There is no difference in hepatic fibrosis rates of patients infected with hepatitis C virus and those co-infected with HIV

A.R. Souza[1,2], C.V. Tovo[1,2], A.A. Mattos[1] and S. Chaves[2]

[1]Curso de Pós-Graduação de Hepatologia, Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, Porto Alegre, RS, Brasil
[2]Hospital Nossa Senhora da Conceição, Porto Alegre, RS, Brasil

Correspondence to: C.V. Tovo, Rua Cel. Aurélio Bitencourt, 115/201, 90430-080 Porto Alegre, RS, Brasil
E-mail: cris.tovo@terra.com.br

Some studies have suggested that human immunodeficiency virus (HIV) infection modifies the natural history of hepatitis C virus (HCV) infection, accelerating the progression of fibrosis and the development of cirrhosis. Our objective was to evaluate the fibrosis progression rate (FPR) in HCV/HIV-co-infected patients, and to identify factors that may influence it. HCV-mono-infected and HCV/HIV-co-infected patients with a known date of HCV infection (transfusion or injection drug use) and a liver biopsy were included. The FPR was defined as the ratio between the fibrosis stage (Metavir score) and the estimated length of infection in years and the result was reported as fibrosis units per year. The factors studied were gender, age at infection, consumption of alcohol, aminotransferase levels, histological activity grade, HCV genotype and viral load, CD4 cell count, HIV viral load, and the use of antiretroviral therapy. Sixty-five HCV-infected (group 1) and 53 HCV/HIV-co-infected (group 2) patients were evaluated over a period of 19 months. The mean FPR of groups 1 and 2 was 0.086 ± 0.074 and 0.109 ± 0.098 fibrosis units per year, respectively (P = 0.276). There was a correlation between length of HCV infection and stage of fibrosis in both groups. The age at infection, the aspartate aminotransferase level (r = 0.36) and the inflammatory activity grade were correlated with the FPR (P < 0.001). No difference in FPR was found between HCV-mono-infected and HCV/HIV-co-infected patients.

Introduction

Infection with hepatitis C virus (HCV) has an estimated prevalence of 3% in the world, meaning that there are around 170 million people chronically infected with HCV, thus representing a serious public health problem (1,2). Studies on the progression of hepatic fibrosis in HCV-infected patients and on the factors responsible for the wide variability shown in its natural history have been emphasized in the literature (3-6). Some investigators have concluded that fibrosis progression is time-dependent and that its estimate is an important piece of information in evaluating the vulnerability of an individual patient and the impact of treatment on the natural history of the disease (3-5).

Some factors identified in individual studies are associated with a greater progression of liver fibrosis: length of infection, age at the time of infection, male gender, abuse of alcohol (3,7), co-infection with hepatitis B virus (8) and with human immunodeficiency virus (HIV) (9), and the presence of elevated alanine aminotransferase (ALT) (10,11).

The natural history of liver fibrosis progression in patients with HCV/HIV co-infection has been studied during the last decade. The high prevalence of HCV/HIV co-infection is ascribed to the shared transmission route, the most important being the use of intravenous drugs, which continues to be the greatest risk for acute HCV infection (12-14). In the United States and Europe, the prevalence of HCV/HIV co-infection is approximately 30% (12). In Brazil, the prevalence depends on the geographic area, with values ranging from 8.9 to 54% (15-18).

Since the prognosis of HIV infection has improved

significantly with antiretroviral (ARV) therapies, chronic liver diseases, initially mere coadjutants in the spectrum of HIV infection, have become prominent and seem bound to be the leading cause of morbidity and mortality in the HCV/HIV-co-infected population (19-22).

Several studies conducted before the introduction of highly active antiretroviral therapy (HAART) have suggested that HIV infection changes the natural history of HCV infection, leading to more rapid progression of liver fibrosis (9,23-28). The progression, which typically takes 30 years or more in patients infected with HCV, could take less than half this time in co-infected patients (12).

The present study was designed to evaluate the fibrosis progression rate (FPR) and the factors influencing this progression in patients infected with HCV and in patients co-infected with HCV and HIV.

Patients and Methods

Patients with HCV infection (group 1) and with HCV/HIV co-infection (group 2) referred to the Gastroenterology Unit of Hospital Nossa Senhora da Conceição, a large public General Hospital in the South of Brazil, for a percutaneous liver biopsy during the period from January 2003 to August 2004 were evaluated. Considering the 45% prevalence of high grade fibrosis by Metavir (29) score (F3/F4) in co-infected patients and of 17% for those mono-infected with HCV (8), the estimated size of the sample was 49 patients in each group, for a 95% confidence interval and 80% statistical power.

The inclusion criteria for consecutively attended patients were positive anti-HCV antibodies (in the mono-infected group), with verification of HCV infection by the polymerase chain reaction (PCR); association of anti-HCV with HIV infection (in the co-infected group) determined by the immunofluorescence test and Western blot; estimated date of viral infection (blood and/or blood product transfusion, or intravenous drug users), with infection considered to occur at first transfusion and at the beginning of intravenous drug use; age between 18 and 70 years, and no previous antiviral treatment for HCV.

Exclusion criteria were patients with risk factors for developing HCV infection other than intravenous drug use or transfusion; with decompensated cirrhosis or hepatocellular carcinoma; pregnant women, and associated hepatitis B infection (HBsAg positive).

The following data were evaluated: gender, age at the time of infection, serum levels of ALT and aspartate aminotransferase (AST), fibrosis and inflammatory activity grades, HCV genotype, viral load of HCV, infection transmission mode, drinking behavior, CD4 cell count, HIV viral load, and antiretroviral treatment.

HCV-RNA was detected by PCR using the Amplicor HCV Test, version 2.0 (Roche Diagnostics, Nutley, NJ, USA; detection limit: 50 IU/mL).

HCV-RNA was quantitated using the Amplicor HCV Monitor™ Test, version 2.0 (Roche Diagnostics; detection limit: 600 IU/mL).

Patients with positive HCV-RNA were tested for genotype by the technique of restriction fragment length polymorphism-PCR.

The CD4 cell count was performed by flow cytometry according to manufacturer instructions (Becton Dickinson, Heidelberg, Germany).

HIV viral load was determined by the *b-DNA* technique (Bayer Corporation, Tarrytown, NY, USA; detection limit: 50 copies/mL).

All patients were submitted to a percutaneous liver biopsy with a Menghini needle. The liver biopsy fragments were embedded in paraffin and stained with hematoxylin-eosin and picrosirius-red and then examined blindly by a pathologist. The biopsy was considered to be satisfactory when the fragments presented at least five complete portal tracts.

For each biopsy, the grades of fibrosis and inflammatory activity were determined using the Metavir classification (29).

The annual FPR was determined by the ratio of fibrosis stage (by the Metavir score) to the estimated duration of infection in years. The results are reported as fibrosis units per year. For example, for a patient with stage F2 fibrosis and length of infection estimated at 15 years, the FPR would be 2/15 = 0.133 fibrosis units per year.

This research was approved by the Ethics Committee of the Nossa Senhora da Conceição Hospital and posed minimum risk for the participants. All patients gave written informed consent to participate in the study.

For statistical analysis, the MS Excel 2000 software was used to store the data and the results were analyzed using the Statistical Package for the Social Sciences (SPSS 8.0). The quantitative variables are presented as means ± SD. ANOVA was used to compare means, followed by the multiple comparisons Tukey test. The qualitative variables are presented as frequency and percentage. To confirm the association between these variables the Pearson chi-square test was used, with the complementary resource of adjusted residues analysis to identify the location of associations. The level of significance was set at 5%.

Results

A total of 118 patients, 65 of whom were mono-infected

with HCV (group 1) and 53 co-infected with HCV/HIV (group 2), were evaluated. Table 1 shows the clinical and laboratory data of the population studied.

Table 2 shows the results of the histological evaluation. There was no statistically significant difference between groups regarding grade of fibrosis and inflammatory activity. Likewise, when more intense fibrosis grades (F3/F4) were evaluated as a whole, there was no difference across groups (36.5 vs 36.5%). Similarly, moderate or severe

Table 1. Characteristics of the study population.

	HCV (N = 65)	HCV/HIV (N = 53)
Mean age (years)	45.86	40.29*
Female gender	55.3%	20.7%**
Transfusion	75.4%	20.75%**
Intravenous drug use	24.61%	79.24%**
Drinking >50 g/day	23.5%	52.0%**
Mean length of infection (years)	20.64	18.73
Mean age at the time of infection (years)	25.18	22.95
Genotype 1	55.90%	51.10%
Genotype 2	1.70%	2.10%
Genotype 3	42.40%	46.80%
HCV-VL (IU/mL)	1,433,842 ± 3,252,323	1,068,236 ± 2,080,291
CD4 (cell/mm^3)	ND	420 ± 172
HIV-VL (IU/mL)	ND	55,802 ± 136,152
ALT (IU/L)	101 ± 51	120 ± 84
AST (IU/L)	61 ± 55	81 ± 31*

Data are reported as means ± SD, percent or years. HCV-VL = hepatitis C virus viral load; ND = not determined; HIV-VL = human immunodeficiency virus viral load; ALT = alanine aminotransferase; AST = aspartate aminotransferase.
*P < 0.05 compared to HCV group (ANOVA). **P < 0.05 compared to HCV group (Pearson χ^2 test).

Table 2. Comparison of liver histopathology (Metavir score).

	HCV (N = 65)	HCV/HIV (N = 53)
Fibrosis stage		
F0	18 (27.69%)	12 (22.64%)
F1	10 (15.38%)	12 (22.64%)
F2	14 (21.53%)	11 (20.75%)
F3	13 (20.00%)	13 (24.52%)
F4	10 (15.38%)	5 (9.43%)
Inflammatory activity		
A0	9 (13.84%)	5 (9.43%)
A1	36 (55.38%)	33 (62.26%)
A2	15 (23.07%)	11 (20.75%)
A3	5 (7.69%)	4 (7.54%)

HCV = hepatitis C virus; HIV = human immunodeficiency virus. There were no statistical differences between groups for fibrosis stage or activity degree (Pearson χ^2 test).

inflammatory activity (A2/A3) evaluated as a whole was present in 33.9% of patients in the mono-infected group and in 30.8% of the HCV/HIV co-infected group, with no statistically significant difference.

Figure 1 shows the relation between length of infection and grade of fibrosis.

The mean FPR for patients with HCV mono-infection and for patients with HCV/HIV co-infection was 0.086 and 0.109 ± 0.098 fibrosis units per year, respectively (P = 0.276). This rate did not show a normal distribution, with a median of 0.090 in both groups.

Among the patients in group 2, 40 were using ARV therapy. None of the patients was on monotherapy. The mean length of ARV treatment was 48.6 months before the liver biopsy. HIV viral load was determined in group 2, being undetectable in 60% of the cases. The mean CD4 cell count was 420 ± 172/mm^3 (54-930/mm^3; Table 1).

Age at the time of HCV infection, inflammatory activity degree, and serum AST level were the factors associated with higher mean FPR (P < 0.001). There was no correlation between gender (P = 0.50), drinking behavior (P = 0.98), mode of transmission (P = 0.91), genotype (P = 0.64), HCV viral load (P = 0.40), total CD4 cell count (P = 0.71), and ARV treatment (P = 0.61).

Discussion

In the last decade, many investigators (9,30) found a greater FPR in HCV/HIV-co-infected patients than in HCV-mono-infected ones; however, recent studies (31,32) did not demonstrate a significant difference between these groups.

The cohort study of Kramer et al. (31) showed that the impact of HCV/HIV co-infection on the progression of liver disease has changed with the arrival of HAART. The incidence of cirrhosis was lower in HCV/HIV-co-infected
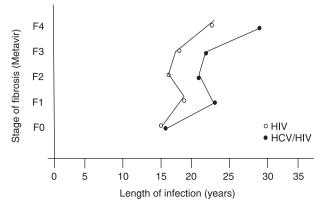

Figure 1. Length of HIV infection vs stage of fibrosis.

patients than in HCV-mono-infected patients, and HCV/HIV co-infection was a significant risk factor for the development of cirrhosis only in the pre-HAART era.

The results of the present study showed a similar FPR for mono- and co-infected patients. Despite the results of previous studies, the lack of difference regarding fibrosis progression between groups is in agreement with a recent report by Brau et al. (32), considering that most co-infected patients included in the present study were young, were on treatment with ARV and presented good immunity, as reflected by CD4 cell count and low HIV viral load (undetectable in 60% of the cases).

Moreover, in the present series, there was no association between co-infection and fibrosis stage. When more intense fibrosis stages (F3/F4) were analyzed, there was no difference between the two groups of patients.

In the evaluation of the various factors implicated in the progression of liver fibrosis, only age at the time of HCV infection, inflammatory activity grade, and AST serum level were significantly correlated with FPR.

Although the co-infected patients were younger, there was no significant difference between groups regarding mean age at the time of infection or mean length of infection.

The role of inflammatory activity as an independent factor for fibrosis progression is a controversial matter (3). Poynard et al. (4) failed to demonstrate any impact of inflammatory activity on the progression of fibrosis, considering the first 20 years after infection. Only the absence of activity was associated with slower progression. However, in more recent studies (33,34), HCV-mono-infected and HCV/HIV-co-infected patients who presented moderate or severe inflammatory activity had more rapid fibrosis progression than those with minimum or absent activity. Likewise, as observed for fibrosis grade, in the present study, there was no difference when histological activity was evaluated in the co-infected group. It is thus impossible to conclude for a greater hepatic involvement in this group.

It is recognized that an AST/ALT ratio above 1 suggests a diagnosis of cirrhosis in patients with chronic HCV infection (35), and some investigators (11,33) have observed a relationship between aminotransferase levels and fibrosis grade. In the present study, patients with more advanced fibrosis presented high AST and ALT levels, but only AST level proved to be correlated with FPR.

Regarding alcohol intake, although the co-infected patients had a greater intake than mono-infected patients, no difference in FPR was observed. However, the fact that the co-infected group consisted of younger patients may have contributed to this result.

Viral aspects have been studied in the context of progress of liver disease caused by HCV, with conflicting results in the literature (3,4,33,36-38). The influence of genotype and of viral load remains in discussion. In the present study, the different HCV genotypes or viral load did not affect the histological findings or FPR in the co-infected or mono-infected group.

Regarding the HCV/HIV-co-infected patients, some aspects such as CD4 cell count and the use of ARV therapy should be highlighted. A CD4 count equal to or below 200/mm^3 is associated with a higher rate of liver fibrosis progression (9,39). Mohsen et al. (33), evaluating HCV/HIV-co-infected patients, failed to find an association between fibrosis and ARV treatment. In the present study, CD4 cells and the use of ARV therapy were not correlated with FPR in co-infected patients. However, the CD4 cell count was below 200/mm^3 in only one patient, reproducing an immunity profile similar to that of immunocompetent patients, and this can be an important reason for the absence of a difference in FPR between groups.

The present study has limitations, the most relevant of which is possibly related to the model used to calculate the FPR in HCV-infected and in HCV/HIV-co-infected patients. Ideally, in the calculation of fibrosis progression, consecutive biopsies should be performed with a long interval between them in patients untreated for HCV, which would be ethically unacceptable. The present model, described elsewhere (3), permits the evaluation of the cumulative effect of fibrosis progression according to the duration of infection. Although previously validated, this model may be criticized since it considers the patient to have no fibrosis at the time of HCV infection. The accuracy of the calculation of the estimated length of infection can also be criticized, as it is based on patient history. In order to adequately analyze the duration of HCV infection, the population evaluated here included only patients with a history of blood transfusion or intravenous drug users, since in this population contamination generally occurs during the first transfusion or in the first year of intravenous drug use in as many as 90% of cases (38). Although not an ideal method, this estimate using a single biopsy remains a useful tool in the study of the natural history of chronic hepatitis C. Besides, the groups presented some differences related to epidemiological aspects that possibly influenced the results when comparative analyses were performed.

In the present study, no difference in FPR was found between HCV-mono-infected and HCV/HIV-co-infected patients. HCV/HIV co-infection had no relevant negative impact on the progress of these patients as compared to HCV-mono-infected ones. However, more studies are necessary to evaluate FPR in co-infected HCV/HIV patients in order to better understand the natural history of HCV infection in this population.

References

1. EASL International Consensus Conference on Hepatitis C. Paris, 26-28, February 1999, Consensus Statement. European Association for the Study of the Liver. *J Hepatol* 1999; 30: 956-961.

2. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C 2002 (June 10-12, 2002). *Gastroenterology* 2002; 123: 2082-2099.

3. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; 349: 825-832.

4. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. *J Hepatol* 2001; 34: 730-739.

5. Chossegros P, Pradat P, Bailly F, Chemello L, Sauleda S, Saracco G, et al. Natural history of chronic hepatitis C: fibrosis progression is not linear. *J Hepatol* 1999; 30 (Suppl 1): 52.

6. de Torres M, Poynard T. Risk factors for liver fibrosis progression in patients with chronic hepatitis C. *Ann Hepatol* 2003; 2: 5-11.

7. Roudot-Thoraval F, Bastie A, Pawlotsky JM, Dhumeaux D. Epidemiological factors affecting the severity of hepatitis C virus-related liver disease: a French survey of 6,664 patients. The Study Group for the Prevalence and the Epidemiology of Hepatitis C Virus. *Hepatology* 1997; 26: 485-490.

8. Benvegnu L, Fattovich G, Noventa F, Tremolada F, Chemello L, Cecchetto A, et al. Concurrent hepatitis B and C virus infection and risk of hepatocellular carcinoma in cirrhosis. A prospective study. *Cancer* 1994; 74: 2442-2448.

9. Benhamou Y, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. *Hepatology* 1999; 30: 1054-1058.

10. Shindo M, Arai K, Sokawa Y, Okuno T. The virological and histological states of anti-hepatitis C virus-positive subjects with normal liver biochemical values. *Hepatology* 1995; 22: 418-425.

11. Mathurin P, Moussalli J, Cadranel JF, Thibault V, Charlotte F, Dumouchel P, et al. Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine transaminase activity. *Hepatology* 1998; 27: 868-872.

12. Soriano V, Sulkowski M, Bergin C, Hatzakis A, Cacoub P, Katlama C, et al. Care of patients with chronic hepatitis C and HIV co-infection: recommendations from the HIV-HCV International Panel. *AIDS* 2002; 16: 813-828.

13. Quaranta JF, Delaney SR, Alleman S, Cassuto JP, Dellamonica P, Allain JP. Prevalence of antibody to hepatitis C virus (HCV) in HIV-1-infected patients (Nice SEROCO cohort). *J Med Virol* 1994; 42: 29-32.

14. Bruno R, Sacchi P, Puoti M, Soriano V, Filice G. HCV chronic hepatitis in patients with HIV: clinical management issues. *Am J Gastroenterol* 2002; 97: 1598-1606.

15. Pavan MH, Aoki FH, Monteiro DT, Goncales NS, Escanhoela CA, Goncales FL Junior. Viral hepatitis in patients infected with human immunodeficiency virus. *Braz J Infect Dis* 2003; 7: 253-261.

16. Segurado AC, Braga P, Etzel A, Cardoso MR. Hepatitis C virus coinfection in a cohort of HIV-infected individuals from Santos, Brazil: seroprevalence and associated factors. *AIDS Patient Care STDS* 2004; 18: 135-143.

17. Ferraz GGS, Menezes JA, Gazineo JL, Passoni LFC, Lessa MPM, Pinto PTA, et al. Prevalência de infecção pelo vírus B e C da hepatite em pacientes com infecção pelo vírus da imunodeficiência humana. *Rev Med HSE* 2002; 36: 6-11.

18. Tovo CV, Dos Santos DE, de Mattos AZ, de Almeida PR, de Mattos AA, Santos BR. Ambulatorial prevalence of hepatitis B and C markers in patients with human immunodeficiency virus infection in a general hospital. *Arq Gastroenterol* 2006; 43: 73-76.

19. Soriano V, Garcia-Samaniego J, Valencia E, Rodriguez-Rosado R, Munoz F, Gonzalez-Lahoz J. Impact of chronic liver disease due to hepatitis viruses as cause of hospital admission and death in HIV-infected drug users. *Eur J Epidemiol* 1999; 15: 1-4.

20. Puoti M, Spinetti A, Ghezzi A, Donato F, Zaltron S, Putzolu V, et al. Mortality for liver disease in patients with HIV infection: a cohort study. *J Acquir Immune Defic Syndr* 2000; 24: 211-217.

21. Monga HK, Rodriguez-Barradas MC, Breaux K, Khattak K, Troisi CL, Velez M, et al. Hepatitis C virus infection-related morbidity and mortality among patients with human immunodeficiency virus infection. *Clin Infect Dis* 2001; 33: 240-247.

22. Soriano V, Martin-Carbonero L, Garcia-Samaniego J, Puoti M. Mortality due to chronic viral liver disease among patients infected with human immunodeficiency virus. *Clin Infect Dis* 2001; 33: 1793-1795.

23. Soto B, Sanchez-Quijano A, Rodrigo L, del Olmo JA, Garcia-Bengoechea M, Hernandez-Quero J, et al. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. *J Hepatol* 1997; 26: 1-5.

24. Di Martino V, Rufat P, Boyer N, Renard P, Degos F, Martinot-Peignoux M, et al. The influence of human immunodeficiency virus coinfection on chronic hepatitis C in injection drug users: a long-term retrospective cohort study. *Hepatology* 2001; 34: 1193-1199.

25. Romeo R, Rumi MG, Donato MF, Cargnel MA, Vigano P, Mondelli M, et al. Hepatitis C is more severe in drug users with human immunodeficiency virus infection. *J Viral Hepat* 2000; 7: 297-301.

26. Soriano V, Rodriguez-Rosado R, Garcia-Samaniego J. Management of chronic hepatitis C in HIV-infected patients. *AIDS* 1999; 13: 539-546.

27. Poles MA, Dieterich DT. Hepatitis C virus/human immunodeficiency virus coinfection: clinical management issues. *Clin Infect Dis* 2000; 31: 154-161.

28. Torriani F, Soriano F. Chronic hepatitis C in HIV-infected individuals. *AIDS Rev* 2000; 2: 168-177.

29. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; 24: 289-293.

30. Ibrahim A, Shpaner A, Nieto J, Saag S. Risk factors of accelerated liver fibrosis in HIV/CHV coinfection: a matched analysis. *Liver Int* 2004; 24: 30.

31. Kramer JR, Giordano TP, Souchek J, Richardson P, Hwang LY, El-Serag HB. The effect of HIV coinfection on the risk of cirrhosis and hepatocellular carcinoma in U.S. veterans with hepatitis C. *Am J Gastroenterol* 2005; 100: 56-63.

32. Brau N, Salvatore M, Rios-Bedoya CF, Fernandez-Carbia A, Paronetto F, Rodriguez-Orengo JF, et al. Slower fibrosis progression in HIV/HCV-coinfected patients with successful HIV suppression using antiretroviral therapy. *J Hepatol* 2006; 44: 47-55.

33. Mohsen AH, Easterbrook PJ, Taylor C, Portmann B, Kulasegaram R, Murad S, et al. Impact of human immuno-deficiency virus (HIV) infection on the progression of liver fibrosis in hepatitis C virus infected patients. *Gut* 2003; 52: 1035-1040.

34. Poynard T, Mathurin P, Lai CL, Guyader D, Poupon R, Tainturier MH, et al. A comparison of fibrosis progression in chronic liver diseases. *J Hepatol* 2003; 38: 257-265.

35. Pratt DS, Kaplan MM. Evaluation of the liver. A: Laboratory tests. In: Schiff ER, Sorrel MF, Maddrey WC (Editors), *Schiff's diseases of the liver*. 9th edn. Philadelphia: Lippincott Williams & Wilkins; 2003. p 221-255.

36. Sabin CA, Telfer P, Phillips AN, Bhagani S, Lee CA. The association between hepatitis C virus genotype and human immunodeficiency virus disease progression in a cohort of hemophilic men. *J Infect Dis* 1997; 175: 164-168.

37. Mangia A, Cascavilla I, Lezzi G, Spirito F, Maertens G, Parlatore L, et al. HCV genotypes in patients with liver disease of different stages and severity. *J Hepatol* 1997; 26: 1173-1178.

38. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology* 1998; 28: 1687-1695.

39. Benhamou Y, Di Martino V, Bochet M, Colombet G, Thibault V, Liou A, et al. Factors affecting liver fibrosis in human immunodeficiency virus- and hepatitis C virus-coinfected patients: impact of protease inhibitor therapy. *Hepatology* 2001; 34: 283-287.