Effect of Tenofovir With and Without Interferon on Hepatitis D Virus Replication in HIV–Hepatitis B Virus–Hepatitis D Virus-Infected Patients

Anders Boyd,¹ Patrick Mialhes,^{2,3} Ségolène Brichler,^{4,5} Caroline Scholtès,^{3,6} Sarah Maylin,⁷ Constance Delaugerre,⁷ Phillipe Chevallier-Queyron,⁶ Emmanuel Gordien,^{4,5} Pierre-Marie Girard,^{1,8,9} and Karine Lacombe^{1,8,9}

Abstract

The effect of tenofovir (TDF) alone or in combination with interferon on hepatitis D virus (HDV) replication is poorly characterized in patients infected with human immunodeficiency virus (HIV), hepatitis B virus (HBV), and HDV. We analyzed triinfected patients undergoing treatment with either TDF alone ($n=13$) or including interferon (IFN) at some point during TDF therapy (TDF+IFN, $n=4$). Linear mixed-effect models were used to estimate the mean change from baseline of HDV-RNA and hepatitis surface antigen (HBsAg) levels during treatment. Patients were followed for a median 31.6 (25–75%-tile: 15.0–47.4) months. In the TDF+IFN group, three initiated IFN-based therapy after a median of 21.7 months (range=10.5–24.9) of lamivudine (LAM)+TDF, while the remaining patient had 46.8 months of prior LAM exposure. Significant decreases in HDV-RNA were observed in both groups [TDF alone: $-0.380 \log_{10}$ copies/ml per year (95% CI: $-0.557, -0.202$) vs. TDF+IFN: $-1.325 \log_{10}$ copies/ml per year (95% CI: $-1.931, -0.720$)], while the HDV-RNA decline overall was significantly faster in patients with TDF+IFN ($p=0.002$). Accordingly, two patients achieved HDV-RNA below the limit of quantification (LOQ: $<1,000$ copies/ml) and one near LOQ (1450 copies/ml), all concomitantly treated with interferon. There were no significant changes in HBsAg levels for either group [TDF alone: $-0.008 \log_{10}$ IU/ml per month (95% CI: $-0.019, 0.004$), TDF+IFN: $-0.011 \log_{10}$ IU/ml per month (95% CI: $-0.037, 0.015$)] and no significant difference in slope between treatment groups ($p=0.8$). Interferon therapy might be more effective after extended previous anti-HBV antiviral exposure among triinfected patients; however, the long-term implications of these findings remain unknown.

Introduction

INFECTION WITH HEPATITIS D VIRUS (HDV) greatly increases the risk of liver cirrhosis, hepatic decompensation, and death in patients also coinfecting with human immunodeficiency virus (HIV) and hepatitis B virus (HBV).¹ Interferon (IFN) is the only treatment recommended in HIV–HBV–HDV-triinfected patients with active HDV replication, yet with low rates of therapeutic success and high risk of side effects; other alternatives need further evaluation.^{2,3} For in-

stance, long-term treatment with potent nucleos(t)ide reverse transcriptase inhibitors (NRTIs) has led to substantial reduction in HDV-RNA and hepatitis B surface antigen (HBsAg) levels, the biological material required for HDV assembly and replication.⁴

Tenofovir (TDF), a commonly used NRTI with dual anti-HIV and anti-HBV activity, exhibits a certain degree of HBsAg suppression⁵ and could therefore affect HDV replication. One recent clinical report in particular has suggested that HDV-RNA replication during acute infection can be

¹Inserm, UMRS707, Paris, France.

²Hospices Civils de Lyon, Hôpital de la Croix-Rousse, Service des Maladies Infectieuses et Tropicales, Lyon, France.

³Université Lyon 1, Lyon, France.

⁴AP-HP, Service de Bactériologie-Virologie-Hygiène, associé au CNR des Hépatites B, C et Delta, Hôpital Avicenne, and Université Paris 13, Bobigny, France.

⁵Inserm, U955, Créteil, France.

⁶Hospices Civils de Lyon, Hôpital de la Croix-Rousse, Laboratoire de Virologie, Lyon, France and Université Lyon 1, Lyon, France.

⁷AP-HP, Service de Virologie, Hôpital Saint-Louis, Paris, France.

⁸Université Pierre et Marie Curie – Paris VI, Paris, France.

⁹AP-HP, Service des Maladies Infectieuses et Tropicales, Hôpital Saint-Antoine, Paris, France.

suppressed while undergoing TDF and lamivudine (LAM) therapy without IFN.⁶ However, relatively larger-scale studies in the HIV-HBV-HDV-infected population have not examined HDV replication specifically after TDF initiation or have included a mix of anti-HBV regimens within which TDF was in the slight majority.

Another major research question for this specific patient population is whether adding IFN after extended periods of anti-HBV therapy has any added effect on HDV replication compared to TDF alone. Among HIV-negative patients, initiating adefovir or TDF in combination with IFN has shown no additional benefit in HDV-RNA decline or clearance rate when compared to IFN alone.⁷⁻⁹ Nevertheless, various pieces of evidence have pointed toward the potential advantage of different IFN + TDF treatment strategies.^{10,11}

We then present an observational, longitudinal, multicenter study on the changes of HBV-DNA and HDV-RNA levels during TDF therapy with or without IFN in 17 HIV-HBV-HDV-triinfected patients. The effect of these treatment strategies on HBsAg quantification was also evaluated.

Materials and Methods

Patients and study design

From 2002 to 2010, HIV-HBV-HDV-triinfected patients, who had initiated treatment with TDF 300 mg/day as part of their combined antiretroviral treatment (cART), were selected for the present study. Inclusion criteria were HIV positivity confirmed by complete Western blot, HBsAg seropositivity for >6 months, an available serum sample at TDF initiation, at least one available serum ≥ 6 months apart during follow-up, and baseline HDV-RNA >1,000 copies/ml. Data were collected prospectively for patients enrolled in the French HIV-HBV Cohort Study¹² ($n=9$) or retrospectively for patients regularly visiting outpatient clinics at Hôpital Saint-Antoine ($n=5$) or Hôpital de la Croix-Rousse ($n=3$). All patients provided written informed consent to participate in the study.

Quantification of HBV and HDV parameters

HBV-DNA levels were quantified using real-time polymerase chain reaction (PCR) assay (PCR-Amplicor; Roche Diagnostic Systems, Meylan, France; detection threshold: 60 IU/ml). HBsAg was quantified using the ARCHITECT HBsAg EIA (Abbott Laboratories, Rungis, France).

HDV-RNA quantification was performed from serum aliquots kept frozen at -80°C using one of two real-time quantitative PCR assays [limit of quantification (LOQ) for HDV-RNA at Hôpital Avicenne¹³ $\sim 1,000$ copies/ml or at Hôpital de la Croix-Rousse¹⁴ ~ 500 copies/ml]. One potential problem with these techniques is the lack of an international standard, which may inadvertently increase between-assay and between-center variability. However, in a recent International Quality Control Survey in 2013, organized by the French national reference laboratory for HDV, samples with various viral loads and genotypes were compared from both laboratories, demonstrating rather comparable results.¹⁵

Evaluation of liver function

All patients had available alanine aminotransferase and aspartate aminotransferase (ALT and AST, respectively) at baseline and end of follow-up. Fibrosis was evaluated using

the Fibrometer score, a logistic function including platelet count, prothrombin time, aspartate transaminase (AST), α_2 -macroglobulin, hyaluronic acid, urea, and age¹⁶ or Fibroscan (Echosens, Paris, France). A subset of 12 patients had one hepatic punch biopsy performed near TDF initiation and three had a second biopsy during treatment. Fibrosis and activity staging were done according to METAVIR classification.¹⁷

Statistical analysis

Correlations were calculated between endpoints using Pearson's correlation for continuous variables above detection thresholds and Spearman's rank correlation for any measure with a dichotomous variable. All comparisons between continuous parameters were done using a Kruskal-Wallis rank test. Linear mixed-effect models were used to estimate the mean change from baseline of HBV-DNA, HBsAg levels, and HDV-RNA (as well as ALT/AST and Fibrometer score) during treatment, accounting for within-patient correlation by an unstructured correlation matrix. Slopes of HBV parameters were compared between patients taking TDF and TDF with IFN by including an interaction term with continuous time and treatment group. All analyses were performed using Stata statistical package (v.12.1,

TABLE 1. DESCRIPTION OF THE STUDY POPULATION AT BASELINE

Study population	N = 17
Demographics	
Age, years ^a	40.4 (36.0–43.6)
Male/female ratio (% males)	15/2 (88.2)
Patients born in Mediterranean or African country ^b	8 (47.1)
HIV characteristics	
Estimated HIV duration, years ^a	9.2 (2.7–16.0)
HIV-RNA, copies/ml ^a	17,328 (101–41,600)
HIV-RNA <50 copies/ml ^b	3 (17.6)
CD4 ⁺ , cells/mm ^a	301 (203–432)
Duration of cART, years [N=12] ^a	4.8 (4.0–7.9)
Viral hepatitis characteristics	
Estimated HBV duration, years ^a	5.7 (2.6–13.7)
Pretreated with lamivudine ^b	7 (41.2)
Duration of prior lamivudine, months ^a	47.3 (35.8–69.9)
Cotreated with lamivudine ^b	9 (52.9)
Cotreated with emtricitabine ^b	5 (29.4)
HBV-DNA <60 UI/ml ^b	7 (41.2)
HBV-DNA, log ₁₀ IU/ml ^{a,c}	3.54 (2.28–6.15)
HDV-RNA, log ₁₀ copies/ml ^a	7.00 (6.75–7.89)
HBsAg quantification, log ₁₀ IU/ml ^a	3.72 (3.01–3.84)
ALT, IU/ml ^a	106 (53–160)
ALT >35 IU/ml ^b	16 (94.1)
AST, IU/ml ^a	84 (62–185)
AST >35 IU/ml ^b	17 (100.0)
HBeAg seropositive ^b	6 (35.3)

^aMedian (25th–75th percentile).

^b n (%).

^cIn patients with detectable HBV-DNA.

cART, combination antiretroviral therapy; HBV, hepatitis B virus; HDV, hepatitis D virus; HBsAg, hepatitis surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

College Station, TX) and significance was determined as a p -value < 0.05 .

Results

Study population

As shown in Table 1, patients were predominately male and almost half were from a region of high HBV endemicity. Patients were HIV infected for an estimated median 9.2 years and had minor immunosuppression. Twelve patients had extensive periods of previous antiretroviral treatment, while the remaining five patients were treatment naive at the time of TDF initiation. The time from first HBsAg-positive serology was 5.7 (25–75 percentile = 2.6–13.7) years and most patients were HBeAg negative ($n = 11$).

Characteristics of baseline antiretroviral and concomitant anti-HBV treatment are given in Table 1. Of the 17 patients followed, 13 (76.5%) had no concomitant IFN-based regimen (herein defined as the “TDF-alone” group). In this group, no one discontinued treatment due to an adverse event. The other four (23.5%) patients were at some point additionally treated with IFN (defined as the “TDF+IFN” group): three initiated either pegylated-IFN ($n = 2$) or standard IFN- α ($n = 1$) after a median of 21.7 months (range = 10.5–24.9) of LAM+TDF treatment, while one initiated pegylated-IFN at the same time as TDF (after 46.8 months of prior exposure to LAM). In this group, IFN was given for the standard 48-week treatment schedule in two patients and an extended duration of 56 weeks in one patient. The remaining patient stopped IFN therapy after 2 weeks due to severe adverse events (rash, swelling, cervical lymphadenopathy).

HBV-DNA viral replication during follow-up

Overall, patients were followed during a median 31.6 (25–75 percentile: 15.0–47.4) months of TDF treatment. Before TDF

initiation, HBV-DNA replication was undetectable in six (46.2%) and one (25.0%) patient(s) in the TDF-alone and TDF+IFN groups, respectively. At the end of follow-up, no difference in obtaining undetectable serum HBV-DNA (viral load < 60 IU/ml) was observed in those without versus those with IFN-based treatment (76.9% vs. 75.0%, respectively, $p = 0.9$). In those patients with detectable HBV-DNA at baseline irrespective of treatment group, HBV-DNA levels significantly decreased at $-0.212 \log_{10}$ IU/ml per month (95% CI: -0.331 , -0.094) during the first 12 months after TDF initiation.

HDV-RNA viral replication during follow-up

The baseline median HDV-RNA level was high (Table 1) with no observable difference between the TDF-alone and TDF+IFN groups (7.09 and 7.09 $\log_{10}$ copies/ml, respectively, $p = 0.4$). In the TDF-alone group (Fig. 1a), HDV-RNA decline was at $-0.380 \log_{10}$ copies/ml per year (95% CI: -0.557 , -0.202). If we compare the overall slope of HDV-RNA decline in the TDF+IFN group, while including the time prior to IFN initiation, we observe a significantly steeper slope than those treated with TDF alone ($-1.325 \log_{10}$ copies/ml per year; 95% CI: -1.931 , -0.720 ; p between groups = 0.002). A drop in HDV-RNA did occur in this group directly after initiating IFN therapy (Fig. 1b). Accordingly, no one in the TDF-alone group achieved a HDV-RNA viral load below the LOQ during follow-up. In the TDF+IFN group, two patients had HDV-RNA levels below the LOQ at the end of follow-up and one other patient reached levels close to the LOQ (1,450 copies/ml). In the patient without virological response, follow-up was extended 18 months after showing no decline in HDV-RNA replication (Fig. 1b). Low compliance in this patient was suspected by the physician, as HBV-DNA viral loads were intermittently between detectable and undetectable levels.

FIG. 1. Hepatitis surface antigen (HBsAg) and hepatitis D virus (HDV)-RNA levels during treatment with tenofovir (TDF). Follow-up of TDF-treated patients without interferon (IFN) are represented in (a) ($n = 13$). Thick lines are used to describe the mixed-effect slopes of change in HBsAg and HDV-RNA during follow-up. Patients treated with both tenofovir and interferon are represented in (b) ($n = 4$). In this figure, the x -axis denotes the time in months from the point at which TDF+IFN combination therapy was initiated. All patients had prior exposure to TDF alone before initiating TDF+IFN, except for one patient whose follow-up is represented in open data points. In both figures, individual lines correspond to the clinical course and dots correspond to the parameter values of each patient.

Changes in HBsAg quantification

Baseline median HBsAg levels were 3.72 log₁₀ IU/ml with no difference between patients undergoing TDF alone and TDF+IFN (3.77 and 3.66 log₁₀ IU/ml, respectively, $p=0.5$). No significant difference in HBsAg decline was observed between patients in the TDF-alone (-0.008 log₁₀ IU/ml per month, 95% CI: $-0.019, 0.004$) vs. the TDF+IFN group (-0.011 log₁₀ IU/ml per month, 95% CI: $-0.037, 0.015$) (Fig. 1a and 1b, $p=0.8$). One of five HBeAg-positive patients with TDF alone lost HBeAg 54.0 months after TDF initiation, while the only HBeAg-positive patient in the TDF+IFN group lost HBeAg after 36.5 months. All 17 patients remained HBsAg positive at the end of follow-up.

Of note, HBsAg was significantly correlated with HBV-DNA levels ($r=0.72$, $p=0.004$), yet weakly correlated with a positive HBV-DNA result (cut off <60 IU/ml, $r=0.13$, $p=0.3$). Both HBV-DNA and HBsAg levels were not significantly correlated with HDV-RNA ($r=0.30$, $p=0.3$ and $r=0.16$, $p=0.3$, respectively).

Liver enzymes and fibrosis during follow-up

Concerning biochemical parameters, all patients had high baseline levels of transaminases (Table 1). Only three patients in the TDF-alone group and one patient in the TDF+IFN group had normalized levels of liver enzymes at the end of follow-up. Yet, ALT/AST levels did decrease on average $-32/-33$ IU/ml and $-36/-43$ IU/ml over a 3-year period for the TDF-alone and TDF+IFN groups, respectively ($p>0.7$). Using noninvasive measures of fibrosis ($n=9$ with Fibrometer and $n=8$ with Fibroscan), fibrosis equivalent levels were distributed as follows: F4 $n=10$, F3 $n=3$, F2 $n=1$, $<F2$ $n=3$. Overall, no evidence of changes in noninvasive scores was observed, whether it was determined from Fibrometer (change in score $+0.001$ per month, 95% CI: $-0.002, 0.004$, $n=9$) or Fibroscan (change $+0.2$ and -1.5 kPa per month during follow-up in two patients). Among the 12 patients with liver biopsy at TDF initiation, most presented with a Metavir fibrosis score at either F3 ($n=3$) or F4 ($n=6$) and an activity score at A2 ($n=7$) or A3 ($n=4$). No change in fibrosis was noted in liver biopsies during treatment ($n=3$), which remained at F3 ($n=1$) or F4 ($n=2$).

Discussion

In a cohort of HIV-HBV-HDV-coinfected patients followed for a median 3 years, HDV-RNA did appear to decrease during treatment. Even though the decline observed specifically in the TDF-alone group was similar to the decline reported in HIV-infected patients undergoing long-term treatment with a variety of anti-HBV NRTIs,⁴ it was nonetheless very slow, with an estimated 3-year drop in HDV-RNA at only -1.2 log₁₀ copies/ml. Likewise, TDF-associated HDV suppression had minimal effects on any clinically relevant outcome. The majority of patients did not completely clear HDV infection and no decrease in liver fibrosis was particularly noted in either precirrhotic or cirrhotic patients.

Importantly, we observed two patients with HDV-RNA below the LOQ and one patient with HDV-RNA very close to this level, all among patients treated with either IFN- α or pegylated-IFN. A continual theme underscored in prior research among HBV-HDV-infected patients is that initiating a

combination of NRTIs, including TDF, to IFN is therapeutically unsuccessful.^{7-9,18,19} Nevertheless, one major feature distinguishes our patient population from previous studies. All patients with IFN had roughly more than a year of previous exposure to an anti-HBV-containing antiretroviral regimen before initiating IFN. Preliminary evidence has suggested that adding IFN therapy may be effective in HBsAg seroconversion after extended periods of NRTI exposure,¹¹ which could potentially reduce HDV-RNA levels. Currently, no other data exist comparing patients with and without intensification after extended periods of NRTI therapy. In addition, therapy with TDF after several months of IFN has resulted in resolution of a case of chronic HDV, suggesting that sequential therapy might be beneficial.¹⁰

Combination therapy with adefovir and interferon has nevertheless been shown to produce a superior decline in HBsAg levels when compared to these treatments individually.⁷ In contrast, we observed no difference in HBsAg decline between treatment groups, possibly owing to certain HIV-related factors. For example, intracellular HBsAg is known to be higher in HIV-HBV than HBV-infected patients,²⁰ wherefore HBsAg may have been released at very high rates during clearance of infected hepatocytes. We have also previously observed in HIV-HBV coinfection that the decline in HBsAg levels is diminished when the CD4⁺ cell count is below 350/mm³ and that HBsAg loss occurs with larger increases in CD4⁺ cell count after treatment initiation.^{5,21} Since immunoreconstitution was generally slow in our cohort (the median maximum change in CD4⁺ count was 180/mm³ after a median of 20.2 months), drastic changes in HBsAg for either treatment group would have been unlikely.

The present study design may not be the most appropriate in evaluating whether the addition of IFN to TDF is beneficial. Adding a treatment arm with IFN alone would have been advantageous in this regard, yet is for the most part contraindicated in triinfection. Considering that most of the very few patients taking TDF and IFN had achieved low HDV-RNA viral loads, this therapeutic strategy may merit further investigation. Fortunately, a clinical trial is currently underway that aims at addressing the effectiveness of TDF+IFN,^{8,22} but only in HIV-negative patients.

One limitation of our study is that only HIV-infected patients with mild immunosuppression were included. It is debatable to what extent the efficacy and tolerance of combination TDF+IFN therapy would be affected in patients with severe immunosuppression. Second, follow-up time may have been too short and thus unable to capture the clinical impact of long-term TDF use on HDV-RNA and HBsAg levels. For example, the lack of extensive follow-up in the TDF+IFN group prevents any conclusions regarding sustained virological response and perhaps the effects observed in this group were only transient. In addition, another study conducted among HIV-HBV-HDV-infected patients, undetectable HDV-RNA was achieved in 19% of patients using a potent combination of lamivudine, TDF, and/or emtricitabine after longer periods (5-8 years).⁴ However, our results stress that 36-month therapy on TDF alone is not a sufficient means of controlling chronic HDV infection, especially when the addition of IFN may be more beneficial. A TDF-containing ARV regimen is also unlikely to curb severe morbidity and mortality, since 16.1% and 40.6% of patients with severe cirrhosis are expected to die

after 5 and 10 years of HDV infection, respectively.²³ Third, there have been no studies validating the use of noninvasive markers in this specific study population, hence making it difficult to judge their accuracy. Finally, the small number of patients limits our conclusions, especially with respect to treatment efficacy.

In conclusion, although TDF is strongly potent against HBV replication, it alone is most likely not sufficient for the effective resolution of HDV infection in HIV-HBV-infected patients. There was a high percentage of patients who achieved low levels of HDV replication when IFN was added after longer periods of anti-HBV treatment, thereby making the evaluation of other treatment combinations strongly warranted in this patient population. Further studies need to determine whether prior anti-HBV therapy should be initiated well before or after IFN, if there is any difference in effectiveness among severely immunodepressed patients, and if quad-infected patients with hepatitis C virus may benefit from this treatment strategy.

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Author Disclosure Statement

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References

- Sheng WH, Hung CC, Kao JH, *et al.*: Impact of hepatitis D virus infection on the long-term outcomes of patients with hepatitis B virus and HIV coinfection in the era of highly active antiretroviral therapy: A matched cohort study. *Clin Infect Dis* 2007;44(7):988–995.
- Soriano V, Grint D, d'Arminio Monforte A, *et al.*: Hepatitis delta in HIV-infected individuals in Europe. *AIDS* 2011;25(16):1987–1992.
- Hughes SA, Wedemeyer H, and Harrison PM: Hepatitis delta virus. *Lancet* 2011;378(9785):73–85.
- Sheldon J, Ramos B, Toro C, *et al.*: Does treatment of hepatitis B virus (HBV) infection reduce hepatitis delta virus (HDV) replication in HIV-HBV-HDV-coinfected patients? *Antivir Ther* 2008;13(1):97–102.
- Maylin S, Boyd A, Lavocat F, *et al.*: Kinetics of hepatitis B surface and envelope antigen and prediction of treatment response to tenofovir in antiretroviral-experienced HIV-hepatitis B virus-infected patients. *AIDS* 2012;26(8):939–949.
- Babiker ZO, Hogan C, Ustianowski A, and Wilkins E: Does interferon-sparing tenofovir disoproxil fumarate-based therapy have a role in the management of severe acute hepatitis delta super-infection? *J Med Microbiol* 2012;61:1780–1781.
- Wedemeyer H, Yurdaydin C, Dalekos GN, *et al.*: Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* 2011;364(4):322–331.
- Yurdaydin C, Wedemeyer H, Caruntu FA, *et al.*: Pegylated-interferon-a-2a plus tenofovir or placebo for the treatment of hepatitis delta: First results of the HIDIT-2 study. *Hepatology* 2012;56(S1):54A.
- Lutz HH and Trautwein C: Reviving pegylated interferon as a therapeutic agent for hepatitis D: No more room for nucleos(t)ides? *Hepatology* 2011;53(6):2131–2133.
- Mansour W, Ducancelle A, Le Gal F, *et al.*: Resolution of chronic hepatitis delta after 1 year of combined therapy with pegylated interferon, tenofovir and emtricitabine. *J Clin Virol* 2010;47(1):97–99.
- Kittner JM, Sprinzl MF, Grambihler A, *et al.*: Adding pegylated interferon to a current nucleos(t)ide therapy leads to HBsAg seroconversion in a subgroup of patients with chronic hepatitis B. *J Clin Virol* 2012;54(1):93–95.
- 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(9):907–915.
- Le Gal F, Gordien E, Affolabi D, *et al.*: Quantification of hepatitis delta virus RNA in serum by consensus real-time PCR indicates different patterns of virological response to interferon therapy in chronically infected patients. *J Clin Microbiol* 2005;43(5):2363–2369.
- Scholtes C, Icard V, Amiri M, *et al.*: Standardized one-step real-time reverse transcription-PCR assay for universal detection and quantification of hepatitis delta virus from clinical samples in the presence of a heterologous internal-control RNA. *J Clin Microbiol* 2012;50(6):2126–2128.
- Brichler S, Le Gal F, Neri-Pinto F, *et al.*: Urgent need for a standardized hepatitis delta virus RNA quantification method: Results of a French National quality control. Paper presented at the Asian Pacific Association for the Study of the Liver Week 2013, 6–10 June, 2013; Singapore.
- Boursier J, Bacq Y, Halfon P, *et al.*: Improved diagnostic accuracy of blood tests for severe fibrosis and cirrhosis in chronic hepatitis C. *Eur J Gastroenterol Hepatol* 2009;21(1):28–38.
- Bedossa P and Poinard T: An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996;24(2):289–293.
- Yurdaydin C, Bozkaya H, Onder FO, *et al.*: Treatment of chronic delta hepatitis with lamivudine vs lamivudine+interferon vs interferon. *J Viral Hepat* 2008;15(4):314–321.
- Triantos C, Kalafateli M, Nikolopoulou V, and Burroughs A: Meta-analysis: Antiviral treatment for hepatitis D. *Aliment Pharmacol Ther* 2012;35(6):663–673.
- Iser DM, Warner N, Revill PA, *et al.*: Coinfection of hepatic cell lines with human immunodeficiency virus and hepatitis B virus leads to an increase in intracellular hepatitis B surface antigen. *J Virol* 2010;84(12):5860–5867.
- Miailhes P, Traubad MA, Pradat P, *et al.*: Impact of highly active antiretroviral therapy (HAART) on the natural history of hepatitis B virus (HBV) and HIV coinfection: Relationship

- between prolonged efficacy of HAART and HBV surface and early antigen seroconversion. *Clin Infect Dis* 2007;45(5): 624–632.
22. Manns M, Heidrich B, and Wedemeyer H: Unmet needs in delta hepatitis. Paper presented at the EASL Monothematic Conference on Delta Hepatitis, 22–24 September, 2010; Istanbul, Turkey.
23. Niro GA, Smedile A, Ippolito AM, *et al.*: Outcome of chronic delta hepatitis in Italy: a long-term cohort study. *J Hepatol* 2010;53(5):834–840.

Address correspondence to:

*Anders Boyd
Service de Maladies Infectieuses et Tropicales
Hôpital Saint-Antoine
184 rue du Faubourg Saint-Antoine
75012 Paris
France*

E-mail: boyd@u707.jussieu.fr