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Research Article

Controlled HIV-HCV Viremia and Immune-Reconstitution are Associated with Slow Progression of Liver Disease in Co-infected Hemophilic Patients After 30 Years of Follow-Up

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1. Abstract

1.1. Introduction and aim: Controversial results have been reported about the progression of liver disease in HIV-HCV coinfected populations. The purpose of this study is to assess long-term liver disease progression in a group of coinfected patients with hemophilia.

1.2. Materials and Methods: From 1995 to 2015, liver disease was assessed through enzyme levels, platelet counts, Hepatitis C and HIV viral loads (VL), and CD4+T cell counts. Evolution of the APRI liver index was used to estimate hepatic disease (APRI > 1.0 indicating severe fibrosis).

1.3. Results: 2005-2015 proportional liver-related mortality was below 17% while AIDS and other causes including hemorrhagic events reached 42% each. APRI index >1.0 was found in 3 of 32 (9%) patients alive, showing significant liver disease after more than 30 years of infection. Analyzing the evolution of liver disease markers, liver enzymes increased significantly only in those patients with detectable HIV and /or HCV VL (for AST and ALT, $p<0.0001$; for GGT, $p=0.001$). HIV suppression and reconstitution of CD4+T cell counts were required to achieve HCV eradication. Through multivariate logistic regression, pre ART (pre-antiretroviral therapy) HIV VL was associated with the development of liver fibrosis (OR=4.755; IC95: 1.057 – 21.387) and with altered liver enzyme values (OR=4.091; IC95: 1.293 – 12.947). No persistent increase in enzyme levels or APRI index was observed in the group controlling HIV and HCV replication and adequate immune recovery.

1.4. Conclusions: The suppression of both viruses, HIV and HCV, together with adequate immune recovery is associated with minimal or slow progression of liver disease.

2. Abbreviations: HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; ART: Antiretroviral therapy; ALT: serum alanine aminotransferase; AST: Aspartate aminotransferase; GGT: γ-Glutamyl-Transpeptidase; VL: viral load; APRI: AST-to-Platelet Ratio Index; ESLD: End stage liver disease

3. Keywords: HIV-HCV co infection, Liver disease, Progression, Hemophilia

4. Introduction

Controversial results have been reported about the progression of Human immunodeficiency virus (HIV) and Hepatitis C virus (HCV) diseases in coinfected populations and the mechanisms leading to advanced liver disease progression in HCV/HIV coinfected subjects remain unclear. The hemophilia population provides a useful model for studying the natural

history of HCV and HIV infections with their associated complications.

Among some cohorts of patients with hemophilia, HIV coinfection accelerated progression to liver disease in the context of chronic HCV infection [1] even though the mechanisms have yet to be fully characterized. Some reports confer a protective role to CD4+ T cell counts, inversely associated with fibrosis, cirrhosis, hepatic decompensation, and/or death due to end-stage liver disease [2, 3]. The lack of CD4 recovery upon antiretroviral therapy (ART) was associated with increased risk of progression to HCV-associated liver disease [4]. However, Collazos et al. demonstrated that the current or past immunological status of HIV-HCV-coinfected patients did not seem to have any significant influence on HCV viral load or on the development of liver fibrosis when adjusting for important covariates [5]. Considering the complex interplay between the 2 viruses, host immunologic response, and treatment, the analysis in coinfected patients is extremely difficult and the controversy in the results remains.

The purpose of this retrospective study is to assess liver disease progression after more than 30 years of infection in a group of HIV/HCV coinfected patients with hemophilia. Long-term follow-up with a close approximation to the date of infection offers a valuable tool to assess disease progression and to evaluate the possible contribution of different viral, immunologic and host factors in this group of patients.

5. Material and Methods

Of the1315 individuals registered at the Hemophilia Foundation (Fundacion de la Hemofilia, Buenos Aires), 211 patients tested positive for HIV antibodies (16%) from 1985 to 1986, when HIV-1 antibody detection reagents became available in Argentina. Subsequently, in 1997, 37% of the population was found to be infected with HCV (772 patients out of 2080 patients, the majority being born before 1985 (629 patients). Only few seroconversions occurred after November 1985, when heat-inactivated factor concentrates became available. A group of 66 HIV/HCV coinfected patients was identified in this population and was included in this study. Data on the type and severity of the bleeding disorder, medical history, death date and cause, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-Glutamyl-Transpeptidase (GGT), cholesterol, platelet counts, and antiviral treatment history were collected from medical files. Serologic data, HCV genotype and viral loads (VL) were performed and/or also collected from records. The CD4+ T-cell count was determined regularly by flow cytometry using commercially available monoclonal antibodies and analyzed with Cell quest software (Beckton Dickinson). Since 1997, HIV-1 and HCV viral loads were measured every 3-6 months with Amplicor HCV monitor version 2.0; range of detection: 600 - 850,000 IU/ mL and Amplicor HIV-1 monitor version 1.5; range of detection: 400 - 750,000 copies/ mL or 50 – 50,000 copies/ mL with the ultrasensitive method (Roche Diagnostics, Branchburg, NJ, USA). Recommendations of the suppliers were followed. Detection limits varied according to the development of the commercial reagents. The distribution of IL28b polymorphism (rs12979860) was evaluated using PCR techniques with subsequent digestion with restriction enzymes previously described by Fabris [6]. The presence of chemokine receptor type 5 (CCR5) delta 32 deletion was also determined by endpoint PCR technique. Both polymorphisms could be linked to HCV and HIV disease progression as shown in different previous reports [7].

This study was conducted in accordance and compliance with the ethical principles of the 1975 Declaration of Helsinki and was approved by the institutional review board of the Academia Nacional de Medicina of Buenos Aires.

5.1. Important considerations

5.1.1. Estimation of the date of seroconversion: The exact date of seroconversion is unknown, however as with many other hemophilic populations, it is estimated to have occurred in most of the patients between 1975 and 1985 for both, HIV and HCV infections. Considering that the commercial factor concentrates were not accessible until 1975 in Argentina, those patients who were born before that year, were considered to have been infected in 1975. For those who were born after 1975, the date of infection was considered to have occurred within the first year of life [8]. Dates of HIV seroconversion were based on previously published studies, establishing 1982 as the median year of seroconversion for hemophilia A and 1983 for hemophilia B [9]. The patients considered for this study did not show any other risk factor for infection at the time, other than their condition of hemophilia [10, 11].

5.2. Liver Disease Progression Assessment

For the assessment of liver disease progression, sequential measurements of AST, ALT, GGT, platelet counts, and cholesterol were considered during long-term follow-up (1995-2015). Clinical conditions stated on their medical records, such as hepatomegaly, splenomegaly, hepatosplenomegaly, esophageal varices, liver failure, and portal hypertension were registered from the patient record. The upper normal levels for AST, ALT and GGT were 38 units/l serum, 40 units/l and 50 IU/l, respectively. Values above these were considered altered. Noninvasive liver indexes APRI (AST-to-Platelet Ratio Index), FORNS and Fib4 were calculated and used as surrogate markers for liver disease progression. The cut-off values were as follows: APRI > 1.0 indicating severe fibrosis; APRI >2.0 indicating cirrhosis; FORNS > 6.9 indicating fibrosis F2, F3 or F4 and FIB-4 > 3.25 for cirrhosis.

Three different time points were considered in the analysis:

- Pre-ART (period before antiretroviral therapy implementation in Argentina (1997).
- 2000 to 2005.

- Post 2005(2006 to 2015). This corresponds to more than 20 years of HCV infection.

6. Statistical Methods

Qualitative analysis was performed using the Chi-square testor Fisher's exact test as appropriate. In addition, odds ratio (OR) was calculated for dichotomous qualitative data along with 95% Confidence Interval (CI). Unpaired Student's t test or Mann-Whitney U test were used as appropriate for testing differences between two groups of quantitative data. For the comparison of variables along the study, quantitative paired data were analyzed by repeated measures ANOVA or Friedman's test. A level of $p<0.05$ was accepted as being statistically significant. The statistical analysis was implemented in IBM SPSS Statistics 21 (IBM Corporation, Armonk, New York, USA). Multivariate logistic models were fitted to assess factors associated with different outcomes: significant fibrosis (APRI score >1.0), altered liver enzymes (ALT, AST) and death. All graphs were made using Graph Pad 8.0 (Graph Pad Software, San Diego California, USA).

7. Results

The study included 66 HIV/HCV coinfected male patients with hemophilia. Some characteristics of the population, such as type and severity of hemophilia, HCV genotypes, duration of infections are displayed in (Table 1).

Table 1: Population characteristics.

Median Age in Years			Range
At study entry	25		14 to 59
At the end of follow-up	42		20 to 70
Type of hemophilia	A	B	
Se/Mo/Mi	28/18/8	7/3/2002	
HBV Markers			
Absent/resolved/non- resolved*	9/40/14		
HCV genotypes			
1/2/3/4/mixed genotypes	44/4/5/1/8		
Duration of infection in years (median)			
HIV	33		14 to 33
HCV	36		19 to 39
Distribution of IL28b			
CC/CT/TT	33/25/8		
Distribution of CCR5 delta32 mutation			
+ +/+ - /- -	0/13/53		

Se: severe, Mo: moderate, Mi: mild; non-resolved*: patients with non-resolved HBV infection include hepatitis B antigen carriers and patients with hepatitis B core antibodies alone. (- /+): absence or presence of delta 32 mutation

Mortality and causes of death: By August 1996, 7 of 66patients had died. Deaths were mainly due to AIDS related complications (6 of 7 patients). Twelve patients died between 1998 and 2005. AIDS development showed to be the major cause of death in the 1995-1997 and 1998-2005 periods in our cohort (Figure 1). Deaths occurred in those patients who could not get access to therapy (before 1997) or those with virus resistance or poor adherence. While liver disease was expected to grow as the duration of HCV infection rose, the results from 2005 to 2015 (12 patients deceased) showed that the proportional mortality was still below 17% compared to AIDS with 42%, or other causes including cardiovascular and hemorrhagic events, 42%. Thus far, liver-related complications are still not the leading cause of mortality in this group of patients.

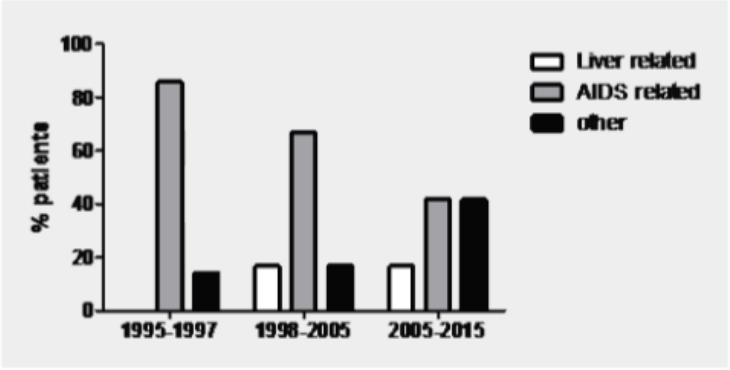

Figure 1: Contribution of human immunodeficiency virus- (HIV-) or AIDS-related, liver-related, and other cause related mortality (percentage of total number of deaths) along the study period.

No homozygosity for CCR5 delta 32 was detected in this cohort. Thirteen of 66 patients showed one copy of the gene encoding the CCR5 delta 32 non-functional receptor. Three deaths of 13 (23%) in the group with heterozygosis occurred only in post 2005 periods, after more than 20 years of infection. In the group lacking the protective polymorphism, 53% died along the study. However, no significant association between the presence of the protective polymorphism and death could be found ($p=0.06$).

The median age at the estimated moment of seroconversion in this population was 9 years for HIV and 3 years for HCV. The inter quartile range was 4 to 18 years for HIV and 1 to 12 years for HCV. No association between death and age at seroconversion was found ($p>0.05$).

Description of HIV disease progression markers over time: HIV viral loads were detectable in 46 patients at pre-ART periods with a median value of 4.25 log (copies/ml plasma)and a range between 2.87 and 5.94 log (copies/ml plasma). Only 5 patients displayed undetectable HIV viral burden in this period. As expected in a population after ART implementation, a gradual decline in HIV viral load ($p<0.001$) and an increase in CD4-T cell counts ($p=0.012$) were observed throughout the study, showing an efficient HIV viral suppression and adequate immune recovery in the majority of the patients.

Description of liver disease markers over time: Analyzing the evolution of hepatic enzymes over time, we observed increasing values of AST, ALT ($p<0.0001$) and GGT ($p=0.01$) (Figure 2). Platelets

did not show significant differences along the study. As mentioned before, APRI, FORNS, and Fib4 liver indexes and their variation over time were used to evaluate liver disease progression in patients. Correlation between APRI-FORNS, APRI-FIB4 and FORNS-FIB4 was r= 0.7; 0.9 and 0.7. Since APRI is the most frequently used and the recommended index, the results presented here are based on this index alone. APRI levels were considered "altered" when greater than 1.0 (indicating fibrosis) or >2.0 (indicating probable cirrhosis). The number of patients with altered liver index increased significantly over time. While only 2 of 49 (4%) patients with altered APRI values (>1.0) were observed in pre ART era, 7 of 47 (15%) individuals showed values >1.0between 2000 and 2005 (4 of them >2.0), and 11of 42 (26.2%) showed values >1.0(with 4 patients >2.0) in time-points after 2005 (p=0.01). Patients with altered APRI showed persistently detectable and high HCV VL all along the study.

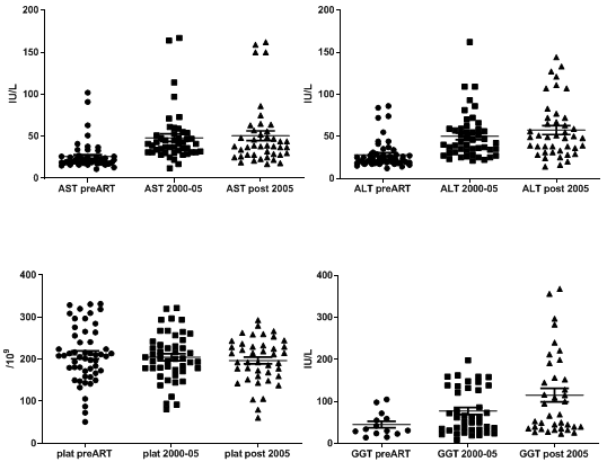

Figure 2: ALT: serum alanine aminotransferase, AST: aspartate aminotransferase, plat: platelets, GGT: γ-Glutamyl-Transpeptidase. Median values and standard errors are shown for each time point. Evolution of liver enzyme levels and platelet counts in the whole population over time.

Patients with HCV and HIV viremia control, at the end of study: 14 patients showed suppression for both HIV and HCV viruses (undetectable viral loads) at the end of the study (post 2005-2015). Analyzing the evolution of liver disease markers over time, no HIV- or HCV-related disease progression was observed in individuals controlling both viruses. They displayed normal values for AST, ALT, GGT, APRI below 1.0 and adequate CD4+T cell counts (significantly higher compared to those without HIV and /or HCV suppression (mean=709, median=600, range=252-1827 cells/mm3; p=0.01) (Table 2). We observed that liver enzymes increased significantly only in those with detectable HIV and/or HCV viral loads (for AST and ALT, p<0.0001; for GGT, p=0.001) (Figure 3).

The same analyses done with HIV suppressed versus HIV non-suppressed individuals showed that lower HIV VL are necessary but not enough for maintaining low levels of liver disease markers. Inside the HIV (-) group, the subgroup with HCV (+) VL, displayed higher enzyme levels and APRI index.

Table 2: Clinical markers in the groups with HIV or HCV viral control.

	HIV(-) HCV(-) n=14			HIV(+) HCV(+) n=10			HIV(+) HCV(-) n=2			HIV(-) HCV(+) n=9			Kruskal Wallis test	Student *t* test
	med	mean	SD	med	mean	SD	med	mean	SD	med	mean	SD	p	p
AST	21	28	21	34	83	102	22	22	3	52	66	61	0.026	na
ALT	25	35	33	38	65	72	35	35	11	74	93	69	0.037	na
GGT	28	30	8.9	131	140	34	25	25	14	389	343	179	0.0003	na
Platelets	233	246	70	219	221	56	255	255	52	193	197	57	0.369	na
CD4+T cell counts	599	709	432	264	283	194	255	255	145	494	632	636	0.019	na
HIV VL				3.9	3.61	1	2.88	2.88	0.42				na	0.436
HCV VL				5.5	5.26	1.3				7	6.72	0.6	na	0.0039
APRI	0.27	0.34	0.27	0.3	0.82	1.1	0.24	0.24	0.08	0.4	1.17	2	0.199	na

med: median; SD Standard deviation

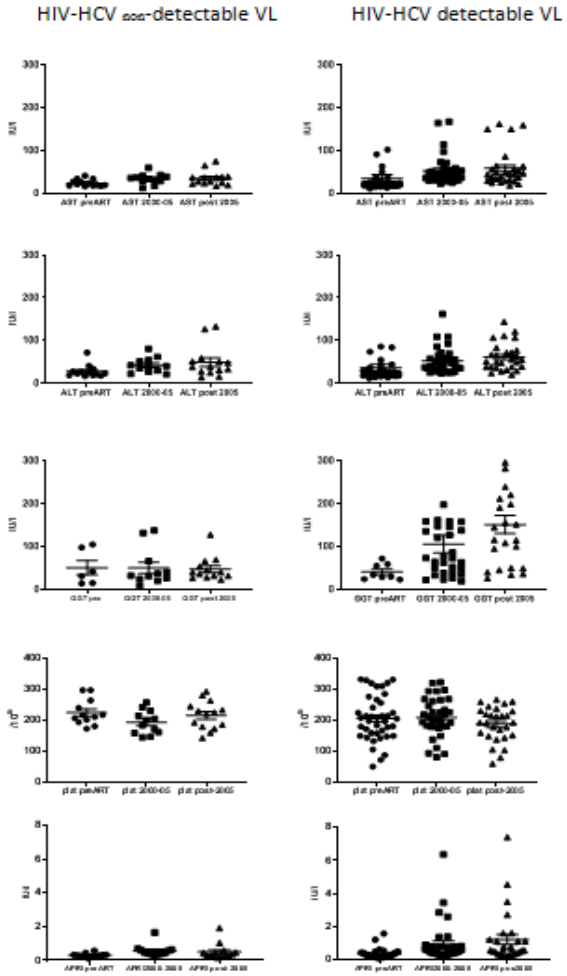

Figure 3: ALT: serum alanine aminotransferase, AST: aspartate aminotransferase, GGT: γ-Glutamyl-Transpeptidase, plat: platelet counts, APRI index. Median values and standard errors are shown for each time point. Evolution of markers in groups with HIV and HCV viral control in different time points

Patients with HCV clearance at the end of study: HCV clearance was observed in 19 patients. Eradication occurred in 17 individuals, several years after successful ART. Patients who displayed gradually lower HIV viral loads (p<0.0001) over time and progressively higher CD4+T cell counts (p=0.02) were those able to eradicate HCV-RNA from plasma at the end of the study. Ten of them were treated with IFN-RBV and the other 7 individuals achieved spontaneous HCV control, after CD4 counts recovery, as we previously reported [12].

Immune recovery preceded HCV negativization in plasma. The time of HCV spontaneous clearance is not certain for the remaining 2 patients.

Analyzing IL28b polymorphisms, the presence of the CC genotype was found in 68% (13 of 19) among the group who eradicated HCV. Its frequency did not reach statistically significance compared with the group with persistent detectable HCV load which was 45%, probably because of the small size of the sample ($\chi 2=3.05$; gl=1; p=0.08; OR=0.37, 95% CI=0.12 to 1.15). It is worth mentioning that 6 of the 7 patients with spontaneous clearance showed IL28b CC genotype, emphasizing the positive impact of this genotype upon HCV disease outcome.

Description of patients alive and followed-up through the end of study: At present, 35 patients are alive. Three of them were lost to follow-up before 2010. Among the remaining individuals, 30 are still under antiretroviral therapy and 23 (77%) controlling HIV infection and maintaining undetectable viral loads with adequate CD4+T cell counts (median=531, Mean=679, range=185 to 2259 cells/mm3). Two patients decided not to receive antiretroviral treatment or did not adhere satisfactorily. They maintained detectable HIV viral loads (median=3.86, mean= 3.64, range= 2.5 to 4.8 log (copies/ml plasma)) and low CD4+ T-cell counts all along the study (median=264, Mean=263, range=72 to 461 cells/mm3).

Altered APRI index (>1.0) was found in 3 of 32 patients alive and still followed-up (9%), showing that they may have developed significant liver fibrosis/cirrhosis after more than 30 years of infection. One of them, current APRI=1.32, with signs of severe chronic hepatitis, presented an F4 stage of fibrosis in a recent transient elastography test. The other 2 patients (APRI=6.32 and APRI=3.28) exhibited hepatic complications specified in their medical files that confirmed the urgent need of antiviral treatment.

8. Discussion

From a population perspective, hemophilia patients with HCV chronic infection have many of the known risk factors associated with rapid fibrosis progression (HIV coinfection, long duration of infection, presence of genotype 1 in most of the patients). The evidence in this study does not support such effect in this cohort. Based on the sequential measurement and calculations of noninvasive laboratory markers along time, this study proved that fibrosis progression is probably slow in a substantive proportion of HIV/HCV-co-infected patients. A low percentage of patients showed sustained altered liver disease markers (9%) and a small number of deaths related to liver cause (17%).Likewise, a recent report among hemophilic patients demonstrated10% of end stage liver disease (ESLD) in HIV positive patients [13]. Another group described that the cumulative incidence of ESLD 35 years after HCV infection was 11.5% in HIV negative patients but 35% in HIV positive patients [14]. Although the size of the present cohort is small, the close monitoring and frequent laboratory measurements ensured an accurate evaluation of the patient status.

Early administration of antiretroviral therapy with successful suppression of HIV viral load may improve overall survival and decrease the risk of dying from liver disease-associated complications by delaying fibrosis progression in patients [15-17], even though other studies have not demonstrated this beneficial effect [18, 19]. Most of the patients in our small cohort could have benefitted from prompt ART administration responding adequately; supporting that preserved immune status is associated with a more benign HCV course and favorable outcomes.

Liver disease progression estimated through APRI index and hepatic enzyme levels was associated with higher pre ART HIV viral loads. In addition, both, low plasma HIV loads and high CD4+T cell counts were necessary to achieve HCV eradication in this study. This observation agrees with our own previous report [12] and with a recent publication in which coinfected patients achieved spontaneous HCV clearance after starting HIV therapy and regaining immune competence [20]. Individuals with hemophilia with a weaker immune system at the time of ART implementation were not able to recover adequately and could not eradicate HCV. Accordingly, in previous reports, impaired immune function and lack of CD4+T cells recovery on antiretroviral therapy was associated with increased risk of progression to HCV-associated liver disease [17, 21].

It is unclear whether HCV-RNA titers affect the liver disease progression and controversial reports have been published. Noh and collaborators found serum HCV-RNA titer as an independent risk factor for the development of HCC but no liver-related mortality [22]. Conversely, in a previous report, the risk of ESLD among intravenous drug users with chronic HCV infection was found to be associated with HCV RNA levels [23].

From our results, individuals with persistently detectable HCV viral load showed a gradual and significant rise of liver enzyme levels and APRI index compared to those with HCV viral clearance. It was not possible to associate the liver disease progression (with altered enzymes or APRI>1.0) to HCV VL in a multivariate analysis, controlling other factors that could affect the progression of the disease. However, when both, HIV and HCV, viruses were suppressed, liver enzymes and APRI index did not show any increase along the study and no signs of liver disease were observed. Adequate CD4+Tcell counts and immune recovery were important characteristics of this group.

A recent revision of the literature showed that elevated GGT is linked to increased risk in many diseases and conditions, including cardiovascular disease, diabetes, metabolic syndrome, and all-cause mortality [24]. GGT activation could be influenced by many factors such as alcohol intake, antiretroviral history, age, weight, physical activity and smoking, among others. In the setting of HCV infection, the

predictive value of GGT showed the most robust association with response to IFN-RBV therapy and with disease outcomes [25, 26]. Almost half of the patients in this cohort displayed altered GGT values after more than 30 years of infection. It is noteworthy that 73% of the patients with HCV persistence in plasma showed augmented values of GGT that were twice as high as the upper reference value (100 IU/l), unlike what happens in people with HCV eradication. The underlying mechanisms of the increase were not explored and were the purpose of this study; however, the cause of this specific release of GGT deserves further investigation since it appears to be linked to HCV VL.

In the setting of chronic HIV/HCV infection, regardless of the stage of liver disease, anti-HCV treatment is recommended. Our results showed that the suppression of both HIV and HCV viremia together with immune reconstitution are associated with slow or minimal progression of liver disease in coinfected hemophilic patients, after 30 years of follow-up.

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