CLINICAL LABORATORY ASSESSMENT OF HEPATITIS C AND HIV COINFECTED PATIENTS ACCORDING TO THE ANTIRETROVIRAL THERAPY RECEIVED

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SUMMARY

During the year of 2001, a retrospective, descriptive study in order to determine the influence of the antiretroviral therapy received by 111 HIV-HCV coinfected patients who had undergone at least one liver biopsy was conduced, 74 of them were treated with a protease inhibitor regimen (WPI), and 37 with a non-protease inhibitor regimen (NPI). The main characteristics found were: a young patient population (mean age 41 years old in both groups), composed in most part of male individuals (74.3% WPI and 51.4% NPI) with previous risk factors for both infections (WPI 93.2% and NPI 89.2%). The most significant findings included AIDS-defining disease (WPI 18.9% and NPI 13.5% of the cases), elevated hepatic enzyme levels (WPI: SGOT 52.1 and NPI 53.2), absence of liver disease-related symptoms (16.2% for both groups), average CD4 count > 350 for both groups (WPI 362.2 and NPI 378.1), predominantly low-grade fibrosis in both populations (0-2 in 63.6% of WPI patients and in 80% of NPI patients), with necro-inflammatory activity ranging from 5-7 in 51.3% and 42.9% of WPI patients and NPI patients, respectively. It is suggested a sequential biopsy to better evaluate the evolution of the hepatic disease, according to the HAART regimen received.

KEYWORDS: Coinfection; HIV; HCV; AIDS; HAART; Liver biopsy.

INTRODUCTION

Considering the common exposure points, there is an important association between the mechanism of transmission of hepatitis C virus (HCV) and human immunodeficiency virus (HIV), mainly in some risk groups, such as intravenous drug users and previously, hemophiliac patients[9,27,29].

As of 1996, when the highly active antiretroviral therapy (HAART) was first used in AIDS patients, potentially fatal co-morbidities, among them HCV infection, have been considered increasingly important[2,15,19].

HCV is a widespread disease worldwide. According to WHO data, 170 million people are infected with this virus in the whole world, 2-4% of total population, and they are at risk for progression to hepatocellular carcinoma[22].

There is no local data concerning HCV infection in Brazil. A research conducted among blood donors in the State of São Paulo found anti-HCV positive serology in 0.84% of the subjects[14]; in São Paulo City a prevalence of 1.42% (0.70-2.12%) was estimated using a predictive statistical inference method based on a randomized sampling stratified by gender, age, region of residence[7].

In HIV positive populations, figures are highly inconsistent, depending on the population studied; based on data from different publications around the world, we can conclude that, depending on the risk factors presented by the population studied, its prevalence may vary. In populations consisting of 100% haemophilic patients, mainly those studied before the availability of serologic tests to identify HCV, the prevalence of coinfection among HIV infected patients is expected to rise to 98% of this population[26,28].

Figures should be less consistent when the population studied is not homogeneous. An example of that are the data from non-homogeneous populations originated from different American and European studies, where the prevalence ranges from 7% in populations with majority of males, current or previous intravenous drug users, and a minor percentage of men who have sex with men and previous history of blood transfusion, as found in studies performed in California, U.S.A. and Toronto, Canada[10,20], to 57% in populations consisting primarily of male individuals, current or previous intravenous drug users, and a minority of men who have sex with men as in a large Spanish study[16].

Previous studies conducted in Brazilian population at the same site where the study of this publication was conducted showed a 17.7% prevalence of coinfection[14].

It is currently accepted that both infections are reciprocally

influenced. This coinfluence was determined even before the introduction of the highly active antiretroviral therapy (HAART) and continues to be a controversial issue, including the decision of treat the virus first[24].

This project was designed as part of a protocol of the Laboratory of Clinical Investigation on Hepatitis (LIM-47) intended to study the evolution of HCV disease in HIV-coinfected patient, according to the antiretroviral therapy given.

PATIENTS AND METHODS

The records of patients followed-up at the Extension Unit for Treatment of HIV/AIDS Patients (Casa da AIDS) University of São Paulo School of Medicine, Clinical Division of Infectious and Parasitic Diseases of Hospital das Clínicas - University of São Paulo School of Medicine (HC-FMUSP) during the year of 2001, were assessed. All procedures were accepted by the Institutional Review Board of Hospital das Clínicas.

We reviewed 3,512 active records so as to establish a coinfection data basis, based on the antibody seropositivity status determined by ELISA-HIV testing, Western Blot and ELISA-HCV. All patients were positive to ELISA-HIV testing and Western Blot, 435 of them had not been evaluated as to the presence of HCV by ELISA. The remaining 3,077 were tested as to the presence of HVC, being found at least one positive serology in 468 patients. These patients were divided into those who have undergone liver biopsy and those who have not. This liver biopsy was performed at the physician's discretion in all patients with diagnosis of HCV infection, and the material was analysed at the pathology laboratory of the HC-FMUSP. The grade of hepatic fibrosis and necroinflammatory activity was determined according to the Brazilian National Consensus on Chronic Hepatitis classification[8]. This procedure was performed in 121 patients, who were divided into two groups for analysis: Group 1 (with protease inhibitor regimen = WPI) and group 2 (with non-protease inhibitor regimen = NPI). Ten patients were excluded because they were HBV seropositive, were not using antiretroviral drugs or had been treated with interferon for HCV Infection.

Laboratory tests were performed at the HC-FMUSP Central Laboratory; the Laboratory of Fundação Pró-Sangue-Hemocentro de São Paulo, the Laboratory of Clinical Investigation on Hepatitis of University of São Paulo School of Medicine (LIM-47) and at private laboratories.

Once the records of patients coinfected with HIV-HCV virus and followed-up at Casa da AIDS were identified, the following data were collected: demographic data: gender, age, race, job; clinical data: alcohol consumption, concomitant diseases, use of hepatotoxic drugs, symptoms suggestive of hepatic disease (such as jaundice, liver enlargement or esophageal varices), potential source of HIV-HCV infection (such as blood transfusion, use of intravenous drug or potentially infected material), adherence to the antiretroviral regimens used; laboratory and viral data: liver biochemistry (SGOT, SGPT, GGT, alkaline phosphatase, TB and F, PT), hematology, CD4 count, HIV viral load, liver biopsy, qualitative PCR for hepatitis C virus.

Each patient was assigned to a different group according to the antiretroviral therapy received for at least six months of study (maximum time was not pre-defined), and the drugs were only considered as a group (non-nucleoside, nucleoside and protease inhibitors).

Data analysis: the qualitative variables were represented by both absolute and relative frequency, and the quantitative ones by average, standard-deviation (sd), median, minimum and maximum values.

RESULTS

Male patients were predominant in both groups, accounting for 74.3% of WPI group and 52.4% of NPI group, as well as Caucasian patients with 80.6% and 94.4% of WPI and NPI groups, respectively.

Ethnic data were not available in the medical chart for 43 patients from WPI group (58%) and 19 patients from NPI group (51%). In average, the age at the time of first biopsy was exactly the same for both groups (41 years old) and the standard-deviation was greater in NPI group (8.3 years). Most patients in both groups had no professional occupation and those who had it accounted for less than 10% in any of the groups.

At least one risk factor, such as blood transfusion, use of illegal intravenous drugs, use of potentially infected material, presence of infected contact, risky sexual behavior or maternal-fetal transmission, was identified in 93.2% of WPI patients and in 89.2% of NPI patients. Risk factors were considered common for both infections.

Alcohol consumption was higher in WPI group (40% used to drink alcohol regularly) than in the NPI group (42.3% did not use to drink alcohol regularly).

During the follow-up period, most patients, either from WPI group (81.1%) or NPI group (86.5%), presented no AIDS-defining infection (according to the so-called Rio de Janeiro/Caracas criteria for AIDS case that is officially recognized by the Brazilian regulations[16]. Most patients from either group did not take hepatotoxic drugs during the clinical follow-up period; WPI (91.9%) and NPI (86.5%).

Low incidence of liver disease-related symptoms was observed for both groups (16.2%).

As showed in Table 2 mean CD4 count for both series was above 350, averaging 362.2 in WPI group and 378.1 in NPI group. There is an apparent inconsistency between mean CD4 count and HIV viral load that is normally inversely correlated: mean for WPI group: 53220.3 and mean for NPI group: 17946.7. In both treatment groups, viral load was consistently high.

Based on the consensus of the Brazilian Society of Pathology, the grade of fibrosis found in liver biopsies was mostly ≤ 2 in both groups; 63.6% and 80% for WPI group and NPI group, respectively.

The level of necro-inflammatory activity seen in liver biopsies, ranged mostly between 5 and 7 for both groups, 51.3% for WPI group and 42.9% for NPI group, respectively.

Table 1
Biochemical and virological parameters assessed in HIV-HCV coinfected patients according to the antiretroviral therapy

Parameters	Normal Ranges	Group	
	Minimum-Maximum male/female(Units)	WPI Average (sd) Minimum-Maximum	NPI Average (sd) Minimum-Maximum
HIV Viral Load	Minimal level of detection: 400 copies/ml	53220.3 (99443.3) 110.0 - 440000.0	17946.7 (21930.9) 100.00 - 58000.0
CD4	493-1.666 copies/UL	362.2 (187.5) 54.0 - 812.0	378.1 (175.3) 128.0 - 673.0
SGOT	10-44/36 (U/L)	52.1 (61.2) 9.0 - 433.0	53.2 (41.1) 10.0 - 136.0
SGPT	10-34/30 (U/L)	60.9 (56.4) 7.0 - 252.0	71.3 (56.2) 10.0 - 261.0
GGT	11(7)-50/32 (U/L)	89.4 (90.4) 8.0 - 385.0	122.9 (150.5) 9.0 - 559.0
Alkaline phosphatase	45/32-122/104 (U/L)	113.2 (54.3) 53.0 - 330.0	104.1 (34.8) 51.0 - 180.0
Total bilirubin	0.2-1.0 (mg/dl)	1.37 (0.8) 0.4 - 3.1	0.93 (0.5) 0.3 - 2.1
Direct bilirubin	0-0.4 (mg/dl)	0.4 (0.2) 0.1 – 1.2	0.3 (0.3) 0.1 – 1.4
Total protein	6.0-8.0 (g/dl)	7.9 (0.8) 6.0 - 9.4	8.0 (0.7) 7.0 - 9.2
Albumin	3.5-5.0 (g/dl)	4.2 (0.6) 3.0 - 5.2	4.3 (0.4) 3.3 - 4.7
Hemoglobin	13/12-18/16 (g/dl)	14.6 (1.4) 11.0 - 17.1	13.6 (1.6) 10.7 - 16.0
Platelet	140.0-450.0 (ml/mm^3)	201015.9 (62081.2) 66000 - 341000	203071.4 (55970.2) 103000 - 309000
Prothrombin time	>70% (%)	12.7 (1.7) 9.5 - 16.9	13.3 (1.4) 10.8 - 15.7

Table 2
Evaluation of fibrosis grade* in liver biopsy of HIV-HCV coinfected patients, according to the antiretroviral therapy received

Fibrosis	Group			
	WPI		NPI	
	n	%	n	%
0 - 2	49	(63.6)	28	(80.0)
3 - 4	28	(36.4)	7	(20.0)
Total	77	(100.0)	35	(100.0)

* measured in accordance with the consensus of the Brazilian Society of Pathology[8].

Table 3
Evaluation of the level of necro-inflammatory activity* in HIV-HCV coinfected patients, according to the antiretroviral therapy received

Inflammation	Group			
	WPI		NPI	
	n	%	n	%
1 - 4	26	(33.3)	14	(40.0)
5 - 7	40	(51.3)	15	(42.9)
8 - 10	12	(15.4)	6	(17.1)
Total	78	(100.0)	35	(100.0)

*Measured as the sum of portal + periportal + parenchymatous changes seen, in accordance with the Consensus of the Brazilian Society of Pathology[8].

DISCUSSION

Currently, there is no consensus on the influence of the different antiretroviral regimens used in HIV treatment of patients coinfected with HCV on the evolution of liver disease caused by HCV[3,11].

The present paper is a preliminary study designed to determine epidemiological, clinical and laboratorial characteristics of the patients treated at Casa da AIDS in São Paulo, followed for at least six months, receiving similar antiretroviral regimens and who had been submitted to at least one liver biopsy at the time of evaluation. The group agrees

with the concept that the best way to assess liver disease caused by HCV is through sequential liver biopsies.

Populations of HIV/HCV coinfection studies are predominantly young; the populations involved in studies conducted in Spain, France and Italy were 40 years old or below[4,6,13]. The population included in this study was in average 41 years old.

Regarding the educational level, studies have shown that in low educational level population, the prevalence of HCV infection is higher than in those subjects with a higher educational level[7]. Most patients included in this study had low educational level, which correlates with low social economical situation.

Identification of risk factors and their significance in coinfection studies: Both virus can be transmitted through percutaneous exposure to infected blood, sexual intercourse, and from the mother to her baby, however the relative efficacy of these routes is variable[23].

Nowadays, the concept of blood transfusion as a risk factor for both viruses has almost disappeared due to the introduction of serological tests in the 80´s, besides syringe exchange programs and educational programs implemented in major cities, originally as an immediate reaction to AIDS epidemy[9,18,25].

Alcohol consumption has been associated with severe toxicity after the use of HAART, as well as to a greater liver fibrosis progression resulting from HCV coinfection[3,17]. It was also considered an independent risk factor associated with the development of hepatotoxicity after six months of treatment with HAART, being observed a great difference in the occurrence of hepatotoxicity in alcohol consumers either with or without coinfection[1].

No difference was observed between both groups as to the use of hepatotoxic drugs during their follow-up period at Casa da AIDS, being only slightly greater in the group receiving protease inhibitors.

Toxicity at mitochondrial level has been described with the concomitant use of Ribavirin in coinfected patients, and mainly associated with the use of nucleoside reverse transcriptase inhibitors[12]. Other drugs are known to interfere with the drugs used in HAART at mitochondrial level, but the quantification of the coinfection injury, has not been fully investigated.

The quantification of this injury is likely impossible, however, it should be considered a potential conflicting variable in future studies.

Finally, our findings regarding low-grade fibrosis seen in most patients from both groups, may be correlated with the lower mean age of the patients, since the investigators who are currently studying coinfection consider that an increase in progression of liver disease caused by HCV infection occurs as of the age of 50[5,13,23] and that the lower fibrosis grades found in younger population are due to the shorter period of time elapsed since the beginning of the infection[11,14,22]. Unfortunately, based on the data currently available in our clinic, it was not possible to clearly determine how HAART therapy could influence the progression of the liver disease in the population studied.

It is suggested that new biopsies are performed in order to determine the difference in liver disease progression in both groups, according to HAART therapy received.

RESUMO

Avaliação clínico-laboratorial de pacientes co-infectados com o vírus da hepatite C e HIV-1 em relação ao tipo de terapia antirretroviral recebida

Durante o ano de 2001, um estudo retrospectivo e descritivo foi efetuado a fim de determinar a influência da terapia antirretroviral recebida por 111 pacientes co-infectados HIV-HCV que tinham se submetido ao menos a uma biopsia hepática. Destes, 74 foram tratados com um regime contendo inibidor da protease (WPI) e 37 com um regime do não contendo inibidor da protease (NPI). As características principais encontradas eram: uma população de pacientes jovem (idade média 41 anos em ambos os grupos), composta na maior parte por indivíduos masculinos (74,3% WPI e 51,4% NPI) com fatores de risco precedentes para ambas as infecções (WPI 93,2% e NPI 89,2%). Os achados mais significativos incluíram doença definidora de AIDS (WPI 18,9% e NPI 13,5% dos casos), nível elevado de enzimas hepáticas (WPI: SGOT 52.1 e NPI 53.2), ausência de sintomas relacionáveis à doença hepática (16.2% para ambos os grupos), CD4 contagem média 350 para ambos os grupos (WPI 362.2 e NPI 378.1), fibrose predominantemente de baixo grau em ambas as populações (0-2 em 63,6% de pacientes de WPI e em 80% de pacientes de NPI), com atividade necro-inflamatória que varia de 5-7 em 51.3% e em 42,9% de pacientes de WPI e de pacientes de NPI, respectivamente. Foi concluído que uma nova biópsia hepática deveria ser executada em todos os pacientes para determinar melhor qual a diferença no avanço da doença em ambos os grupos.

REFERENCES

1. ACETI, A.; PASQUAZZI, C.; ZECHINI, B.; DE BAC, C. & LIVERHAART GROUP - Hepatotoxicity development during antiretroviral therapy containing protease inhibitors in patients with HIV: the role of hepatitis B and C virus infection. **J. Acquir. Immune. Defic. Syndr., 29:** 41-48, 2002.

2. BARNES, E.; WEBSTER, G.; WHALLEY, S. & DUSHEIKO, G. - Predictors of a favorable response to alpha interferon therapy for hepatitis C. **Clin. Liver Dis., 3:** 775-791, 1999.

3. BENHAMOU, Y.; BOCHET, M.; DI MARTINO, V. *et al*. - Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. **Hepatology, 30:** 1054-1058, 1999.

4. BENHAMOU, Y.; DI MARTINO, V.; BOCHET, M. *et al*. - Factors affecting liver fibrosis in human immunodeficiency virus-and hepatitis C virus-coinfected patients: impact of protease inhibitor therapy. **Hepatology, 34:** 283-287, 2001.

5. BORGIA, G.; REYNAUD, L.; GENTILE, I. & PIAZZA, M. - HIV and hepatitis C virus: facts and controversies. **Infection, 31:** 232-240, 2003.

6. DE BONA, A.; SITIA, G.; UBERTI-FOPPA, C. *et al*. - Impact of HAART on liver histology of HIV/HCV coinfected patients. **J. biol. Regul. Homeost. Agents, 17:** 195-197, 2003.

7. FOCACCIA, R.; DA CONCEICAO, O.J.; SETTE Jr., H. *et al*. - Estimated prevalence of viral hepatitis in the general population of the municipality of São Paulo, measured by a serologic survey of a stratified, randomized and residence-based population. **Braz. J. infect. Dis. 2:** 269-284, 1998.

8. GAYOTO, L.C. & COMITÉ SBP/SBH - Visão histórica e consenso nacional sobre a classificação das hepatites crônicas. Projeto do Clube de Patologia Hepática da Sociedade Brasileira de Patologia aprovada pela Sociedade Brasileira de Hepatologia. **GED, 19:** 137-140, 2000.

9. HAHN, J.A.; PAGE-SHAFER, K.; LUM, P.J.; OCHOA, K. & MOSS, A.R. - Hepatitis C virus infection and needle exchange use among young injection drug users in San Francisco**. Hepatology, 34:** 180-187, 2001.

10. HAYASHI, P.H.; FLYNN, N.; McCURDY, S.A. *et al*. - Prevalence of hepatitis C virus antibodies among patients infected with human immunodeficiency virus. **J. med. Virol., 33:** 177-180, 1991.

11. KLEIN, M.B.; LALONDE, R.G. & SUISSA, S. - The impact of hepatitis C virus coinfection on HIV progression before and after highly active antiretroviral therapy**. J. Acquir. Immune Defic. Syndr., 33:** 365-372, 2003.

12. LAFEUILLADE, A.; HITTINGER, G. & CHADAPAUD, S. - Increased mitochondrial toxicity with ribavirin in HIV/HCV coinfection**. Lancet, 357:** 280-281, 2001.

13. MARTINEZ-SIERRA, C.; ARIZCORRETA, A.; DIAZ, F. *et al*. - Progression of chronic hepatitis C to liver fibrosis and cirrhosis in patients coinfected with hepatitis C virus and human immunodeficiency virus. **Clin. infect. Dis.. 36:** 491-498, 2003. Epub Jan 31, 2003.

14. MENDES-CORREA, M.C.; BARONE, A.A. & GUASTINI, C. - Hepatitis C virus seroprevalence and risk factors among patients with HIV infection. **Rev. Inst. Med. trop. S. Paulo, 43:** 15-19, 2001.

15. MURAKAMI, J.; SHIMIZU, Y.; KASHII, Y. *et al*. - Functional B-cell response in intrahepatic lymphoid follicles in chronic hepatitis C. **Hepatology, 30:** 143-150, 1999.

16. NEGREDO, E.; DOMINGO, P.; SAMBEAT, M.A.; RABELLA, N. & VAZQUEZ, G. - Influence of coinfection with hepatitis viruses on human immunodeficiency plasma viral load. **Arch. intern. Med., 159**: 2367-2368, 1999.

17. NUNEZ, M.; LANA, R.; MENDOZA, J.L.; MARTIN-CARBONERO, L. & SORIANO, V. - Risk factors for severe hepatic injury after introduction of highly active antiretroviral therapy. **J. Acquir. Immune Defic. Syndr., 27:** 426-431, 2001.

18. ORLAND, J.R.; WRIGHT, T.L. & COOPER, S. - Acute hepatitis C. **Hepatology, 33:** 321-327, 2001.

19. PIROTH, L.; DUONG, M.; QUANTIN, C. *et al*. - Does hepatitis C virus co-infection accelerate clinical and immunological evolution of HIV-infected patients? **AIDS, 12:** 381-388, 1998.

20. QUAN, C.M.; KRAJDEN, M.; GRIGORIEW, G.A. & SALIT, I.E. - Hepatitis C virus infection in patients infected with the human immunodeficiency virus**. Clin. infect. Dis., 17:** 117-119, 1993.

21. REVISÃO DA DEFINIÇÃO nacional de caso de Aids em indivíduos com 13 anos de idade ou mais, para fins de vigilância epidemiológica. Accessed in internet http://www.aids.gov.br/udtv/link203.htm

22. SULKOWSKI, M.S. & THOMAS, D.L. - Hepatitis C in the HIV-infected patient. **Clin. Liver Dis., 7:** 179-194, 2003.

23. SULKOWSKI, M.S. & THOMAS, D.L. - Hepatitis C in the HIV-infected person. **Ann. intern. Med., 138:** 197-207, 2003.

24. TAMALET, C. & COLSON, P. - Reciprocal influence of HIV and HCV infections in co-infected patients and the involvement of HAART. **Clin. Microbiol. Infect., 9**: 159-160, 2003.

25. TONG, M.J.; EL-FARRA, N.S.; REIKES, A.R. & CO, R.L. - Clinical outcomes after transfusion-associated hepatitis C. **New Engl. J. Med., 3**32: 1463-1466, 1995.

26. TROISI, C.L.; HOLLINGER, F.B.; HOOTS, W.K. *et al*. - A multicenter study of viral hepatitis in a United States hemophilic population. **Blood, 81**: 412-418, 1993.

27. VAN BEEK, I.; DWYER, R.; DORE, G.J.; LUO, K. & KALDOR, J.M. - Infection with HIV and hepatitis C virus among injecting drug users in a prevention setting: retrospective cohort study. **Brit. med. J., 317:** 433-437, 1998.

28. YEE, T.T.; GRIFFIOEN, A.; SABIN, C.A.; DUSHEIKO, G. & LEE, C.A. - The natural history of HCV in a cohort of haemophilic patients infected between 1961 and 1985. **Gut, 47:** 845-851, 2000.

29. ZHANG, C.; YANG, R.; XIA, X. *et al*. - High prevalence of HIV-1 and hepatitis C virus coinfection among injection drug users in the southeastern region of Yunnan, China. **J. Acquir. Immune Defic. Syndr., 29:** 191-196, 2002.