Available online at www.sciencedirect.com
SCIENCE DIRECT®
Antiviral Research 63 (2004) 123–131
Antiviral Research
www.elsevier.com/locate/antiviral

Hepatitis B and C viral load changes following initiation of highly active antiretroviral therapy (HAART) in patients with advanced HIV infection☆

David M. Asmuth[a,*], Michael P. Busch[b,c], Megan E. Laycock[b,c], Beth A. Mohr[d], Leslie A. Kalish[d], Charles M. van der Horst[e], Viral Activation Transfusion Study (VATS) Group[1]

[a] *Department of Internal Medicine, University of California—Davis Medical Center, Sacramento, CA, USA*
[b] *Blood Centers of the Pacific, San Francisco, CA, USA*
[c] *Blood Systems, Inc., Scottsdale, AZ, USA*
[d] *New England Research Institutes, Watertown, MA, USA*
[e] *Department of Medicine, University of North Carolina Medical School, Chapel Hill, NC, USA*

Received 27 August 2003; accepted 29 March 2004

Abstract

Chronic infection with either hepatitis B (HBV) or hepatitis C virus (HCV) is frequently present in patients seropositive for human immunodeficiency virus (HIV) because of shared routes of transmission. With the advent of highly active antiretroviral therapy (HAART) regimens capable of controlling HIV replication and dramatically prolonging the survival of HIV-infected patients, the impact of co-morbid infections such as HBV and HCV has come into focus. Several studies have monitored HBV or HCV viral loads following initiation of HAART, with disparate results. The effects of HAART on hepatitis B and C plasma viral loads ($n = 9$ and 32, respectively) and on liver enzyme levels were studied in a large cohort of prospectively studied subjects with advanced stage HIV disease. Comparing the mean pre- and post-HAART levels, there was an estimated increase of (a) 1.40 $\log_{10}$ from 4.83 to 6.24 $\log_{10}$ for HBV plasma viral load ($P = 0.07$), (b) 0.74 $\log_{10}$ from 6.38 to 7.12 $\log_{10}$ for HCV plasma viral load ($P = 0.001$), and (c) 19.4 U/L from 37.4 to 56.8 U/L for serum alanine aminotransferase ($P < 0.001$). While the number of subjects co-infected with HIV and HBV was limited, these data collected in a population of advanced stage HIV-infected patients raises questions regarding the interactions of these viruses with each other and the host immune system and has implications regarding the order in which antiviral therapies are initiated.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Antiretroviral therapy; Hepatitis B; Hepatitis C; Human immunodeficiency virus; Viral load

1. Introduction

Chronic infection with either hepatitis B (HBV) or hepatitis C virus (HCV) is frequently present in patients seropositive for human immunodeficiency virus (HIV) because of shared routes of transmission. An estimated 10 and 30% of HIV-infected persons are co-infected with HBV or HCV, respectively (Perrillo et al., 1986; Bodsworth et al., 1991; Poles and Dieterich, 2000; Sulkowski et al., 2000a; Tedaldi et al., 2003b). Although the effect of HBV and HCV co-infection on HIV disease progression is uncertain, it is believed that HBV does not substantially alter HIV disease (Koblin et al., 1992; Gilson et al., 1997). Whereas some investigators have observed that HCV co-infection may be associated with a more rapid rate of HIV disease progression (Bonacini and Puoti, 2000; Greub et al., 2000; Daar et al., 2001) compared to HCV seronegative HIV positive patients, others have not observed significant differences between the two groups (Sulkowski et al., 2002a). Conversely, HIV co-infection reduces seroconversion and control of HBV infection (Gilson et al., 1997; Rodriguez-Mendez et al., 2000; Dore and Cooper, 2001; Martinez, 2001) and is associated with higher HCV viral loads and progression to cirrhosis (Eyster et al., 1994; Rockstroh et al., 1996; Martinez, 2001; Puoti et al., 2001; Sulkowski et al., 2002a) compared to HIV seronegative patients with the respective viral hepatitis. With the advent of highly active antiretroviral therapy (HAART) regimens capable of controlling

☆ This work was supported in part by NHLBI (see Appendix A), as well as by reagents and testing by Roche Molecular Systems.
* Corresponding author. Tel.: +1-916-734-8695; fax: +1-916-734-7766.
E-mail address: dasmuth@ucdavis.edu (D.M. Asmuth).
[1] VATS study group membership is detailed in Appendix A.

0166-3542/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2004.03.006

HIV replication and dramatically prolonging the survival of HIV-infected patients, the impact of co-morbid infections such as HBV and HCV with prolonged natural histories of their own has come into focus. HIV/HCV co-infected patients who respond to HAART have similar survival rates as those who are infected with HIV alone (Macias et al., 2002; Tedaldi et al., 2003a). However, major aspects under consideration include the potential morbidity and mortality that progressive hepatic disease may contribute in this population, as well as potential interactions of the viruses and the effects each may have on specific antiviral therapeutic interventions considered. For instance, liver failure has become a prominent cause of morbidity and death among hospitalized patients with HIV infection (Greub et al., 2000; Puoti et al., 2000b; Sulkowski et al., 2002a). In addition, hepatotoxicity to antiretroviral medications, a leading cause of severe adverse events leading to treatment discontinuation, is highly associated with co-infection with either HBV or HCV (den Brinker et al., 2000; Sulkowski et al., 2000b; Wit et al., 2002; Sulkowski et al., 2002b). In the setting of hepatic enzyme elevations coincident with antiretroviral therapy for HIV, it is important to know whether this is due to (a) direct drug toxicity in the environment of liver injury, (b) augmented HBV/HCV specific T-lymphocyte activity following immune reconstitution, or (c) a flare-up of hepatitis viral replication resulting from unknown mechanisms. While the larger clinical studies cited above did not, several small studies have monitored HBV or HCV viral loads following initiation of HAART in an attempt to shed light on this important issue. In addition, these smaller studies have yielded disparate results (Rockstroh et al., 1997; Rutschmann et al., 1998; Zylberberg et al., 1998; Ragni and Bontempo, 1999; Fialaire et al., 1999; Perez-Olmeda et al., 2000; Yokozaki et al., 2000; Puoti et al., 2000a; Manegold et al., 2001). In order to better characterize these effects, we undertook to measure the association between the initiation of HAART and HBV and HCV viral loads in a large cohort of prospectively enrolled subjects with advanced HIV.

The Viral Activation Transfusion Study (VATS) enrolled 531 HIV-infected, anemic subjects at eleven geographically dispersed centers into a randomized, double-blind clinical trial of non-leukoreduced versus leukoreduced red blood cell transfusion (Busch et al., 1996). The hypothesis of the study was that donor leukocytes from packed red blood cells reduced survival in recipient HIV-seropositive subjects and increased the HIV-RNA viral load in recipients following transfusion. Secondary endpoints included activation of multiple viruses (such as HIV, HBV and HCV), measured at weekly and then quarterly timepoints following transfusion. No significant increases of these viruses were demonstrated in the plasma of subjects receiving either non-leukoreduced (NLR) or leukoreduced (LR) allogeneic blood products (Collier et al., 2001; Asmuth et al., 2003). Because HAART was introduced during the study period, we were able to conduct additional analyses of the effect of HAART on subsequent death and opportunistic infections in this cohort of very advanced HIV-infected subjects (Murphy et al., 2001). We now report the effect of the initiation of HAART on plasma viral loads of HBV and HCV in the subset of subjects who were co-infected with HIV and either HBV or HCV.

2. Methods

2.1. Selection of subjects and timepoints

Selection of antiretroviral regimens was at the discretion of the primary care provider, except that new antiretroviral medications were discouraged immediately following the transfusion episode. Only patients who began HAART after entering VATS were considered for this analysis. The definition of HAART therapy for the study required at least three antiretroviral agents with at least one of which being a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor. Timepoints before HAART was initiated were classified as “pre-HAART.” All time points subsequent to the initiation of a regimen that met the definition of HAART, irrespective of whether the subject continued to take the prescribed therapy or not, were classified as “post-HAART.” This was done to approximate an intention-to-treat analysis. All medications were recorded at each quarterly visit, but adherence to antiretroviral therapy was not recorded.

The IRB committees of the collaborating institutions approved VATS, and informed consent was obtained from all participants in compliance with human experimentation guidelines of the US Department of Health and Human Services.

Selection of subjects for this report was performed in three steps. First, 523 of the 531 VATS subjects were screened for hepatitis B and C antibodies at baseline. (The other eight had insufficient baseline samples.) Samples were screened for hepatitis B total antibody using the Hepatitis B Virus Core Antigen (Recombinant) Corzyme Assay (Abbott Laboratories, Abbott Park, IL, USA) and for hepatitis B surface antigen using the Ortho HBsAg ELISA 2.0 (Ortho-Clinical Diagnostics, Inc. (Raritan, NJ, USA). Samples were also screened for hepatitis C using the Ortho HCV Version 3.0 ELISA (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA). All samples were tested in singlicate only due to volume constraints, and considered reactive if the singlicate test was reactive.

Second, transcription mediated amplification (TMA) testing was performed for selected subjects to confirm the presence of the hepatitis virus. For hepatitis B, subjects who were HBcAb positive/HBsAg negative or HBcAb negative/HBsAg positive received HBV DNA TMA testing by the Procleix HBV Discriminatory Assay (Gen-Probe Incorporated, San Diego, CA, USA). For hepatitis C, if the HCV antibody test was positive, Chiron dHCV TMA testing was performed on individual samples using the Procleix HCV Discriminatory Assay (Gen-Probe Incorporated). For the

HCV antibody negative subjects, it was performed in pools, followed by individual tests for subjects in a positive pool. TMA testing was not performed if a baseline blood sample was unavailable or if testing would have depleted volume needed for viral load testing. Such subjects were dropped from further consideration. HCV antibody negative subjects who lacked samples for these reasons were also dropped. Subjects were considered positive for HBV if they were HBcAb and HBsAg positive or HBV DNA TMA positive, and were considered positive for HCV if they were dHCV TMA positive or if they were HCV antibody positive without sufficient blood volume for the dHCV TMA test but with at least one follow-up phlebotomy.

Finally, subjects positive for HBV or HCV who started HAART after baseline, and who had at least one pre-HAART quarterly bleed and at least two post-HAART quarterly phlebotomies after the initiation of HAART, were eligible for analyses. HBV and HCV viral load testing was performed on all available quarterly bleeds up to 2 years before the initiation of HAART and all quarterly bleeds at most 9 months after HAART. HBV viral load was evaluated using the COBAS Amplicor HBV Monitor assay (Roche Diagnostics, Branchburg, NJ, USA). HCV viral load was evaluated using the COBAS Amplicor HCV Monitor 2.0 Assay (Roche Diagnostics). Viral load for each virus was reported as copies/mL. ALT was measured at timepoints that were selected for HBV or HCV viral load testing. If there was not enough volume for both ALT and HBV and/or HCV viral load testing, testing was prioritized as follows: (i) HBV, (ii) HCV, (iii) ALT.

The HIV RNA level was measured using a reverse transcriptase polymerase chain reaction assay with a lower limit of quantification of 200 copies/mL (Amplicor Monitor Assay; Roche Molecular Systems, Branchburg, NJ, USA).

2.2. Statistical methods

A random effects model with robust variance estimates was used to model change in viral load and ALT over time, assuming linear changes over time, but with separate slopes and intercepts pre- and post-HAART. A simpler random effects model was then fit to compare the mean levels pre- versus post-HAART, by assuming flat slopes but different mean levels pre- and post-HAART. The random effects models adjusted for correlated observations caused by taking repeated longitudinal measurements within each patient. Hepatitis B and C viral loads data were $\log_{10}$ transformed before analysis.

3. Results

3.1. Available data and patient characteristics

Table 1 shows the number of subjects and timepoints with HBV and HCV viral load data and ALT data. Table 2 shows demographics and pre-HAART clinical characteristics of patients analyzed for HBV or HCV loads. For approximately half of the subjects with HBV data, all or nearly all values were below the limit of quantification (<200 copies/mL).

Table 1
Number of patients and timepoints with hepatitis B viral load, hepatitis C viral load, and alanine aminotransferase (ALT) data analyzed

	Hepatitis B	Hepatitis C	ALT[a]
Number of patients	9	32	45
pre-HAART			
Number of timepoints	25	69	110
Mean (range)	2.8 (1, 9)	2.2 (1, 6)	2.4 (1, 9)
Number of patients w/>1 timepoint	5	19	28
post-HAART			
Number of timepoints	23	79	109
Mean (range)	2.6 (2, 3)	2.5 (2, 4)	2.4 (2, 4)

[a] Includes all patients with hepatitis B or C viral loads analyzed, plus an additional eight hepatitis B infected patients with last pre-HAART and all post-HAART viral loads below limit of quantification. Four patients are co-infected with hepatitis B and C.

Table 2
Demographic and pre-HAART laboratory characteristics

	Hepatitis B ($N = 9$)	Hepatitis C ($N = 32$)
Demographic characteristics		
Age, median (quartiles)	39 (33, 40)	39 (34, 43)
Sex (%)		
Male	89	66
Female	11	34
Race (%)		
Black	22	41
White	67	44
Hispanic	0	13
Other	11	3
HIV risk group (%)[a]		
Injection drug use	33	75
Men who have sex with men	56	34
Heterosexual contact	22	44
Blood product recipient	0	0
Other/unknown	11	0
Last pre-HAART values[b]		
CD4 (cells/mm^3)	118 (30, 149)	33 (9, 127)
HIV RNA ($\log_{10}$ copies/mL)	4.26 (4.11, 5.21)	4.67 (3.43, 5.20)
HBV DNA ($\log_{10}$ copies/mL)	4.85 (3.71, 5.28)	NA
HCV RNA ($\log_{10}$ copies/mL)	NA	6.50 (6.07, 7.50)
Alanine aminotransferase (U/L)	36 (24, 45)	35 (20, 47)

NA: not applicable.
[a] Patients could have multiple risk factors.
[b] Median (quartiles).

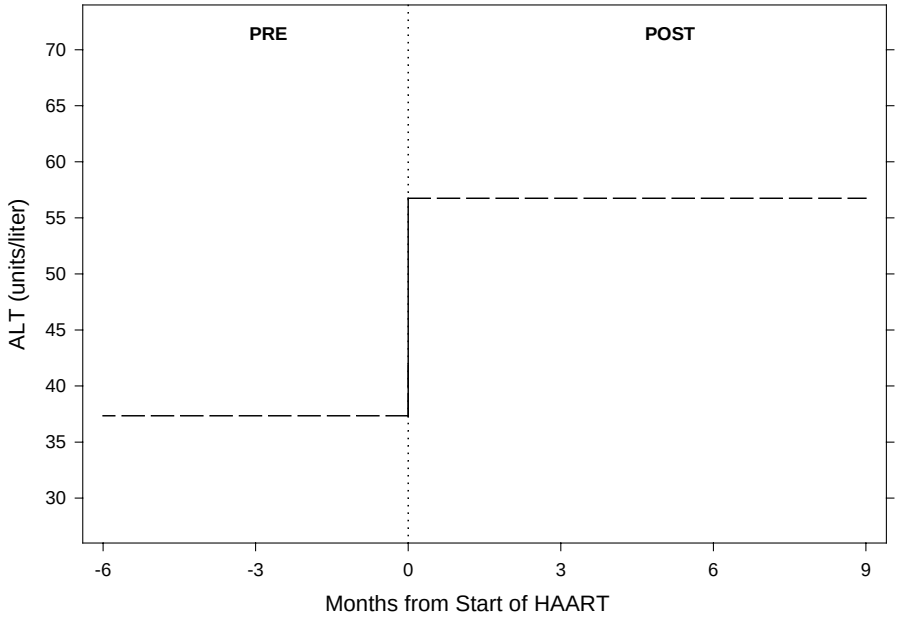

Fig. 1. Average alanine aminotransferase (ALT) levels pre- and post-HAART.

Subjects with all post-HAART observations and the last pre-HAART observation below the limit of quantification were excluded from analysis of HBV ($N = 8$). In total, there were 48 timepoints in 9 subjects analyzed for HBV and 148 timepoints in 32 subjects analyzed for HCV. Four subjects were in both the HBV and HCV cohorts analyzed.

The analysis of ALT levels included all subjects and timepoints with available HBV and/or HCV data (including those excluded from the HBV analysis because their values were below the limit of quantification). In total, there were 219 timepoints in 45 subjects analyzed for ALT.

3.2. ALT

The analysis of ALT included the HBV and HCV cohorts. The overall pattern was a flat slope pre-HAART followed by post-HAART levels that were generally higher. The two slopes were not significantly different ($P = 0.11$). Comparing the mean pre- and post-HAART levels, there was a mean increase of 19.4 U/L (95% CI 8.9–29.9, $P < 0.001$), from 37.4 U/L to 56.8 U/L (Fig. 1). Results were unchanged if the HBV or HCV cohorts were analyzed separately.

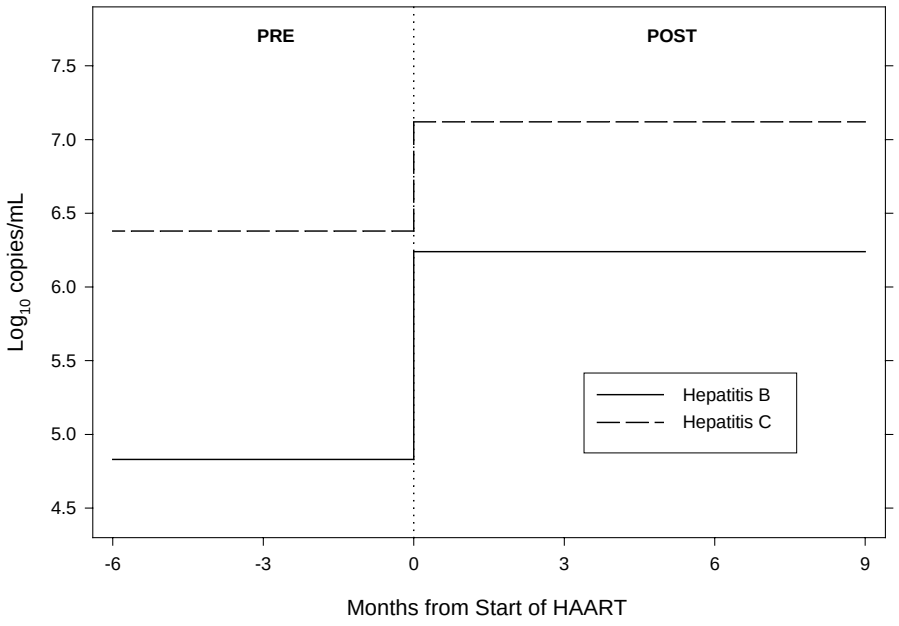

Fig. 2. Average hepatitis B and C viral load levels pre- and post-HAART.

3.3. Hepatitis B

A total of 9 subjects had one or more quantifiable post-HAART measurements and/or a quantifiable final pre-HAART measurement. Only five of these nine subjects had two or more pre-HAART measurements, i.e., only five could contribute information on within-patient change over time pre-HAART.

The estimated pre-HAART slope was significantly negative (−0.45 logs per month, $P < 0.001$) but this result could be an artifact of the very small sample size ($N = 5$). The post-HAART slope was essentially flat (0.02 $\log_{10}$ per month, $P = 0.85$). Discounting the significant pre-HAART slope and noting the flat post-HAART slope, we compared the overall mean pre- and post-HAART levels. There was an estimated increase of 1.40 $\log_{10}$, from 4.83 logs to 6.24 $\log_{10}$, but the change was not statistically significant (95% CI −0.05 to 2.86, $P = 0.07$) (see Fig. 2).

3.4. Hepatitis C

The estimated pre- and post-HAART slopes for hepatitis C viral load were both nearly flat and were not significantly different from each other ($P = 0.15$). Comparing the overall mean pre-HAART and post-HAART levels, there was an estimated increase of 0.74 $\log_{10}$ (95% CI 0.32–1.15, $P = 0.001$), from 6.38 $\log_{10}$ to 7.12 $\log_{10}$ (see Fig. 2).

3.5. HIV-1 RNA and CD4 T-lymphocyte counts

The estimated pre- and post-HAART slopes for HIV-1 RNA were nearly flat. Comparing the overall mean pre-HAART and post-HAART viral loads, there was an estimated decrease of −1.02 log10 (95% CI −1.35 to −0.69, $P < 0.001$).

The overall pattern for $CD4^+$ counts was an essentially flat slope pre-HAART ($P = 0.48$), followed by an increasing slope post-HAART of an average of 4.5 cells per month, $P = 0.04$).

4. Discussion

Assessed in the context of previous reports on the effect of HAART initiation on HBV and HCV plasma viral loads in smaller cohorts, a possible pattern emerges that may help define the interaction between HIV and hepatitis viral infection in the setting of antiretroviral treatment. Subjects identified for these analyses were very advanced with respect to their HIV infection (Table 2). At the last visit before HAART started, the median $CD4^+$ T-lymphocyte count was 33 cells/mm^3 for the HCV cohort and 118 cells/mm^3 for the HBV cohort. Among the 32 subjects analyzed for HCV, 9 (28%) died. Among the 9 subjects analyzed for HBV, 2 (22%) died on study. Indeed, ALT, a surrogate marker for hepatocyte injury, increased over the period of observation from 37.4 to 56.8 U/L. Drug toxicity or increased viral-induced inflammation could be contributing to this increase. Reports in the literature monitoring changes in HBV or HCV viral load following initiation of HAART draw from cohorts of AIDS patients with advanced immunodeficiency as well as several reporting observations during early and intermediate HIV disease.

With respect to the impact of HAART on HBV viral load, reports are scarce in the literature. Manegold et al. (2001) report their findings in two patients with initial pre-therapy $CD4^+$ T-cell counts <100 cells/mm^3, who had fully recovered from HBV infection evidenced by the presence of HBsAb. Both patients developed clinical hepatitis with the appearance of HBV DNA in the plasma and disappearance of HBsAb early during HAART therapy as HIV RNA became undetectable and $CD4^+$ T-cell counts rose. In both cases, the clinical hepatitis and HBV viremia spontaneously resolved with the reappearance of HBsAb within 4 months. In the present study of HBV/HIV co-infected subjects, which had nine evaluable subjects, the median $CD4^+$ T-cell count was 118 cells/mm^3. In contrast to the experience reported by Manegold et al. (2001), plasma HBV DNA increased by 1.40 $\log_{10}$ following the initiation of HAART (though not statistically significant) and the slope of the plasma HBV DNA was flat following HAART. In addition, among the nine evaluable subjects, there was only one subject who had an undetectable pre-HAART HBV viral load. This patient subsequently developed measurable HBV DNA by TMA.

A greater breadth of information, albeit highly variable, is available regarding the effect of the initiation of HAART on HCV viral load. Yokozaki et al. (2000) report their experience with 25 Japanese hemophiliacs co-infected with HIV and HCV and a mean $CD4^+$ T-cell count above 300 cells/mm^3 who were treated with HAART. Six months after the initiation of HAART, the mean HCV RNA plasma viral load had fallen by approximately 50% from 9×10^6 eq./mL. Two subjects were observed to have developed persistently undetectable HCV RNA. Interestingly, prior to the initiation of antiretroviral medications, there was no correlation between $CD4^+$ T-cell count and HCV viral load, in sharp contrast to the experience of others (Sulkowski et al., 2000a). Similarly, in a report from Spain, 16 subjects whose mean $CD4^+$ T-cell count rose 210 cells/mm^3 over the 12-month period following the initiation of HAART, HCV RNA plasma viral load fell a mean of 1 $\log_{10}$ and became undetectable in 4 months (Perez-Olmeda et al., 2000). However, in a population of 12 co-infected injection drug users (IDU) followed in Italian clinics with a mean baseline $CD4^+$ T-cell count of 338 ± 93 cells/mL, HCV RNA levels transiently rose during the first month of treatment, returning to baseline by the end of the observation period of 12 weeks (Puoti et al., 2000a). Similarly, in a population of 20 co-infected subjects consisting of hemophiliacs (60%) and IDU (35%) with a mean baseline $CD4^+$ T-cell count of 119 ± 118 cells/mm^3, HCV RNA did not change after 9 months of HAART (Zylberberg et al., 1998).

Conversely, Ragni and Bontempo (1999) treated 21 co-infected hemophiliacs in the US with a median baseline $CD4^+$ T-cell count of 152 cells/mm^3 with HAART and observed a 61% rise in HCV RNA at week 96. Rutschmann et al. reported their experience with 19 Swiss co-infected mainly IDU subjects with a mean baseline $CD4^+$ T-cell count of 63 cells/mm^3 (Rutschmann et al., 1998). A transient but significant rise of 0.4 $\log_{10}$ HCV RNA was observed that returned to baseline levels after 17 weeks. In addition, the initially elevated ALT levels over baseline also returned to baseline levels after 32 weeks. The confounding variable of several studies was the uncontrolled effect of mode of HIV transmission (i.e., through intravenous contamination versus sexual contact) and HCV genotype (types 3 and 4 predominate in Asian populations). No subjects in the current report acquired HIV through infusion of contaminated clotting factors. The rise in HCV RNA was sustained in our cohort of very advanced AIDS subjects with $CD4^+$ T-cells of 33 cells/mm^3 over a 9-month period of observation.

The effect of HAART on hepatitis viral load appears to be further related to the patient's immune state when placed on HAART. Patients whose immune systems are partially preserved at the time of HAART initiation or who rapidly reconstitute their immune system as evidenced by a sharp sustained rise in circulating $CD4^+$ T-lymphocyte cell counts appear to gain relative control of HCV viremia, ranging from stable viral loads to clearance of measurable HCV. Conversely, patients with advanced immunodeficiency who have a temporary or partial response to HAART are noted to have persistently elevated HCV plasma viral loads, as in the case of the present study. Indeed, in the present study, there was only a decrease of 1.02 $\log_{10}$ in HIV-RNA with an essentially flat slope post-HAART that was accompanied by an increase in $CD4^+$ T-lymphocyte count of 4.5 cells/mm^3 per month. As mentioned above, an intention to treat approach was taken in classifying subjects into the HAART treatment group by analyzing all subjects who were initiated with HAART as though they were maintained on therapy. This contrasts with the other studies where only subjects continuing therapy were maintained in the analysis. This hypothesis is consistent with the findings from Chung et al. (2002) that monitored the HCV plasma viral load following the institution of HAART in several clinical trials spanning a broad range of initial $CD4^+$ T-cell counts. The analysis included observations of 100 HCV/HIV co-infected individuals. While the mean entry $CD4^+$ T-cell count was between 243 and 424 cells/mm^3 in the studies included in the analysis, subjects' HCV plasma viral load increased 0.43 and 0.59 $\log_{10}$ IU/mL at weeks 16 and 48 for baseline $CD4^+$ T-cell counts <350 cells/mm^3, but only 0.26 and 0.1 $\log_{10}$ IU/mL at weeks 16 and 48 for baseline $CD4^+$ T-cell counts >350 cells/mm^3.

Several theories describe the dichotomized pattern of HCV plasma viral load responses to HAART initiation. A component of the initial rise in HCV plasma viral load could represent the immune reconstitution syndrome for all groups. This syndrome is poorly described, but is associated with viral reactivation in the case of Varicella Zoster Virus and is noted for an acute inflammatory response in the case of *Mycobacterium avium* complex infection (Shelburne et al., 2002). In addition, HCV is known to replicate in peripheral blood lymphocytes as well as hepatocytes (Cribier et al., 1996a; Laskus et al., 2000). Thus, the initial rise in HCV plasma viral load following the initiation of HAART therapy may relate to both the immune dysregulation associated with the immune reconstitution syndrome and increased targets available for HCV infection in the extra-hepatic compartment. Given the known role of the adaptive immune system in the control of HCV infection (Battegay, 1996), subsequent immune recovery and control of HCV infection in patients with less advanced HIV infection would be expected, as suggested by Valdez et al. in a study of HCV specific T-lymphocyte function in a cross section of co-infected subjects (Valdez et al., 2000). This does not explain why patients with very advanced HIV infection would experience persistent elevations in HCV plasma viral load following the introduction of antiretroviral therapy. These events may be related to the impact of antiretroviral therapy on innate immune system pathways. Whereas endogenous interferon levels correlate with control of HCV infection (Cribier et al., 1996b) and may impact the success of recombinant interferon-α therapy (Pirisi et al., 1997), effective HAART therapy is known to result in decreases of endogenous interferon levels (Mildvan et al., 1992; Kelleher et al., 1997). In this setting, HAART may disrupt host immune response to HCV at the level of cytokine interaction and innate immune system mechanisms that potentially would play a more vital role in patients with advanced HIV disease and little measurable HCV specific T-lymphocyte function.

With questions like these being raised, further study into the interaction between HIV and hepatitis viruses is warranted to better understand the immunopathogenesis of these viruses and the effect of HAART therapy on hepatitis virus disease progression or control. While the clinical significance of plasma viral load elevations remains uncertain (Rosenberg, 2001), important questions of viral–viral interactions are raised that could have clinical implications. Our data, however, begin to address the issue of when antiviral therapies should be initiated. For a patient population such as ours with advanced HIV disease, antiviral therapy for HBV and HCV (when new agents become available) should be initiated simultaneously or prior to antiretroviral therapy.

Acknowledgements

The Viral Activation Transfusion Study is the responsibility of the persons detailed in Appendix A. We would like to thank the patients who participated in the study for their efforts, and the transfusion service personnel and nurses at each medical center, without whose assistance the study could not have been accomplished.

Appendix A

Clinical sites

Case Western Reserve University, Cleveland, OH (N01-HB-57115): Michael Lederman, MD; Roslyn Yomtovian, MD; Michael Chance, RN; Donna Hendrix, RN

Georgetown University, Washington, DC (N01-HB-57116): Princy N. Kumar, MD; S. Gerald Sandler, MD; Karyn Hawkins, RN

Miriam Hospital/Brown University, Providence, RI (N01-HB-57117): Timothy P. Flanigan, MD; Joseph Sweeney, MD; Maria D. Mileno, MD; Melissa Di Spigno, RN; Michelle Dupuis, MT (SSB)

Mt. Sinai School of Medicine, New York, NY (N01-HB-57118): Henry S. Sacks, PhD, MD; Kala Mohandas, MD; Frances R. Wallach, MD; Letty Mintz, ANP

Ohio State University, Columbus, OH (N01-HB-57119): Michael F. Para, MD; Melanie S. Kennedy, MD; Jane Russell, RN; Dave Krugh, MT

University of California, San Diego, CA (N01-HB-57120): Thomas A. Lane, MD; W. Christopher Mathews, MD; Peggy Mollen-Rabwin, RN

University of California, San Francisco, CA (N01-HB-57121): Edward L. Murphy, MD, MPH; Steven G. Deeks, MD; Maurene Viele, MD; Chaolun Han, MD; Joanne Moore, MT (ASCP) SBB

University of North Carolina, Chapel Hill, NC (N01-HB-57122, RR00046): Charles van der Horst, MD; Meera Kelley, MD; Mark E. Brecher, MD; Linh Ngo, FNP

University of Pittsburgh, Pittsburgh, PA (N01-HB-57123): John W. Mellors, MD; Darrell J. Triulzi, MD; Deborah K. McMahon, MD; Sharon Riddler, MD

University of Texas Medical Branch, Galveston, TX (N01-HB-57124): Richard B. Pollard, MD; David M. Asmuth, MD; Janice Curry, PA-C; Gerald Shulman, MD

University of Washington/Puget Sound Blood Center, Seattle, WA (N01-HB-57125): Ann Collier, MD; Terry Gernsheimer, MD; Dee Townsend-McCall, RN; Jill Corson, RN

Central laboratory

Blood Centers of the Pacific, San Francisco, CA (N01-HB-57126): Michael P. Busch, MD, PhD; Tzong-Hae Lee, MD, PhD; W. Lawrence Drew, MD, PhD (UCSF Mt. Zion Medical Center, San Francisco); Megan Laycock

Coordinating center

New England Research Institutes, Watertown, MA (N01-HB-57127): Leslie A. Kalish, ScD; Susan F. Assmann, PhD; Jane D. Carrington, RN, BS; Margot S. Kruskall, MD (Beth Israel Deaconess Medical Center, Harvard Medical School, Boston MA); Ruth Eisenbud, BA

Sponsoring agency

National Heart, Lung and Blood Institute: George J. Nemo, PhD, project officer; Paul R. McCurdy, MD; Dean Follmann, PhD

Steering Committee Chair

Paul V. Holland, MD, BloodSource, Sacramento, CA

Data Safety Monitoring Board

Jeffrey McCullough, MD (Chair), University of Minnesota, Minneapolis, MN; Victor DeGruttola, ScD; Peter Frame, MD; Janice G. McFarland, MD; Ronald T. Mitsuyasu, MD; Elizabeth J. Read, MD; Dorothy E. Vawter, PhD

References

Asmuth, D.M., Kalish, L.A., Laycock, M.E., Murphy, E.L., Mohr, B.A., Lee, T.-H., Gallarda, J., Giachetti, C., Dollard, S.C., van der Horst, C.M., Grant, R.M., Busch, M.P., 2003. Hepatitis B and C virus, human T-cell leukemia virus I/II, and human herpes virus-8 activation following allogeneic red blood cell transfusion in patients with advanced HIV-1 infection. Transfusion 43, 451–458.

Battegay, M., 1996. Immunity to hepatitis C virus: a further piece of the puzzle. Hepatology 24, 961–963.

Bodsworth, N.J., Cooper, D.A., Donovan, B., 1991. The influence of human immunodeficiency virus type 1 infection on the development of the hepatitis B virus carrier state. J. Infect. Dis. 163, 1138–1140.

Bonacini, M., Puoti, M., 2000. Hepatitis C in patients with human immunodeficiency virus infection: diagnosis, natural history, meta-analysis of sexual and vertical transmission, and therapeutic issues. Arch. Intern. Med. 160, 3365–3373.

Busch, M.P., Collier, A., Gernsheimer, T., Carrington, J.D., Flanigan, T.P., Kashkari, M., Kennedy, M., Kumar, P.N., Lane, T.A., Mellors, J.W., Mohandas, K., Pollard, R.B., Viele, M., Yomtovian, R., Holland, P.V., McCurdy, P.R., 1996. The Viral Activation Transfusion Study (VATS): rationale, objectives, and design overview. Transfusion 36, 854–859.

Chung, R.T., Evans, S.R., Yang, Y., Theodore, D., Valdez, H., Clark, R., Shikuma, C., Nevin, T., Sherman, K.E., 2002. Immune recovery is associated with persistent rise in hepatitis C virus RNA, infrequent liver test flares, and is not impaired by hepatitis C virus in co-infected subjects. AIDS 16, 1915–1923.

Collier, A.C., Kalish, L.A., Busch, M.P., Gernsheimer, T., Assmann, S.F., Lane, T.A., Asmuth, D.M., Lederman, M.M., Murphy, E.L., Kumar, P., Kelley, M., Flanigan, T.P., McMahon, D.K., Sacks, H.S., Kennedy, M.S., Holland, P.V., 2001. Leukocyte-reduced red blood cell transfusions in patients with anemia and human immunodeficiency virus infection: the Viral Activation Transfusion Study: a randomized controlled trial. JAMA 285, 1592–1601.

Cribier, B., Rey, D., Uhl, G., Schmitt, C., Libbrecht, E., Vetter, D., Lang, J.M., Kirn, A., Stoll-Keller, F., 1996a. Quantification of hepatitis C virus RNA in peripheral blood mononuclear cells: a comparison between patients chronically infected by HCV and patients coinfected by HIV. Res. Virol. 147, 325–332.

Cribier, B., Schmitt, C., Rey, D., Lang, J.M., Kirn, A., Stoll-Keller, F., 1996b. Role of endogenous interferon in hepatitis C virus (HCV) infection and in coinfection by HIV and HCV. Res. Virol. 147, 263–266.

Daar, E.S., Lynn, H., Donfield, S., Gomperts, E., Hilgartner, M.W., Hoots, W.K., Chernoff, D., Arkin, S., Wong, W.Y., Winkler, C.A., 2001. Relation between HIV-1 and hepatitis C viral load in patients with hemophilia. J. Acquir. Immune Defic. Syndr. 26, 466–472.

den Brinker, M., Wit, F.W., Wertheim-van Dillen, P.M., Jurriaans, S., Weel, J., van Leeuwen, R., Pakker, N.G., Reiss, P., Danner, S.A., Weverling, G.J., Lange, J.M., 2000. Hepatitis B and C virus co-infection and the risk for hepatotoxicity of highly active antiretroviral therapy in HIV-1 infection. AIDS 14, 2895–2902.

Dore, G.J., Cooper, D.A., 2001. The impact of HIV therapy on co-infection with hepatitis B and hepatitis C viruses. Curr. Opin. Infect. Dis. 14, 749–755.

Eyster, M.E., Fried, M.W., Di Bisceglie, A.M., Goedert, J.J., 1994. Increasing hepatitis C virus RNA levels in hemophiliacs: relationship to human immunodeficiency virus infection and liver disease. Multicenter Hemophilia Cohort Study. Blood 84, 1020–1023.

Fialaire, P., Payan, C., Vitour, D., Chennebault, J.M., Loison, J., Pichard, E., Lunel, F., 1999. Sustained disappearance of hepatitis C viremia in patients receiving protease inhibitor treatment for human immunodeficiency virus infection. J. Infect. Dis. 180, 574–575.

Gilson, R.J., Hawkins, A.E., Beecham, M.R., Ross, E., Waite, J., Briggs, M., McNally, T., Kelly, G.E., Tedder, R.S., Weller, I.V., 1997. Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. AIDS 11, 597–606.

Greub, G., Ledergerber, B., Battegay, M., Grob, P., Perrin, L., Furrer, H., Burgisser, P., Erb, P., Boggian, K., Piffaretti, J.C., Hirschel, B., Janin, P., Francioli, P., Flepp, M., Telenti, A., 2000. Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. Lancet 356, 1800–1805.

Kelleher, A.D., Al Harthi, L., Landay, A.L., 1997. Immunological effects of antiretroviral and immune therapies for HIV. AIDS 11 (Suppl. 1), S149–S155.

Koblin, B.A., Taylor, P.E., Rubinstein, P., Stevens, C.E., 1992. Effect of duration of hepatitis B virus infection on the association between human immunodeficiency virus type-1 and hepatitis B viral replication. Hepatology 15, 590–592.

Laskus, T., Radkowski, M., Piasek, A., Nowicki, M., Horban, A., Cianciara, J., Rakela, J., 2000. Hepatitis C virus in lymphoid cells of patients coinfected with human immunodeficiency virus type 1: Evidence of active replication in monocytes/macrophages and lymphocytes. J. Infect. Dis. 181, 442–448.

Macias, J., Melguizo, I., Fernandez-Rivera, F.J., Garcia-Garcia, A., Mira, J.A., Ramos, A.J., Rivera, J.M., Pineda, J.A., 2002. Mortality due to liver failure and impact on survival of hepatitis virus infections in HIV-infected patients receiving potent antiretroviral therapy. Eur. J. Clin. Microbiol. Infect. Dis. 21, 775–781.

Manegold, C., Hannoun, C., Wywiol, A., Dietrich, M., Polywka, S., Chiwakata, C.B., Gunther, S., 2001. Reactivation of hepatitis B virus replication accompanied by acute hepatitis in patients receiving highly active antiretroviral therapy. Clin. Infect. Dis. 32, 144–148.

Martinez, E.H., 2001. Hepatitis B and hepatitis C co-infection in patients with HIV. Rev. Med. Virol. 11, 253–270.

Mildvan, D., Machado, S.G., Wilets, I., Grossberg, S.E., 1992. Endogenous interferon and triglyceride concentrations to assess response to zidovudine in AIDS and advanced AIDS-related complex. Lancet 339, 453–456.

Murphy, E.L., Collier, A.C., Kalish, L.A., Assmann, S.F., Para, M.F., Flanigan, T.P., Kumar, P.N., Mintz, L., Wallach, F.R., Nemo, G.J., 2001. Highly active antiretroviral therapy decreases mortality and morbidity in patients with advanced HIV disease. Ann. Intern. Med. 135, 17–26.

Perez-Olmeda, M., Garcia-Samaniego, J., Soriano, V., 2000. Hepatitis C viraemia in HIV-HCV co-infected patients having immune restoration with highly active antiretroviral therapy. AIDS 14, 212.

Perrillo, R.P., Regenstein, F.G., Roodman, S.T., 1986. Chronic hepatitis B in asymptomatic homosexual men with antibody to the human immunodeficiency virus. Ann. Intern. Med. 105, 382–383.

Pirisi, M., Fabris, C., Toniutto, P., Falleti, E., Tisminetzky, S.G., Gerotto, M., Soardo, G., Vitulli, D., Del Forno, M., Baralle, F., Bartoli, E., 1997. Endogenous interferon-alpha concentration and outcome of interferon treatment in patients with chronic hepatitis C. Dig. Dis. Sci. 42, 767–771.

Poles, M.A., Dieterich, D.T., 2000. Infections of the liver in HIV-infected patients. Infect. Dis. Clin. North Am. 14, 741–759.

Puoti, M., Bonacini, M., Spinetti, A., Putzolu, V., Govindarajan, S., Zaltron, S., Favret, M., Callea, F., Gargiulo, F., Donato, F., Carosi, G., 2001. Liver fibrosis progression is related to CD4 cell depletion in patients coinfected with hepatitis C virus and human immunodeficiency virus. J. Infect. Dis. 183, 134–137.

Puoti, M., Gargiulo, F., Roldan, E.Q., Chiodera, A., Palvarini, L., Spinetti, A., Zaltron, S., Putzolu, V., Zanini, B., Favilli, F., Turano, A., Carosi, G., 2000a. Liver damage and kinetics of hepatitis C virus and human immunodeficiency virus replication during the early phases of combination antiretroviral treatment. J. Infect. Dis. 181, 2033–2036.

Puoti, M., Spinetti, A., Ghezzi, A., Donato, F., Zaltron, S., Putzolu, V., Quiros-Roldan, E., Zanini, B., Casari, S., Carosi, G., 2000b. Mortality for liver disease in patients with HIV infection: a cohort study. J. Acquir. Immune Defic. Syndr. 24, 211–217.

Ragni, M.V., Bontempo, F.A., 1999. Increase in hepatitis C virus load in hemophiliacs during treatment with highly active antiretroviral therapy. J. Infect. Dis. 180, 2027–2029.

Rockstroh, J.K., Spengler, U., Sudhop, T., Ewig, S., Theisen, A., Hammerstein, U., Bierhoff, E., Fischer, H.P., Oldenburg, J., Brackmann, H.H., Sauerbruch, T., 1996. Immunosuppression may lead to progression of hepatitis C virus-associated liver disease in hemophiliacs coinfected with HIV. Am. J. Gastroenterol. 91, 2563–2568.

Rockstroh, J.K., Theisen, A., Kaiser, R., Spengler, U., Sauerbruch, T., 1997. Antiviral combination therapy decreases HIV-load but does not alter hepatitis C virus (HCV)-replication [abstract 735]. Hepatology 26, 735.

Rodriguez-Mendez, M.L., Gonzalez-Quintela, A., Aguilera, A., Barrio, E., 2000. Prevalence, patterns, and course of past hepatitis B virus infection in intravenous drug users with HIV-1 infection. Am. J. Gastroenterol. 95, 1316–1322.

Rosenberg, P.M., 2001. Hepatitis C: a hepatologist's approach to an infectious disease. Clin. Infect. Dis. 33, 1728–1732.

Rutschmann, O.T., Negro, F., Hirschel, B., Hadengue, A., Anwar, D., Perrin, L.H., 1998. Impact of treatment with human immunodeficiency virus (HIV) protease inhibitors on hepatitis C viremia in patients coinfected with HIV. J. Infect. Dis. 177, 783–785.

Shelburne III, S.A., Hamill, R.J., Rodriguez-Barradas, M.C., Greenberg, S.B., Atmar, R.L., Musher, D.W., Gathe Jr., J.C., Visnegarwala, F., Trautner, B.W., 2002. Immune reconstitution inflammatory syndrome: emergence of a unique syndrome during highly active antiretroviral therapy. Medicine (Baltimore) 81, 213–227.

Sulkowski, M.S., Mast, E.E., Seeff, L.B., Thomas, D.L., 2000a. Hepatitis C virus infection as an opportunistic disease in persons infected with

human immunodeficiency virus. Clin. Infect. Dis. 30 (Suppl. 1), S77–S84.

Sulkowski, M.S., Moore, R.D., Mehta, S.H., Chaisson, R.E., Thomas, D.L., 2002a. Hepatitis C and progression of HIV disease. JAMA 288, 199–206.

Sulkowski, M.S., Thomas, D.L., Chaisson, R.E., Moore, R.D., 2000b. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. JAMA 283, 74–80.

Sulkowski, M.S., Thomas, D.L., Mehta, S.H., Chaisson, R.E., Moore, R.D., 2002b. Hepatotoxicity associated with nevirapine or efavirenz-containing antiretroviral therapy: role of hepatitis C and B infections. Hepatology 35, 182–189.

Tedaldi, E.M., Baker, R.K., Moorman, A.C., Alzola, C.F., Furhrer, J., McCabe, R.E., Wood, K.C., Holmberg, S.D., 2003a. Influence of coinfection with hepatitis C virus on morbidity and mortality due to human immunodeficiency virus infection in the era of highly active antiretroviral therapy. Clin. Infect. Dis. 36, 363–367.

Tedaldi, E.M., Hullsiek, K.H., Malvestutto, C.D., Arduino, R.C., Fisher, E.J., Gaglio, P.J., Jenny-Avital, E.R., McGowan, J.P., Perez, G., 2003b. Prevalence and characteristics of hepatitis C virus coinfection in a human immunodeficiency virus clinical trials group: the Terry Beirn Community Programs for Clinical Research on AIDS. Clin. Infect. Dis. 36, 1313–1317.

Valdez, H., Anthony, D., Farukhi, F., Patki, A., Salkowitz, J., Heeger, P., Peterson, D.L., Post, A.B., Asaad, R., Lederman, M.M., 2000. Immune responses to hepatitis C and non-hepatitis C antigens in hepatitis C virus infected and HIV-1 coinfected patients. AIDS 14, 2239–2246.

Wit, F.W., Weverling, G.J., Weel, J., Jurriaans, S., Lange, J.M., 2002. Incidence of and risk factors for severe hepatotoxicity associated with antiretroviral combination therapy. J. Infect. Dis. 186, 23–31.

Yokozaki, S., Takamatsu, J., Nakano, I., Katano, Y., Toyoda, H., Hayashi, K., Hayakawa, T., Fukuda, Y., 2000. Immunologic dynamics in hemophiliac patients infected with hepatitis C virus and human immunodeficiency virus: influence of antiretroviral therapy. Blood 96, 4293–4299.

Zylberberg, H., Chaix, M.L., Rabian, C., Rouzioux, C., Aulong, B., Brechot, C., Viard, J.P., Pol, S., 1998. Tritherapy for human immunodeficiency virus infection does not modify replication of hepatitis C virus in coinfected subjects. Clin. Infect. Dis. 26, 1104–1106.