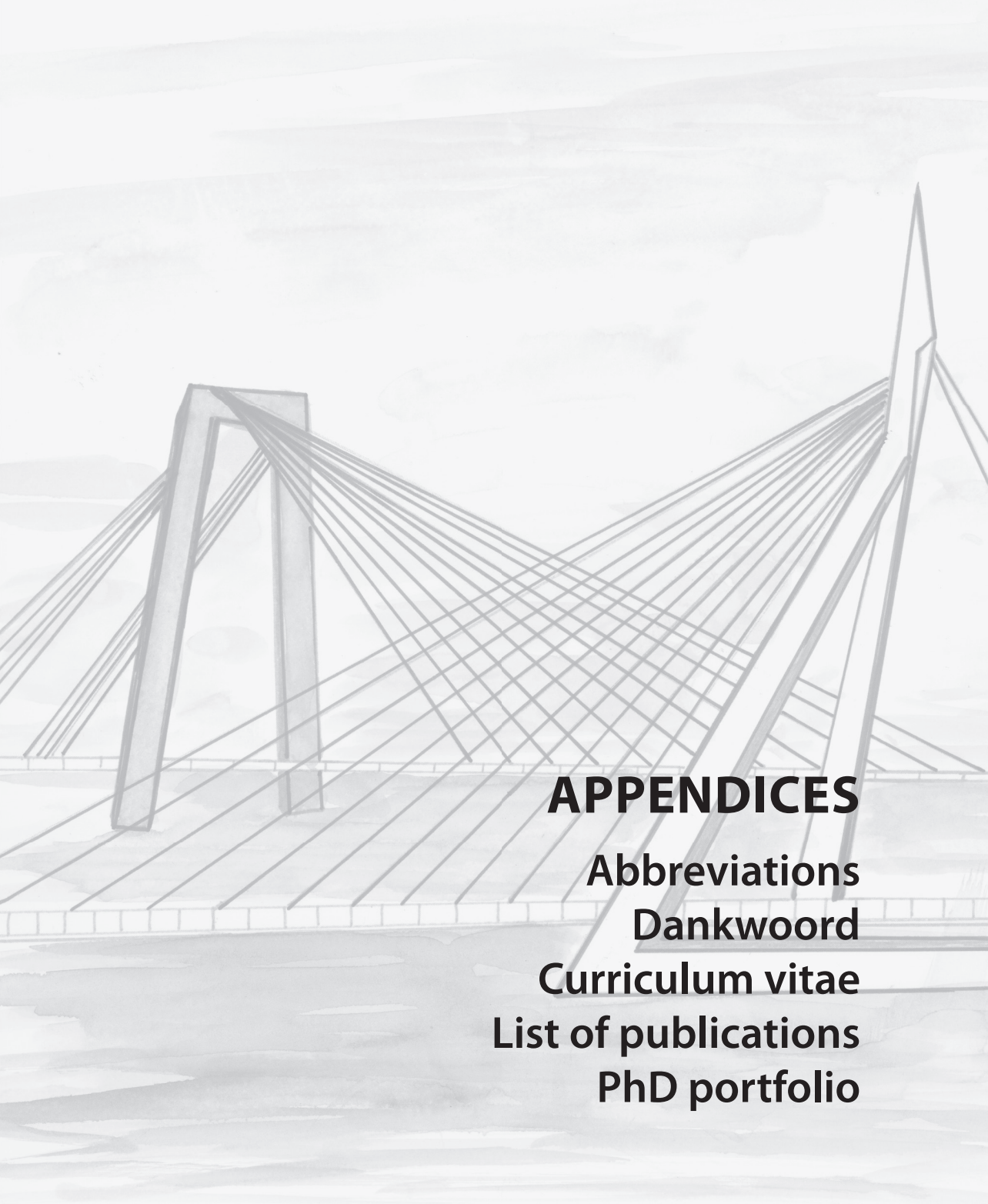
Door het wereldwijd gebruik van HAART is de kans op overlijden van HIV geïnfecteerde patiënten met 80% verminderd. Wanneer patiënten tevens lijden aan een chronische HBV infectie openbaren nu ook vaker de lange termijn complicaties van deze infectie zich. Leverziekten zijn op dit moment bij HIV geïnfecteerde patiënten een belangrijke oorzaak geworden van niet aan AIDS gerelateerde sterfte. De doelstellingen van dit proefschrift zijn om de klinische implicaties van de preventie en behandeling van HBV infecties bij HIV geïnfecteerde patiënten te beschrijven.

Om diverse redenen is het een uitdagende gebeurtenis om HBV vaccinatie te verstrekken aan HIV geïnfecteerde patiënten. In verband met de hoge prevalentie onder mannen die seks hebben met mannen (MSM) vormt HBV infectie een voortdurend aandachtspunt en is primaire preventie in deze groep van groot belang. Ten tweede blijkt het volgen van het standaard hepatitis B vaccinatieschema in de praktijk lastig en moeilijk te volbrengen met als gevolg onvolledige vaccinatie en verminderde bescherming tegen HBV infectie. Ten derde is uit eerder onderzoek gebleken dat HIV geïnfecteerde patiënten een verminderde respons op HBV vaccinatie laten zien ten opzichte van individuen met een ongestoorde afweer. In ***hoofdstuk 2*** beschrijven we de resultaten van een grote prospectief gerandomiseerde studie waarin we de toepasbaarheid en effectiviteit van een versneld hepatitis B vaccinatieschema (0, 1 en 3 weken) vergelijken met het standaard vaccinatieschema (0, 4 en 24 weken). De resultaten laten zien dat de compliantie van het versnelde schema significant beter is dan van het standaard schema. De effectiviteit is echter alleen vergelijkbaar bij de patiënten met een $CD4^+$-celgetal > 500 cellen/mm^3. Voor alle HIV geïnfecteerde patiënten geldt dat een betere respons wordt bereikt indien patiënten HAART gebruiken, een ondetecteerbare HIV-RNA load hebben en gedurende langere tijd ingesteld zijn op HAART. Bovendien hebben vrouwen en jongere patiënten een betere respons. Totdat meer duidelijkheid is verkregen over de effectiviteit van HBV vaccinatie bij HIV geïnfecteerde patiënten, is het van groot belang om de respons in deze populatie te documenteren middels een anti-HBs titer. Dit in tegenstelling tot de algemene populatie waarbij dit niet noodzakelijk is. Ongeveer 50% van ons HIV geïnfecteerde patiënten cohort respondeerde op de initiële HBV vaccinatie serie. In een poging om een hogere respons te bereiken hebben we alle non-responders (anti-HBs titer = 0 IU/L) nogmaals 3 keer gevaccineerd met een maandelijks interval en met een dubbele dosis vaccin. In ***hoofdstuk 3*** beschrijven wij de resultaten van deze revaccinatie studie. Een additionele 51 % respons werd bereikt in de HIV geïnfecteerde non-responders groep. Een respons met dubbele dosis hepatitis B vaccin werd vaker gevonden bij patiënten jonger dan 40 jaar oud. Deze respons was onafhankelijk van de virale load, terwijl bij patiënten ouder dan 40 jaar de respons geassocieerd was met een ondetecteerbare HIV-RNA load. Uiteindelijk bereikten wij een totale respons van 75% op HBV vaccinatie in HIV geïnfecteerde patiënten.

In ***hoofdstuk 4*** hebben wij de relatie onderzocht tussen de respons op HBV en influenza vaccinatie bij HIV geïnfecteerde patiënten. We vonden een trend tot een hogere titer in de HBV vaccinatie responders vergeleken met de HBV non-responders. Dit gold zowel voor de pre- als voor de postinfluenza vaccinatie bepalingen. Het verschil in respons op HBV en influenza vaccinatie bij HIV geïnfecteerde patiënten wordt waarschijnlijk veroorzaakt door een defect in het immuunsysteem van de gastheer, wat niet weergegeven wordt door de hoogte van het $CD4^+$-celgetal, en niet door het vaccin zelf.

Sinds november 2002 is in Nederland een HBV vaccinatie programma gericht op hoog risicogedrag groepen, zoals MSM, gestart. Ondanks dit programma is de prevalentie van HBV onder HIV geïnfecteerde patiënten nog steeds 8%. Bij HIV/HBV gecoïnfecteerde patiënten bestaat de mogelijkheid om een behandeling te geven met tenofovir en lamivudine als onderdeel van hun HAART regime. Beide middelen zijn werkzaam tegen zowel HIV als HBV. In ***hoofdstuk 5*** beschrijven wij de virale kinetiek van de tenofovir en lamivudine combinatie-behandeling gedurende de eerste 24 weken bij gecoïnfecteerde patiënten met een wild type HBV en een virus met al resistentie tegen lamivudine. Wij toonden in deze studie aan dat de virale daling een bifasisch patroon vertoont. Alleen gedurende de eerste fase, die minder dan 7 dagen in beslag neemt, was er een statistisch verschil in respons tussen het wild type en het resistente HBV. Na 24 weken therapie was de daling van het virus in beide groepen vergelijkbaar. Dit is een belangrijke observatie aangezien veel met lamivudine voorbehandelde HIV/HBV gecoïnfecteerde patiënten deze YMDD variant bij zich dragen. Deze studie toonde aan dat toevoegen van tenofovir aan het HAART regime een vergelijkbare daling van het HBV in beide groepen gaf. De volgende vraag die zich opdrong was of deze gunstige resultaten op korte termijn ook voor de lange termijn gelden. In ***hoofdstuk 6*** beschrijven we in een groot HIV/HBV gecoïnfecteerd patiënten cohort de lange termijn effectiviteit van tenofovir als onderdeel van de antiretrovirale behandeling. Uit deze retrospectieve studie bleek dat ongeveer 90% van de patiënten een ondetecteerbare HBV-DNA load bereikt heeft na 5 jaar follow-up. Er was geen verschil tussen patiënten met of zonder lamivudine resistentie op baseline. Bovendien hebben wij geen resistentie kunnen aantonen tegen tenofovir in ons cohort.

Concluderend: screenen op HBV infectie is erg belangrijk bij HIV geïnfecteerde patiënten. In patiënten die een negatieve HBV serologie hebben, moet preventie middels vaccinatie worden nagestreefd. Dit is vooral van belang omdat het beloop van een chronische leverziekte bij HIV/HBV gecoïnfecteerde patiënten versneld is. In het geval van een chronische HBV infectie bij een HIV geïnfecteerde patiënt zou het HAART regime tenofovir en lamivudine (of emtricitabine) moeten bevatten omdat deze combinatie heeft laten zien dat de lange termijn effecten, het resistentie profiel en het veiligheidsprofiel goed zijn.



APPENDICES

Abbreviations
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Abbreviations

ADV	adefovir
AIDS	acquired immunodeficiency syndrome
AL(A)T	alanine aminotransferase
Anti-HBc	antibodies against hepatitis B core antigen
Anti-HBe	antibodies against hepatitis B envelop antigen
Anti-HBs	antibodies against hepatitis B surface antigen
AZT	zidovudine
BMI	body mass index
cccDNA	covalently closed circular DNA
CDC	Centers of Disease Control and Prevention
DNA	deoxyribonucleic acid
eGFR	estimated glomerular filtration rate
ETV	entecavir
GMT	geometric mean titre
HA	haemagglutinin
HAART	highly active antiretroviral therapy
HAV	hepatitis A virus
HBV	hepatitis B virus
HBeAg	hepatitis B envelop antigen
HBsAg	hepatitis B surface antigen
HI	haemagglutination inhibition
HIV	human immunodeficiency virus
HCV	hepatitis C virus
IFN	interferon
LAM	lamivudine
LTFU	lost to follow-up
MITT	modified intention to treat
MSM	men having sex with men
NRTI	nucleos(t)ide reverse transcriptase inhibitor
NNRTI	non-nucleos(t)ide reverse transcriptase inhibitor
PCR	polymerase chain reaction
PEG-IFN	pegylated interferon
PI	protease inhibitor
RNA	ribonucleic acid
TDF	tenofovir
VR	virologic response
YMDD	thyrosine methionine aspartate aspartate

Dankwoord

Lang heb ik gedacht dat het schrijven van het dankwoord het leukste en makkelijkste onderdeel van het boekje zou zijn. Inmiddels weet ik dat het wel het leukste onderdeel is om te schrijven, maar of het ook het makkelijkste was??? De afgelopen jaren heb ik regelmatig het gevoel gehad dat ik de draaiende bordjes op stokjes nog maar net hoog kon houden. Gelukkig kwam er in deze periodes ook hulp van anderen.

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veranderde de boemeltrein in een denderende HSL trein, waar Nicole moeiteloos opsprong en meereed. Tirza, heel veel succes met je eigen boekje, het blijft jammer dat je niet de infectieziekten als toekomst ziet! Nicole, heel veel succes met je opleiding, als je in Breda gaat werken, schrijf ik me bij jou in de praktijk in! Dames, heel veel dank en we gaan nog eens gezellig eten.

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De dames van de virologie Cobi Kerkhof, Hélène Korff, Soeranie Moeljosoewito, Darina Weggemans, Sandra Scherbeijn, Suzanne Diepstraten, nooit, maar dan ook nooit was het een probleem om nabepalingen te verrichten. Nog een keer het sample uit de vriezer, opgestuurde samples verwerken, altijd vriendelijk werden de kruisjes op de formulieren gezet en de bepalingen uitgevoerd. Veel dank voor deze prettige samenwerking.

Mijn collega HIV behandelaren in den lande, mijn directe collega infectiologen en de fellows infectiologie die de afgelopen jaren in het Erasmus MC gewerkt hebben, dank ik voor hun deelname aan de multicenter studies.

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dat ik de academie toch wel begon te waarderen. Vanaf het begin hebben we veel samen meegemaakt en doorstaan. Dat maakt dat de vriendschap zo waardevol is. Hoop dat we nog vaak met de gezinnen op vakantie kunnen gaan. Volgend jaar 2e lustrum!

Mijn (schoon)familie, dank voor de getoonde interesse in de afgelopen jaren. En broertje, valt de dikte mee? Mijn schoonmoeder, Anneke de Vries, wil ik bedanken voor het ontwerp van de kaft. Het is niet altijd gemakkelijk voor een niet kunstenaar om uit te leggen wat ze wil met als gevolg veel werk voor de kunstenaar, maar het resultaat is prachtig.

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Curriculum Vitae

De auteur van dit proefschrift werd geboren op 9 mei 1964 te Zwijndrecht. Na het behalen van het eindexamen in 1983 aan het Gymnasium Erasmianum te Rotterdam, werd zij in 1985, na het toepassen van de hardheidsclausule, toegelaten tot de studie Geneeskunde aan de Rijksuniversiteit Leiden. Het doctoraalexamen werd behaald in augustus 1990 en het artsexamen in januari 1993. In 1991 deed zij keuzeonderwijs getiteld "Prevalence of Hepatitis B and C virus infection in various liver diseases at Garankuwa Hospital" aan de Medical University of Southern Africa te Pretoria onder supervisie van Prof.dr. C.F. van der Merwe. Alvorens te gaan werken in mei 1993 als arts-assistent geneeskunde niet in opleiding (AGNIO) in het Havenziekenhuis te Rotterdam, werkte zij drie maanden als arts-assistent op de afdeling Eerste Hulp en Interne Geneeskunde in het Academisch Ziekenhuis Paramaribo in Suriname. Na een periode van twee jaar AGNIO, verrichtte zij in de tweede helft van 1995 onderzoek getiteld "Investigations into patients with fever in rural Kenya" in het Memisa ziekenhuis Mumias te Kenia. In 1996 begon zij aan de opleiding tot internist in het Havenziekenhuis (opleider Dr. A.G.C. Bauer) en vervolgens in het Erasmus MC te Rotterdam (opleider Prof.dr. M.A.D.H. Schalekamp en Prof.dr. H.A.P. Pols). Per 1 januari 2002 is zij geregistreerd als internist. Vanaf 1 mei 2001 specialiseerde zij zich in het aandachtsgebied Infectieziekten (opleider Dr. S. de Marie) en op 9 mei 2003 vond de registratie tot internist-infectioloog plaats. Snel daarna begon zij met het klinisch onderzoek bij HIV geïnfecteerde patiënten onder supervisie van Dr. M.E. van der Ende, Dr. R.A. de Man en in een later stadium ook onder begeleiding van Prof.dr. H.L.A. Janssen. Dit onderzoek heeft uiteindelijk geleid tot de totstandkoming van dit proefschrift. Zij is getrouwd met Jeroen de Vries en zij hebben twee kinderen.

List of publications

1. **Sluys TEMS**, van der Ende ME, Swart GR, van den Berg JW, Wilson JH. Body composition in patients with acquired immunodeficiency syndrome: a validation study of bioelectric impedance analysis. JPEN 1993;17(5):404-406.
2. Van Daele PL, Zanen AL, de Ronde W, **de Vries-Sluijs TEMS**, Hayes DP. Severe hypokalemia with paralysis in a patient with distal renal tubular acidosis as an initial expression of Sjögren's syndrome. Ned Tijdschr Geneeskd, 2002;146(5):218-221.
3. **de Vries-Sluijs TEMS**, Dieleman JP, Arts D, Huitema AD, Beijnen JH, Schutten M, van der Ende ME. Low nevirapine plasma concentrations predict virological failure in an unselected HIV-1-infected population. Clin Pharmacokinet 2003;42(6):599-605.
4. Den Hollander JG, **de Vries-Sluijs TEMS**, Warris A, Schneider AJ, Hartwig NG, van der Ende ME. Een vergeten test, een levenslang vonnis! Ned Tijdschr Obstr & Gynaecol 2004;117:112-115.
5. **de Vries-Sluijs TEMS**, van der Eijk AA, Hansen BE, Osterhaus AD, de Man RA, van der Ende ME. Wild type and YMDD variant of hepatitis B virus: no difference in viral kinetics on lamivudine/tenofovir therapy in HIV-HBV co-infected patients. J Clin Virol 2006;36(1):60-63.
6. **de Vries-Sluijs TEMS**, Hansen BE, van Doornum GJ, Springeling T, Evertsz NM, de Man RA, van der Ende ME. A prospective open study of the efficacy of high-dose recombinant hepatitis B rechallenge vaccination in HIV-infected patients. J Infect Dis. 2008;197(2):292-294.
7. **de Vries-Sluijs TEMS**, Hansen BE, van Doornum GJJ, Kauffmann RH, Leyten EMS, Mudrikova T, Brinkman K, den Hollander JG, Kroon FP, Janssen HLA, van der Ende ME, de Man RA. A Randomized controlled study of accelerated versus standard hepatitis B vaccination in HIV positive patients. Conditionally accepted in Journal of Infectious Diseases.
8. **de Vries-Sluijs TEMS**, Gelinck LBS, Lindner T, van Doornum GJJ, Rimmelzwaan GF, van der Ende ME. The response to Influenza compared to Hepatitis B vaccination in HIV-infected individuals. Submitted for publication.
9. **de Vries-Sluijs TEMS**, Reijnders JGP, Hansen BE, Zaaijer HL, Prins JM, Pas SD, Schutten M, Hoepelman IM, Richter C, Mulder JW, de Man RA, Janssen HLA, van der Ende ME. Long-term therapy with tenofovir is effective for patients co-infected with HIV and HBV. Gastroenterology 2010, in press.

PhD portfolio

Name PhD student:	T.E.M.S. (Dorine) de Vries-Sluijs
Erasmus MC Department:	Internal Medicine, section Infectious Diseases
PhD period:	2004 - 2010
Promotor:	Prof.dr. H.L.A. Janssen
Copromotoren:	Dr. M.E. van der Ende
	Dr. R.A. de Man

In-depth courses

- Teach-the-Teacher. Desiderius School, 2006
- HIV masterclass. Virology education, Utrecht, 2004/2005
- Hepatitis Masterclass. Virology eduction, Utrecht/Antwerp, 2009

Oral and poster presentations

- Viral dynamics during Lamivudine/Tenofovir therapy in HIV/HBV co-infected patients. Wetenschapsdagen Erasmus MC, Goes, 2005 *(poster presentation)*
- Viral dynamics during Lamivudine/Tenofovir therapy in HIV/HBV co-infected patients. Wetenschappelijke voorjaarsvergadering VIZ en NVAB, Amsterdam, 2005 *(oral presentation)*
- Hepatitis B vaccinatie bij risicogroepen. Wetenschappelijke voorjaarsvergadering NATEC/NVAB, Amsterdam, 2005 *(oral presentation)*
- Hepatitis B vaccinaties bij HIV-geïnfecteerde patiënten. Wetenschapsdagen Erasmus MC, Goes, 2006 *(poster presentation)*
- Hepatitis B vaccinatie bij risicogroepen. Wetenschappelijke voorjaarsvergadering VIZ en NVAB-NATEC, Leiden, 2006 *(oral presentation)*
- HIV/HBV co-infecties, retrospectieve cohortstudie 2002-2006. Symposium HIV en HBV: maakt de dokter het verschil?, Amsterdam, 2007 *(oral presentation)*
- Efficacy of high dose recombinant Hepatitis B rechallenge vaccination in HIV-infected patients. CROI 2007, Los Angeles, USA, 2007 *(poster presentation)*
- Long term efficacy of combination therapy Lamivudine/Tenofovir in HIV/HBV co- infected patients. CROI 2007, Los Angeles, USA, 2007 *(poster presentation)*
- A prospective open study on the efficacy of high dose recombinant Hepatitis B rechallenge vaccination in HIV-infected patients. Wetenschappelijke najaarsvergadering NVAB, Scheveningen, 2008 *(oral presentation)*
- A prospective open study on the efficacy of high dose recombinant Hepatitis B rechallenge vaccination in HIV-Infected patients. Wetenschapsdagen Erasmus MC, Antwerp, Belgium, 2008 *(poster presentation)*

- HIV/HBV co-infection. Avondsymposium Infectieziekten Erasmus MC, HIV: onbekend maakt onbemind?, Rotterdam, 2008 *(oral presentation)*
- Lamivudine (Emtricitabine)/Tenofovir in HIV/HBV co-infected patients. Symposium HIV en HBV: maakt de dokter het verschil?, Rotterdam, 2008 *(oral presentation)*
- Accelerated Hepatitis B vaccination schedule in HIV-infected patients. Wetenschappelijke midwintervergadering, NVAB Utrecht, 2010 *(oral presentation)*
- Accelerated Hepatitis B vaccination schedule in HIV-infected patients. Wetenschapsdagen Erasmus MC, Antwerp, Belgium, 2010 *(poster presentation)*
- Five year tenofovir therapy is associated with maintained HBV response and renal toxicity in HIV/HBV co-infected patients. CROI 2010, San Francisco, USA, 2010 *(poster presentation)*
- Accelerated Hepatitis B vaccination schedule in HIV-infected patients. CROI 2010, San Francisco, USA, 2010 *(poster presentation)*

Conferences and courses

- Derde Landelijke Hepatitisweek, Amersfoort, 2004
- Boerhaave cursus nascholing Infectieziekten, Noordwijkerhout, 2004
- NIV 16e Internistendagen, Maastricht, 2004
- HIV-2, virologie, epidemiologie en behandeling, Rotterdam, 2004
- First international workshop on HIV and Hepatitis Co-infecton, Amsterdam, 2004
- Boerhaave cursus prikken en afweer uitgediept. Praktische vaccinatievraagstukken, Leiden, 2005
- Avondsymposium Infectieziekten: Invasieve schimmelinfecties: nieuwe middelen, nieuwe uitdagingen, Rotterdam, 2005
- Voorjaarsvergadering VIZ en NVAB, Amsterdam, 2005
- NIV 17e Internistendagen, Maastricht, 2005
- Boerhaave cursus nascholing Infectieziekten, Noordwijkerhout, 2005
- IDSA, San Francisco, USA, 2005
- Second international workshop on HIV and Hepatitis Co-infecton, Amsterdam, 2006
- Avondsymposium Infectieziekten: Lijninfecties en de IC, Hemato- of Oncologiepatiënt: zelfde probleem, andere aanpak?, Rotterdam, 2006
- NIV 18e Internistendagen, Maastricht, 2006
- PAOG-Heyendael nascholingscursus Internationale gezondheidszorg, Nijmegen, 2006
- IDSA, Toronto, Canada, 2006
- Regionale Refeeravond IC, Rotterdam, 2006
- Symposium HIV en HBV: maakt de dokter het verschil?, Amsterdam, 2007
- CROI, Los Angeles, USA, 2007
- Boerhaave cursus nascholing Infectieziekten, Noordwijkerhout, 2007
- NIV 19e Internistendagen, Maastricht, 2007
- EACS, Madrid, Spain, 2007

- The 1st Netherlands Conference on HIV Pathogenesis, Prevention and Treatment (NCHIV 2007), Amsterdam, 2007
- CROI, Boston, USA, 2008
- EASL conference: Hepatitis B and C virus resistance to antiviral therapies,
- Paris, France 2008
- Tweede Rotterdamse internistendag, Rotterdam, 2008
- Avondsymposium Infectieziekten: HIV: onbekend maakt onbemind?, Rotterdam, 2008
- Symposium HIV en HBV: maakt de dokter het verschil?, Rotterdam, 2008
- Zwangerschap en Infecties, Ede, 2008
- ICAAC/IDSA, Washington, USA, 2008
- Wetenschappelijke najaarsvergadering VIZ/ NVMM, Amsterdam, 2008
- Avondsymposium Infectieziekten: Dokters in debat, Rotterdam, 2009
- Derde Rotterdamse internistendag, Rotterdam, 2009
- NIV 21e Internistendagen, Maastricht, 2009
- ESCMID, Helsinki, Finland, 2009
- Diagnostiek bij vermoeden van een afweerstoornis, Breda, 2009
- Avondsymposium Infectieziekten: Timing is everything, Rotterdam, 2009
- CROI, San Francisco, USA, 2010
- Vierde Rotterdamse internistendag, Rotterdam, 2010
- HIV en psychische klachten: kennis voor de toekomst, Amsterdam, 2010
- Nieuwe inzichten in invasieve mycosen, Rotterdam, 2010

Teaching activities

- HIV and HBV during pregnancy, training masters obstetricians, Rotterdam, 2006
- Antibiotic use and the host, training chemists, Utrecht, 2004, 2005
- HIV: epidemiology, natural course and treatment, training nurses, Rotterdam, 2005, 2006

Awards

- Young Investigators Award, NVAB 2010

Grants

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