Facultad de Medicina

Desarrollo y validación de un índice prónostico de la respuesta del Virus de la Hepatitis C al tratamiento con Interferón pegilado y Ribavirina en pacientes coinfectados por el Virus de la Inmunodeficiencia Humana

Tesis Doctoral

Jose Medrano Laporte

Madrid 2010

Dedicado a Amaya, a Asier y a toda mi familia

Agradecimientos:

A Vicente Soriano, por dirigir esta tesis y compartir conmigo su entusiasmo por la investigación.

A Jose María Peña, por su apoyo decidido en el proyecto.

A todos los compañeros del Servicio de Enfermedades Infecciosas del Hospital Carlos III, y a su jefe, el Dr Juan González-Lahoz.

A Salvador Resino por su apoyo y su generosidad.

A todos los compañeros con los que he compartido años de residencia en la Fundación Jiménez Díaz.

A todos los compañeros del “Service de Maladies Infectieuses et tropicales” del CHU Bichat, Paris y los del “Service de Réanimation Médicale” del CHU-Pellegrin, Burdeos.

Al profesor Salmi y los docentes del Instituto de Salud Pública de Burdeos.

Presentación:

Los trabajos que conforman esta Tesis Doctoral son el resultado de una línea de investigación que finaliza en el desarrollo de una herramienta pronóstica que permite estimar de manera individualizada la probabilidad de curación de la hepatitis crónica C en pacientes coinfectados por el Virus de la Inmunodeficiencia humana. Esta herramienta está disponible gratuitamente en www.investigacion-clinica.org/pisvr.xls

El primer artículo "***Hepatitis C virus (HCV) treatment uptake and changes in the prevalence of HCV genotypes in HIV-HCV coinfected patients***", es un estudio epidemiológico que describe la cohorte de pacientes coinfectados por el VIH y la hepatitis C tratados en el Hospital Carlos III con Interferon pegilado y Ribavirina. El período de seguimiento se inicia en el año 2000, fecha en la que el Interferón pegilado está disponible en el Hospital, y finaliza en enero 2009. A través del análisis de las prescripciones anuales de tratamiento y de la incidencia/prevalencia de los distintos genotipos presentes en la cohorte se demuestra un impacto del tratamiento en la distribución de los diferentes genotipos a lo largo de la década. Este estudio demuestra, además, una progresión de la enfermedad hepática en los pacientes que no se curaron de la hepatitis C. De esta cohorte surgen las diferentes poblaciones a estudio de los artículos principales y relacionados con esta tesis.

El segundo artículo, "***Rate and timing of hepatitis C virus relapse after a successful course of pegylated interferon plus ribavirin in HIV-infected and HIV-uninfected patients***", selecciona un grupo de pacientes que alcanzan viremia indetectable tras completar un ciclo completo de tratamiento con Interferon pegilado y ribavirina y analiza las causas de recidiva en pacientes con y sin coinfección por el VIH. Para ello, se identificó y caracterizó la cohorte de pacientes monoinfectados por el VHC tratados durante el mismo período en el servicio de Digestivo del Hospital Carlos III. Además de corroborar la asociación entre las variables predictivas de recidiva ya conocidas en aquel momento (fibrosis hepática, carga viral basal y genotipo del VHC), este estudio analizó, a través de un estudio filogenético, el caso particular de 3 pacientes con rebrote de carga viral C mas allá de la semana 24. Aunque se demostró que los pacientes coinfectados por el VIH presentan una tasa de recidiva mayor que los pacientes monoinfectados, el estudio no demostró que la infección por el VIH fuera un factor independiente de recidiva en pacientes con infección crónica por el virus de la hepatitis C.

El tercer artículo, "***Modeling the probability of sustained virological response to peginterferon-ribavirin therapy in HCV-HIV coinfected patients***", desarrolla un modelo predictivo con las variables que mejor predicen la respuesta virológica sostenida en pacientes tratados de la hepatitis C y que

tienen coinfección por el VIH. Además de las variables predictivas citadas en los artículos anteriores, el modelo incorpora la determinación de un polimorfismo genético recientemente descrito, el snp rs12979860. Este gen es responsable de la síntesis endógena de interferón ʎ y la homocigosis CC se asocia fuertemente a respuesta virológica sostenida. El modelo resultante se validó en una cohorte independiente de pacientes procedentes de los hospitales Virgen del Rocío de Córdoba y Virgen de Valme de Sevilla.

Las referencias de estos artículos son las siguientes:

1. **Medrano J**, Resino S, Vispo E, Madejon A, Labarga P, Tuma P. Hepatitis C virus (HCV) treatment uptake and changes in the prevalence of HCV genotypes in HIV-HCV coinfected patients. ***J Viral Hepat*** **2010 (in press)**

2. **Medrano J**, Barreiro P, Resino S, Tuma P, Rodriguez V, Vispo E, et al. Rate and timing of hepatitis C virus relapse after a successful course of pegylated interferon plus ribavirin in HIV-infected and HIV-uninfected patients. ***Clin Infect Dis*** **2009**; 49:1397-1401

3. **Medrano J,** Karin Keulen, Norma Rallón, Juan Macías, Salvador Resino et al. Modeling the probability of sustained virological response to peginterferon-ribavirin therapy in HCV-HIV coinfected patients. **(*Submitted to CID).***

Artículos relacionados:

Otros artículos publicados por el grupo de investigación en la misma línea, son los siguientes:

1. Tuma P, **Medrano J**, Resino S, Vispo E, Madejón A, Sánchez-Piedra C et al. Different incidence of liver cirrosis in HIV-infected patients with chronic hepatitis B or C in the HAART era. Journal of viral hepatitis 2010 (in press)
2. Rallon NI, Naggie S, Benito JM, **Medrano J**, Restrepo C, Goldstein D, et al. Association of a single nucleotide polymorphism near the interleukin-28B gene with response to hepatitis C therapy in HIV/hepatitis C virus-coinfected patients. AIDS 2010; 24:F23-9.
3. Tuma P, Jarrin I, Del Amo J, Vispo E, **Medrano J**, Martin-Carbonero L, et al. Survival of HIV-infected patients with compensated liver cirrhosis. AIDS 2010; 24:745-753.
4. Labarga P, Vispo E, Barreiro P, Rodriguez-Novoa S, Pinilla J, Morello J, **Medrano J** et al. Rate and predictors of success in the retreatment of chronic hepatitis C virus in HIV/hepatitis C Virus coinfected patients with prior nonresponse or relapse. J Acquir Immune Defic Syndr 2010; 53:364-368.
5. Morello J, Soriano V, Barreiro P, **Medrano J**, Madejon A, Gonzalez-Pardo G, et al. Plasma ribavirin trough concentrations at week 4 predict hepatitis C virus (HCV) relapse in HIV-HCV-coinfected patients treated for chronic hepatitis C. Antimicrob Agents Chemother 2010; 54:1647-1649.
6. Blanco F, Barreiro P, Ryan P, Vispo E, Martin-Carbonero L, Tuma P, **Medrano J** et al. Risk factors for advanced liver fibrosis in HIV-infected individuals: role of antiretroviral drugs and insulin resistance. J Viral Hepat 2010;.
7. Soriano V, Vispo E, Labarga P, **Medrano J**, Barreiro P. Viral hepatitis and HIV co-infection. Antiviral Res 2010; 85:303-315.
8. Soriano V, Tuma P, Vispo E, Labarga P, Fernandez JV, **Medrano J**, et al. Hepatitis B in HIV patients: what is the current treatment and what are the challenges? J HIV Ther 2009; 14:13-18.
9. **Medrano J**, Barreiro P, Tuma P, Vispo E, Labarga P, Blanco F, et al. Risk for immune-mediated liver reactions by nevirapine revisited. AIDS Rev 2008; 10:110-115.

Para optar a la mención de Doctorado Europeo, esta tesis está redactada en Inglés. El resumen y las conclusiones están redactados en castellano.

Abreviaturas

DAA	Direct antiviral agent
CDC	centre for diaseases and control
RNA	ribonucleic acid
EVR	early virological response
SVR	sustained virological response
EOT	end of treatment
IDU	Intravenous drug user
RVR	rapid virological response
HCC	hepatocarcinoma
PISVR	predictive index for sustained virological response
AUC-ROC	area under the curve / receiver operating curve
DOR	diagnostic odds ratio
LTFU	lost to follow up
AST	aspartate amino transferase
ALAT	alanine amino transferase
SNP	single nucleotide polymorphism
HAART	hightly active antiretroviral therapy
HIV	human immunodeficiency virus
HCV	hepatitis C virus
RBV	ribavirin

General Index

Index for tables

Index for figures

Index for Annexes

1. Background

A. Hepatitis C and HIV

Of the 34 million people currently living with HIV worldwide around 20% (7 million) has chronic hepatitis C (Figure 1 and Figure 2). This population is mainly represented by individuals with past history of intravenous drug use, hemophiliacs and recipients of contaminated blood (Figure 3)

Figure 1 Prevalence of Hepatitis C worlwide

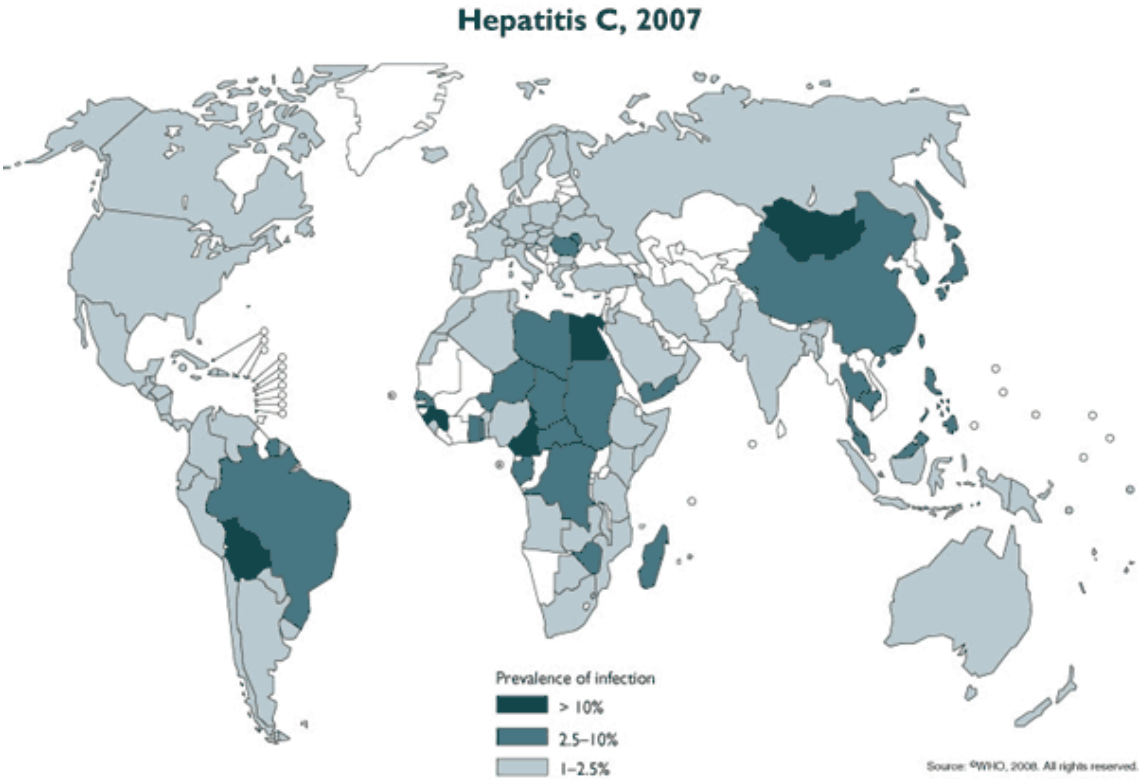


Figure 2 Estimated number of individuals with HIV, HBV and HCV worldwide in 2010

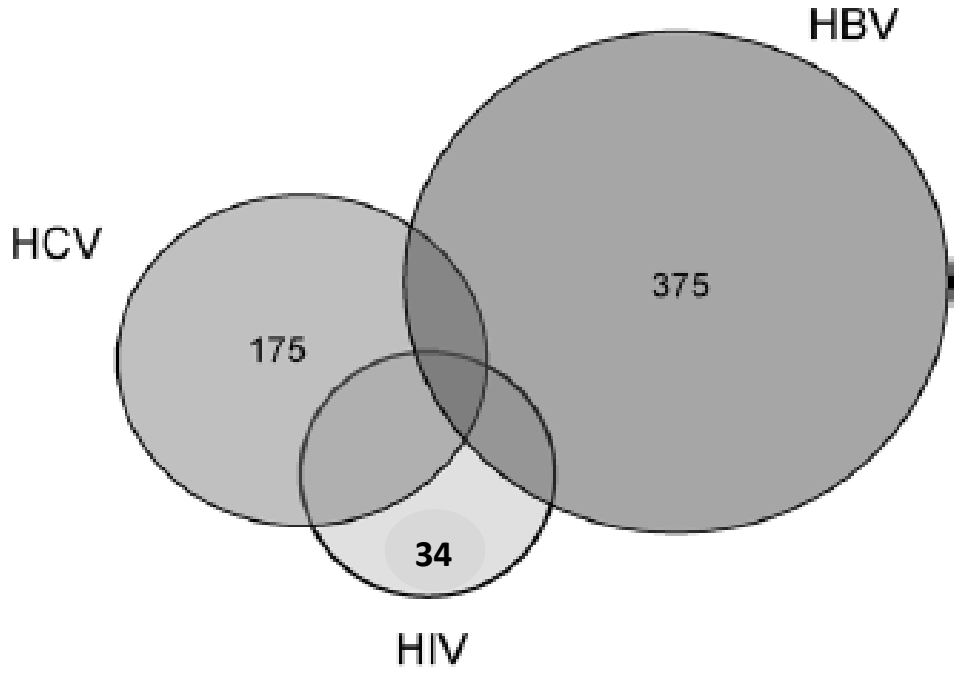
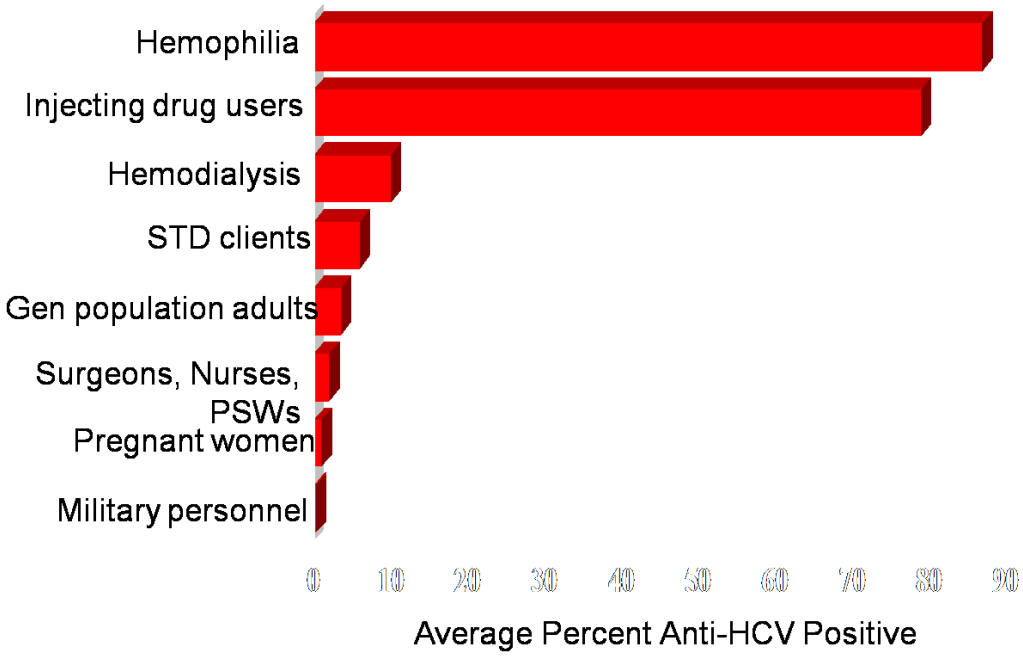


Figure 3 Prevalence of HCV infections in different risk categories. CDC.



1. Natural history of HCV-related liver disease in HIV+ patients

Besides experiencing an increased risk of chronification following initial HCV infection, HIV+ individuals with chronic hepatitis C show a faster progression of liver fibrosis [7]. On average, nearly half of patients have developed liver cirrhosis after 25 years of HCV infection. Low CD4 counts enhance the hepatic fibrogenesis process in co-infected patients, and therefore early introduction of HAART in these patients is warranted [8].

2. Treatment of chronic hepatitis C in HIV+ patients

It has become a priority for at least two reasons. Firstly, progression to end-stage liver disease occurs more rapidly in this population [7, 9]. Secondly, the tolerance of antiretroviral agents is much poor in the presence of underlying chronic hepatitis C, with a greater risk of hepatotoxicity [10, 11]. Successful treatment of chronic hepatitis C can revert these drawbacks. Indeed, clearance of HCV has been associated with a regression of liver fibrosis [12, 13] and with a reduced risk of antiretroviral-related hepatotoxicity [14].

3. Selection of HIV+ candidates for HCV therapy

Virological features such as HCV genotype and HCV load largely influence the response to HCV therapy. However, viral factors rarely determine who should be considered as good candidate for HCV therapy. Host factors, including extent of liver fibrosis, CD4 counts, and patient's motivation are the most important parameters that should determine who should receive HCV therapy (see Table 1). More recently, specific host genetic factors have also demonstrated to largely influence treatment outcomes. Of note, a genetic polymorphism near the interleukin 28B gene, encoding interferon gamma 3, has been associated with a two-fold chance in response to pegIFN+RBV. The susceptibility allele is more common in Caucasians than blacks, contributing to explain at least in part the differences in response rates seen between these ethnic groups [15-18].

a) Liver fibrosis

The extent of hepatic fibrosis is the best prognostic factor of disease progression in patients with chronic hepatitis C, and therefore its consideration is worth before indicating HCV therapy. Liver biopsy has been for many years the only tool to assess hepatic fibrosis. However, its invasive nature with occasional serious and even life-threatening complications, sampling error and inherent heterogeneity of hepatic fibrosis, low acceptance by most patients, and relatively elevated cost, have prompted the development of non-invasive tools for staging hepatic fibrosis. These are currently split into two major categories, ultrasound techniques, such as elastometry (FibroScan), and serum biochemical indexes (i.e. Fibrotest, APRI, SHASTA, FIB-4, hyaluronic acid, etc.). These tools are generally accurate to discriminate between lack of fibrosis and advanced fibrosis, and less precise to distinguish between intermediate fibrosis stages. Their predictive value is particularly good for advanced hepatic fibrosis and cirrhosis. However, serum fibrosis markers are generally less reliable in co-infected patients, given the inflammatory nature of HIV disease and/or the frequent prescription of drugs in this population which may interfere with some fibrosis markers in the blood. This is the case for bilirubin elevations due to atazanavir, gamma-glutamyl-transpeptidase (GGT) abnormalities with non-nucleoside reverse transcriptase inhibitors, or cholesterol elevations using most ritonavir-boosted protease inhibitors. In contrast, liver fibrosis staging using elastometry seems to be more reliable in this setting, avoiding such interference [19]. Elastometric measurements can be made in 10 min, be repeated periodically, are inexpensive, and had more than 90% positive predictive value for advanced liver fibrosis. When the diagnosis of any hepatic disease is clear by other means, as occurs with chronic hepatitis C testing positive for serum HCV–RNA, the need for a liver biopsy to stage hepatic fibrosis and guide treatment decisions, is currently no longer justified in most instances. The higher response to pegIFN–RBV with respect to standard interferon, the faster progression of HCV-related liver disease in the HIV population, and the opportunity for assessing viral response at earlier time-points and identify who will and who will not respond to therapy, all favour the prescription of anti-HCV therapy avoiding a liver biopsy in most cases [20]. Patients with repeatedly normal liver enzymes might benefit from HCV therapy. Few studies have been conducted so far in coinfected patients with normal ALT. Less than 10% of this population show persistently normal liver enzymes [21]. Exposure to antiretroviral drugs, alcohol abuse and other conditions explain the lower rate of normal ALT in HIV patients with chronic hepatitis C. On the other hand, significant liver fibrosis has been reported in up to 25–40% of co-infected patients with normal ALT and “silent” cirrhosis in nearly 15% of them [22].

HIV/HCV co-infected patients with liver decompensation (ascites, gastrointestinal bleeding, hepatic encephalopathy, etc.) should not be treated

with pegIFN, given their higher risk for developing serious side effects. These patients should be assessed for liver transplantation. However, patients with compensated cirrhosis (Child-Pugh class A) must be treated with pegIFN plus RBV, because their chance of response is currently relatively high (25%) and ultimately these patients will benefit more than any other from HCV clearance. In contrast with HIV disease, chronic hepatitis C can be cured and there is conclusive data supporting that undetectable serum HCV–RNA 6 months after completion of treatment really reflects eradication of HCV infection [23].

b) CD4 count

Old therapeutic trials using IFN monotherapy concluded that the efficacy of HCV therapy depended of baseline CD4 cell counts. More recently, a subanalysis of the APRICOT trial has shown that treatment responses are less as lower is the baseline CD4 percentage. Candidates to receive HCV therapy ideally should have more than 200–350 CD4+ T cells/mm3, a feasible threshold for most patients if antiretroviral therapy is used appropriately. In patients with CD4 counts below 200 cells/mm3 and already under HAART, the decision to treat HCV infection must be made taking into account other factors, such as the estimated length of HCV infection, the severity of liver disease, the extent of HIV suppression, and classical predictors of response to HCV therapy such as HCV genotype and viral load (Table 1). It should be kept in mind that toxicities of pegIFN and/or RBV as well as poorer responses may be more frequent in severely immune deficient patients. In general, HCV therapy should be deferred in individuals with less than 200 CD4+ T cells/mm3, mainly because concerns on toxicity and since the response might be much poor. Moreover, IFN generally causes a decline in the CD4 count, which may put these patients at further risk for developing opportunistic infections. Therefore, in drug-naïve co-infected patients with low CD4 counts, antiretroviral therapy should be considered at front. Once CD4 cells have raised and plasma HIV–RNA is under control, the prescription of HCV therapy should be reassessed [6]. Conversely, in antiretroviral-naïve individuals with good CD4 counts, hepatitis C should be treated first. These patients will further benefit from an improved tolerance of antiretroviral drugs [14].

c) Patient’s motivation

Individuals with prior history of serious neuropsychiatric disorders should not be treated, because IFN can exacerbate these conditions. Patients currently engaged in heavy alcohol intake and/or illegal drug addiction practices should delay anti-HCV treatment, whereas all efforts should be devoted to put them into detoxification programs. Patients on methadone are acceptable candidates for HCV therapy. However, up to one-third of them may need adjustments in

methadone dosage. This is generally due to psychological demands rather than to pharmacological interactions between anti-HCV drugs and methadone. Ideally, a multidisciplinary team, including experts in addiction medicine and psychologists/psychiatrics, should take care of these patients. In summary, all HIV persons with chronic HCV infection should be considered at front as potential candidates for HCV therapy, given their higher risk of progression to end-stage liver disease compared to HIV-negative patients and their increased risk of liver toxicity after beginning antiretroviral therapy. The timing for HCV treatment should be decided on an individual basis. Severe neuropsychiatric disorders, alcohol and drug abuse generally contraindicate HCV treatment. However, methadone use and non-decompensated cirrhosis are not contraindications for therapy. Treatment of patients with CD4 counts below 200 cells/mm3 or low CD4 percentages is less effective and potentially risky; therefore, it should generally not be advised.

B. Predictors of response to HCV therapy in HIV+ patients

1. Baseline variables

Serum HCV–RNA and HCV genotype are the main baseline predictors of SVR to pegIFN–RBV in co-infected as in HCVmonoinfected patients. Several other variables, however, may influence treatment responses, although generally in less extent. As shown in

Table 1, they can be grouped in three categories. Especial attention has recently been paid to the negative impact of insulin resistance on HCV treatment response [24]. Insulin resistance is quite prevalent in co-infected patients at least in part due to the use of certain antiretrovirals, as ritonavir-boosted protease inhibitors. Therefore, prevention of insulin resistance and/or its adequate management (even considering treatment with insulin-sensitiser agents when indicated) might improve HCV treatment outcomes in co-infected patients.

Table 1 Factors associated with sustained virological response to HCV therapy in HIV infected patients

Host	Virus	Treatment
• Genetic (White ethnicity)	• Genotypes 2/3	• Adequate peginterferon dose
• Younger age	• Low baseline HCV RNA	• Weight-based ribavirin dose
• Minimal liver fibrosis	• Undetectable HCV–RNA at week 4	• Good adherence
• Low body mass index		• No concurrent didanosine or zidovudine
• Lack of insulin resistance		• Use of hematopoietic growth factors when needed
• Lack of hepatic steatosis		
• Higher CD4 count		
• No polysubstance abuse		
• No psychiatric disease		

2. Treatment compliance

As in HCV-monoinfected patients, treatment adherence should be encouraged as much as possible. The “80/80/80” rule is equally valid in co-infected patients, meaning that subjects who take more than 80% of pegIFN and of RBV doses during at least 80% of planned period of therapy respond significantly better than the rest. Therefore, adequate selection of treatment candidates, psychological and/or psychiatric support, and use of growth factors to avoid dose reductions of either pegIFN and/or RBV, all must be encouraged in order to keep on adequate doses of anti-HCV medications the majority of patients. Since depressive symptoms are a major obstacle for completion of a full course of therapy in a significant proportion of patients it is worthy to familiarise doctors taking care of co-infected patients with their appropriate use. Management of mild–moderate depression will result in improved SVR rates. Recognition of major depressive symptoms, however, should prompt to refer patients to a psychiatrist and stop HCV therapy.

3. HCV kinetics

Changes in serum HCV–RNA in response to pegIFN–RBV is a reliable indicator of treatment efficacy. The availability of sensitive quantitative tools to closely monitor HCV decays under treatment has allowed recognition of early time-points with high predictive value of SVR. Overall, the early virological response to HCV therapy splits patients into those sensitive and those refractory to therapy. Nearly 20% of HCV-monoinfected subjects do not show a significant reduction in HCV viremia (defined as a decline >1 log) during the first month of pegIFN–RBV, and this figure increases up to 30% in co-infected patients [25]. In virological responders, the best positive predictive value for SVR is achieved when a negative serum HCV–RNA is attained at week 4 of therapy (rapid virological response, RVR), while the best negative predictive value for SVR is seen when HCV–RNA drops <2 logs at week 12 [23, 26, 27]. Higher baseline HCV–RNA levels in co-infected patients with respect to HCV-monoinfected individuals may explain why they achieve less frequently undetectable HCV viremia at week 4 and therefore less often SVR [28]. Alternatively, co-infected patients might show slower HCV decays on HCV therapy [27]. Interestingly, the latter could be overcome at least partially using higher RBV doses [29]. The so-called “2-log stopping rule” refers to the strong predictive value of non-response assessing week 12 virological response. The lack of achieving HCV–RNA declines >2 logs (early virological response, EVR) permits the premature discontinuation of HCV therapy, avoiding side effects and costs, when there is no chance of eradicating HCV infection. Fortunately, this rule works in coinfected as well as in HCV-monoinfected patients [23, 26, 27]. On the other

hand, a negative serum HCV–RNA 6 months after completing anti- HCV therapy, which defines SVR, correlates with the long-term clearance of serum HCV as well as with histological and clinical improvements in most patients [12, 13, 21].

C. Optimal pegylated interferon and ribavirin dosing

Adequate exposure to RBV is crucial to maximise responses to HCV therapy [29], particularly in HIV-co-infected patients [11, 30]. Weight-based dosing seems to well balance the highest efficacy and the lowest limiting toxicities of the drug, namely anaemia. Pharmacokinetic studies have shown a good correlation between RBV plasma levels and HCV–RNA responses [31]. Thus, the use of fixed low doses of RBV (800mg per day) in the old trials conducted in co-infected patients could explain their low SVR [26, 27]. The use of higher RBV doses (1000–1200 mg/day) in the PRESCO trial has confirmed this assumption, since the overall SVR in this trial (50%) is the highest reported so far for co-infected patients Figure 4 [32, 33].
shows the proportion of patients achieving SVR in pivotal trials as a function of distinct RBV doses and HIV status. The benefits of adequate RBV exposure seems to be particularly important in co-infected patients and is not limited to those infected with HCV-1/4, and expand to genotype 3 [33]. In HCV-monoinfected individuals a flat RBV dose of 800 mg/day seems to be enough for genotype 3, as long as therapy is provided for at least 24 weeks. However, shorter periods of therapy seem to require greater RBV doses in order to minimize relapses [34, 35]. The efficacy of higher doses of pegIFN in co-infected patients has been explored in a few studies. In the CORAL-1 trial, the administration of 270_g/week of pegIFN alpha-2a for the first 4 weeks did not improve the early virological response, either considering the proportion of patients with undetectable HCV load at week 4 or with >2 log reductions in HCV–RNA at week 12, as compared with the administration of standard doses (180µg weekly) [36]. However, the size of the study population in that study was relatively small and nearly half of patients carried non-1 HCV genotypes. In contrast, data from some studies conducted in HCV monoinfected individuals have suggested that there is a subset of patients who may benefit from exposure to higher pegIFN doses and therefore this issue still warrants further investigation.

D. Optimal duration of HCV therapy

Studies conducted in HCV-monoinfected patients have shown that RVR, defined as undetectable HCV load at week 4, in patients treated with pegIFN–RBV is the best predictor of SVR and may allow a safety shorten therapy. Accordingly, treatment for only 12- 16 weeks in patients with HCV genotype 3

[34, 35] or for only 24 weeks in HCV genotype 1 [37, 38] have been proposed for patients with RVR. The picture seems to be slightly different in co-infected patients, in whom, however, this high predictive value of SVR in subjects experiencing RVR has also been reproduced [39]. First, HCV load is generally higher in this population, which could explain why a lower proportion of them reach undetectable viremia at week 4 [28]. Second, HCV clearance driven by interferon could be delayed in the HIV setting [27]. Third, the relapse rate upon completion of treatment might be increased in co-infected patients. This was the case for 24 weeks of therapy in HCV-2/3 in earlier trials [23]. For all these reasons, older guidelines recommended that duration of treatment in co-infected patients should be of 48 weeks regardless HCV genotype [40]. Recent studies, however, have questioned these simple views. In a retrospective study conducted in co-infected patients with HCV- 2/3, the subset of them who reached undetectable HCV–RNA at week 4 could safely stop therapy at week 24, with minimal risk of relapse [41]. On the other hand, a retrospective substudy of the APRICOT trial has shown that patients with HCV genotype 1 with low baseline HCV–RNA and RVR obtained high rates of SVR (61%) and did not relapse, suggesting that shorten periods of therapy could have been enough for those patients. Overall, all these data support shorter periods of therapy on the basis of viral response at week 4 in HCV-2/3 co-infected patients. In some patients with slow virological response, extended periods of treatment may permit to achieve SVR [42]. Recognition of detectable viremia at week 4 seems to identify a subset of patients with HCV-1/4 which may benefit from longer duration of therapy as long as it proves to be effective (>2 log drop in HCV–RNA at week 12 followed by undetectable viremia at week 24) [43, 44]. However, the main problem with extended periods of therapy is compliance [33, 44]. This concern can be particularly problematic in co-infected individuals, given that a poor tolerance of the medication has largely impacted negatively on outcomes [26]. The 2007 guidelines from the HCV–HIV International Panel ([10, 45] recommend the therapeutic algorithm recorded in Figure 5. This algorithm has been later endorsed by the European AIDS Clinical Society guidelines [46]. Shorter periods of therapy (24 weeks) could be advised in patients with HCV-2/3 with RVR, as long as HCV load is low, there is good adherence, no advanced hepatic fibrosis exists, and weight-based RBV dosing is provided. For the rest of HCV-2/3 patients, 48 weeks of therapy could still be advisable. In patients with HCV-1/4, extension of treatment beyond 48 weeks could be recommended in the absence of RVR if the medication is well tolerated. However, as previously noted, high drop-out rates might limit the benefit of this strategy [33].

E. Antiretroviral drugs during HCV therapy

Nucleoside reverse transcriptase inhibitors are the backbone of most current antiretroviral regimens. These compounds mimic physiological nucleosides and enter phosphorylation pathways within the cells causing inhibitory competition and chain termination when incorporated into the nascent viral nucleic acid synthesis. Interactions between antiretrovirals and RBV, which is a guanosine analogue, have been a matter of concern for a while, since in most trials conducted so far more than 75% of HIV/HCV-co-infected patients have received pegIFN–RBV along with antiretroviral medications. In earlier studies, only the enhanced risk of anaemia using concomitantly zidovudine (AZT) was the main focus of attention. Use of recombinant erythropoietin has been recommended to counterbalance this deleterious additive hematological side effect, which otherwise may force to reduce RBV doses in a substantial proportion of patients. On the other hand, RBV enhances the intracellular phosphorylated metabolites of didanosine, increasing the risk of mitochondrial toxicities, including pancreatitis, lactic acidosis and hepatic decompensation [47, 48]. The loss of subcutaneous fat typically linked to stavudine may be exacerbated during pegIFN–RBV therapy, mimicking rapidly progressive lipoatrophy [49]. Rather than RBV, it seems to be pegIFN the main agent responsible for this deleterious effect, although an enhanced synergistic mitochondrial toxicity of RBV and stavudine over the subcutaneous fat tissue has not been ruled out.

Recent reports have underlined that abacavir may compromise the activity of RBV and therefore might reduce the efficacy of hepatitis C therapy [47, 50]. Both drugs are guanosine analogues and may compete in their phosphorylation pathways within the cells. This observation has important therapeutic considerations; moreover, it provides further insights about the mechanism of action of RBV. Although still a matter of controversy, these data indirectly but strongly support that RBV is acting as a true antiviral agent against HCV, rather than exerting immune mediated effects. In this regard, the antiviral effect of RBV against HCV may follows the pattern already well demonstrated against other RNA viruses. With respect to tenofovir, it has no deleterious interactions with RBV, since no interference with the antiviral activity of RBV or an increased risk of nephrotoxicity has been shown [51].

F. Management of non-responders and relapsers

As result of a wide prescription of pegIFN+RBV in chronic hepatitis C patients, there is a growing pool of patients who did not respond or relapsed to a prior course of treatment. This circumstance is also recognised in co-infected patients, especially in places where hepatitis C therapy has been actively provided for the last decade. Non-responders and relapsers can be classified

into three groups (see Table 2). Those exposed to suboptimal therapies in the past and show advanced liver fibrosis should be re-assessed and optimal pegIFN plus RBV regimens must be offered again for at least 1 year. In patients who discontinued the medication due to limiting toxicities (e.g. severe anaemia or depression), adequate support with haematopoietic growth factors or antidepressants must be encouraged. Finally, for the growing number of patientswhoshowed virological failure treated with adequate drug dosing and regimens, the only good advise is to avoid potential hepatotoxic factors (e.g. alcohol) and wait for the new antivirals against HCV.

Table 2 Classification of and interventions for HCV/HIV-co-infected patients nonresponders/relapsers to prior interferon-based therapies

Category	Recommended intervention
Suboptimal prior treatment schedules: • *Interferon (monotherapy or with ribavirin)* • *Low ribavirin dosing* • *Short length of therapy*	Re-treatment using combination therapy with peginterferon plus weight-based ribavirin doses
Limiting toxicities and poor adherence	Optimal support (psychiatric, pharmacists, use of hematopoietic growth factors)
Virological failure	Wait until new antivirals come to the market

G. Prospects of new HCV drugs for HIV/HCV co-infected patients

The advent of new antiviral drugs against HCV is eagerly awaited by many HIV+ patients with chronic hepatitis C. Many of them are relatively young, show significant liver fibrosis and have already failed a course of pegIFN–RBV. A few considerations merit attention before a widely introduction of direct antiviral agents (DAA) in the co-infected population (Table 3).

Table 3 Considerations for the use of new HCV antivirals in HIV/HCV-co-infected patients

1. Higher HCV load.
2. Higher rate of HCV-1a than -1b.
3. Greater proportion of HCV genotypes 3 and 4.
4. Potential drug interactions with antiretroviral agents.

Baseline characteristics of hepatitis C in HIV+ patients differ from HIV-negative persons. Higher viral load, greater prevalence of HCV genotypes 3 and 4, more frequent HCV-1a than -1b, and concomitant use of antiretroviral agents may largely influence the performance of STAT-C drugs in the co-infected population. Many of these drugs are less or not effective against HCV genotypes other than HCV-1 [52]. Moreover, in the case of HCV proteaseinhibitors, natural polymorphisms may account for a lower proportion of susceptibility in HCV-1a than -1b variants [53]. This observation coupled with the greater baseline HCV viremia and the potential for drug interactions, have discouraged the use of STAT-C drugs in co-infected patients until now. However, studies with close monitoring of early viral response have already been initiated. Table 4 and Table 5 summarize the main DAA in clinical development and record the main characteristics of the main different drug families.

Table 4 New antivirals against HCV

Protease inhibitors	Polymerase inhibitors	
	Nucleoside analogues	Non-nucleoside analogues
• Ciluprevir*	• Valopicitabine*	• HCV-796*
• ITMN-191/R-7227	• R-1626/R-1479*	• VHC-916
• Telaprevir	• R-7128/PSI-6130	• XTL-2125*
• Boceprevir	• MK-0608	• ANA-598
• GS-9132/ACH-806*	• IDX-184	• GS-9190*
• BI-1335	• VHC-759	• Filibuvir (PF-554)
• TMC-435	• BI-127	
• MK-7009	• MK-3281	
• SCH-900518		

Telaprevir and boceprevir are the two HCV protease inhibitors in more advanced stages of clinical development. Approval is expected for 2011 [54]. In coinfected patients, trials have been initiated with caution and drug development will move slowly following each of the steps in HCVmonoinfected individuals. Moreover, it must be kept in mind that at least initially each of the new antivirals against HCV will be given along with pegIFN+RBV. Moreover, combinations with first generation HCV protease inhibitors and non-nucleoside analogues will only be active against HCV genotype 1, leaving few options for other HCV variants (exclusively with polymerase inhibitors)

Table 5 Main differential features of new direct anti-HCV drugs.

Protease inhibitors	Nucleoside	Non-nucleoside inhibitors
• Interact with the catalytic triad • Genotype-dependent activity for some drugs • Rapid selection of resistance	• Analogues of natural substrates • Need to be phosphorylated • Inhibitory competition • Chain terminators • Similar activity against all genotypes • High genetic barrier to resistance	• 5 distinct target sites at the polymerase • Allosteric inhibition • Genotype-dependent activity • Rapid selection of resistance • Polymorphisms may influence susceptibility

H. Multiple viral hepatitis and HIV

The prevalence of multiple viral hepatitis (HBV/HCV, HBV/HDV, HCV/HBV/HDV) in HIV patients is below 5% in developed countries, but higher than in the general population. In patients with HDVAb+, replication of this virus uniformly predominates over others, with low or undetectable HBV and/or HCV viremia, and rapid progression to cirrhosis [55]. Patients carrying HBV/HCV infections seem to present a reciprocal inhibition of virus replication, predominating one virus over the other. Moreover, this predominance may occasionally fluctuate over time, with one virus taking over the other intermittently. However, in patients with severe immunosuppression, replication of all hepatitis viruses may occur simultaneously. In most HIV+ patients with relatively good immune status, viral interference seems to favour HCV over HBV replication rather than vice versa [56]. However, it is noteworthy that the proportion of subjects with HCV-Ab showing negative serum HCV–RNA is much higher in patients carrying HBsAg [57]. Progression of liver disease seems to be further accelerated in HIV+ patients dually co-infected with HBV and HCV [58] . Moreover, these individuals are more prone to develop HCC [59]. Liver-related mortality is increased in HIV+ patients with multiple viral hepatitis as compared with those with HBV or HCV monoinfection [60]. A few studies have examined the efficacy and safety of IFN–RBV in patients with dual HBV/HCV infections. Overall, most studies have concluded that results are similar. There is little information of the efficacy of pegIFN–RBV in HIV+ patients co-infected with HBV/HCV. Moreover, few data exist regarding the influence of anti-HBV medications on HCVreplication inHBV/HCVdually infected patients. The treatment of all replicating viruses should be pursued, mainly in patients with advanced liver fibrosis. During therapy of one virus, replication of the other should be actively monitored since reactivations of latent infections may occur [61].

Figure 4 Proportion of patients with sustained virological response in three different large trials in HIV-positive and HIV-negative patients using low or weight-based ribavirin (RBV) doses (intent-to-treat analysis).

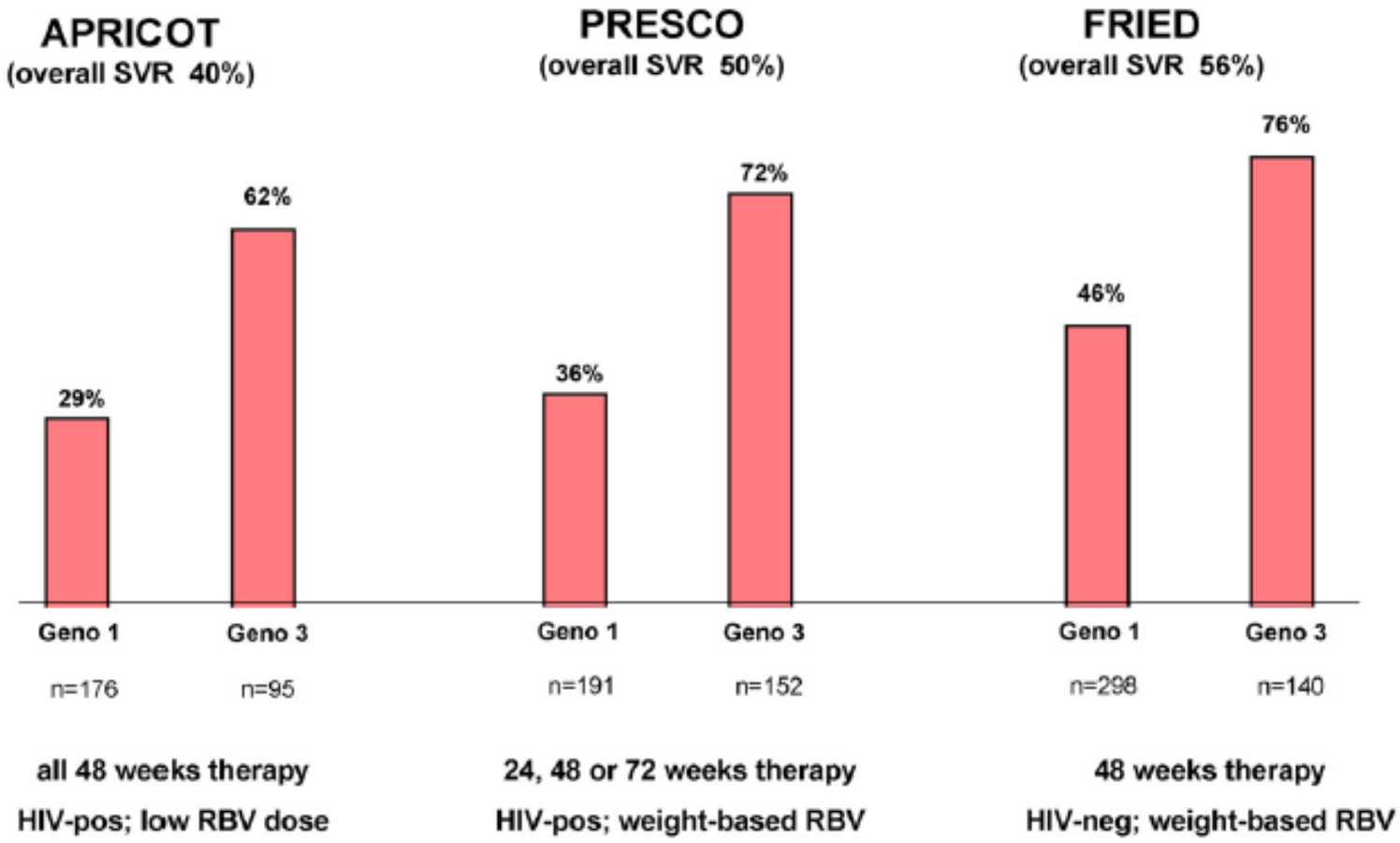
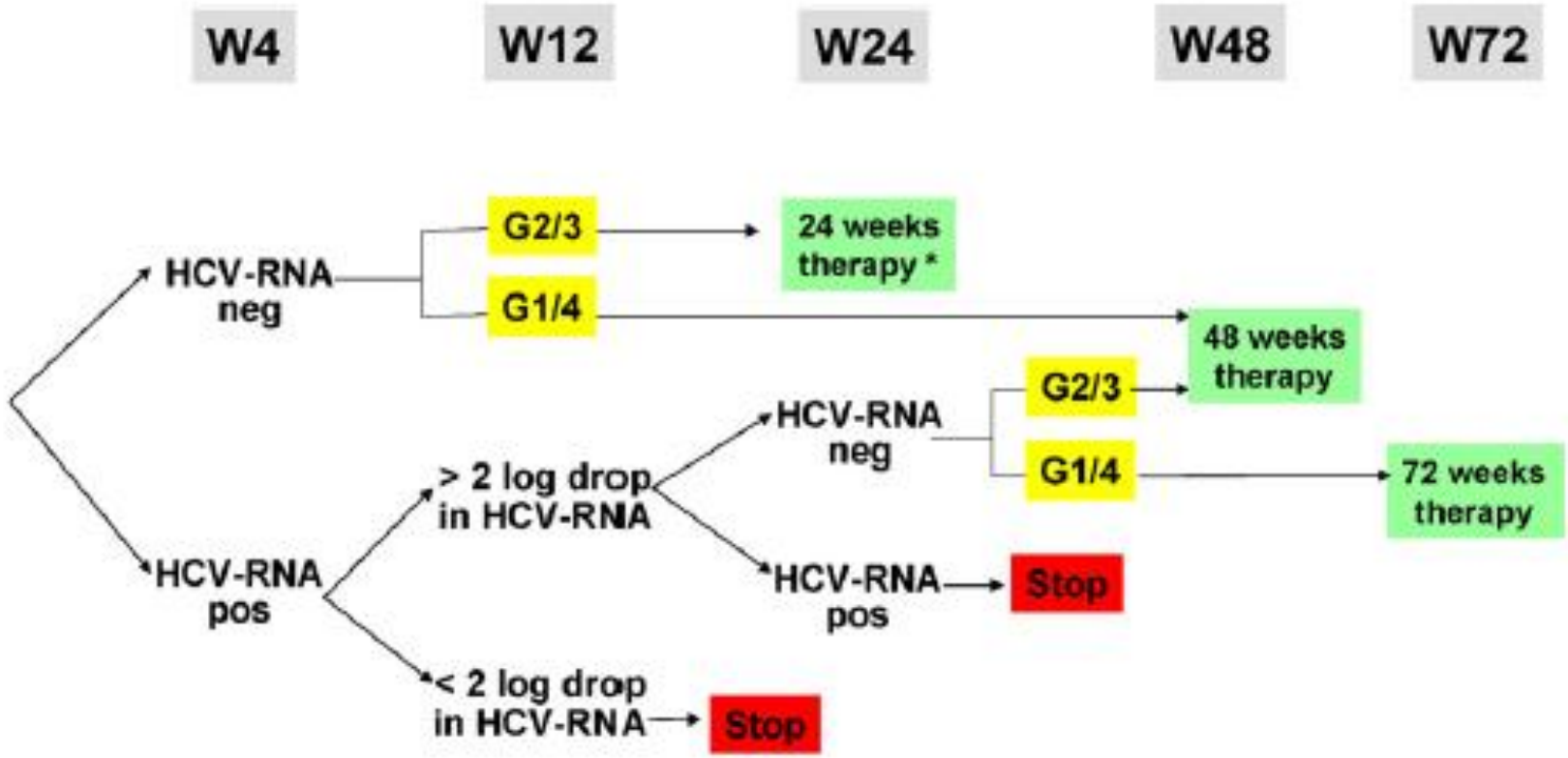


Figure 5 Proposed optimal duration of HCV therapy in HCV/HIV-co-infected patients.



* *In patients with baseline low viral load and minimal liver fibrosis.*

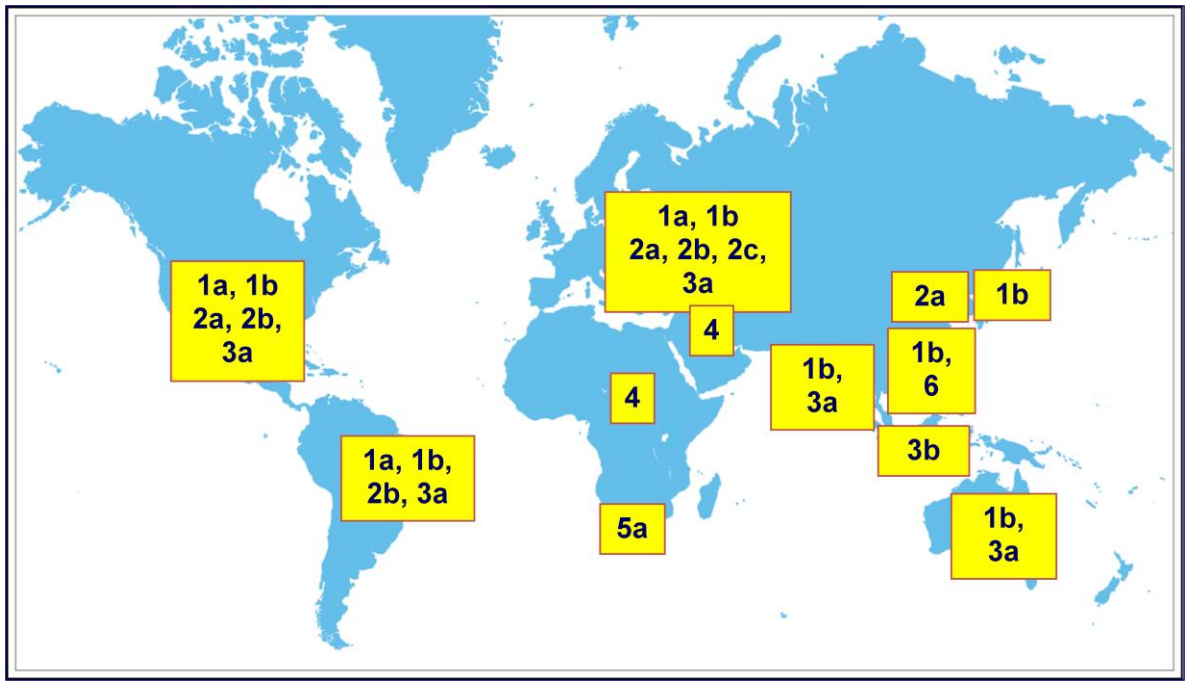
2. Clinical relevance of coinfection

A. Trends in the prevalence of HCV genotypes over time

Because of shared routes of transmission, coinfection with HIV and HCV is relatively common [1,2]. Progression of liver disease is accelerated in coinfected individuals [7, 62]. In Western countries, where highly active antiretroviral therapy (HAART) is widely available, liver disease due to HCV infection has become a leading cause of morbidity and mortality in HIV-infected individuals [5-8].

Six major HCV genotypes (numbered 1 to 6) have been described, of which 1 to 4 are by far predominant in Western countries. Differences in the distribution of HCV genotypes and subtypes in distinct group populations and geographical regions have been associated with differences in natural history and treatment response [63, 64] .

Figure 6 HCV genotype prevalence worldwide.



Most cross-sectional studies have failed to detect any significant change in the prevalence of HCV genotypes in a given region over time. This is most likely due to the limited number of new HCV infections, the relatively low rate of HCV cure with treatment and the large pool of HCV carriers [10,11[65]]. However, as hepatitis C therapy has been increasingly used in some areas and/or risk populations, it would be expected an impact on the most recent distribution of HCV genotypes, since some HCV variants (ie, HCV-2 and HCV-3) are more susceptible to pegylated interferon-ribavirin (pegIFN-RBV) than others (ie, HCV-1 and HCV-4) [33, 63, 66, 67] . If this trend is proven, the recognition of a steadily accumulation of difficult-to-treat patients may stress the need for

prioritizing new anti-HCV drugs in the coinfected population [68], in whom progression to end-stage liver disease occurs faster and a substantial proportion of them already have cirrhosis [69]. Moreover, the characterization of the virological profile of the currently alive HIV-HCV coinfected population must be considered as particularly important, since some of the new drugs against HCV only or mainly target HCV genotype 1.

B. HIV status as a prognostic factor for HCV relapse

Treatment with pegylated interferon (pegIFN) plus ribavirin (RBV) permits to achieve sustained virological response (SVR) in 30-70% of patients with chronic hepatitis C virus (HCV) infection. Viral genotype and co-morbidities, as HIV coinfection, are important determinants of different outcomes following HCV therapy [1-5]. Although still a matter of some controversial, HCV eradication is presumed to have occurred in most patients who attain undetectable serum HCV-RNA at the end of treatment and persist negative 24 weeks thereafter [6]. In comparison with chronic hepatitis C patients who remain untreated or those who do not achieve SVR, treated individuals with SVR show over time persistently normal values of liver enzymes, improvements in liver histology [7,8] and no or minimal liver-related morbidity and mortality [9,10]. The chances for HCV reappearance once SVR has been attained seem to be very rare and HCV reinfection rather than late HCV relapse seems to be more common [11,12].

In HCV-monoinfected patients, HCV relapses generally occurs early after discontinuation of successful therapy, within the first 12 weeks in 98% of cases in one study [13]. Given that HIV coinfection is frequently seen in patients with chronic hepatitis C and HIV-associated immune abnormalities might hypothetically impair definitive HCV clearance following hepatitis C therapy, we examined the rate, timing and predictors of HCV relapse in a relatively large group of HIV-HCV coinfected patients treated with pegIFN plus RBV. This information is particularly relevant now that clinical trials testing the efficacy of new hepatitis C drugs have begun in both HCV-monoinfected and HCV-HIV coinfected patients, and there is pressure for moving definitions of treatment success to earlier time points upon completion of therapy.

C. Applicability of a prognostic score to predict HCV clearance

Hepatitis C virus (HCV) infects more than 175 million people worldwide [70]. In western countries, HCV is the leading cause of end-stage liver disease and

hepatocellular carcinoma, as well as the main indication for liver transplantation [70]. HCV and HIV-1 share routes of transmission and establish chronic infections; therefore coinfection is relatively common (15-40%) [6]. The course of HCV-associated liver disease is accelerated in dually infected individuals [71, 72], and thereby HCV has emerged as an important cause of morbidity and mortality in persons infected with HIV-1 [73], especially since successful antiretroviral therapy has dramatically reduced the rate of opportunistic illnesses. Current therapy for chronic hepatitis C is based on a combination of peginterferon-α (pegIFN) and ribavirin (RBV) administered for 6 to 18 months, depending on viral kinetics and genotype [74]. Unfortunately, the medications are poorly tolerated and results in low response rates, with only half of the patients achieving HCV clearance. This figure is lower in HIV/HCV-coinfected patients [6, 75]. Thus, identification of predictors of treatment success is desirable in order to select the best candidates for currently available therapy and for encouraging a course of HCV therapy in this population, which unfortunately remains largely untreated in most places [76-78].

It is well established that infection due to HCV genotypes 2 or 3, low serum HCV-RNA and null or minimal liver fibrosis are the best predictors of response to therapy [79]. Recently, three independent genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) around the IL28B gene (coding for IFN-λ-3) that are strongly associated with treatment outcomes in HCV-monoinfected individuals [17, 80-83]. The SNP with the strongest association, rs12979860, is located in chromosome 19q13, 3kb upstream of the IL28B gene. In patients infected with HCV genotype 1, the rs12979860 CC genotype is associated with a more than two fold greater rate of sustained virological response (SVR) than the CT or TT genotypes, regardless their HIV status [80, 84]. Endogenous production of IFN-λ is crucial in HCV clearance but pathways and relationship with host genetics are still not well understood.

Identifying the prognostic factors associated with good response at baseline and constructing diagnostic tools to predict outcomes should help clinicians to aim HCV therapy towards potentially curable patients [85-88].

3. Hypothesis and Objectives:

A. Hypothesis

Hypothesis was made that HCV clearance can be estimated prior to initiate therapy with Pegylated Interferon plus Weight based Ribavirin with a non-invasive score in HIV-HCV coinfected patients.

B. Objectives:

Each objective was followed by an original article and will be presented in the results section.

1. First objective

To describe the cohort of coinfected patients on regular follow- up at Hospital Carlos III and to describe Hepatitis C treatment uptake during the period 2000-2009.

The following steps were required:

1. To describe the demographic characteristics of the patients on regular follow-up at Hospital Carlos III during the period 2000-2008.
2. To describe the annual incidence of coinfected patients who entered the cohort during the period 2000-2008
3. To describe the annual mortality in the cohort.
4. To describe annual uptake of Pegylated Interferon and Ribavirin among coinfected patients.
5. To describe the annual rate of sustained virological response in the cohort.
6. To describe the annual prevalence of coinfected patients viremic por HCV.
7. To estimate the impact of HCV therapy among the prevalence of HCV genotypes and subtypes in the cohort

2. Second objective

To analyze the predictive factors associated with HCV clearance, including HIV status and to compare rates of HCV relapse among HIV infected and uninfected patients

The following steps were required:

1. To determine the prognostic value of HIV coinfection for HCV relapse
2. To assess the prognostic value of a single nucleotide polymorphism for SVR
3. To assess the predictive value of liver stiffness for SVR
4. To determine the predictive value of other host and viral variables

3. Third objective

Third objective was the development and validation of a prognostic score to predict Sustained Virological response in HIV-HCV coinfected patients treated with PegInterferon plus Weight based Ribavirin.

Two steps were required:

1. The development of the score with a local cohort
2. The external validation with an independent cohort

The objectives reported before are expressed with the 3 following questions:

First objective:

How the cohort of coinfected patients treated at Hospital Carlos III looks like?

Second objective:
Do coinfected patient have higher rates of HCV relapse and is HIV coinfection an independent factor associated to HCV relapse?

Third objective:
What would be the accuracy of a score constructed with the strongest predictive variables associated to sustained virological response?

4. Methods

A. Study designs

1. For the primary objective

To construct a predictive index of sustained virological response (PISVR), the study population was selected from a cohort of HIV-HCV coinfected patients with regular follow-up at Hospital Carlos III (HCIII), Madrid (development cohort). Characteristics of the whole cohort and HCV therapy uptake will be described through the secondary objective. To obtain an external validation of the PISVR, this index was tested on a population of HIV-HCV coinfected patients treated during the same period at the Hospital Universitario de Valme (HUV), Seville (validation cohort). The score was constructed and validated with a diagnostic study.

2. For the other objectives

We conducted a retrospective analysis of all patients with chronic hepatitis C, naïve for interferon, who initiated treatment with subcutaneous pegIFN, alpha-2a or alpha-2b, plus oral ribavirin, between January 2001 and January 2008, at a single tertiary hospital located in Madrid, Spain. Both HIV-positive and negative patients were included. HBsAg positive patients were excluded from this analysis. Criteria for indication of hepatitis C therapy followed international guidelines, both in HCV-monoinfected and HIV/HCV coinfected patients. We did a longitudinal retrospective cohort study.

B. Regulatory and Ethical considerations

To participate in the diagnostic study, written informed consent for genetic testing was obtained from all individuals attended at Hospital Carlos III (Annex 4), and the study protocol was evaluated and approved by the hospital ethics committee (Annex 1). The epidemiological part of the study was in accordance to current regulatory laws.

C. Study population:

1. Internal cohorts, from the Infectious diseases Unit of Hospital Carlos III

a) Main cohort:

All consecutive HIV-HCV coinfected individuals on regular follow-up at the Infectious diseases Unit were enrolled in a dynamic cohort as soon as HCV genotype was available (**¡Error! No se encuentra el origen de la referencia.**). Patients with a follow-up shorten than one year, including those entering the cohort in year 2008 were excluded. The decision to exclude individuals with short follow-up was taken to avoid consideration of patients only seen sporadically (ie, subjects from other centres asking for second medical opinion) and not being regular attenders at our institution. The main baseline demographic information was collected retrospectively. Clinical and outcome data, including exposure and response to hepatitis C therapy were obtained from the main clinical database and further validated checking pharmacy records.

b) Relapsers cohort

We conducted a retrospective analysis of all patients from the main cohort to determine the prognostic factors associated with HCV relapse. For the purpose of this study, only patients who attained serum HCV-RNA levels <10 IU/mL at the planned end-of-therapy (EOT) were analysed. PegIFN alpha-2a had been given subcutaneously at doses of 180 μg per week and pegIFN alpha-2b had been prescribed at doses of 1.5 μg/Kg per week. Oral RBV dosing was adjusted to weight (~13 mg per kg); in all instances was 1000 mg/day if <75 Kg and 1200 mg/day if >75 Kg. Treatment had been given for 48 weeks, although in patients with HCV genotypes 2 or 3 infection who had attained rapid virological response (RVR, serum HCV-RNA <10 IU/mL at week 4), treatment was given for only 24 weeks since year 2006. A cohort of HCV monoinfected patients with similar characteristics was also identified (2.a).

c) Developmental cohort

From the main cohort (a)), we selected 159 individuals who initiated therapy with pegIFN-RBV and had validated outcomes. This population was treated from November 2004 to December 2008. Inclusion criteria included patients for whom HCV genotype was done at our institution, were IFN-naïve, who had benefited from liver stiffness assessment 6 months before treatment and could be tested for rs12979860. To avoid a selection bias in the IL28b testing, patients without stored blood samples were tested prospectively. Patients with poor drug compliance and/or who discontinued therapy due to side effects were excluded, as were patients with HBV coinfection.

2. External cohorts, from other units

a) HCV Monoinfected patients from the Gastroenterology Unit of the Hospital Carlos III, Madrid.

The purpose of this study was to assess the prognostic factors related to HCV relapse, both in mono and coinfected patients. For this purpose, only patients who attained serum HCV-RNA levels <10 IU/mL at the planned end-of-therapy (EOT) were analysed.

b) HIV-HCV Coinfected patients from the Unit of Infectious diseases of Hospital Universitario de Valme, Seville (validation cohort)

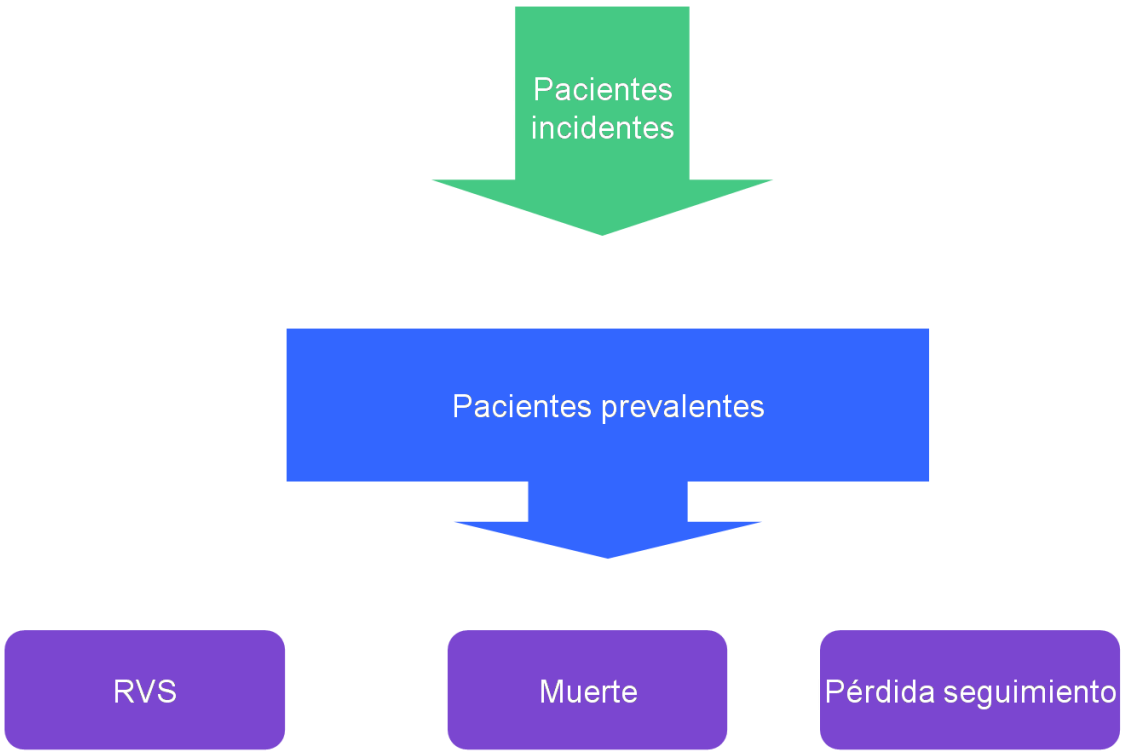
In all, 154 patients completed therapy during the same period at the Unit of Infectious Diseases, Hospital Universitario de Valme Seville, Spain. Inclusion criteria were the same as in the developmental cohort. In this cohort, 68 patients benefited from a liver biopsy but were not assessed for liver stiffness and were excluded. Finally, 86 patients were eligible for the study. Table 15 reports clinical data available for this group.

D. Follow-up:

1. In the dynamic cohort

In order to establish a dynamic cohort of HCV viremic HIV patients and then analyse trends in the annual distribution of HCV genotypes, as trends in other characteristics, the date of the first hospital visit was considered as the entry date in the cohort.

Figure 7 Flow chart resulting in prevalent patients



For patients who achieved a sustained virological response (SVR) after a course of pegIFN-RBV therapy, the end of treatment date was considered as the time of exit from the cohort, since these patients no longer remained HCV viremic. The rest of viremic untreated patients and those who experienced HCV relapse upon treatment discontinuation continued to be considered as active in the cohort until the date of the last hospital visit (Figure 7). In patients for whom visits were lost at a given time, mortality was checked at the national registry from the Spanish Institute of Statistics.

2. In the prognostic studies

In clinical routine, follow up was done for each 3 months during and after therapy.

E. Outcomes

1. Sustained virological response

Sustained virological response was the primary outcome and was defined as undetectable serum HCV-RNA 24 weeks after the end of treatment. The rest of the patients were considered relapsers [75] and were excluded from the SVR group. For the purpose of this study, relapsers were considered along with non-responders (NR), who were patients who experienced suboptimal virological response during the treatment period and for these reasons did not complete the planned duration of therapy. Patients with poor drug compliance and/or who discontinued therapy due to side effects were excluded from the NR group.

2. Relapse.

The main end-points of the study were to determine the prevalence and timing of HCV-RNA rebounds in patients with EOT response, with longitudinal assessments of serum HCV-RNA at least at weeks 12, 24, 36 and 48 weeks upon completion of hepatitis C therapy. Three types of HCV relapse were defined: i) early, when HCV-RNA rebounds occurred between EOT and week 12; ii) late, when recognized between weeks 12 and 24; and iii) very late, when occurring beyond week 24. Late relapses had to show serum HCV-RNA <10 IU/mL at week 12 and detectable viremia at week 24. In very late relapses, HCV-RNA <10 IU/mL had to be proven at week 24 and being positive any time thereafter. Given the retrospective nature of the study, when virological data were not available at all time points following completion of therapy, testing was made on -70ºC stored plasma specimens.

3. Non response.

For the purpose of these studies, non responders were defined as treated patients who couldn´t achieve a sustained virological response. This definition included relapsers and patients who experienced breaktrougt and suboptimal response at week 12.

4. Lost-to-follow-up and Deaths

In patients for whom visits were lost at a given time, mortality was checked at the national registry from the Spanish Institute of Statistics. Patients not registered as dead in the national database and not present in the HCIII during 2008 were considered as lost to follow up.

F. Explicative variables

1. Host related

a) Demographic characteristics

Variables which may influence HCV relapses were investigated comparing patients who achieved SVR (serum HCV-RNA <10 IU/mL 24 weeks after EOT) and subjects with HCV-RNA rebound at any time point following completion of therapy. The pharmacy database was initially consulted to retrieve patients that had initiated hepatitis C therapy with pegIFN plus RBV. Demographics, laboratory values and treatment outcomes were then obtained from the clinical database.

b) Comorbidities

(1) HIV coinfection

Most important variables were collected. Viral load at baseline, suppressive HAART, CD4 count, CD4 nadir.

(2) Liver fibrosis

The extent of liver fibrosis was measured, in both cohorts, using transient elastography by FibroScan (Echosens®, Paris, France). Details about this non-invasive method, the examination procedure, and correlation of liver fibrosis estimates with liver biopsy have been reported elsewhere [89-91]. The median value of all tests is expressed in Kilopascal (kPa). In clinical practice, advanced liver fibrosis (severe fibrosis or cirrhosis, corresponding to METAVIR scores F3 and F4) is defined for liver stiffness values $\geq$9.5 kPa, according to previous reports from both HCV-monoinfected and HIV/HCV-coinfected patients [19, 92].

As transient elastometry was available at our institution only after year 2004, estimation of liver fibrosis stage in all patients recruited in the study before was made using a composite of three different serum biochemical indexes: APRI index [AST / upper limit of normal (ULN) x 100 / platelet count (10^9/L)] [19], FIB-4 [age x AST [IU/L] / (platelet count [10^9/L]) x (ALT [IU/L])$^{1/2}$)] [20] and Forns [7.811 - 3.131 * ln(platelet count) + 0.781 * ln(GGT) + 3.467 * ln(age) – 0.014 * (cholesterol)] [21].

(3) Alcoholism

Patients were asked by alcohol consumption at each visit. Result were categorized as >60gr/day, <60 gr/day and any-consumption.

c) Single nucleotide polymorphism *rs12979860*

For patients from Hospital Carlos III, genotyping was performed by the Duke Institute for Genome Sciences and Policy. Genotyping was conducted in a blinded fashion on DNA specimens collected from each individual, using the 5' nuclease assay with allele specific TaqMan probes (ABI TaqMan allelic discrimination kit and the ABI7900HT Sequence Detection System (Applied Biosystems, Carlsbad, CA, USA) . For patients from HUV, similar primers and procedures were done at a local laboratory.

2. HCV related

a) HCV viral load

In both cohorts plasma HCV-RNA was measured using a real-time PCR assay (COBAS TaqMan, Roche, Barcelona, Spain), whose lower limit of detection is 10 IU/mL.

b) HCV genotypes and subtypes

HCV genotyping was performed using a commercial RT-PCR hybridization assay (Versant HCV Genotype v2.0 LiPA, Siemens, Barcelona, Spain), which maximally reduces the chances of HCV genotype misclassification.

c) Phylogenetic analyses

In order to investigate the source of HCV-RNA rebounds beyond week 24 upon completion of therapy and try to elucidate whether HCV recurrences or reinfections had occurred, phylogenetic trees were constructed in which viral sequences obtained at the time of last detectable viremia on therapy and at the time of first HCV rebound off therapy were compared. As control, similar analyses were performed examining viral sequences obtained from patients who experienced early HCV relapses. In all instances a fragment of the viral E1/E2 coding region was amplified and directly sequenced using a commercial DNA sequencing kit (Applied Biosystems, Foster City, CA). The comparison of sequences was performed using the Clustal X Multiple Sequence Analysis Program, and phylogenetic trees were constructed using the DNASTAR Lasergene Software and TreeView (Win32 version 1.6.6).

d) HCV therapy

In both cohorts, treatment regimens included pegIFN alpha 2a or 2b at standard doses (180 μg/week or 1.5 μg/kg/week, respectively) plus weight-adjusted RBV (1000 mg/day for patients weighing <75 kg and 1200 mg/day for patients weighing >75 kg). In accordance with international guidelines [6] , patients with

HCV genotypes 1 or 4 received either 48 or 72 weeks of treatment, and patients with HCV genotype 3 received 24 or 48 weeks of treatment, according to virological response at week 4. Early stopping rules were applied for subjects with suboptimal virological response at weeks 12 and 24 [6].

G. Statistical methods

Statistical methods are adapted to the objectives of the study.

Overall, results are presented as medians, percentile 25 and percentile 75 for continuous variables and as frequencies and percentages for categorical data. Categorical data and proportions were analyzed using the chi-squared test or Fisher's exact test, as required. The Student T-test was used to compare the means of the two groups with normal distributions.

Epidemiological study

In the epidemiological study, trends were analysed using a simple linear regression model. Proportion of genotypes/subtypes was the dependent variable and calendar year the non-dependent variable

Prognostic study

Descriptive statistics were expressed as mean and standard deviation (SD); and percentages for continuous and categorical variables, respectively. Baseline characteristics were compared in HIV-positive versus HIV-negative patients using the chi-square test and non-parametric tests, as needed. Univariate and multivariate logistic regression analyses were performed to calculate the odds ratio (OR) and 95% confidence intervals (95% CI) for variables associated with HCV relapses. Only variables reaching p values below 0.2 in the univariate analysis were used in the multivariate stepwise regression analysis.

Most statistical analyses were made by an independent and external statistician, who was blind to the main variables associated with outcomes.

Multiple association tests were conducted using univariate logistic regression to indentify the independent variables associated with the primary outcome (SVR). In the last analysis we included all variables that were statistically significant ($p<0.05$) in the univariate analysis. A forward stepwise logistic regression analysis was conducted with a p-value for entry and exit of 0.05 and 0.10 respectively.

Diagnostic study

We developed an index to predict SVR via a logistic probability function that we have denominated predictive index of sustained virological response (PISVR). The accuracy and the predictive values of the PISVR were obtained and compared by calculating the areas under the receiver operating characteristic

curves (AUC-ROCs) for the development and validation cohorts. We evaluated several cut-offs for the PISVR; to obtain a higher sensitivity (Se) and negative predictive value (NPV), the lowest cut-off was established at 0,25. To obtain a higher specificity (Sp) and positive predictive value (PPV) the highest cut-off was 0,75. Finally, we analyzed an “optimal” cut-off near the maximum sensitivity and specificity, at 0.5. We also calculated the diagnostic odds ratio (DOR) which expresses the strength of the association between test result and disease: it is the ratio of the odds of a positive result in a person with the target condition compared to a person without the condition (18). A DOR of 1 suggests that the test provides no diagnostic evidence. Finally, we calculated the likelihood ratios (LR) which describe how many times a person with the target condition is more likely to have a particular test result than a person without that condition. LRs contribute to change, after the test has been made, the probability that a target condition is present. Binary tests have two LRs, positive and negative (LR+, LR). A LR of 1 indicates no diagnostic value.

All tests were two-tailed with P values <0.05 considered to be significant. Statistical analysis was performed by SPSS 16.0 software (SPSS INC, Chicago, IL, USA) and STATA 9.1.

5. Results

A. First article: Hepatitis C virus (HCV) treatment uptake and changes in the prevalence of HCV genotypes in HIV-HCV coinfected patients.

Journal of Viral Hepatitis, 2010 doi:10.1111/j.1365-2893.2010.01309.x

Hepatitis C virus (HCV) treatment uptake and changes in the prevalence of HCV genotypes in HIV/HCV-coinfected patients

J. Medrano,[1] S. Resino,[2] E. Vispo,[1] A. Madejón,[1] P. Labarga,[1] P. Tuma,[1] L. Martín-Carbonero,[1] P. Barreiro,[1] S. Rodriguez-Novoa,[3] I. Jiménez-Nacher[3] and V. Soriano[1] [1]*Department of Infectious Diseases & CIBERehd, Hospital Carlos III, Madrid, Spain;* [2]*Research Unit, Instituto de Salud Carlos III, Majadahonda, Spain; and* [3]*Pharmacy Unit, Hospital Carlos III, Madrid, Spain*

Received November 2009; accepted for publication February 2010

SUMMARY. The efficacy of current hepatitis C therapy in HIV/HCV-coinfected patients is largely dependent on HCV genotype. The annual prevalence of HCV genotypes/subtypes and their influence on HCV clearance with antiviral treatment were examined in a dynamic cohort of HIV/HCV-coinfected patients followed up in Madrid since 2000. Patients entered the cohort at first visit and left the cohort when HCV clearance was achieved with HCV therapy or when follow-up was interrupted for any reason, including death. A total of 672 HIV/HCV-coinfected patients constituted the cohort. The mean follow-up time was 5.5 years, corresponding to 4108 patient-years. Mean age at entry was 37 years, and 73% were men and 86% were intravenous drug users. Overall distribution of HCV genotypes was as follows: 57.1% HCV-1 (1a: 29.2%, 1b: 20.4%, unknown: 7.6%), 1.3% HCV-2, 25.4% HCV-3 and 15.9% HCV-4. A total of 274 (40.8%) patients were treated with peginterferon–ribavirin, of whom 116 (42.3%) achieved HCV clearance following 1–3 courses of therapy. The proportion of HCV-1/4 rose from 71.7% in 2000 to 76.8% in 2008, whereas the proportion of HCV-2/3 fell from 28.1% in 2000 to 23.2% in 2008. The yearly prevalence increased for HCV-1 (R^2: 0.92, b: 0.59, $P < 0.001$) and HCV-4 (R^2: 0.77, b: 0.33, $P < 0.005$) and conversely diminished for HCV-3 (R^2: 0.94, b: −0.82, $P < 0.001$). In summary, the prevalence of HCV-1 and HCV-4 has increased over the last decade in HIV/HCV-coinfected patients, whereas conversely it has declined for HCV-3, in association with the wider use of HCV therapy (41%) in this population.

Keywords: HCV genotypes, hepatitis C, HIV, pegylated interferon, treatment uptake.

INTRODUCTION

Because of shared routes of transmission, coinfection with HIV and HCV is relatively common [1,2]. Progression of liver disease is accelerated in coinfected individuals [3,4]. In Western countries, where highly active antiretroviral therapy (HAART) is widely available, liver disease as a result of HCV infection has become a leading cause of morbidity and mortality in HIV-infected individuals [5–8].

Six major HCV genotypes (numbered 1–6) have been described, of which 1–4 are by far the most predominant in Western countries. Differences in the distribution of HCV genotypes and subtypes in distinct group populations and geographical regions have been associated with differences in natural history and treatment response [9,10].

Most cross-sectional studies have failed to detect any significant change in the prevalence of HCV genotypes in a given region over time. This is most likely because of the limited number of new HCV infections, the relatively low rate of HCV cure with treatment and the large pool of HCV carriers [10,11]. However, as hepatitis C therapy has been increasingly used in some areas and/or risk populations, it would be expected to have an impact on the most recent distribution of HCV genotypes, because some HCV variants (i.e. HCV-2 and HCV-3) are more susceptible to pegylated interferon–ribavirin (pegIFN-RBV) than others (i.e. HCV-1 and HCV-4) [9,12–14]. If this trend is proven, the recognition of a steady accumulation of difficult-to-treat patients may stress the need for prioritizing new anti-HCV drugs in the coinfected population [15], in whom progression to end-stage liver disease occurs faster and a substantial proportion of them already have cirrhosis [16]. Moreover, the characterization of the virological profile of the currently alive HIV/HCV coinfected population must be considered as particularly

Abbreviations: HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; PCR, polymerase chain reaction; pegIFN-RBV, pegylated interferon–ribavirin; SVR, sustained virological response.

Correspondence: Dr Vincent Soriano, Department of Infectious Diseases, Hospital Carlos III, Calle Sinesio Delgado 10, 28029 Madrid, Spain. E-mail: vsoriano@dragonet.es

important, because some of the new drugs against HCV only or mainly target HCV genotype 1.

PATIENTS AND METHODS

Study population

All consecutive HIV/HCV coinfected individuals on regular follow-up at a reference HIV/AIDS clinic located in Madrid, Spain, were enrolled as soon as the HCV genotype was available. Patients with a follow-up of <1 year, including those entering the cohort in year 2008, were excluded. The decision to exclude individuals with short follow-up was taken to avoid consideration of patients only seen sporadically (i.e. subjects from other centres asking for second medical opinion) and not being regular attendees at our institution. The main baseline demographic information was collected retrospectively. Clinical and outcome data, including exposure and response to hepatitis C therapy, were obtained from the main clinical database and further validated checking pharmacy records.

To establish a dynamic cohort of HCV/HIV patients and then analyse the annual distribution of HCV genotypes, the date of the first hospital visit was considered as the entry date in the cohort. For patients who achieved a sustained virological response (SVR) after a course of pegIFN-RBV therapy, the end of treatment date was considered as the time of exit from the cohort, because these patients were no longer HCV viremic. The rest of the viremic untreated patients and those who experienced HCV relapse upon treatment discontinuation continued to be considered as active in the cohort until the date of the last hospital visit. For patients who stopped their visits at a given time, mortality records were checked at the national registry from the Spanish Institute of Statistics.

Virological assessment

Serum HCV-RNA was initially tested in all HCV antibody positive specimens using a commercial assay (HCV Cobas Amplicor; Roche, Madrid, Spain). In viremic samples, serum HCV-RNA was subjected to reverse transcription using a commercial kit (Promega Corporation, Madison, WI, USA) following the manufacturer's instructions. The cDNA obtained was amplified by polymerase chain reaction (PCR) using conserved primers from the 5′-noncoding region of HCV provided in the LIPA HCV-RNA Amplification Kit (Innogenetics, Ghent, Belgium). PCR products were genotyped using a reverse hybridization line probe assay (INNOLIPA HCV III; Innogenetics).

Liver fibrosis assessment

Estimates of liver fibrosis were obtained measuring hepatic stiffness using transient elastography [17,18], routinely performed since year 2004 in all patients. Liver stiffness values <7.1, between 7.2 and 9.5, 9.6 and 12.5, and >12.6 KPa were classed as Metavir scores F0F1, F2, F3 and F4, respectively.

Statistical analysis

The chi square test was used to compare the proportions in the annual distribution of HCV genotypes between calendar years, and P values below 0.05 were considered significant. Trends were analysed using a simple linear regression model with ANOVA. Proportion of genotypes/subtypes was the dependent variable and calendar year the nondependent variable. All statistical analyses were performed using SPSS v15.0 software (SPSS Inc, Chicago, IL, USA).

RESULTS

Baseline characteristics

From a total of 672 HIV/HCV coinfected patients who entered the dynamic cohort, 489 (73%) patients were men. At entry, mean age was 36.6 (±5.8) years. Most individuals (94.8%) were native Spaniards, and the main mode of transmission was intravenous drug use (86.5%).

The overall distribution of HCV genotypes in the study cohort over the entire decade was as follows: HCV-1 57.1% (1a: 29.2%, 1b: 20.4%, unknown subtype: 7.6%), HCV-2 1.3%, HCV-3 25.4% and HCV-4 15.9%. One subject was infected with HCV genotype 6 and none with genotype 5.

Estimates of liver fibrosis using transient elastography were available for 545 (81.1%) of the whole HIV/HCV coinfected study population. At the first assessment in patients with active HCV infection, the distribution of Metavir scores was as follows: F0F1 in 311 (57.1%) patients, F2 in 83 (15.2%), F3 in 57 (10.5%) and F4 in 94 (17.2%). Overall, 27.8% of patients could be considered as having advanced liver fibrosis (Metavir score estimates F3-F4). During the study period, 419 patients underwent longitudinal liver stiffness assessments. After a mean time of 2.8 years (±0.98) from the first measurement, the distribution of Metavir scores in the population was as follows: F0F1 in 111 (26.5%), F2 in 94 (22.4%), F3 in 77 (18.4%) and F4 in 137 (32.7%). At the last assessment, 51.1% of patients had advanced liver fibrosis (Metavir score estimates F3–F4).

HCV genotypes in cohort incident patients

A total of 403 patients were present in the cohort before year 2000, and 268 patients entered the dynamic cohort during the study period (Fig. 1). There was a steady decline in the number of new HCV-infected patients entering the cohort over the study period (86 entered in year 2000 and 16 in year 2007). As shown in Fig. 2a, the proportion of incident genotype 4 increased 3% annually (R^2: 0.67, b: 2.98, P = 0.01).

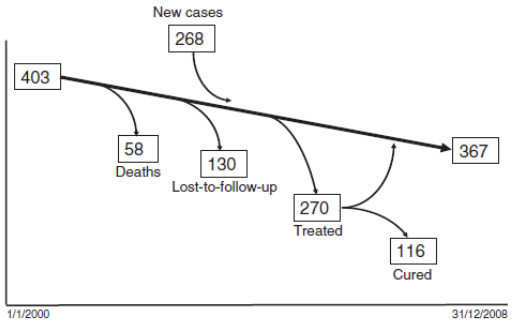


Fig. 1 Dynamic cohort of HIV/HCV coinfected patients – study population flow chart.

HCV genotypes in patients leaving the cohort

A total of 274 (40.8%) of 672 coinfected patients in the cohort were treated with pegIFN-RBV. Among them, 161 (58.8%) completed the planned duration of hepatitis C therapy. Overall, 116 patients achieved SVR (intent-to-treat rate: 42.3%; on-treatment rate: 72%). It should be noted that this rate of HCV clearance occurred after a first course of therapy in two-thirds of patients, while in the rest SVR resulted from repeated (one or two additional) courses.

The overall mean time of follow-up was 5.5 years, corresponding to 4108 patient-years. During the study period, 188 patients left the cohort for other reasons than cure following hepatitis C therapy (116; 38.1%). A total of 58 (19.1%) patients died, and 130 (42.7%) were lost to follow-up (Fig. 2b).

Figure 3 records the yearly distribution of HCV genotypes among patients who left the cohort because of HCV clearance following hepatitis C therapy. It must be noted that HCV clearance with treatment was achieved more than twofold more frequently in patients infected with HCV-2/3 (57/83; 68.7%) than in HCV-1/4 carriers (59/191; 30.9%) ($P < 0.001$).

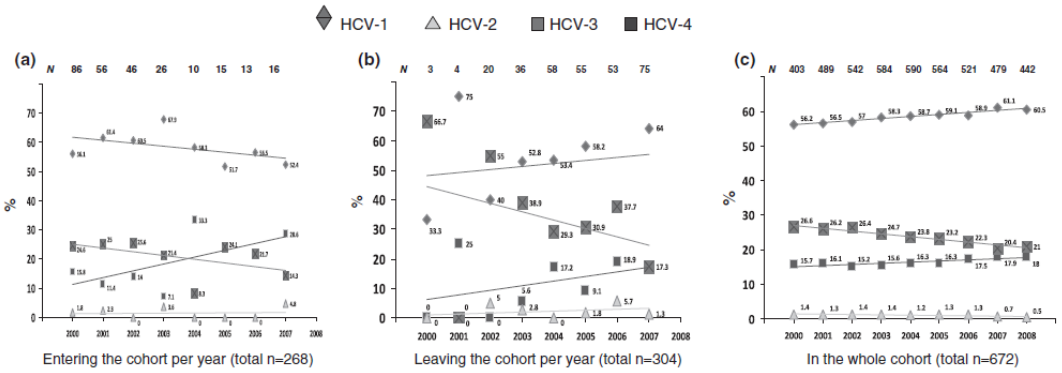


Fig. 2 Annual proportion of HCV genotypes. (a) Entering the cohort per year (total $n = 268$). (b) Leaving the cohort per year (total $n = 304$). (c) In the whole cohort (total $n = 672$).

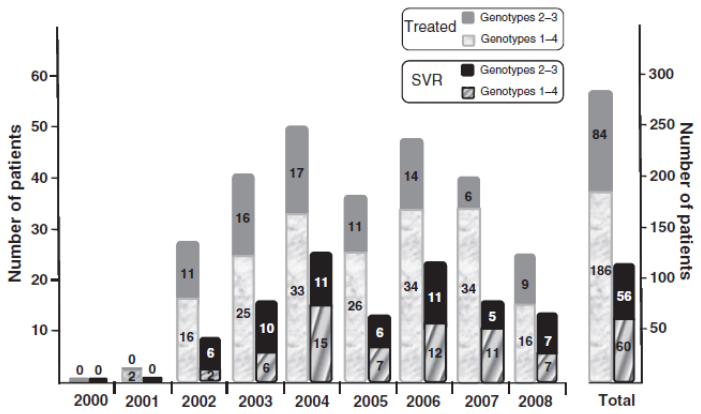


Fig. 3 Annual uptake of pegInterferon plus ribavirin therapy and HCV clearance in the cohort, according to HCV genotype.

Table 1 Trends in the prevalence of HCV genotypes (and subtypes) in the dynamic cohort from 2000 to 2008

HCV genotype (or subtype)	B coefficient	IC 95%	R^2	*P*
1	0.59	043 to 0.74	0.92	<0.001
2	−0.09	−0.16 to −0.03	0.63	0.010
3	−0.82	−1.0 to −0.65	0.94	<0.001
4	0.33	0.17 to 0.49	0.77	0.002
1a	0.47	0.28 to 0.66	0.83	0.001
1b	−0.13	−0.22 to 0.19	0.03	0.884

HCV genotype prevalence by calendar

As a result of entries and exits into the dynamic cohort (Figs 2a,b), the yearly prevalence of HCV genotypes showed significant variations. The final distribution of HCV genotypes in 2008 was as follows: 60.5% HCV-1 (1a: 31.3%, 1b: 20.4%, unknown subtype: 8.7%), 0.5% HCV-2, 21% HCV-3 and 18% HCV-4.

There was an increase in HCV genotypes 1 and 4 from 72% in 2000 to 78.5% in 2008 ($P = 0.041$). Conversely, as shown in Fig. 2c, there was a decline in HCV genotypes 2 and 3 from 28% in 2000 to 21.5% in 2008 ($P = 0.047$). Moreover, the analysis (Table 1) revealed an increase of 0.59% in the annual prevalence for genotype 1 [IC_{95} (0.43–0.74), R^2: 0.92, $P < 0.001$], an increase of 0.33% for genotype 4 [IC_{95} (0.17; 0.49), R^2: 0.77, $P = 0.002$] and 0.47% for subtype 1a [IC_{95} (0.28; 0.66), R^2: 0.83, $P = 0.001$]. Conversely, a decrease of 0.82% was noticed in the annual prevalence for HCV genotype 3 [IC_{95} (−1.00; −0.65), R^2: 0.94, $P < 0.001$].

DISCUSSION

Liver-related complications are currently among the most frequent causes of hospitalization and death in HIV-infected patients in Western countries [5]. Chronic hepatitis C is increasingly leading to end-stage hepatic disease in this population given that other causes of hepatic complications, as drug-associated hepatotoxicity or decompensated cirrhosis owing to chronic hepatitis B, have declined with the use of safer antiretroviral drugs or potent anti-HBV agents (e.g. tenofovir) respectively [19].

In contrast with other cohorts, hepatitis C therapy had been given to a relatively high proportion (41%) of HIV/HCV coinfected patients in our cohort. In parallel with this wide use of hepatitis C therapy and associated benefit in terms of HCV clearance in a fraction of treated patients (42% in our cohort), we recently begun to appreciate that hospital admissions and deaths owing to HCV-related liver disease have stabilized at our clinic [20]. The relatively large uptake of hepatitis C therapy in our cohort contrasts with rates below 10% in studies performed in North America [21,22], but it is in agreement with what is currently seen in other European countries [19,23,24]. For instance, recent French surveys have reported rates of 36% [19,23], and data from EuroSIDA acknowledge an overall exposure to hepatitis C therapy of 27% in HIV/HCV coinfected patients [24]. Differences in patient characteristics, easy access to medication (including free or cheap provision) and physician's expertise in managing hepatitis C in HIV+ individuals may explain these differences.

Distinct HCV genotypes have been associated with differences in geographical distribution, risk group category, natural history and, more importantly, in treatment response to pegIFN-RBV therapy [9]. HCV genotype 1 is generally the most prevalent in Western countries across all distinct risk groups and displays a low susceptibility to pegIFN-RBV. Treatment response in HCV genotype 4 patients is generally as poor as in HCV genotype 1 carriers [12]. In contrast, HCV genotype 3 is relatively frequent among intravenous drug users, has been associated with liver steatosis and is more susceptible to pegIFN-RBV therapy [9]. In our study population of HIV/HCV coinfected individuals, mainly former intravenous drug users, HCV-1 was recognized at entry in 57% of patients while HCV-3 was seen in 25%. Interestingly, HCV-4, which is believed to have been introduced in Southern Europe from North Africa, was recognized in 16% of our study population. As shown in Fig. 2a, it was the only HCV genotype showing a growing incidence over time, reflecting that a growing proportion of new HCV infections in HIV+ individuals are caused by this HCV variant coming from North Africa.

The change in the prevalence of HCV genotypes seen at our cohort since year 2000 was driven mainly by two factors. Firstly, HCV clearance was achieved more frequently in patients infected with HCV-2/3 than in those carrying HCV-1/4. This observation is in agreement with prior therapeutic studies conducted in coinfected individuals [13,25–27]. On the other hand, the number of incident cases and the proportion of them infected with HCV-3 have declined in recent times. This fact is in agreement with recent reports acknowledging that chronic hepatitis C has significantly diminished among newly diagnosed HIV-infected individuals in Spain as a consequence of a dramatic reduction in intravenous drug use [28].

The main implication of the shift in HCV genotypes seen in our study is that there is a progressive enrichment of difficult-to-treat patients within the currently alive HIV/HCV coinfected population. This aspect was further emphasized in our study by the fact that the proportion of patients with advanced liver fibrosis (Metavir F3-F4) increased from 28% to 51% after a mean follow-up of 2.8 years. It is noteworthy that, in our cohort, HCV therapy had more impact than mortality in clearance of HCV infection. Thus, the changing

HCV genotype distribution was largely driven by a differential efficacy of pegIFN-RBV therapy in patients with HCV-2/3 compared to HCV-1/4.

Our study has several limitations, including its retrospective design, the lack of information regarding trends in HCV genotype distribution in a similar HCV-monoinfected population, the fact that data were recorded at one single centre and the potential for biases derived from a different uptake of hepatitis C treatment in subjects carrying distinct HCV genotypes. Despite these weaknesses, the information provided in the study is valuable, given the expectations raised by the prospects of using the new STAT-C drugs in this population. While most nucleoside analogues developed as HCV polymerase inhibitors will work across all HCV genotypes, most protease inhibitors and non-nucleoside polymerase inhibitors are target primarily HCV genotype 1 [14]. In our cohort, 40% of HIV/HCV coinfected patients in 2008 did not carry HCV genotype 1. Moreover, a relatively high proportion of coinfected patients in our cohort had failed hepatitis C therapy. It must be expected that this subset of patients will benefit less from the new anti-HCV drugs, because these compounds will initially be used in combination with pegIFN-RBV, to which these patients already had shown null or less susceptibility. The encouraging data from the PROVE-3 trial, however, which has been conducted in HCV-monoinfected patients with prior nonresponse or relapse, may challenge this point, as more than half of patients achieved cure with a combination of telaprevir plus pegIFN-RBV [29]. A third consideration is that HCV-1 subtype 1a continues to be more frequent than subtype 1b in HIV/HCV coinfected individuals, with a significant increase over time, and natural polymorphisms associated with reduced susceptibility to HCV protease inhibitors are more frequent in subtype 1a than in 1b [30]. Altogether, our results suggest that the burden of HCV-related liver disease presumably will not be halted soon in the HIV/HCV coinfected population following the arrival of new antivirals against HCV infection. Only a fraction of this population, which overall already depicts advanced liver fibrosis in more than a quarter of cases, will be expected to benefit from these new drugs.

ACKNOWLEDGEMENTS

This work was funded in part by grants from Fundacion Investigacion y Educacion en SIDA (IES), Agencia Lain Entralgo, Red de Investigacion en SIDA (RIS, project ISCIII-RETIC RD06/006) and the European NEAT network.

AUTHOR CONTRIBUTIONS

JM and VS designed the study. JM and SR did the statistical analyses. EV, PL, PT, LMC and PB assisted in the recording of clinical data. AM was responsible for the recording of virological results. SRN and IJN assisted providing therapeutic information for individuals enrolled in the cohort. JM and VS wrote the manuscript.

REFERENCES

1 Alter M, Seeff L, Bacon B, Thomas D, Rigsby M, Di Bisceglie A. Testing for hepatitis C virus infection should be routine for persons at increased risk for infection. *Ann Intern Med* 2004; 141: 715–717.
2 Soriano V, Puoti M, Sulkowski M *et al.* Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel. *AIDS* 2007; 21: 1073–1089.
3 Thomas D, Astemborski J, Rai R *et al.* The natural history of hepatitis C virus infection: host, viral, and environmental factors. *JAMA* 2000; 284: 450–456.
4 Martin-Carbonero L, Benhamou Y, Puoti M *et al.* Incidence and predictors of severe liver fibrosis in HIV infected patients with chronic hepatitis C – a European collaborative study. *Clin Infect Dis* 2004; 38: 128–133.
5 Weber R, Sabin C, Friis-Moller N *et al.* Liver-related deaths in persons with the HIV: the D:A:D study. *Arch Intern Med* 2006; 166: 1632–1641.
6 Tedaldi E, Peters L, Neuhaus J *et al.* Opportunistic disease and mortality in patients coinfected with hepatitis B or C virus in the strategic management of antiretroviral therapy (SMART) study. *Clin Infect Dis* 2008; 47: 1468–1475.
7 Lewden C, May T, Rosenthal E *et al.* Changes in causes of death among adults infected by HIV between 2000 and 2005: The "Mortalite 2000 and 2005" surveys (ANRS EN19 and Mortavic). *J Acquir Immune Defic Syndr* 2008; 48: 590–598.
8 Bonacini M, Louie S, Bzowej N, Wohl A. Survival in patients with HIV infection and viral hepatitis B or C: a cohort study. *AIDS* 2004; 18: 2039–2045.
9 Pawlotsky J. Hepatitis C virus genetic variability: pathogenic and clinical implications. *Clin Liver Dis* 2003; 7: 45–66.
10 Soriano V, Mocroft A, Rockstroh J *et al.* Spontaneous viral clearance, viral load, and genotype distribution of hepatitis C virus (HCV) in HIV-infected patients with anti-HCV antibodies in Europe. *J Infect Dis* 2008; 198: 1337–1344.
11 Pizzillo P, Almasio P, Ferraro D, Craxi A, Di S. HCV genotypes in Sicily: is there any evidence of a shift? *J Med Virol* 2009; 81: 1040–1046.
12 Martin-Carbonero L, Puoti M, Garcia-Samaniego J *et al.* Response to pegylated interferon plus ribavirin in HIV-infected patients with chronic hepatitis C due to genotype 4. *J Viral Hepat* 2008; 15: 710–715.
13 Nunez M, Miralles C, Berdun MA *et al.* Role of weight-based ribavirin dosing and extended duration of therapy in chronic hepatitis C in HIV-infected patients: the PRESCO trial. *AIDS Res Hum Retroviruses* 2007; 23: 972–982.
14 Pol S, Soriano V. Management of chronic hepatitis C virus infection in HIV-infected patients. *Clin Infect Dis* 2008; 47: 94–101.
15 Soriano V, Peters M, Zeuzem S. New therapies for hepatitis C virus infection. *Clin Infect Dis* 2009; 48: 313–320.
16 Castellares C, Barreiro P, Martin-Carbonero L *et al.* Liver cirrhosis in HIV-infected patients: prevalence, aetiology and clinical outcome. *J Viral Hepat* 2008; 15: 165–172.

17 Macías J, Recio E, Vispo E *et al.* Application of transient elastometry to differentiate mild from moderate to severe liver fibrosis in HIV/HCV co-infected patients. *J Hepatol* 2008; 49: 916–922.
18 Kirk G, Astemborski J, Mehta S *et al.* Assessment of liver fibrosis by transient elastography in persons with hepatitis C virus infection or HIV-hepatitis C virus coinfection. *Clin Infect Dis* 2009; 48: 963–972.
19 Larsen C, Pialoux G, Salmon D *et al.* Prevalence of hepatitis C and hepatitis B infection in the HIV-infected population of France, 2004. *Euro Surveill* 2008; 13: 1–4.
20 Núñez-Fernández C, Martín-Carbonero L, Valencia ME *et al.* Liver complications have reached a plateau as cause of hospital admission and death in HIV patients in Madrid. *AIDS Res Hum Retroviruses* 2009; 25: 383–385.
21 Mehta S, Genberg B, Astemborski J *et al.* Limited uptake of hepatitis C treatment among injection drug users. *J Community Health* 2008; 33: 126–133.
22 Grebely J, Raffa J, Lai C *et al.* Low uptake of treatment for hepatitis C virus infection in a large community-based study of inner city residents. *J Viral Hepat* 2009; 16: 352–358.
23 Cacoub P, Halfon P, Rosenthal E *et al.* Treatment of hepatitis C virus in HIV-infected patients in ''real life'': modifications in two large surveys between 2004 and 2006. *J Hepatol* 2008; 48: 5–7.
24 Mocroft A, Vogel M, Beniowski M *et al.* Has the uptake of treatment for chronic hepatitis C virus infection in HIV-positive patients in Europe changed over time? 12th European AIDS Conference. Cologne, Germany. November 11-14 2009 (PS2/3). *HIV Med* 2009; 10(Suppl. 2): 2.
25 Torriani F, Rodriguez-Torres M, Rockstroh J *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N Engl J Med* 2004; 351: 438–450.
26 Carrat F, Bani-Sadr F, Pol S *et al.* Pegylated interferon alfa-2b vs standard interferon alfa-2b, plus ribavirin, for chronic hepatitis C in HIV-infected patients. *JAMA* 2004; 292: 2839–2848.
27 Laguno M, Murillas J, Blanco JL *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for treatment of HIV/HCV co-infected patients. *AIDS* 2004; 18: F27–F36.
28 Perez-Cachafeiro S, del Amo J, Iribarren JA *et al.* Decrease in serial prevalence of coinfection with hepatitis C virus among HIV-infected patients in Spain, 1997–2006. *Clin Infect Dis* 2009; 48: 1467–1470.
29 McHutchison J, Manns M, Muir A *et al.* PROVE-3 final results and 1-year durability of SVR with telaprevir-based regimen in HCV-1 infected patients with prior non-response, viral breakthrough or relapse to peginterferon-alfa 2a/b and ribavirin therapy. *Hepatology* 2009; 50(Suppl.): 334–335.
30 Kuntzen T, Timm J, Berical A *et al.* Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naive patients. *Hepatology* 2008; 48: 1769–1778.

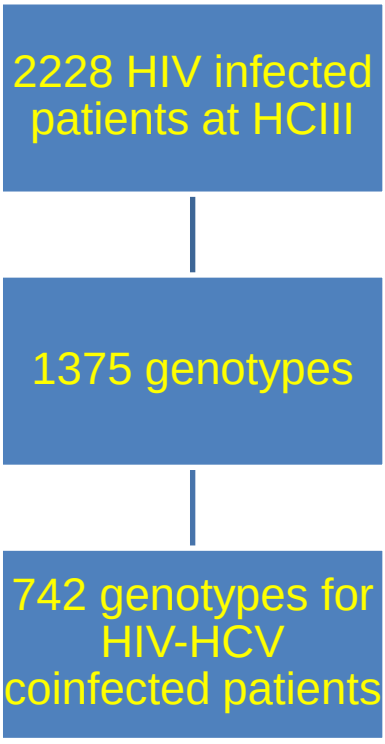
From a total of 2228 HIV infected patients identified at HCIII, 742 were HCV coinfected and 672 were on regular follow-up (Figure 8). A total of 403 patients were present in the cohort before year 2000 and 268 patients entered the dynamic cohort during the study period since then (Figure 9).

1. Baseline characteristics

From a total of 672 HIV-HCV coinfected patients who entered the dynamic cohort, 489 (73%) patients were male. At entry, mean age was 36.6 (±5.8) years. Most individuals (94.8%) were native Spaniards and the main mode of transmission was intravenous drug use (86.5%). Table 6

The overall distribution of HCV genotypes in the study cohort over the entire decade was as follows: HCV-1 57.1% (1a: 29.2%, 1b: 20.4%, unknown subtype: 7.6%), HCV-2 1.3%, HCV-3 25.4% and HCV-4 15.9%. One subject was infected with HCV genotype 6 and none with HCV genotype 5.

Figure 8 Flow chart of HIV infected patients identified at HCIII resulting in the main cohort.



Estimates of liver fibrosis using transient elastography were available for 545 (81.1%) of the whole HIV-HCV coinfected study population. At the first individual assessment in patients with active HCV infection, the distribution of Metavir scores was as follows: F0F1 in 311 (57.1%) patients, F2 in 83 (15.2%), F3 in 57 (10.5%) and F4 in 94 (17.2%). Overall, 27.8% of patients could be considered as having advanced liver fibrosis (Metavir score estimates F3-F4). During the study period, 419 patients underwent longitudinal liver stiffness assessments. After a mean time of 2.8 years ($\pm$0.98) from the first measurement, the distribution of Metavir scores in the population was as follows: F0F1 in 111 (26.5%), F2 in 94 (22.4%), F3 in 77 (18.4%) and F4 in 137 (327%). At the last assessment, 51.1% of patients had advanced liver fibrosis (Metavir score estimates F3-F4).

Table 6 Baseline characteristics and virological response to HCV-therapy of HIV-HCV coinfected patients on regular follow-up at HC-III (2000-2008).

Variables	
No.	672
Age (years)	36.6 ±5.8
Male	489 (73%)
Origin	
Spain	453 (67.4%)
Other	219 (32.6%)
Mode of infection	
IDU	468 (69.6%)
Htx	26 (3.9%)
MSM	34 (5.1%)
Others/Unknown	144 (21.4%)
Liver stiffness	
< 7.1 KPa	311 (46.3%)
7.1 – 9.5 KPa	83 (12.4%)
9.5 – 12.5 KPa	57 (8.5%)
≥ 12.5 KPa	94 (14%)
Not known	127 (18.9%)
HCV-therapy	
ITT	274 (40.8%)
SVR	119 (43.4%)
No-SVR	155 (56.6%)
On-T	
Not Completed	113 (41.2%)
Completed	161 (58.8%)
SVR	119 (73.9%)
No SVR	42 (26.1%)

2. HCV genotypes in cohort incident patients

A total of 403 patients were present in the cohort before year 2000 and 268 patients entered the dynamic cohort during the study period since then (Figure 9). There was a steadily decline in the number of new HCV-infected patients entering the cohort over the study period; 86 entered in year 2000 and 16 in year 2007 (Figure 10). As shown in Figure 11 **,** the proportion of incident HCV genotypes 4 increased 3% annually (R^2: 0.67, b: 2.98, p=0.01). Table 7 summarizes trends in the proportion of HCV genotypes entering the cohort.

Table 7 Summary of trends in the entries of HCV genotypes in the cohort of coinfected patients. N= 268 patients.

HCV genotype	coef b*	IC 95%	R^2	p value
1	-1,9	-4,82; 0,10	0,30	0,16
2	-0,32	-0,85; 0,22	0,26	0,19
3	0,75	-2,84; 1,32	0,11	0,40
4	2,98	0,90; 5,07	0,67	*0,01*

* Linear regression model. B coef means the annual change in the proportion of the genotype.

Figure 9 Dynamic cohort of HIV-HCV coinfected patients – study population flow chart

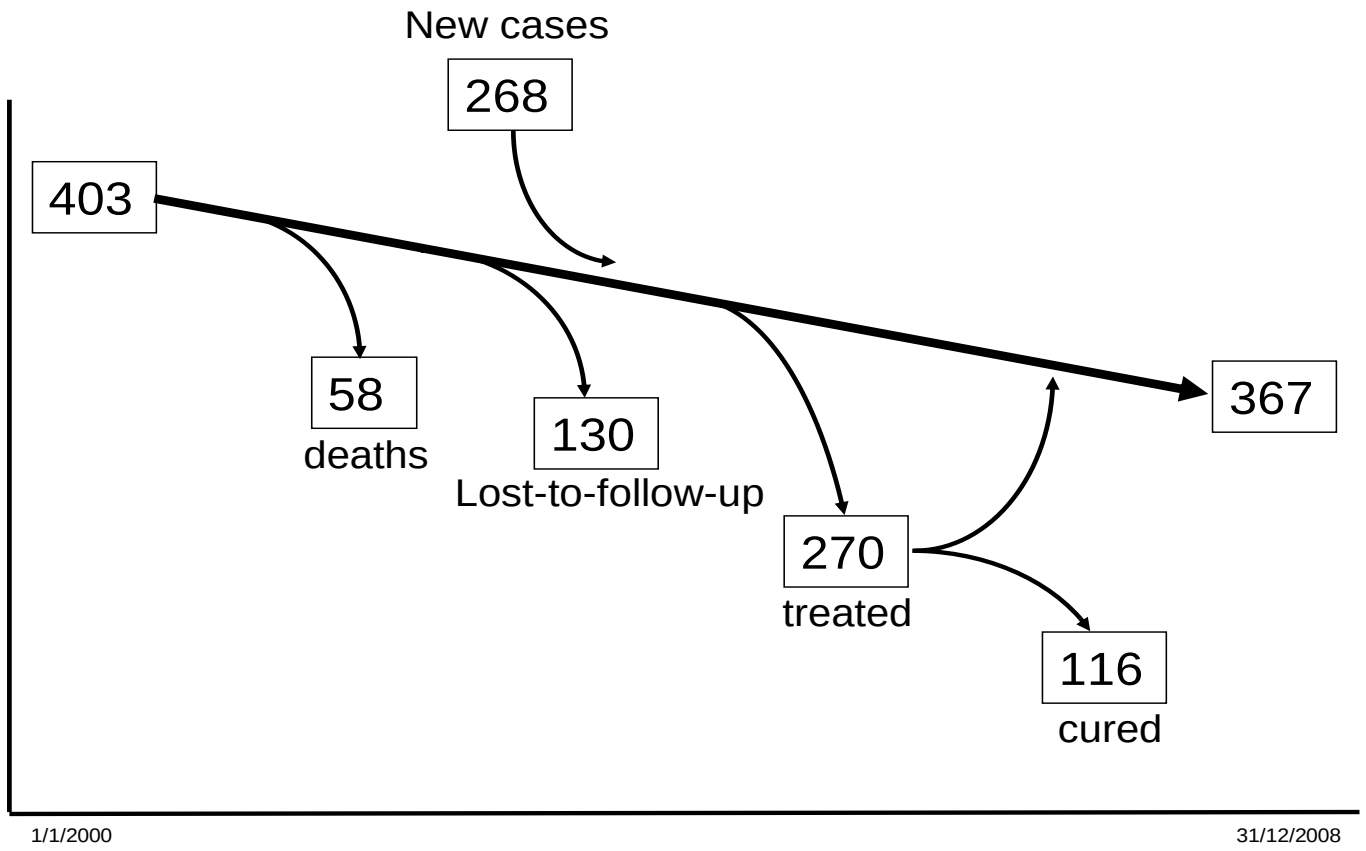


Figure 10 Absolute number of HIV-HCV coinfected patients entering the cohort

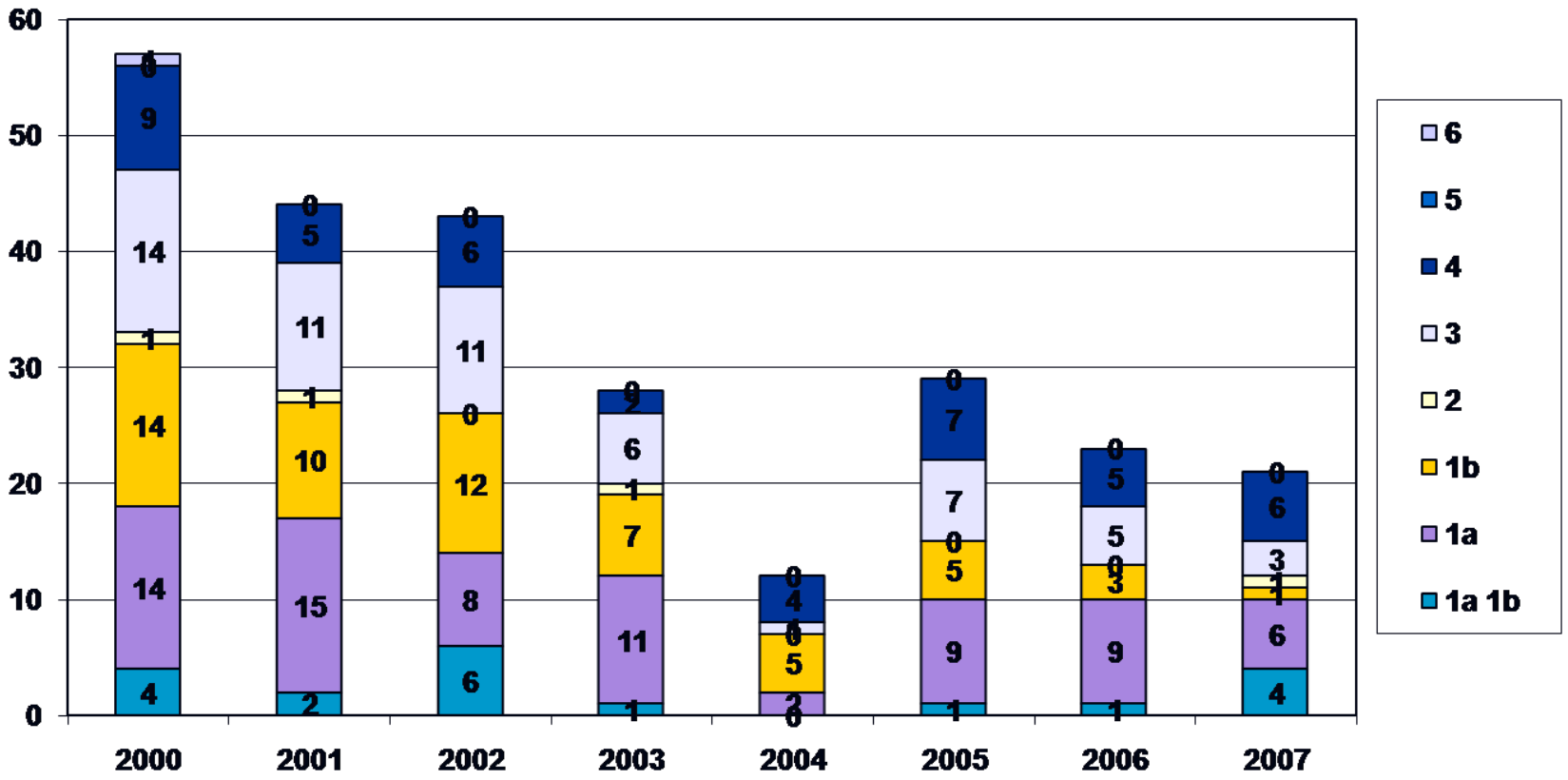


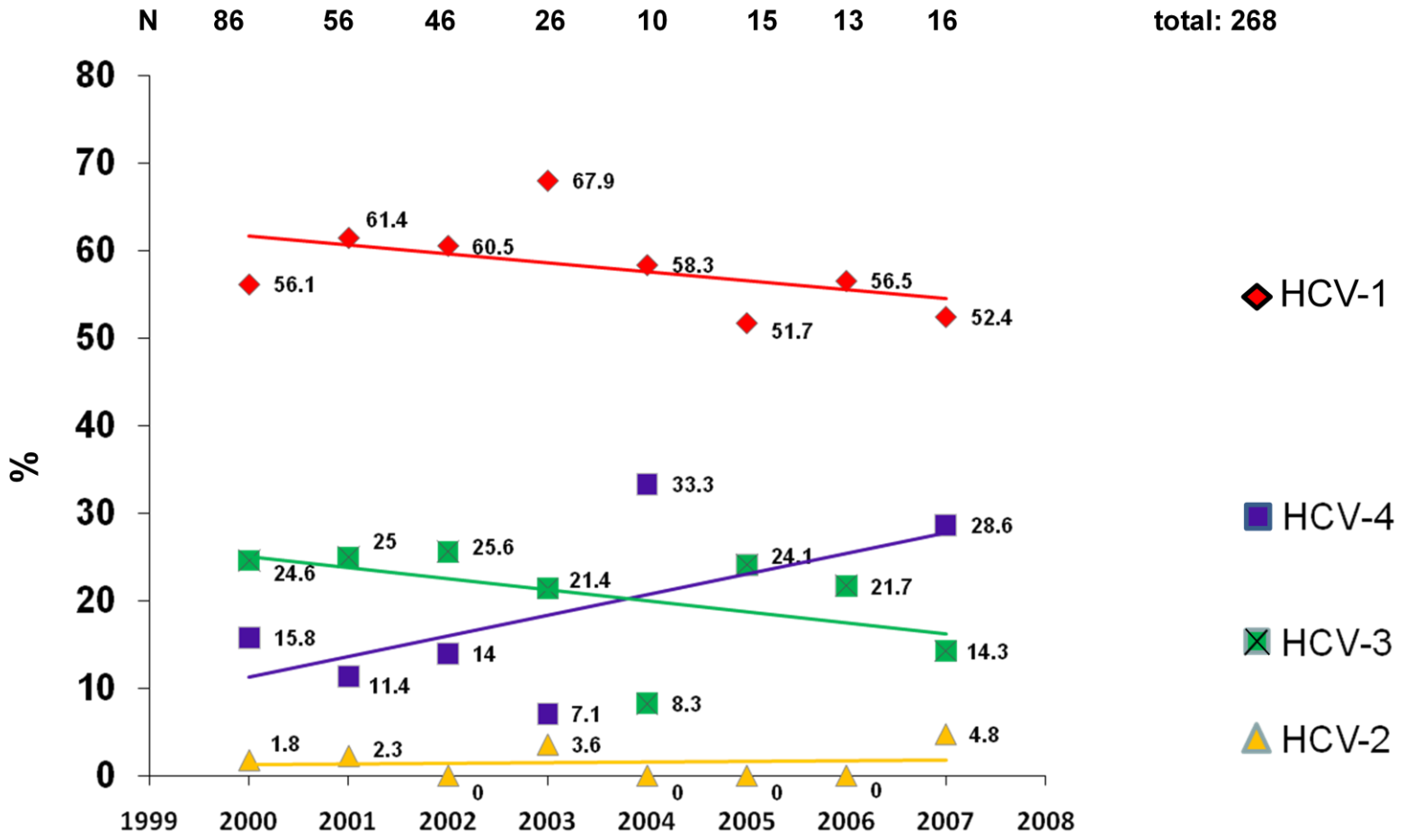
Table 2. Distribution of HCV genotypes in 257 coinfected patients entering the cohort.

	2000	2001	2002	2003	2004	2005	2006	2007	Overall	Pº
No.	57	44	43	28	12	29	23	21	257	
HCV genotypes										
1*	4 (7.0%)	2 (4.5%)	6 (14.0%)	1 (3.6%)	-	1 (3.4%)	1 (4.3%)	4 (19.0%)	19.0 (7.4%)	0.128
1a	14 (24.6%)	15 (34.1%)	8 (18.6%)	11 (39.3%)	2 (16.7%)	9 (31.0%)	9 (39.1%)	6 (28.6%)	74.0 (28.8%)	0.473
1b	14 (24.6%)	10 (22.7%)	12 (27.9%)	7 (25.0%)	5 (41.7%)	5 (17.2%)	3 (13.0%)	1 (4.8%)	57.0 (22.2%)	0.100
2	1 (1.8%)	1 (2.3%)	-	1 (3.6%)	-	-	-	1 (4.8%)	4.0 (1.6%)	0.475
3	14 (24.6%)	11 (25.0%)	11 (25.6%)	6 (21.4%)	1 (8.3%)	7 (24.1%)	5 (21.7%)	3 (14.3%)	58.0 (22.6%)	0.252
4	9 (15.8%)	5 (11.4%)	6 (14.0%)	2 (7.1%)	4 (33.3%)	7 (24.1%)	5 (21.7%)	6 (28.6%)	44.0 (17.1%)	0.171
6	1 (1.8%)	-	-	-	-	-	-	-	1.0 0.4%)	0.475
Genotype groups										
1-4	41 (71.9%)	32 (72.7%)	32 (74.4%)	21 (75.0%)	11 (91.7%)	22 (75.9%)	18 (78.3%)	17 (81.0%)	194.0 (75.5%)	0.302
2-3	15 (26.3%)	12 (27.3%)	11 (25.6%)	7 (25.0%)	1 (8.3%)	7 (24.1%)	5 (21.7%)	4 (19.0%)	62.0 (24.1%)	0.357

º P-value significance for differences between 2000 and 2007

* Unknown subtype for genotype

Figure 11 Annual proportion of HIV-HCV coinfected patients entering the cohort, according to their HCV genotypes. N= 268



3. HCV genotypes in patients leaving the cohort

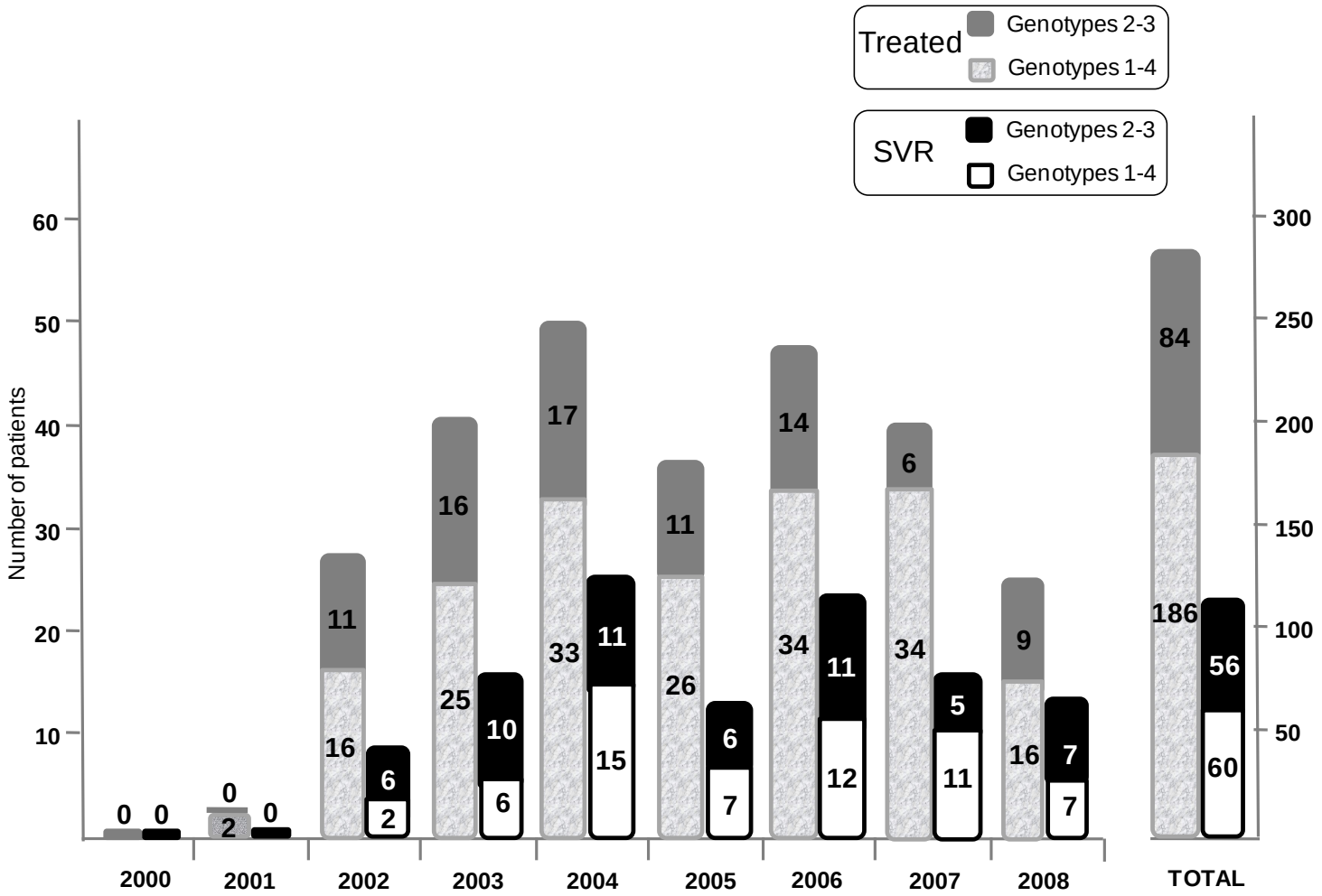
The overall mean time of follow-up was 5.5 years, corresponding to 4,108 patients-years.

a) Annual SVR rate

A total of 274 (40.8%) out of 672 coinfected patients in the cohort were treated with pegIFN-RBV (Figure 12). Among them, 161 (58.8%) completed the planned duration of a course of hepatitis C therapy. Overall, 116 of patients achieved SVR (intent-to-treat rate: 42.3%; on-treatment rate: 72%). It should be noted that this rate of HCV clearance occurred after a first course of therapy in two thirds of patients, while in the rest sustained virological response resulted from repeated (one or two additional) courses of hepatitis C therapy.

Figure 12 records the yearly distribution of HCV genotypes among patients who left the cohort due to HCV clearance following hepatitis C therapy. It must be noted that HCV clearance with treatment was achieved more than two-fold more frequently in patients infected with HCV-2/3 (57/83; 68.7%) than in HCV-1/4 carriers (59/191; 30.9%) ($p<0.001$).

Figure 12 Annual uptake of PegInterferon plus Ribavirin therapy and HCV clearance in the cohort, according to HCV genotype.



b) Death & lost-to-follow-up

During the study period, 188 patients left the cohort for other reasons than cure following hepatitis C therapy (116; 38.1%). A total of 58 (19.1%) patients died and 130 (42.7%) were lost-to-follow-up (Table 8). No significance was found (Figure 13 and Figure 14).

Figure 13 Trends in patients leaving the cohort.

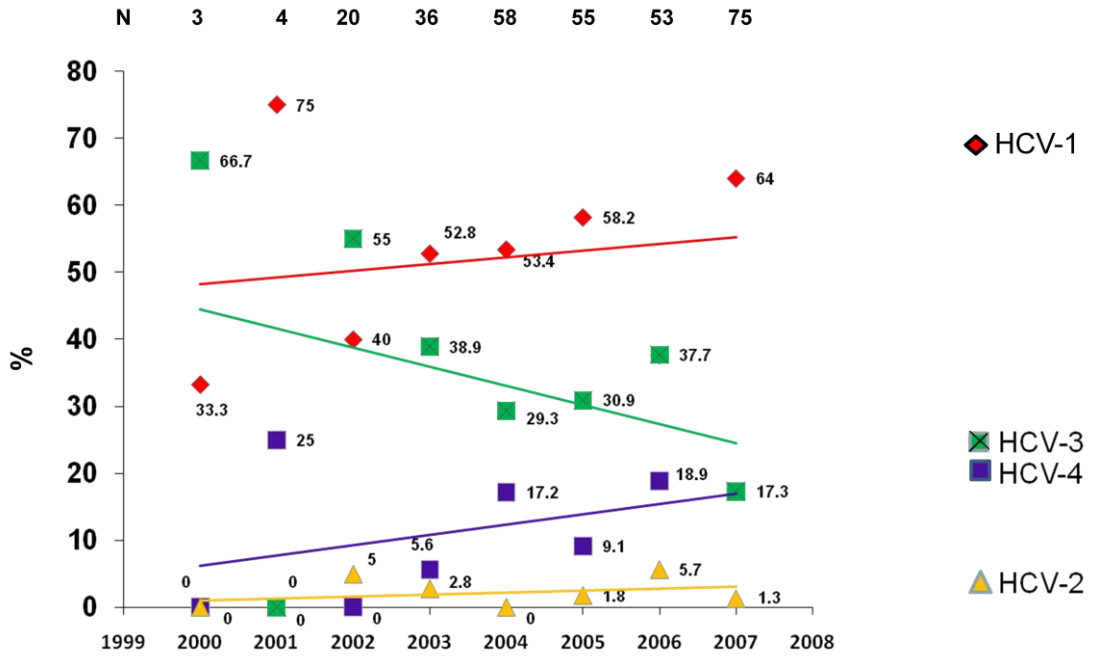


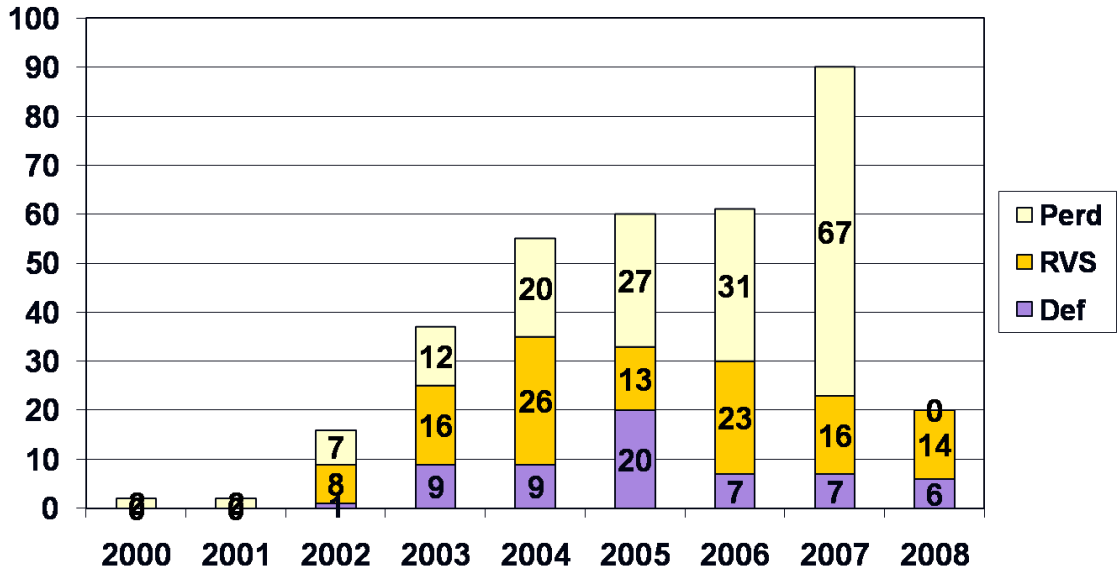
Table 8 Distribution of HCV genotypes in 343 patients leaving the cohort according to SVR, mortality and lost-to-follow-up.

	2000	2001	2002	2003	2004	2005	2006	2007	2008	Overall	pº
Total No.	2	2	16	37	55	60	61	90	20	343	
SVR*	-	-	8 (50.0%)	16 43.2%)	26 (47.3%)	13 (21.7%)	23 (37.7%)	16 (17.8%)	14 (70%)	116 (33.8%)	0.387
1-4	-	-	4 (50.0%)	7 43.8%)	17 (65.4%)	5 (38.5%)	9 (39.1%)	10 (62.5%)	7 (50.0%)		
2-3	-	-	4 (50.0%)	9 (56.3%)	9 (34.6%)	8 (61.5%)	14 (60.9%)	6 (37.5%)	7 (50%)		
Mortality*	-	-	1 (6.3%)	9 24.3%)	9 (16.4%)	20 (33.3%)	7 (11.5%)	7 (7.8%)	6 (30%)	59 (17.2%)	0.174
1-4	-	-	1 (100%)	6 (66.7%)	5 (55.6%)	16 (80%)	6 (85.7%)	4 (57.1%)	3 (50%)		
2-3	-	-	-	3 (33.3%)	4 (44.4%)	4 (20%)	1 (14.3%)	3 (42.9%)	3 (50%)		
Lost follow up*	2 (100%)	2 (100%)	7 (43.8%)	12 32.4%)	20 (36.4%)	27 (45.0%)	31 (50.8%)	67 (74.4%)	-	168 (49.0%)	0.062
1-4	-	1 (50%)	3 (42.9%)	11 (91.7%)	16 (80%)	18 (66.7%)	18 (58.1%)	54 80.6%)	-		
2-3	2 (100%)	-	4 (57.1%)	1 (8.3%)	4 (20%)	9 (33.3%)	3 (41.9%)	13 (19.4%)	-		

* Percentages were calculated by the total number of patients who left the cohort this year

º P-value significance for differences between 2000 and 2008

Figure 14 Absolute number of patients leaving the cohort according to SVR, LTFU and Death



4. HCV genotype prevalence by calendar

As result of entries and exits into the dynamic cohort (Figure 7**)**, the yearly prevalence of HCV genotypes showed significant variations. The final distribution of HCV genotypes in 2008 was as follows: 60.5% HCV-1 (1a: 31.3%, 1b: 20.4%, unknown subtype: 8.7%), 0.5% HCV-2, 21% HCV-3 and 18% HCV-4 (Table 9).

There was an increase in HCV genotypes 1 and 4 from 72% in 2000 to 78.5% in 2008 (p=0.041). Conversely, as shown in Figure 15, there was a decline in HCV genotypes 2 and 3 from 28% in 2000 to 21.5% in 2008 (p=0.047). Moreover, the analysis of trends (Table 10) in the prevalence revealed an increase of 0.59% in the annual prevalence for genotype 1 (IC_{95} [0.43 to 0.74], R^2: 0.92, p<0.001), an increase of 0.33% for genotype 4 (IC_{95} [0.17; 0.49], R^2: 0.77, p=0.002), and 0.47% for subtype 1a (IC_{95} [0.28; 0.66], R^2: 0.83, p=0.001). Conversely a decrease of 0.82% was noticed in the annual prevalence for HCV genotype 3 (IC_{95} [-1.00; -0.65], R^2: 0.94, p<0.001(Table 10 and Figure 16).

Table 9 Annual prevalence of HCV genotypes in the study population

	2000	2001	2002	2003	2004	2005	2006	2007	2008	Overall	p-value
No.	541	584	619	632	609	584	545	507	452	672	
Genotypes											
1*	41 (7.6%)	43 (7.4%)	49 (7.9%)	50 (7.9%)	47 (7.7%)	46 (7.9%)	45 (8.3%)	44 (8.7%)	39 (8.6%)	51 (7.6%)	0.312
1a	156 (28.8%)	170 (29.1%)	177 (28.6%)	185 (29.3%)	178 (29.2%)	172 (29.5%)	165 (30.3%)	156 (30.8%)	141 (31.2%)	196 (29.2%)	0.229
1b	106 (19.6%)	116 (19.9%)	126 (20.4%)	129 (20.4%)	129 (21.2%)	119 (20.4%)	108 (19.8%)	100 (19.7%)	89 (19.7%)	137 (20.4%)	0.483
2	8 (1.5%)	9 (1.5%)	8 (1.3%)	9 (1.4%)	8 (1.3%)	8 (1.4%)	7 (1.3%)	6 (1.2%)	4 (0.9%)	9 (1.3%)	0.095
3	144 (26.6%)	155 (26.5%)	165 (26.7%)	163 (25.8%)	150 (24.6%)	141 (24.1%)	127 (23.3%)	113 (22.3%)	101 (22.3%)	171 (25.4%)	0.069
4	85 (15.7%)	90 (15.4%)	94 (15.2%)	96 (15.2%)	97 (15.9%)	98 (16.8%)	93 (17.1%)	88 (17.4%)	78 (17.3%)	107 (15.9%)	0.284
6	1 (0.2%)	1 (0.2%)	-	-	-	-	-	-	-	1 (0.1%)	-
Genotype groups											
1-4	388 (71.7%)	419 (71.7%)	446 (72.1%)	460 (72.8%)	451 (74.1%)	435 (74.5%)	411 (75.4%)	388 (76.5%)	347 (76.8%)	491 (73.1%)	*0.041*
2-3	152 (28.1%)	164 (28.1%)	173 (27.9%)	172 (27.2%)	158 (25.9%)	149 (25.5%)	134 (24.6%)	119 (23.5%)	105 (23.2%)	180 (26.8%)	*0.047*

Table 10 Summary of trends in the prevalence of HCV genotype and subtypes in the cohort of coinfected patients. N= 671 patients

HCV genotype/subtype	coef b*	IC 95%	R^2	p value
1	0,59	0,43; 0,74	0,92	*<0,001*
2	-0,09	-0,16; -0,03	0,63	*0,010*
3	-0,82	-1,00; -0,65	0,94	*<0,001*
4	0,33	0,17; 0,49	0,77	*0,002*
1a/b	0,13	0,04; 0,22	0,64	0,010
1a	0,47	0,28; 0,66	0,83	*0,001*
1b	-0,13	-0,22; 0,19	0,03	0,884
2-3	-0,92	-1,12; -0,71	0,94	*<0,001*
1-4	0,92	0,71; 1,12	0,94	*<0,001*

* Linear regression model. B coef means the annual change in the proportion of the genotype or subtype.

Figure 15 Absolute prevalence of HIV-HCV coinfected patients

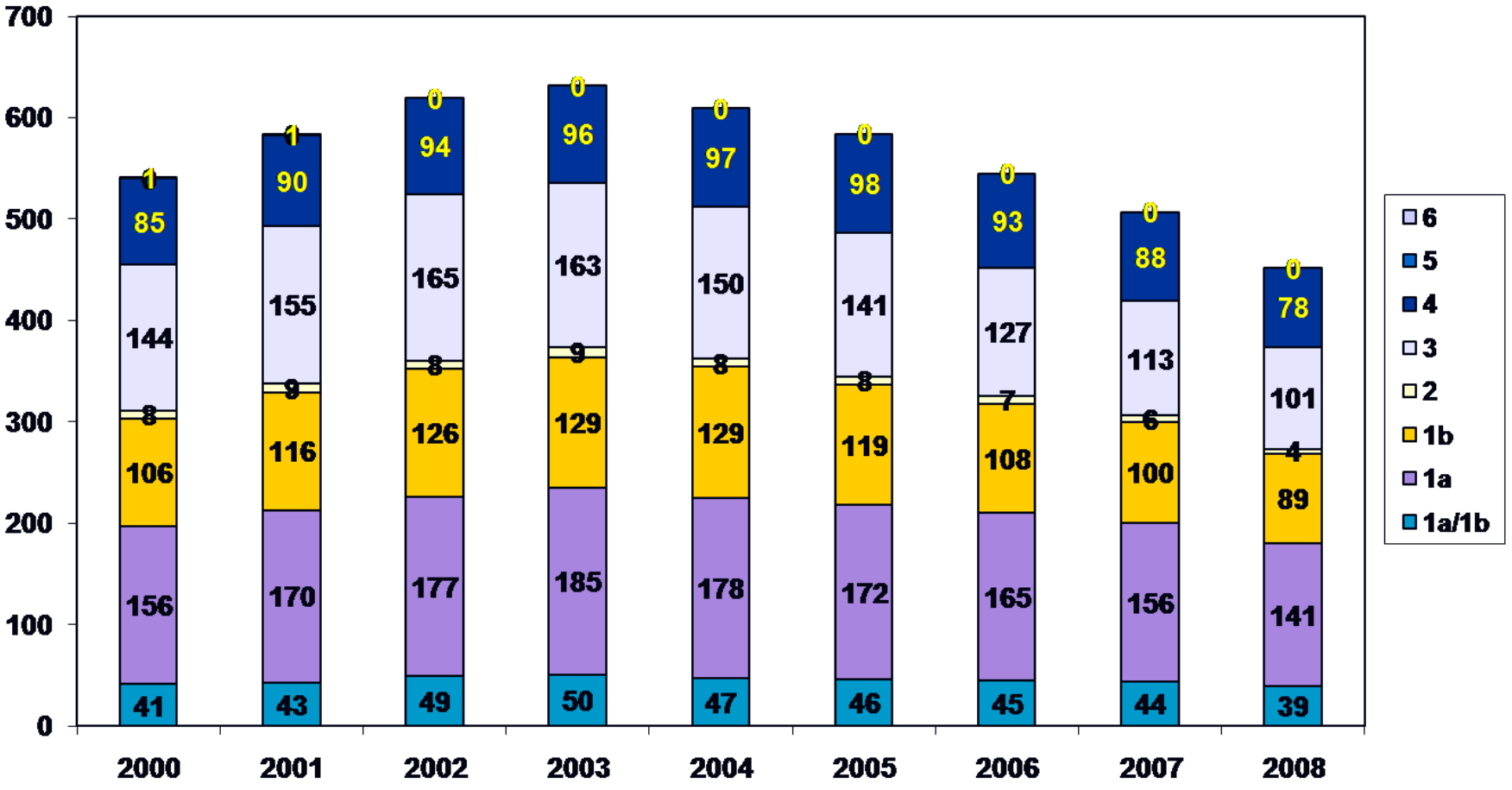
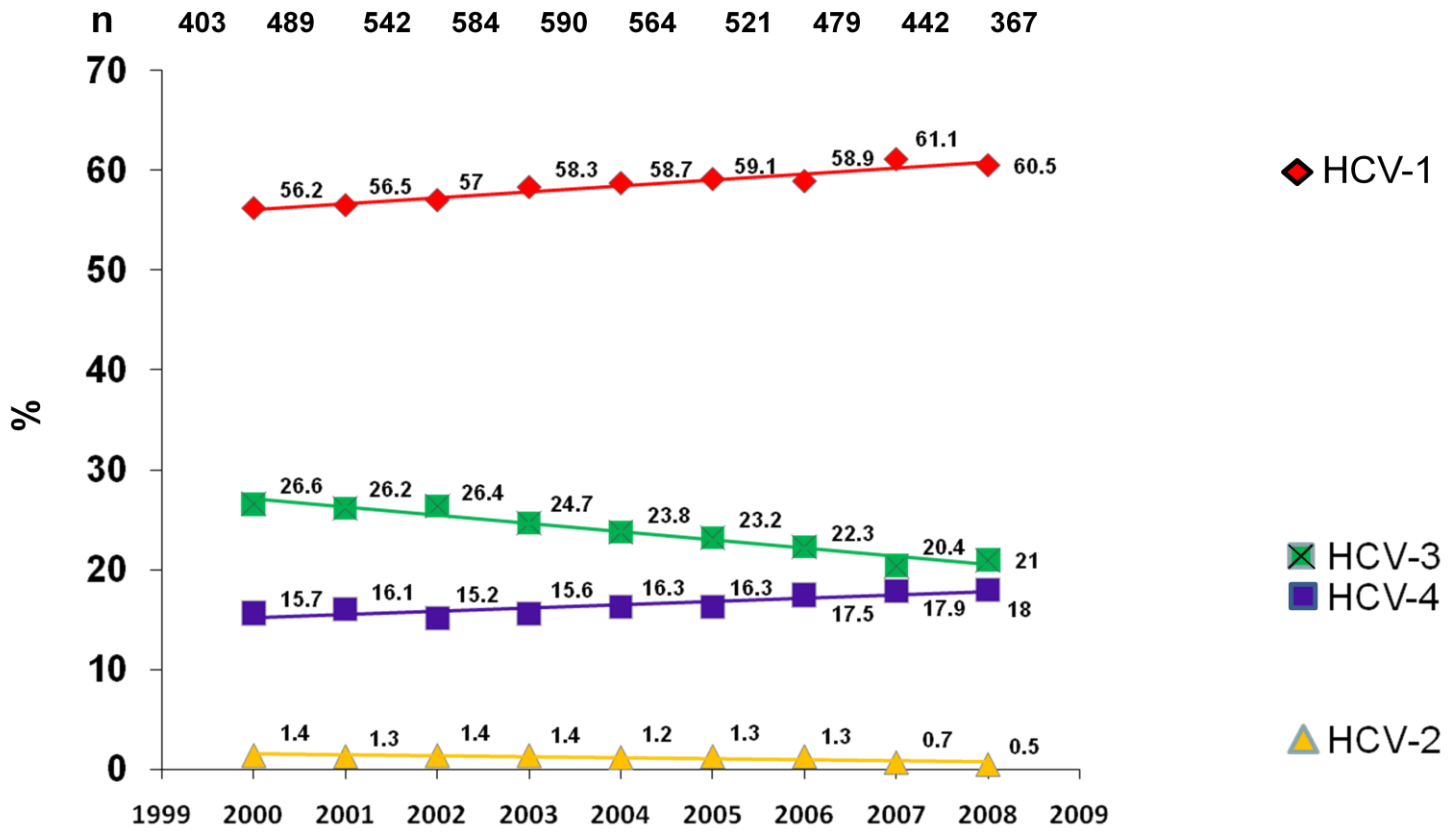


Figure 16 Annual prevalence of HCV genotypes in a cohort of 672 coinfected patients.



B. Second article: Rate and timing of hepatitis C virus relapse after a successful course of pegylated interferon plus ribavirin in HIV-infected and HIV-uninfected patient.

Rate and Timing of Hepatitis C Virus Relapse after a Successful Course of Pegylated Interferon plus Ribavirin in HIV-Infected and HIV-Uninfected Patients

José Medrano,[1] Pablo Barreiro,[1] Salvador Resino,[4] Paula Tuma,[1] Violeta Rodríguez,[1] Eugenia Vispo,[1] Pablo Labarga,[1] Antonio Madejón,[1,2] Javier García-Samaniego,[2] Inmaculada Jiménez-Nácher,[3] Luz Martín-Carbonero,[1] and Vincent Soriano[1]

[1]Department of Infectious Diseases, [2]Service of Hepatology and CIBERehd, and [3]Service of Pharmacy, Hospital Carlos III, and [4]Laboratory of Molecular Epidemiology of Infectious Diseases, National Centre for Microbiology, Instituto de Salud Carlos III, Madrid, Spain

Information on the rate and timing of hepatitis C virus (HCV) relapse after treatment with pegylated interferon plus ribavirin is scarce. Among 604 patients treated for chronic hepatitis C, the 386 who were human immunodeficiency virus (HIV) positive attained an end-of-treatment response less frequently and experienced relapse more often than did the 218 who were HIV negative. However, episodes of HCV relapse occurred before week 12 in most cases, regardless of HIV status.

Treatment with pegylated interferon (peginterferon) plus ribavirin attains a sustained virological response (SVR) in nearly half of patients with chronic hepatitis C virus (HCV) infection. Viral genotype and comorbidities, such as human immunodeficiency virus (HIV) coinfection, are important determinants of response [1–5]. HCV eradication is presumed to occur in most patients attaining undetectable serum HCV RNA at the end of treatment (EOT) and remain negative for 24 weeks thereafter [6]. Patients with a sustained virological response normalize liver enzyme levels, improve liver histology [7, 8], and show minimal liver-related morbidity and mortality [9, 10]. The odds of HCV reappearance once a sustained virological response has been attained are very low [11, 12].

Received 28 February 2009; accepted 27 May 2009; electronically published 8 October 2009.

Reprints or correspondence: Dr Vincent Soriano, Dept of Infectious Diseases, Hospital Carlos III, Calle Sinesio Delgado 10, 28029 Madrid, Spain (vsoriano@dragonet.es).

Clinical Infectious Diseases 2009; 49:1397–1401

1058-4838/2009/4909-0018$15.00
DOI: 10.1086/630205

In HCV-monoinfected patients, relapses generally occur soon after discontinuation of successful therapy—within the first 12 weeks in 98% of cases in one study that used standard interferon [13]. Given that HIV coinfection is frequent in patients with chronic hepatitis C and that hypothetically HIV-associated immune abnormalities might impair definitive HCV clearance after hepatitis C therapy, we examined the rate and timing of HCV relapse in coinfected patients treated with peginterferon plus ribavirin. This information is particularly relevant now that clinical trials testing the efficacy of new hepatitis C drugs have begun, and there is pressure for changing definitions of treatment success to earlier time points after completion of therapy [14, 15].

Methods. We conducted a retrospective analysis of all patients with chronic hepatitis C who were interferon naive and who initiated treatment with peginterferon (alfa-2a or alfa-2b) plus oral ribavirin from 2001 through 2008 at a reference hospital in Madrid. Patients who were positive for hepatitis B surface antigen (HBsAg) were excluded. Criteria for indication of hepatitis C therapy were in accordance with international guidelines, both in HCV-monoinfected and HIV/HCV-coinfected patients [16, 17].

Only patients who attained a serum HCV RNA level <10 IU/mL at the planned EOT were analyzed. Standard doses of both peginterferon modalities were given. Oral ribavirin dosage was adjusted to weight (1000 mg/day if <75 kg and 1200 mg/day if ⩾75 kg). Treatment was administered for 48 weeks except in patients infected with HCV genotypes 2 or 3 experiencing rapid virological response (week 4 serum HCV RNA level, <10 IU/mL) since 2006, who were given treatment for only 24 weeks.

The main end points were the prevalence and timing of HCV RNA rebound in patients with an EOT response, determined on the basis of longitudinal assessments of serum HCV RNA level at weeks 12, 24, 36, and 48 after completion of hepatitis C therapy. Three types of HCV relapse were considered: (1) early, defined as an HCV RNA rebound occurring between the EOT and week 12; (2) late, defined an HCV RNA rebound occurring between weeks 12 and 24; and (3) very late, defined an HCV RNA rebound occurring beyond week 24.

To investigate the source of HCV RNA rebounds beyond week 24 after completion of therapy and to try to elucidate whether HCV recurrences or reinfections had occurred, phylogenetic trees were constructed in which viral sequences obtained at the time of last detectable viremia before or during therapy and at the time of first HCV RNA rebound after com-

Table 1. Main Characteristics of the Study Population

Characteristic	HCV monoinfected	HIV/HCV coinfected	*P*
No. of patients initially treated	218	386	
EOT response	132 (60.5)	143 (37)	<.001
Male sex	82 (62)	103 (72)	.3
Mean age ± SD, years	46 ± 0.8	42 ± 0.4	<.001
Risk group			<.001
Injection drug user	89 (67)	124 (87)	
Other/unknown	43 (33)	19 (13)	
HCV infection			
Mean HCV RNA level ± SD, log IU/mL	6.0 ± 0.1	5.9 ± 0.1	.5
HCV RNA level >500,000 IU/mL	83 (63)	87 (61)	.7
HCV genotypes 1 or 4	97 (73)	85 (59)	<.001
Liver parameters			
Grade 3–4 elevation in liver enzyme level	9 (8)	18 (13)	.4
Mean AST/ALT ratio ± SD	0.7 ± 0.1	0.8 ± 0.2	.02
Mean FIB-4 score ± SD, U	0.91 ± 0.09	1.30 ± 0.12	.02
Mean APRI score ± SD, U	1.76 ± 0.12	2.13 ± 0.15	.07
Metavir fibrosis score estimates			.04
F2 or lower	42 (72)	40 (54)	
F3	6 (11)	19 (25)	
F4	10 (17)	16 (21)	
HCV therapy			
Pegylated interferon			<.001
Alfa-2a	118 (89)	98 (68)	
Alfa-2b	14 (11)	45 (32)	
Mean ribavirin dose ± SD, mg/day	1171 ± 14	1128 ± 23	.6
HIV infection			
Mean HIV RNA level ± SD, log copies/mL	…	3.3 ± 2.9	
HIV RNA level <50 copies/mL	…	107 (77)	
Mean CD4 cell count ± SD, cells/μL	…	575 ± 24	
CD4 cell count >500 cells/μL	…	67 (52)	
Receiving HAART	…	85 (59)	
Abacavir	…	24 (18)	
Zidovudine	…	6 (4)	
Tenofovir	…	41 (30)	
Experienced HCV relapse	29 (22)	47 (32.9)	.04

NOTE. Data are no. (%) of patients, unless otherwise indicated. Values for characteristics refer only to patients with an end-of-treatment (EOT) response. ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; SD, standard deviation.

pletion of therapy were compared. A fragment of the viral E1/E2 coding region was analyzed.

Results. A total of 616 patients with chronic hepatitis C had initiated treatment with peginterferon plus ribavirin during the 7-year study period. Twelve patients were excluded from further analyses because not enough follow-up had been completed. Of the remaining 604 patients, 386 (64%) were HIV positive, and 218 (36%) were HIV negative. Of this initial treated population, 329 patients (54.5%) experienced early virological failure or viral breakthrough during treatment, discontinued prematurely because of adverse effects, or were lost to follow-up before reaching the planned EOT. Overall, only 275 (45.5%) of the original treated population completed the planned duration of hepatitis C therapy and had undetectable serum HCV RNA, and these patients comprised the final population for our analysis. Of the patients, 76 (27.6%) experienced an HCV RNA rebound. The main characteristics of the patients who attained an EOT response are summarized in Table 1. The population was segregated into 132 HCV-monoinfected patients and 145 HIV/HCV-coinfected patients.

The rate of EOT response was statistically significantly lower (37% vs 60.5%) and relapses more frequent (32.9% vs 22%)

in coinfected than in HCV-monoinfected patients (Table 1). When the analysis of HCV relapses was stratified according to HCV genotype (Figure 1*A*), it becomes clear that HCV genotypes 1–4 relapsed more frequently overall than did HCV genotypes 2–3, particularly in HIV/HCV-coinfected patients (41.2% vs 12.5%; $P = .001$). On the other hand, HCV relapses in patients infected with HCV genotypes 1–4 occurred more often in HIV/HCV-coinfected patients than in HCV-monoinfected patients (41.2% vs 24.7%; $P = .02$). This difference was not observed for HCV genotypes 2–3.

The time of HCV RNA rebound could be examined in detail in 71 of 76 patients who experienced relapse (for 5 patients there was no result from week 12 after EOT, nor were there stored specimens for retrospective testing). In these patients, HCV relapse could be either early or late. For the rest, Figure 1*B* depicts the time of HCV relapse, stratified according to HIV status. In all but 3 instances (68/71 [95.8%]) relapses occurred during the early period (before week 12 after EOT). No cases of late relapses (between weeks 12 and 24) were recognized in our series. However, 3 individuals showed a rebound in HCV RNA level at late time points. The main characteristics of these 3 patients with very late relapses are summarized in Table 2. Two occurred in HIV/HCV-coinfected patients, one carrying HCV-1b and another HCV-3a. The HCV-monoinfected individual was infected with HCV-1b. All 3 patients experienced relapsed beyond 24 weeks but before 1 year of completion of HCV therapy, and the same initial HCV genotype was found at the time of HCV RNA rebound. Significant genetic distances in the phylogenetic analysis were found for the 2 patients infected with HCV-1b (data not shown). In contrast, the HIV/HCV-coinfected patient with HCV-3a at both baseline and rebound showed closely related viral sequences.

Discussion. To our knowledge, this is the first study to address the rate and timing of HCV relapse in patients with chronic hepatitis C treated with peginterferon plus ribavirin by comparing HCV-monoinfected and HIV/HCV-coinfected patients. Two previous studies of HCV-monoinfected patients had examined the timing of HCV relapses and concluded that they were very rare beyond week 12 [13, 18]; however, patients in these studies had been treated with standard interferon, with or without ribavirin. The use of peginterferon has been shown to increase the chances of EOT response and to minimize HCV relapses [2]. On the other hand, prescription of weight-based ribavirin (avoiding lower ribavirin exposure) has also been associated with a lower rate of HCV relapse [19–22]. Thus, our study population is somewhat unique for exploration of the rate and timing of relapse using the currently recommended treatment for chronic hepatitis C.

In the present study, the overall rate of EOT response was significantly higher among HCV-monoinfected patients than among HIV/HCV-coinfected patients (60.5% vs 37%). On the other hand, HCV relapses occurred significantly more frequently in coinfected than in HCV-monoinfected individuals (32.9% vs 22%). All these observations occurred despite EOT responses and HCV relapses being more frequent in those infected with HCV genotypes 1–4 than with genotypes 2–3, and the later were significantly more prevalent in coinfected than in HCV-monoinfected patients.

The time of HCV relapse did not differ significantly between HCV-monoinfected and coinfected patients. Most patients who experienced relapse did so within the first 12 weeks after discontinuation of peginterferon plus ribavirin. This information is important in 2 ways. First, late HCV relapse does not seem to occur more frequently with the current therapy for hepatitis

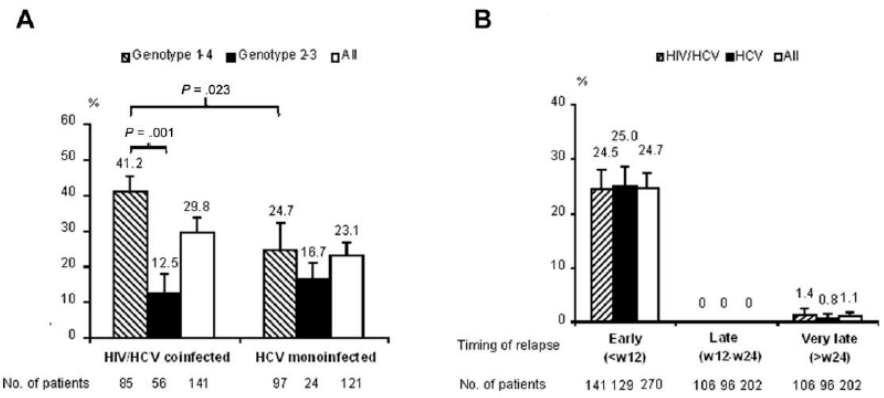


Figure 1. Distribution of episodes of hepatitis C virus (HCV) relapse by HCV genotype (*A*) and timing after end of treatment (*B*), according to human immunodeficiency virus (HIV) status. Note that 9 patients were excluded from the analyses because of lack of information on HCV genotype or missed HCV RNA measurements between the end of treatment and week 24.

Table 2. Main Characteristics of Patients with Very Late Hepatitis C Virus (HCV) RNA Rebound

Characteristic	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Female
Age, years	49	43	44
Weight, kg	68	74	45
Risk group	Unknown	IDU	IDU
Laboratory test result at baseline			
Hemoglobin level, mg/dL	12.8	17.3	15.0
AST level, IU/L	36	31	229
ALT level, IU/L	47	60	149
Platelet count, cells/μL	148,000	205,000	144,000
Liver stiffness, kPa	NA	4.4	26.3
HCV infection			
HCV RNA level before therapy, IU/mL	654,000	276,000	414,000
HCV genotype before therapy	1b	1b	3a
HCV therapy			
Total duration, weeks	53	52	30
Pegylated interferon, μg/week	Alfa-2b at 120	Alfa-2a at 180	Alfa-2a at 180
Ribavirin, mg/day	1000	1200	1000
Drug-dose reduction	No	No	No
HIV infection	No	Yes	Yes
HIV RNA level, copies/mL	NA	<50	3222
CD4 cell count, cells/μL	NA	247	416
CD4 cell percentage	NA	19	16
Concomitant HAART	NA	3TC, d4T, NVP	3TC, TDF, LPV-r
HCV relapse			
Weeks after EOT with HCV RNA level <10 IU/mL	51	32	44
HCV RNA level at relapse, IU/mL	34,000	117,000	2,100,000
HCV genotype at relapse	1b	1b	3a

NOTE. 3TC, lamivudine; d4T, stavudine; EOT, end of treatment; IDU, injection drug user; HAART, highly active antiretroviral therapy; LPV-r, lopinavir-ritonavir; NA, not available or not applicable; NVP, nevirapine; TDF, tenofovir.

C than with standard interferon, with or without ribavirin [13, 18]. On the other hand, HIV coinfection does not seem to increase the risk of HCV relapse beyond week 12 after completion of hepatitis C therapy. In any situation, most HCV relapses occur within the first 12 weeks after discontinuation of therapy.

Recurrence of HCV replication beyond 24 weeks after treatment discontinuation was seen in 3 patients. Although there is some controversy as to the existence of HCV reservoirs, the lack of integration of HCV genetic material in any stable form within infected cells [23, 24] makes it very unlikely that such reservoirs explain late HCV relapses. Phylogenetic analyses suggested that HCV reinfection occurred in 2 patients infected with HCV-1b and that recurrence of the original HCV-3a infection might have occurred in the third subject between months 9 and 12 after treatment discontinuation. However, we cannot exclude the possibility that the latter patient could have been exposed again to the same source that caused her original infection. Several of her relatives (2 brothers and a husband) were former injection drug users. In a recent study of homosexual men infected with HIV who experienced acute hepatitis C and were successfully treated with peginterferon plus ribavirin [12], HCV recurrence beyond 24 weeks was observed in 8 subjects; in 6 of these subjects, phylogenetic analyses showed quite distant sequences and, therefore, supported the notion that HCV reinfections had occurred. However, in the remaining 2 subjects HCV sequences were closely related to the original ones, and exposure to the original source (rather than relapse) was the most likely explanation [25].

In summary, HCV relapse after successful peginterferon plus ribavirin therapy is more frequent in HIV/HCV-coinfected patients than in HCV-monoinfected patients. Regardless of HIV status, HCV relapse is more common in patients infected with HCV genotypes 1–4 than with genotypes 2–3 and almost always occurs within the first 12 weeks after discontinuation of treatment. Most HCV recurrences beyond week 24 are reinfections.

Acknowledgments

Financial support. This work was funded in part by grants from Fundación Investigación y Educación en SIDA, Red de Investigación en SIDA (RETIC 006), and the European AIDS Treatment Network. S.R. was supported by grants from Fondo de Investigación Sanitaria (PI05/2411, PI07/90201, and PI08/0738), Instituto de Salud Carlos III (UIPY 1467/07), and Fundación Investigación y Prevención del SIDA en España (36650/07).

Potential conflicts of interest. All authors: no conflicts.

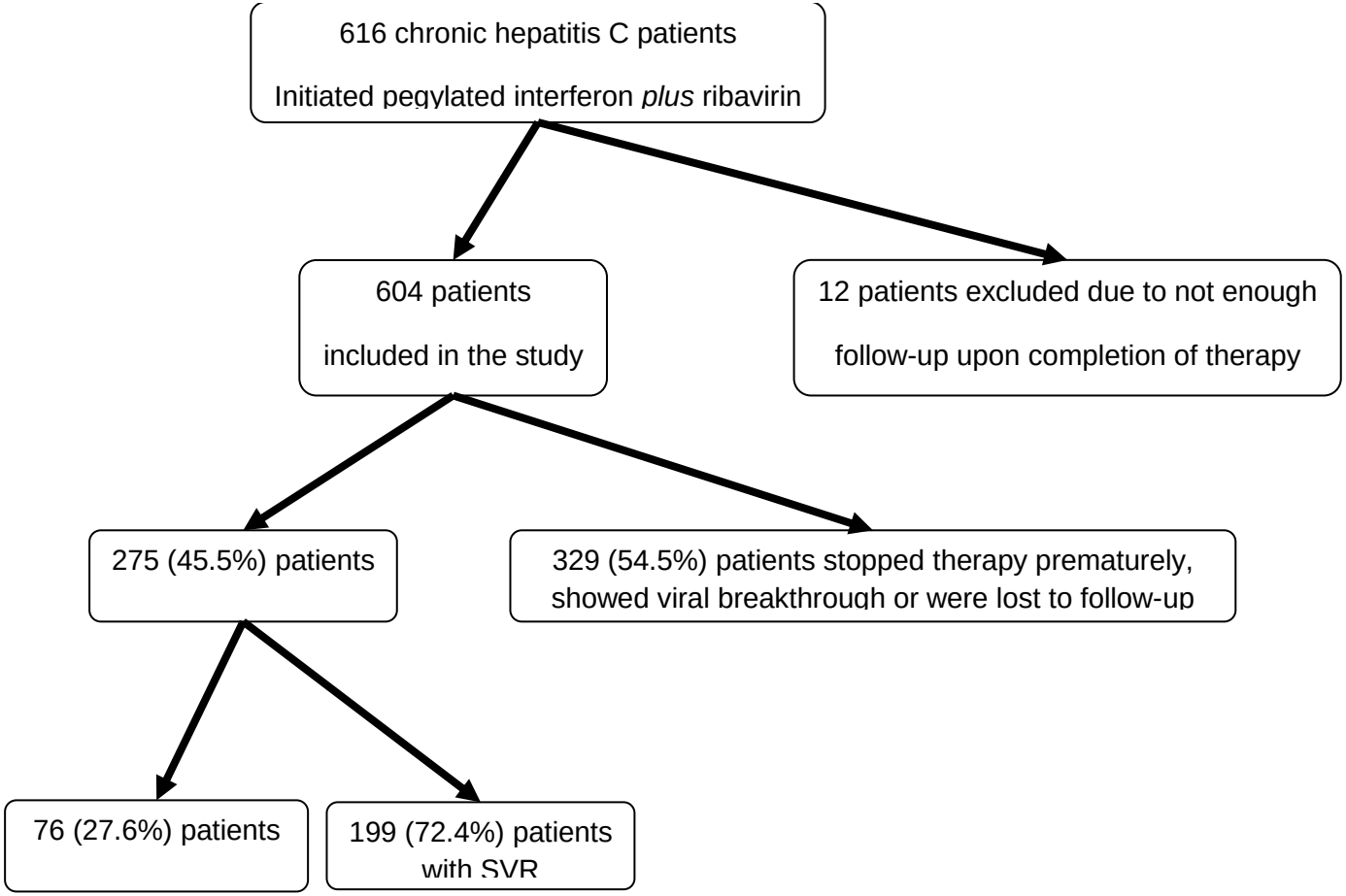
References

1. Manns M, McHutchison J, Gordon S, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet **2001**; 358: 958–65.
2. Fried M, Shiffman M, Reddy K, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med **2002**; 347:975–82.
3. Jacobson I, Brown R, Freilich B, et al. Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. Hepatology **2007**; 46:971–81.
4. Dore G, Torriani F, Rodriguez-Torres M, et al. Baseline factors prognostic of sustained virological response in patients with HIV-hepatitis C virus co-infection. AIDS **2007**; 21:1555–9.
5. Martin-Carbonero L, Nunez M, Marino A, et al. Undetectable hepatitis C virus RNA at week 4 as predictor of sustained virological response in HIV patients with chronic hepatitis C. AIDS **2008**; 22:15–21.
6. Marcellin P, Boyer N, Gervais A, et al. Long-term histologic improvement and loss of detectable intrahepatic HCV-RNA in patients with chronic hepatitis C and sustained response to interferon alpha therapy. Ann Intern Med **1997**; 127:875–81.
7. Poynard T, McHutchison J, Manns M, et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology **2002**; 122:1303–13.
8. Barreiro P, Labarga P, Martín-Carbonero L, et al. Sustained virological response following HCV therapy is associated with non-progression of liver fibrosis in HCV/HIV-coinfected patients. Antivir Ther **2006**; 11: 869–77.
9. Reichard O, Glaumann H, Fryden A, Norkrans G, Wejstal R, Weiland O. Long-term follow-up of chronic hepatitis C patients with sustained virological response to alpha-interferon. J Hepatol **1999**; 30:783–7.
10. Soriano V, Maida I, Nunez M, et al. Long-term follow-up of HIV-infected patients with chronic hepatitis C virus infection treated with interferon-based therapies. Antivir Ther **2004**; 9:987–92.
11. Veldt B, Saracco G, Boyer N, et al. Long term clinical outcome of chronic hepatitis C patients with sustained virological response to interferon monotherapy. Gut **2004**; 53:1504–8.
12. Jones R, Brown D, Nelson M, et al. Hepatitis C viremia following sustained virological response to pegylated interferon and ribavirin in HIV+ men who have sex with men: reinfection or late relapse [abstract LB61]? In: Program and abstracts of the 15th Conference on Retroviruses and Opportunistic Infections (Boston). University of California, San Diego, **2008**.
13. Zeuzem S, Heathcote E, Shiffman M, et al. Twelve weeks of follow-up is sufficient for the determination of sustained virologic response in patients treated with interferon alpha for chronic hepatitis C. J Hepatol **2003**; 39:106–11.
14. Perelson A, Herrmann E, Micol F, Zeuzem S. New kinetic models for the hepatitis C virus. Hepatology **2005**; 42:749–54.
15. Soriano V, Peters M, Zeuzem S. New therapies for hepatitis C virus infection. Clin Infect Dis **2009**; 48:313–20.
16. Yee H, Currie S, Darling J, Wright T. Management and treatment of hepatitis C viral infection: recommendations from the Department of Veterans Affairs Hepatitis C Resource Center program and the National Hepatitis C Program office. Am J Gastroenterol **2006**; 101:2360–78.
17. Soriano V, Puoti M, Sulkowski M, et al. Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel. AIDS **2007**; 21:1073–89.
18. McHutchinson J, Poynard T, Esteban-Mur R, et al. Hepatic HCV RNA before and after treatment with interferon alone or combined with ribavirin. Hepatology **2002**; 35:688–93.
19. Rodríguez-Torres M, Ríos-Bedoya C, Ortiz-Lasanta G, Purcell-Arevalo D, Marxuach-Cuétara A, Jiménez-Rivera J. Weight affects relapse rates in Latinos with genotype 2/3 chronic hepatitis C treated with peginterferon alfa-2a 180 mcg/week and ribavirin 800 mg/day for 24 weeks. J Med Virol **2008**; 80:1576–80.
20. Nuñez M, Marino A, Miralles C, et al. Baseline serum hepatitis C virus (HCV) RNA level and response at week 4 are the best predictors of relapse after treatment with pegylated interferon plus ribavirin in HIV/HCV-coinfected patients. J Acquir Immune Defic Syndr **2007**; 45:439–444.
21. Shiffman M, Salvatore J, Hubbard S, et al. Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. Hepatology **2007**; 46:371–6.
22. Morello J, Rodríguez-Novoa S, Barreiro P, et al. Ribavirin plasma levels at week 4 predicts HCV relapse in HIV/HCV coinfected patients treated for HCV [abstract 842]. In: Program and abstracts of the 15th Conference on Retroviruses and Opportunistic Infections (Montreal). **2009**.
23. Soriano V, Perelson A, Zoulim F. Why are there different dynamics in the selection of drug resistance in HIV and hepatitis B and C viruses? J Antimicrob Chemother **2008**; 62:1–4.
24. Maylin S, Martinot-Peignoux M, Moucari R, et al. Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. Gastroenterology **2008**; 135:821–9.
25. Low E, Vogel M, Rockstroh J, Nelson M. Acute hepatitis C in HIV-positive individuals. AIDS Rev **2008**; 10:245–53.

A total of 616 patients with chronic hepatitis C had initiated treatment with pegIFN plus weight-based RBV during the 7-year study period at our institution. Twelve patients were excluded from further analyses since not enough follow-up had been completed at the time the current analysis was done.

Figure 17 depicts schematically the allocation of patients included in the analyses. From the remaining 604 patients, 386 (64%) were HIV-positive and 218 (36%) HIV-negative. From this initial treated population, 329 (54.5%) patients experienced early virological failure, viral breakthrough during treatment, discontinued prematurely due to side effects or were lost to follow-up before attaining the planned EOT. Overall, 275 (45.5%) of the original treated population completed the planned duration of hepatitis C therapy and had undetectable serum HCV-RNA, and constituted the final population for the analysis. Of them, 76 (27.6%) experienced HCV relapse.

Figure 17 Flow chart of the study population in the prognostic study.



The main characteristics of patients who attained EOT response are summarised in Table 11. The population was segregated into 132 HCV-monoinfected and 145 HIV/HCV co-infected patients. HIV-infected patients were significantly younger and more frequently former intravenous drug users, as compared with HCV-monoinfected individuals. The proportion of HCV genotypes 1 or 4 was lower in coinfected patients but conversely advanced liver fibrosis was more frequently recognised in coinfected compared to HCV-monoinfected patients. RBV dosing administered did not differ significantly comparing both groups of patients. PegIFN alpha-2a was the most frequent modality administered in both groups, but pegIFN alpha-2b was prescribed significantly more frequent in coinfected than HCV-monoinfected patients. With respect to HIV features in coinfected individuals, most were under antiretroviral therapy, had undetectable plasma HIV-RNA and CD4 counts above 500 CD4 cells/mm^3.

Table 11 Pre-treatment characteristics of chronic hepatitis C patients who attained end-of-treatment response following a course of pegylated interferon plus ribavirin therapy.

	HCV monoinfected	**HIV/HCV coinfected**	**p**
No.	132	143	
Male gender (%)	82 (62)	103 (72)	0.3
Mean age (years)	46±0.8	42±0.4	<0.001
Risk group (%)			
IDU	89 (67)	124 (87)	<0.001
Other / Unknown	43 (33)	19 (13)	
HCV infection			
HCV-RNA (log IU/mL)	6.0±0.1	5.9±0.1	0.5
HCV-RNA >500,000 IU/mL	83 (70)	87 (68)	0.7
HCV genotypes 1 or 4	97 (80)	85 (60)	<0.001
Liver parameters			
Grade 3-4 LEE (%)	9 (8)	18 (13)	0.4
Mean AST/ALT (IU/ml)	0.7±0.1	0.8±0.2	0.02
FIB-4 score (units)	0.91±0.09	1.30±0.12	0.02
APRI score (units)	1.76±0.12	2.13±0.15	0.07
Metavir fibrosis score estimates (%)			
F≤2	42 (72)	40 (54)	
F3	6 (11)	19 (25)	0.04
F4	10 (17)	16 (21)	
HCV therapy			
PegIFN alfa-2a / alfa-2b (%)	118/14 (89/11)	98/45 (68/32)	<0.001
Mean RBV dose (mg per day)	1,171±14	1,128±23	0.6
HIV infection			
Mean HIV-RNA (log copies/mL)	--	3.3±2.9	
HIV-RNA <50 copies/mL (%)	--	107 (77)	
Mean CD4 count (cells/mm^3)	--	575±24	
CD4 count >500 cells/mm^3 (%)	--	67 (52)	
Under HAART (%)	--	85 (59)	
Abacavir (%)	--	24 (18)	
Zidovudine (%)	--	6 (4)	
Tenofovir (%)	--	41 (30)	

HCV relapse

The overall outcome of HCV therapy differed significantly comparing HCV monoinfected and coinfected patients (Table 12). The overall rate of EOT response was significantly lower (37% vs 60.5%) and the relapse rate was higher (32.9% vs 22%) in coinfected compared to HCV-monoinfected patients.

Table 12 Outcome following pegylated interferon plus ribavirin therapy in the study population, by HIV status

	HCV monoinfected	**HIV/HCV Coinfected**	**p**
No. of patients treated	218	386	-
EOT response (%)	132 (60.5)	143 (37)	<0.001
HCV relapse (%)	29 (22)	47 (32.9)	0.04

End-of-treatment (EOT) response: serum HCV-RNA <10 IU/mL at completion of HCV therapy; hepatitis C virus (HCV) relapse: serum HCV-RNA >10 IU/mL any time after EOT response.

When the analysis of HCV relapses was stratified according to HCV genotypes (Figure 18 and Figure 19), it becomes clear that HCV genotypes 1 or 4 overall

relapsed more frequently than HCV genotypes 2 or 3, particularly in HIV/HCV coinfected patients (41.2% vs 12.5%, p=0.001). On the other hand, HCV relapses in patients infected with HCV genotypes 1 or 4 occurred significantly more frequently in coinfected than HCV-monoinfected patients (41.2% vs. 24.7%; p=0.02). This difference was not observed for HCV genotypes 2 or 3.

Figure 18 Absolute number of patients with HCV relapse, according to HIV status and HCV genotype

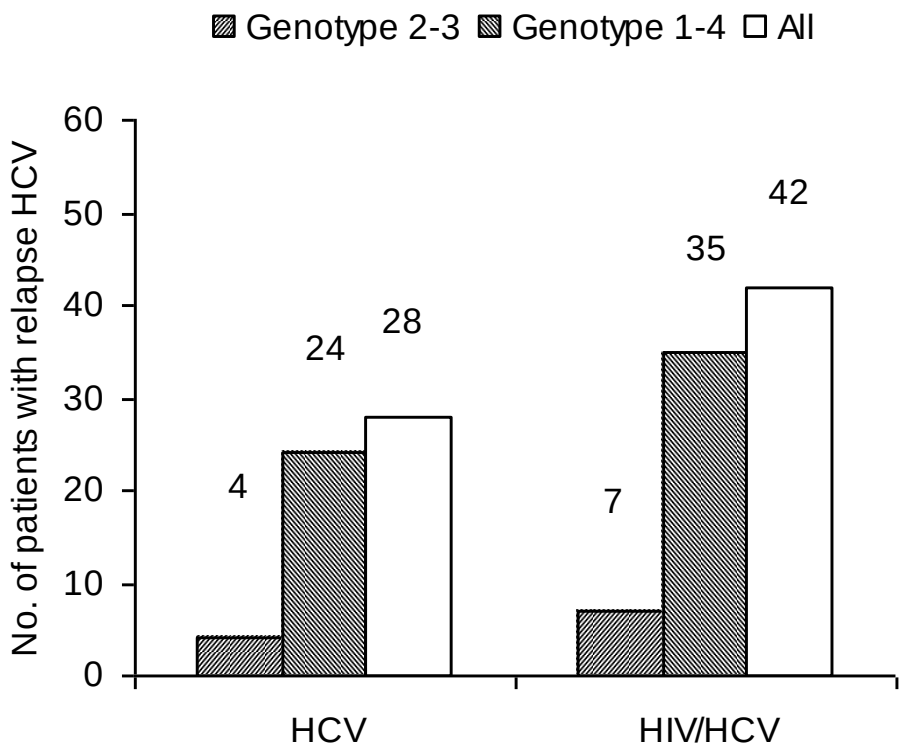
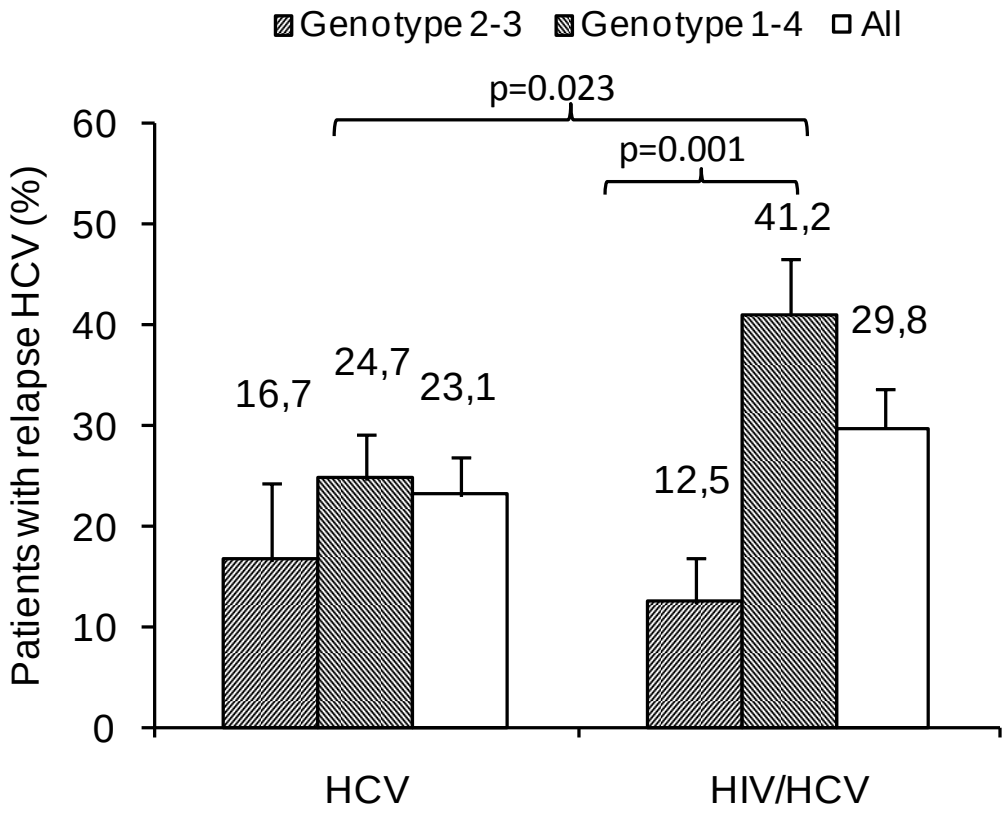


Figure 19 HCV relapse according to HIV status and HCV genotype.



The time of HCV rebound could be examined in detail in 71 out of 76 relapsers (Figure 20), since in 5 patients there was no result from week 12 after EOT neither stored specimens from that time for retrospective testing. In these cases, HCV relapse could be either early or late.

Figure 20 Absolute number of patients with HCV relapse according to HIV status and time to relapse

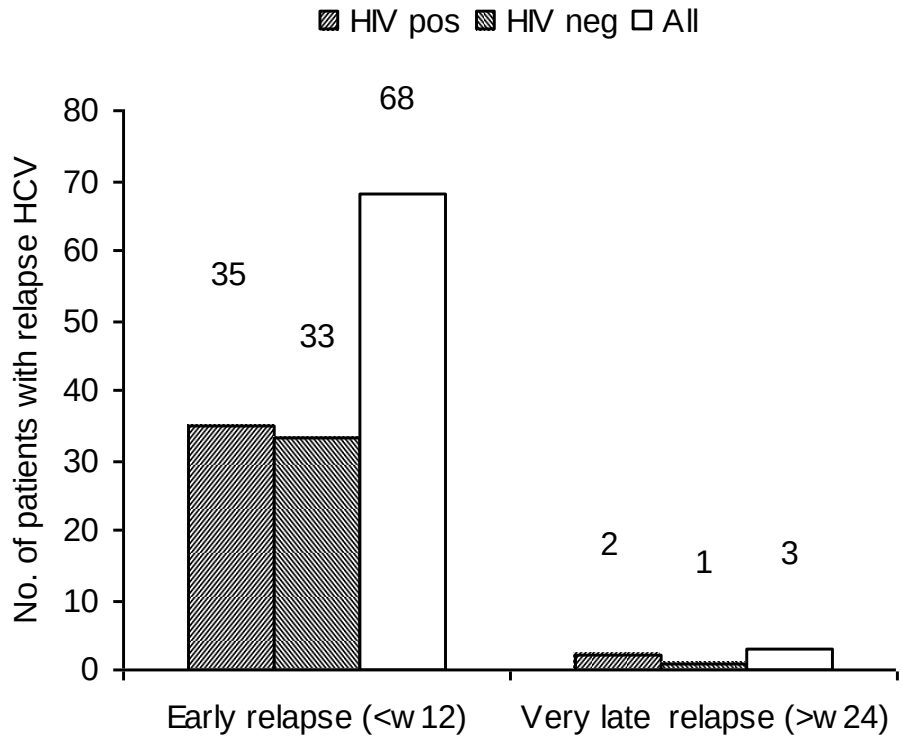
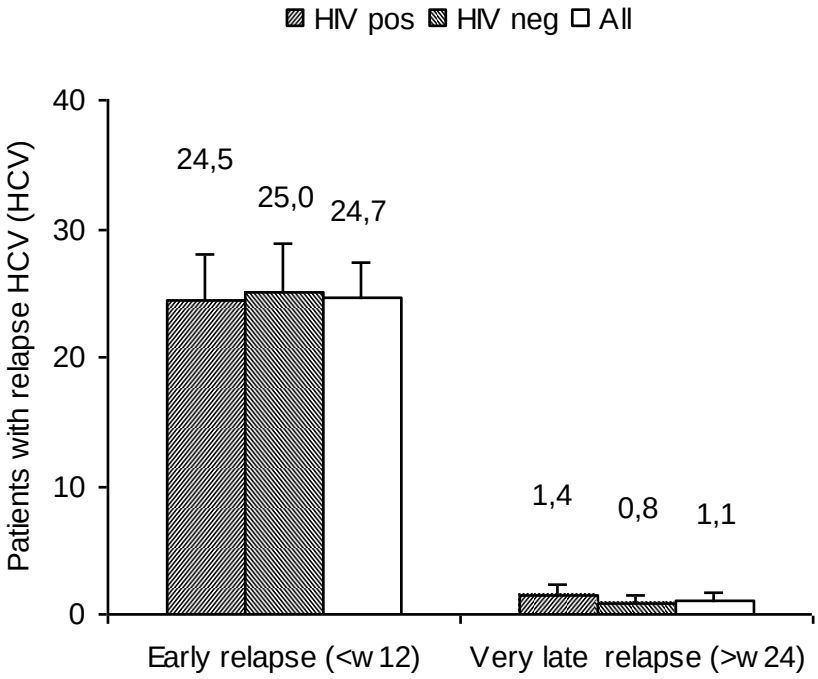


Figure 21 Proportion of HCV relapse according to HIV status and time to relapse



For the rest, **(**

Figure 21**)** depicts the time for HCV relapse, stratified according to HIV status. It is noteworthy that in all (68/71; 95.8%) but three instances, all HCV relapses occurred during the early period (before week 12 after EOT). No cases of late relapses (between weeks 12 and 24) were recognised in our series (Figure 21 and Figure 22).

However, from the remaining 195 subjects with serum HCV-RNA negative at week 24, 3 individuals showed a rebound in HCV-RNA at late time points. The main characteristics of these 3 patients with very late relapses are summarised in Table 13. Two occurred in HIV/HCV coinfected patients, one carrying HCV genotype 1b and another with genotype 3a. The HCV-monoinfected individual was infected with HCV genotype 1b. All three cases relapsed beyond 24 weeks but before 1 year upon completion of HCV therapy and the same initial HCV genotype was found at the time of HCV rebound.

Figure 22 Distribution of episodes of HCV relapse by HCV genotype (a) and lag after end of treatment (b), according to HIV status.

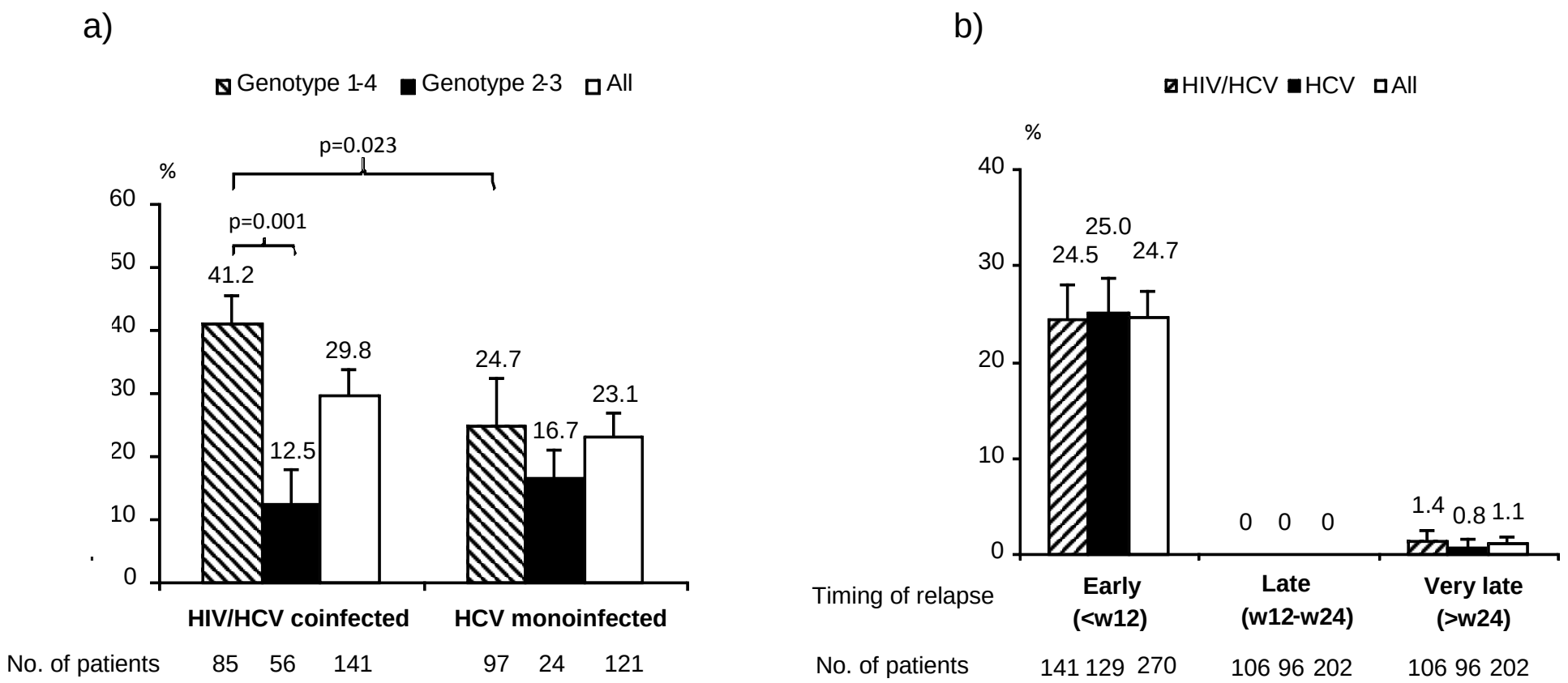


Table 13 Main characteristics of patients with very late HCV relapse.

	#1	#2	#3
Gender	Male	Male	Female
Age (years-old)	49	43	44
Weight (kg)	68	74	45
Risk group	Unknown	IDU	IDU
Lab-test at baseline			
• Hb (mg/dL)	12.8	17.3	15.0
• AST/ALT (IU/L)	36/47	31/60	229/149
• Platelets (per µL)	148,000	205,000	144,000
• Liver stiffness (kPa)	NA	4.4	26.3
HCV infection			
• HCV-RNA level pretherapy (IU/mL)	654,000	276,000	414,000
• HCV genotype pretherapy	1b	1b	3a
HCV therapy			
• Total duration (weeks)	53	52	30
• PegIFN (µg per week)	Alfa-2b (120)	Alfa-2a (180)	Alfa-2a (180)
• RBV (mg per day)	1,000	1,200	1,000
• Drug dose reduction	No	No	No
HIV infection	No	Yes	Yes
• HIV-RNA (copies/mL)	NA	<50	3,222
• CD4 count (cells/µL)	NA	247 (19%)	416 (16%)
• Concomitant HAART	NA	3TC, d4T, NVP	3TC, TDF, LPV-r
HCV relapse			
• Weeks after EOT with HCV-RNA <10 IU/mL	51	32	44
• HCV-RNA level at relapse (IU/mL)	34,000	117,000	2,100,000
• HCV-genotype at relapse	1b	1b	3a

IDU, intravenous drug user; Hb, haemoglobin; PegIFN, pegylated interferon; RBV, ribavirin; HAART, highly active antiretroviral therapy; 3TC, lamivudine; d4T, stavudine; TDF, tenofovir; NVP, nevirapine; LPV-r, lopinavir-ritonavir; EOT, end of treatment

Univariate and multivariate analyses (RR [95% CI p) were conducted to explore which factors were associated with HCV relapse in the study population. Given the wide baseline differences between HCV-monoinfected and coinfected patients, the analyses were done separately for each group. In the HCV-monoinfected population, no significant predictors of HCV relapse could be identified (data not shown). In contrast, HCV genotypes 1 or 4 (7.08 [2.48-20.17] 0.001) and greater serum HCV-RNA levels (1.99 [1.09-3.67] 0.026) emerged as independently associated with an increased risk of HCV relapse in HIV/HCV coinfected patients (Table 14).

Table 14 **Predictors of HCV relapse in HIV/HCV coinfected patients**

Variables	Univariate analysis		Multivariate analysis	
	RR (95% CI)	**p**	**RR (95% CI)**	**p**
Age (per year)	1.01 (0.93-1.09)	0.87		
Male gender	0.69 (0.31-1.51)	0.36		
HCV genotypes 1 or 4	4.90 (1.98-12.07)	0.001	7.08 (2.48-20.17)	0.001
HCV-RNA (per log IU/mL)	1.95 (1.07-3.45)	0.028	1.99 (1.09-3.67)	0.026
PegIFN alpha 2a vs 2b	1.69 (0.76-3.85)	0.21		
RBV dose (per mg daily)	1.01 (1.00-1.02)	0.11		
AST/ALT (per IU/ml)	0.55 (0.13-2.23)	0.41		
FIB-4 (per unit)	1.02 (0.83-1.25)	0.82		
APRI (per unit)	1.04 (0.82-1.33)	0.71		
Liver elasticity (kPa)	0.96 (0.89-1.05)	0.42		
Metavir fibrosis score (per grade)	0.89 (0.58-1.37)	0.61		

RR, relative risk; CI, confidence interval; PegIFN, pegylated interferon; RBV, ribavirin; kPa, kilopascal units.

Very late HCV rebounds

Phylogenetic analyses were conducted in the three patients with very late HCV rebounds in order to provide further insights about whether it was a truly HCV relapse and discharge HCV reinfection. Despite the same original HCV genotype was responsible for the late HCV reappearance in all three patients, significant genetic distances were found for the two patients infected with HCV genotype 1b (Figure 23). In contrast, the HIV/HCV coinfected patient with HCV genotype 3a at baseline and rebound showed viral sequences closely related (Figure 24).

Figure 23 Phylogenetic tree in relapsing patients #1 and #2 and controls matched by genotype (HCV-1b). Sample A refers to the last specimen with detectable serum HCV-RNA under pegIFN plus RBV therapy, and sample B to first detectable HCV-RNA at rebound after successful EOT

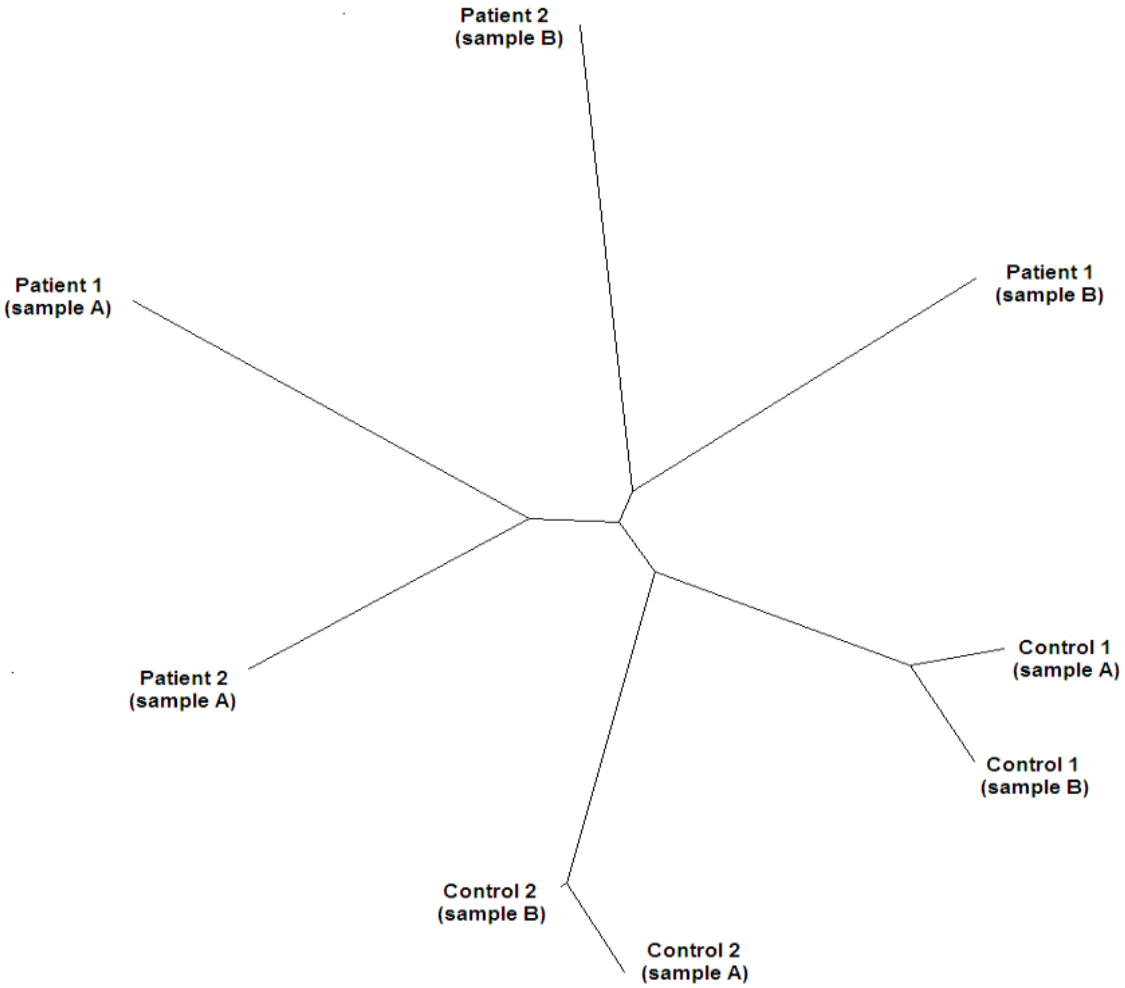
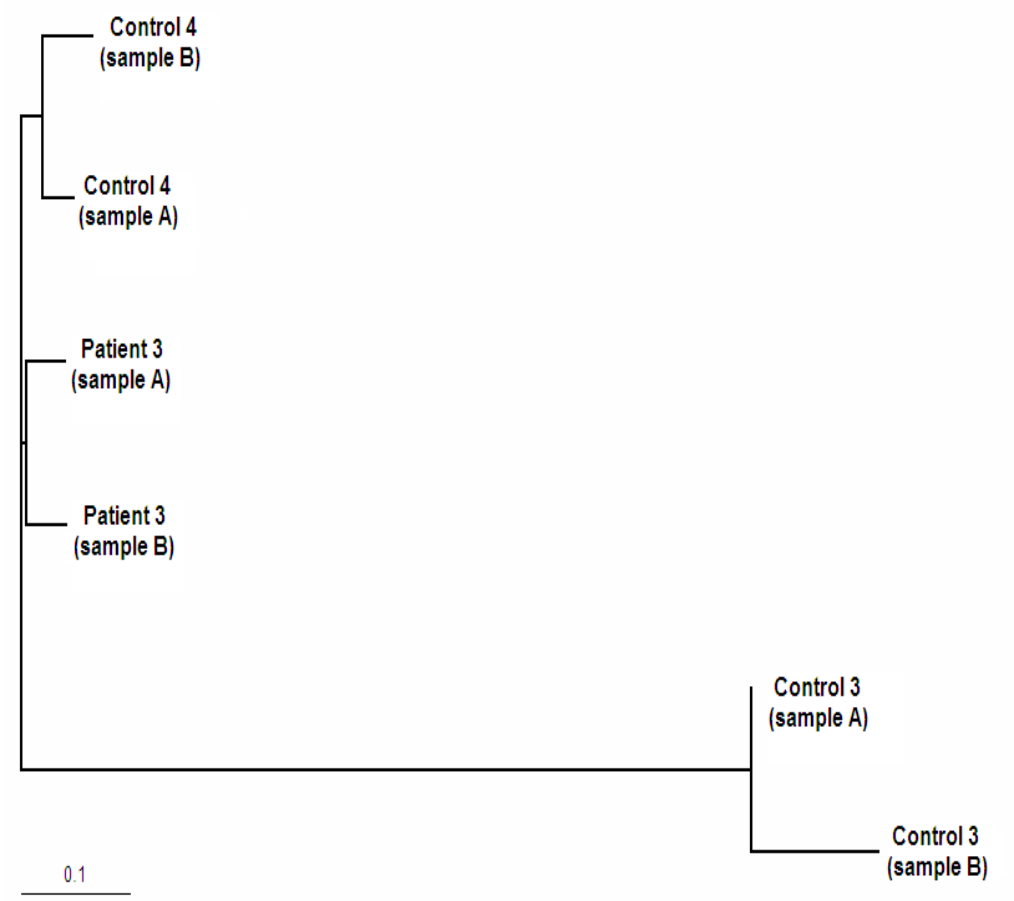


Figure 24 Phylogenetic tree in the third relapsing patients (#3) and controls matched by genotype (HCV-3a). Sample A refers to the last specimen with detectable serum HCV-RNA under pegIFN plus RBV therapy, and sample B to first detectable HCV-RNA at rebound after successful EOT.



C. Third article: Modeling the probability of sustained virological response to peginterferon-ribavirin therapy in HCV-HIV coinfected patients.

Madrid, April 25th 2010

Dear Editor,

Please find attached a manuscript to be considered for publication in **Clinical Infectious Diseases** as an Original contribution. The authors accept the uniform requirements for manuscripts submitted to biomedical journals. All authors have contributed to the work, and have read and accepted the current version of the manuscript. We acknowledge no conflicts of interest with this work.

Looking forward to hearing from you soon.

Sincerely yours,

Dr. Vincent Soriano

Modeling the probability of sustained virological response to peginterferon-ribavirin therapy in HCV-HIV coinfected patients

Jose Medrano[1], Karin Keulen[2], Norma Rallón[1], Juan Macías[2], Salvador Resino[3], Jose Miguel Benito[1], Carlos Sánchez-Piedra[1], Aida Calvino[3], Susanna Naggie[4],

Eugenia Vispo[1], Pablo Barreiro[1], John McHutchison[4], Juan Antonio Pineda[2] and Vincent Soriano[1]

[1]Department of Infectious Diseases, Hospital Carlos III, Madrid. Spain.

[2]Infectious Diseases Unit, Hospital Universitario de Valme, Seville, Spain.

[3]Laboratory of Molecular Epidemiology of Infectious Diseases, National Centre of Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid. Spain.

[4]Duke Clinical Research Institute, Durham, NC, USA.

Key words: HCV, HIV, IL28B, score, interferon, ribavirin, coinfection

Running title: Modeling treatment response in HIV/HCV coinfection

Correspondence: Dr. Vincent Soriano, Department of Infectious Diseases, Hospital Carlos III, Calle Sinesio Delgado 10, Madrid 28029, Spain. Phone +34 91 4532500; Fax +34 91 7336614; E-mail: vsoriano@dragonet.es

Abstract

Background: A single nucleotide polymorphism (SNP) near the *IL28B* gene (rs12979860) strongly predicts sustained virological response (SVR) to peginterferon-ribavirin (pegIFN-RBV) treatment in chronic hepatitis C virus (HCV) infection. Given that therapy is poorly tolerated and response is lower in HIV/HCV-coinfected patients, the recognition of predictors of response is a high priority in this population.

Methods: A non-invasive score was built based on the probability of achieving SVR in a group of 159 HIV/HCV patients treated at one clinic in Spain. Then, it was validated using data from another 86 coinfected individuals treated at another clinic. Only individuals who had completed a course of pegIFN-RBV therapy and had validated outcomes were considered.

Results: The final score only included four variables, two host-related (IL28B rs12979860 SNP and liver stiffness) and to HCV-related (genotype and viral load). The area under the receiver operating characteristic (AUROC) curve was 0.89 in the building group and 0.85 in the validation group.

Conclusion: The probability of achieving SVR with pegIFN-RBV therapy in HIV/HCV-coinfected patients can reliably be estimated at baseline using a score that includes four non-invasive parameters.

Key words: hepatitis C, HIV, coinfection, pegylated interferon, IL28B, score, model.

Introduction

Hepatitis C virus (HCV) infects more than 175 million people worldwide [1]. In western countries, HCV is the leading cause of end-stage liver disease and hepatocellular carcinoma, as well as the main indication for liver transplantation [1]. Both HCV and HIV-1 share routes of transmission and establish chronic infections; therefore coinfection is relatively common. Overall 20-25% of HIV-infected individuals worldwide suffer from chronic hepatitis C, with large differences mainly depending on the risk group category [2]. The course of HCV-associated liver disease is accelerated in dually infected individuals [3,4], and thereby HCV has emerged as an important cause of morbidity and mortality in this population [5], especially since successful antiretroviral therapy has dramatically reduced the rate of opportunistic illnesses.

Current therapy for chronic hepatitis C is based on a combination of peginterferon-α (pegIFN) and ribavirin (RBV) generally administered for 6 to 18 months, depending on viral kinetics and genotype [6]. Unfortunately, the medication is poorly tolerated and overall only 50-60% of patients are cured. Of note, this figure is lower in HIV/HCV-coinfected patients [2,7]. Thus, the identification of predictors of treatment success is particularly desirable in this population in order to ensure adequate selection of the best candidates for HCV therapy, which largely remains untreated in most places [8-10].

The best predictors of response to current HCV therapy are infection due to HCV genotypes 2 or 3, low serum HCV-RNA and null or minimal liver fibrosis [11]. Recently, three independent genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) around the *IL28B* gene as strongly associated with treatment outcomes in HCV-monoinfected individuals [12-16]. The SNP with the strongest association, rs12979860, is located in chromosome 19, 3Kb upstream of the *IL28B* gene. In patients infected with HCV genotype 1, the rs12979860 CC genotype is associated with more than two-fold greater rate of sustained virological response (SVR) than the CT or TT genotypes. Similar findings have recently been reproduced in HIV/HCV-coinfected patients [17]. Modelling the impact of baseline predictors of treatment outcome incorporating this new genetic threat may bring clinicians the opportunity to make more adequate treatment decisions in HIV/HCV-coinfected patients [18-21].

Patients and Methods

To construct a clinically helpful predictive index of SVR, a population of HIV/HCV-coinfected patients with regular follow-up at Hospital Carlos III, Madrid, was initially used as the development cohort. The main characteristics of the whole cohort and HCV therapy uptake have already been described elsewhere [21]. For the external validation of the predictive index, an independent population of HIV/HCV coinfected patients treated during the same

period at another large clinic, Hospital Universitario de Valme, Seville, was then evaluated (validation cohort). To participate in the study, written informed consent for genetic testing was obtained from all individuals, and the study protocol was evaluated and approved by the hospital ethics committees.

Development cohort

From an initial cohort of 672 HIV/HCV-coinfected patients on regular follow-up at Hospital Carlos III [21], a total of 159 individuals who had initiated therapy with pegIFN-RBV and had validated outcomes were selected. Patients with poor drug compliance and/or who had discontinued therapy due to side effects were excluded, as were patients with HBV coinfection. Treatment had been provided between November 2004 and December 2008. Inclusion criteria required being IFN-naïve, have baseline liver fibrosis assessment using elastometry, serum HCV-RNA and genotyping. Lastly, all subjects had to be tested for the rs12979860 SNP. For the last result, peripheral blood cell specimens were recorded from all patients after obtaining informed consent.

Validation cohort

From a total of 154 HIV/HCV-coinfected patients who similarly had completed a course of HCV therapy during the same period at Hospital Universitario de Valme Seville, 86 patients with the same inclusion criteria were eligible for the study.

Hepatitis C therapy

In both cohorts, treatment regimens included pegIFN alpha 2a or 2b at standard doses (180 μg/week or 1.5 μg/kg/week, respectively) plus weight-adjusted RBV dosing (1000 mg/day for patients weighting <75 kg and 1200 mg/day for patients weighting >75 kg). In accordance with international guidelines [2] , patients with HCV genotypes 1 or 4 received either 48 or 72 weeks of treatment, and patients with HCV genotype 3 received 24 or 48 weeks of treatment, according to virological response at week 4. No patients were infected with HCV genotype 2. Early stopping rules were applied for subjects with suboptimal virological responses at weeks 12 and 24 [2].

Treatment outcomes

SVR was the primary outcome and was defined as undetectable serum HCV-RNA 24 weeks after completion of treatment. For the purpose of this study, relapsers were considered along with non-responders, who were patients who experienced suboptimal virological response during the treatment period and for this reason did not complete the planned duration of therapy. As previously mentioned, patients with poor drug compliance and/or who discontinued therapy prematurely due to side effects were excluded from the study.

HCV viremia and genotyping

In both cohorts of patients, serum HCV-RNA was measured using a real-time PCR assay (COBAS TaqMan, Roche, Barcelona, Spain), whose lower limit of detection is 10 IU/mL. HCV genotyping was performed using a commercial RT-PCR hybridization assay (Versant HCV Genotype v2.0 LiPA, Siemens, Barcelona, Spain), which maximally reduces the chances of HCV genotype misclassification [ ].

Liver fibrosis staging

The extent of hepatic fibrosis was measured in both cohorts using transient elastography by FibroScan (Echosens®, Paris, France). Details about this non-invasive method, the examination procedure, and correlation of hepatic fibrosis estimates with liver biopsy findings have been reported elsewhere [22-24]. Liver stiffness values are expressed in Kilopascals (kPa). For clinical purposes, advanced liver fibrosis and cirrhosis, corresponding to METAVIR scores F3 and F4, was defined for liver stiffness values $\geq$9.5 kPa, following the results of evaluations performed in both HCV-monoinfected and HIV/HCV-coinfected patients [25,26].

rs12979860 SNP genotyping

For patients from Hospital Carlos III, IL28B genotyping was performed at the Duke Institute for Genome Sciences and Policy. Genotyping was conducted in a blinded fashion on DNA specimens collected from each individual, using the 5' nuclease assay with allele specific TaqMan probes, as previously described [12,18]. For patients from the validation cohort, similar primers and conditions were used at a local laboratory.

Statistical methods

Overall, results are presented as medians, percentiles 25 and 75 for continuous variables, and as frequencies and percentages for categorical data. Analysis of normality was performed using the Kolmogorov-Smirnov test. Categorical data and proportions were analyzed using the chi-squared test or the Fisher's exact test, as required. The Student T-test was used to compare the means of the two groups with normal distributions. while the Man-Whitney test was used to compare variables with non-normal distributions.

Multiple association tests were conducted using univariate logistic regression to identify independent variables associated with SVR, the primary outcome. In the last analysis we included all variables with p values <0.05 in the univariate

analysis. Then, a forward stepwise logistic regression analysis was conducted with a p value for entry and exit of 0.05 and 0.10, respectively. Thereafter, an index to predict SVR via a logistic probability function was built. The accuracy and the predictive values of this index were obtained and compared by calculating areas under the receiver operating characteristic (AUC-ROC) curves for both the development and validation cohorts.

Several cut-offs were tested for evaluating the predictive index of SVR. For obtaining a higher sensitivity and negative predictive value (NPV), the lowest cut-off was established at 0.25. Conversely, for obtaining a higher specificity and positive predictive value (PPV), the highest cut-off was 0.75. Finally, a search for the most "optimal" cut-off fitting the best sensitivity and specificity was established at 0.5. The diagnostic odds ratio (DOR), which expresses the strength of the association between test result and disease, was also determined. Briefly, DOR is the ratio of the odds of a positive result in a given person with the target condition compared to a person without it [18]. A DOR of 1 suggests that the test provides no diagnostic evidence. Finally, the likelihood ratios (LR), which describe how many times a person with the target condition is more likely to have a particular test result compared to a person without the condition, were also calculated. LRs contribute to change, after the test has been made, the probability that a target condition is present. Binary tests have two LRs, positive and negative (LR+, LR-). A LR of 1 indicates no diagnostic value.

All tests were two-tailed with only p values <0.05 considered as significant. All statistical analyses were performed using the SPSS v16.0 software (SPSS Inc, Chicago, IL, USA) and STATA 9.1 (Lakeway, Houston, TX, USA).

1. Patients

HIV/HCV coinfected patients were divided into: a) an estimation group (159 cases; 64.9%) form HCIII (Madrid) which were used to develop the predictive index; and b) a validation group (86 cases; 35.1%) from HUV (Seville) which were used to confirm its power to predict outcome in different subsets of patients (Figure 25). Both groups were well-matched for most baseline characteristics (Table 15).

Figure 25 Datasets used in the development (estimation group) and external validation (validation group) of the predictive index of sustained virological response (PISVR). ROC, receiver-operating characteristics; SVR, sustained virological response.

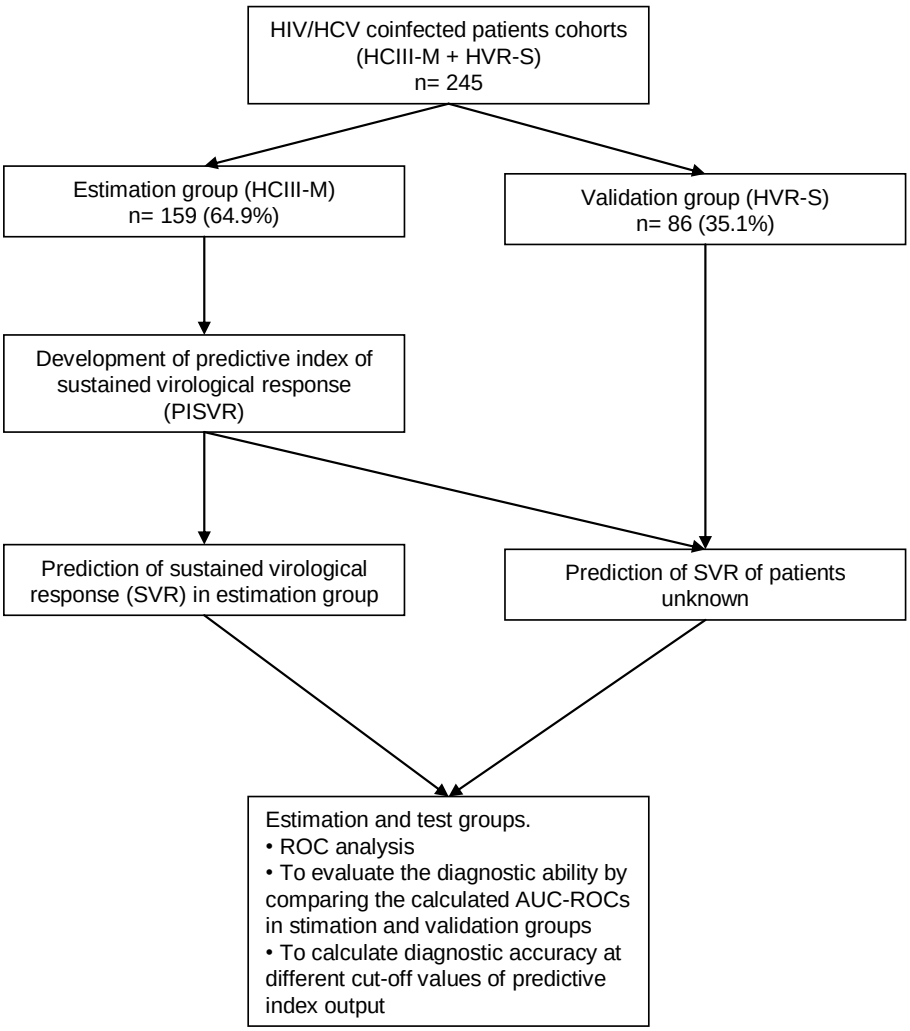


Table 15 Main characteristics of the HIV/HCV-coinfected study population.

	Estimation group	Validation group	p
No. patients	159	86	
Male gender (%)	121 (74.7)	72 (82.8)	0.14
Median age (years)	42 (39 ; 45.2)	42.3 (39.9 ; 45.8)	0.26
Prior intravenous drug use	134 (82.7)	74 (85.1)	0.64
On antiretroviral therapy	127 (80.2)	73 (85.1)	0.33
Liver fibrosis estimates (Metavir stages)			
F0-F1	96 (59.3)	30 (34.5)	0.002
F2	22 (13.6)	21 (24.1)	0.03
F3	20 (12.3)	16 (18.4)	0.09
F4	24 (14.8)	20 (23.0)	0.11
Median liver stiffness (Kpa)	6.7 (4.9 ; 9.9)	8.8 (6.5 ; 13.2)	0.03
HIV markers			
Baseline CD4 count (cells/µL)	477 (363 ; 650)	479 (373 ; 662)	0.54
Median plasma HIV-RNA (log cop/mL)	1.7 (1.7 ; 1.8)	1.8 (1.7 ; 4.2)	0.62
HCV markers			
HCV genotypes 1-4	112 (69.1)	58 (66.7)	0.69
HCV genotypes 2-3	50 (30.9)	29 (33.3)	0.69
Serum HCV-RNA >850,000 cp/ml	49 (30.2)	50 (57.5)	<0.001

In colour, variables with statistically significant differences.

2. Predictive index of sustained virological response (PISVR)

In the estimation group, we identified several variables associated with SVR by forward stepwise logistic regression analysis (Table 16) and theses variables were included to develop the PISVR.

Table 16 Predictors of sustained virological response to peginterferon-ribavirin therapy in HIV/HCV-coinfected patients (stepwise multivariate logistic regression analysis).

	OR	95% CI	p
HCV genotypes 1-4	0.212	0.078; 0.577	0.002
Serum HCV-RNA (log IU/mL)	0.186	0.091; 0.381	<0.001
Liver stiffness (Kilopascals)	0.920	0.865; 0.977	0.007
rs12979860 (CT or TT) SNP	0.170	0.065; 0.442	<0.001

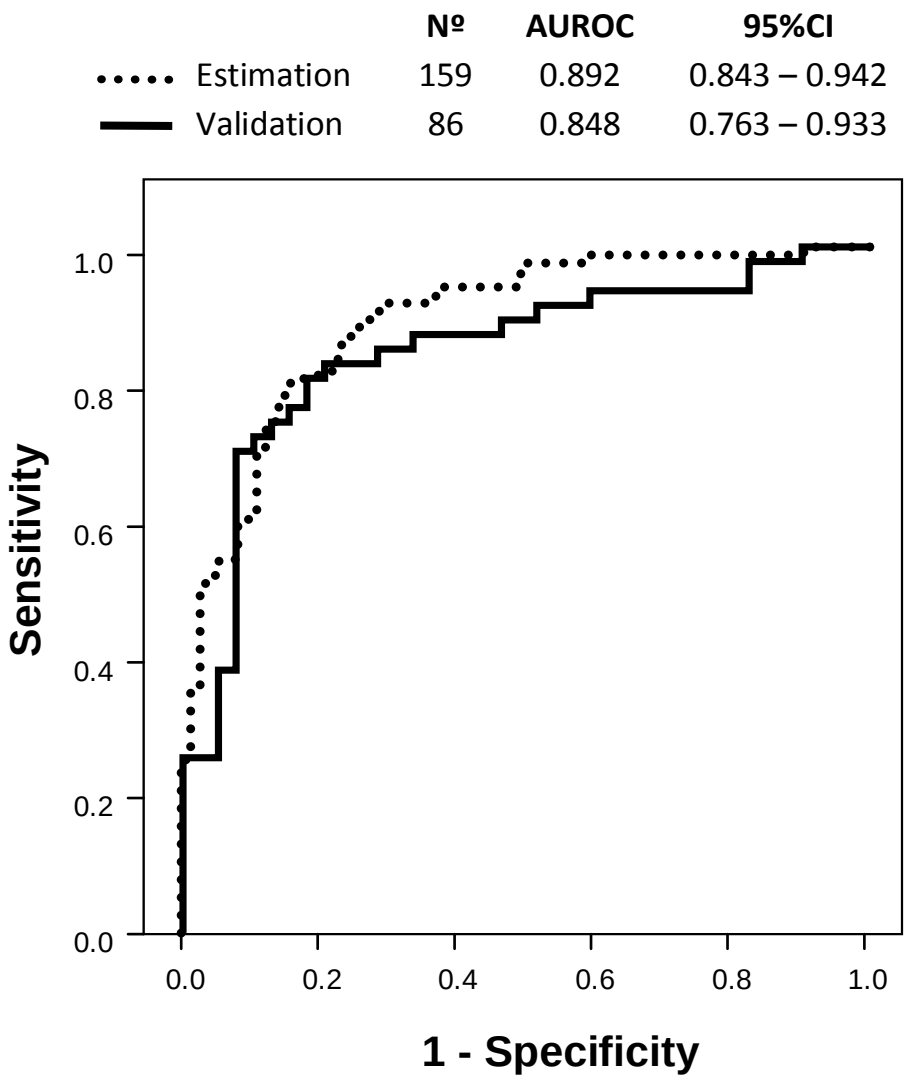
OR, odds ratio; 95% CI, 95% confidence interval; SNP, single nucleotide polymorphism.

Moreover, we found similar AUC-ROCs values of PISVR for the estimation and validation group. (Figure 27)

Figure 26 Probabilistic transformation of the logistic model

$$\Pr(SVR) = \frac{1}{1 + e^{-\left(13.940 + \left(\text{-}1.549 \times HCV\ genotype\ \ 1\&4\right) + \left(\text{-}1.682 \times Log_{10}\ HCV\ VL\right) + \left(\text{-}0.084\text{x Stiffness}\right) + \left(\text{-}1.772\text{x rs12979860(CT or TT)}\right)\right)}}$$

Figure 27 Diagnostic performance of the predictive index of SVR in the estimation and validation groups.



.

3. Diagnostic accuracy of PISVR

With the very low PISVR cutoff point (<0.25) in the estimation group, 41 patients were correctly identified (true negatives (TN) without SVR), and only 5 patients were misclassified (false negatives (FN) with SVR) **(**Table 17**).** We found the presence of Non-SVR with 89% certainty. The LR(-) was 0.10 and the DOR was near 20. For the validation group, Se, NPV, LR(-) and DOR values were similar to the values for the estimation group.

With the high PISVR cutoff (>0.75) to the estimation group, 52 patients were correctly identified (true positive (TP) with SVR), and only 6 patients were misclassified (false positive (FP) without SVR). We found the presence of SVR with 89.7% certainty. The LR(+) was over 7 and the DOR was over 15. For the validation group, Sp, NPV, LR(-) and DOR values were similar to the values for the estimation group.

When we applied the optimal PISVR cutoff (>0.50) to the estimation group, 129 patients were correctly identified (70 patients were TP and 59 patients were TN), and only 30 patients were misclassified (14 patients were FP and 15 patients were FN). We found the presence or absence of SVR with 83.3% and 78.7% certainty respectively. The DOR was over 15. For the validation group, Se, Sp, PPV, NPV, LR(+), LR(-) and DOR values were similar to the values for the estimation group.

Table 17 Diagnostic accuracy and predictors of sustained virological response in the study population

Cutoff	True positive	False positive	True negative	False negative	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	DOR (95% CI)
Estimation group (n=159)											
0.25	81	32	41	5	94.2 (87.1 – 97.5)	56.2 (44.8 - 67)	71.7 (62.8 – 79.2)	89.1 (77 - 95.3)	2.15 (1.65 - 2.8)	0.10 (0.04 - 0.25)	20.76 (7.5 - 57.3)
0.5	70	14	59	16	81.4 (71.9 – 88.2)	80.8 (70.3 - 88.2)	83.3 (73.9 – 89.8)	78.7 (68.1 - 86.4)	4.24 (2.62 - 6.87)	0.23 (0.15 - 0.36)	18.44 (8.3 - 40.9)
0.75	52	6	67	34	60.5 (49.9 – 70.1)	91.8 (83.2 - 96.2)	89.7 (79.2 – 95.2)	66.3 (56.7 - 74.8)	7.36 (3.35 - 16.14)	0.43 (0.33 - 0.57)	17.08 (6.7 - 43.7)
Validation group (n=86)											
0.25	43	23	16	4	91.5 (80.1 – 96.6)	41 (27.1 - 56.6)	65.2 (53.1 – 75.5)	80 (58.4 - 91.9)	1.55 (1.18 - 2.04)	0.21 (0.08 - 0.55)	7.48 (2.2 - 25)
0.5	40	12	27	7	85.1 (72.3 – 92.6)	69.2 (53.6 - 81.4)	76.9 (63.9 – 86.3)	79.4 (63.2 - 89.7)	2.77 (1.7 – 4.5)	0.22 (0.11 - 0.44)	12.86 (4.5 - 36.8)
0.75	31	3	36	16	66 (51.7 – 77.8)	92.3 (79.7 - 97.3)	91.2 (77 – 97)	69.2 (55.7 - 80.1)	8.57 (2.84 - 25.93)	0.37 (0.24 - 0.56)	23.25 (6.2 - 87.3)

6. Discussion

The accurate prediction of the likelihood of response to pegIFN-RBV treatment before initiating therapy is of much interest for the identification of potentially curable HIV/HCV-coinfected patients. For this purpose, independent variables predicting treatment outcomes must be identified. Ideally, they should be easy to obtain and the predictive index must be validated in an independent cohort, showing high accuracy and being cost-effective [85]. As for any other diagnostic or prognostic tool, the accuracy of the score reported in our study was largely dependent on the pre-test probability of presenting or achieving the outcome [86, 101]. In this regard, the appropriate selection of patients and therapeutic management according to international guidelines was essential for making adequate estimates of the probability of HCV clearance. In real life, baseline co-morbidities such as alcohol abuse, neuropsychiatric disorders, active drug abuse, or development of serious adverse events on treatment, prior failure to IFN-based therapies, etc. could act as confounding variables interfering and reducing the power of outcomes prediction using our index. Our predictive model was built based on a population that had excluded non-adherent patients and withdrawals due to side effects, and only evaluated subjects who completed a course of therapy. Thus, selection of candidates, ensuring maximal drug adherence and adequate management of side effects must remain a cornerstone to optimize the chances of SVR to pegIFN-RBV and in this way similarly maximize the predictive value of our index.
Given that our model was developed in HIV/HCV-coinfected populations, in whom other conditions interfering with treatment outcomes are generally more frequent than in HCV-monoinfected persons (e.g., insulin resistance, drug interactions, immunodeficiency, etc.) [3,7,28,29], it is reasonable to assume that our predictive model might perform even better in the latest group. Studies testing this hypothesis in HCV-monoinfected individuals and estimating the predictive value of the HIV status to achieve HCV clearance are warranted to properly estimate outcomes with prognostic scores in HCV infected patients, HIV coinfected or not.

The four variables included in our final model have already shown to predict achievement of SVR in many other studies [80, 102, 103], although prior attempts to model SVR using older predictors of response are scarce [32]. In our knowledge ours is the first attempt to evaluate the predictive value of the newest treatment outcome predictors in conjunction in a single score based on non-invasive tools prior to initiating therapy. Of note, genetic IL28B testing and liver fibrosis assessment using elastometry are incorporated for the first time. The use of elastometry instead of histology for liver fibrosis staging has several advantages besides its non-invasive nature. Over the last few years, liver stiffness assessment has shown to accurately predict SVR, HCV-related complications and mortality in chronic hepatitis C patients [104]. Moreover, there is no doubt that the wide linear range of values using elastometry (from 0 to 74) may allow more accurate assessment of SVR prediction than the few

non-linear histologic fibrosis stages (i.e., F0 to F4 using the Metavir score)[105]. The fact that accuracy of prognostic scores depends on the strength of its predictive variables over etiological considerations led Liver Stiffness to fit better in the model while rendering it more easily available and cost-effective[87].

The values obtained using our proposed predictive index express a probability that ranges from 0 to 1, giving clinicians and patients a tailored estimate of the chances to clear HCV if a course of therapy is conducted according to current recommendations. In our opinion, taking into account host´s and viral genetics, in combination with liver stiffness, allows accurate prediction of SVR at baseline and allows making more adequate treatment decisions in clinical routine if candidates are correctly identified.

Useful information may also arise from the analysis of the accuracy associated to each cutoff we reported in our study and may have an impact in clinical practice.

First utility, as for the majority of the prognostic tools used in clinical practice, is to stratify outcomes and, in our case, the risk of non-response[85, 101]. In this sense, the cutoffs proposed in our study can classify the chances of clearing HCV as high, intermediate and low when probabilities predicted by the model are superior to 0.75, between 0.75 and 0.50, or inferior to 0.50. The last category could be used, for example, to detect patients with a difficult-to-treat profile and to plan a specific therapy or a multidisciplinary approach.

External validation is an essential step and represents another scenario where the cutoffs may help to compare scores through the analysis of their sensitivities, specificities, LR+, LR- and their DOR [106-109]. These parameters, oppositely as for PPV and NPV, reflects the accuracy of the score and are independent of the local pretest probability of achieving the outcome. They also reflect the strength of the prediction and can easily determine which score is the most accurate and the most pertinent for a specific population.

The most challenging question is to determine if cutoffs or risk categories can help to determine the time when therapy with PegylatedInterferon and Ribavirin should be initiated in HCV infected patients. As we reported before, our score can identify easy, intermediate and difficult-to-treat patients. On the other hand, current guidelines recommend therapy when mild fibrosis is established and when, theoretically, patients have already started to lose chances of achieving HCV clearance [110]. Initiating therapy prior to the establishment of end-organ disease is a preemptive approach associated with higher chances of achieving HCV clearance that could be encouraged by prognostic scores but should be evaluated in longitudinal studies.

In summary, the predictive SVR index proposed here based on four non-invasive parameters at baseline is an important step, which may be of great

value for making adequate treatment decisions in HIV/HCV-coinfected patients. Based on this tool, current hepatitis C therapy could be encouraged in subjects without advanced liver fibrosis when the chances of response are the greatest. Conversely, advice to wait for new treatment options, including direct acting antivirals against HCV may be more appropriate for subjects with mild liver fibrosis and minimal chances of response to pegIFN-RBV. As with any other diagnostic tools used in clinical practice, misclassification may occur and understanding the limits of estimated outcomes with predictive indexes is crucial prior to its widespread use.

7. Resumen

A. Introducción

El virus de la hepatitis C y el Virus de la Inmunodeficiencia humana comparten vías de transmisión. Por este motivo la coinfección por el VIH y la Hepatitis C es relativamente frecuente [1].

La enfermedad hepática producida por el virus de la hepatitis C se ve acelerada cuando el VHC y VIH coexisten. Así, en los países en los que la terapia antirretroviral de alta eficacia (TARGA) está disponible, la enfermedad hepática debida al VHC es una de las principales causas de morbilidad y mortalidad.

De los 6 genotipos del VHC descritos, los genotipos 1-4 son los predominantes en los países del oeste. Variaciones en la distribución mundial de los genotipos han sido asociadas con diferencias en la historia natural de la enfermedad y en las tasas de respuesta al tratamiento.

Conocer la prevalencia local de los genotipos del Virus de la hepatitis C tiene interés para identificar a los pacientes potencialmente candidatos a recibir los nuevos antivirales que están en fase de desarrollo, y que actúan principalmente sobre el genotipo 1.

Predecir la respuesta del VHC antes de iniciar el tratamiento permite estratificar las probabilidades de curación e individualizar la terapia antiviral.

B. Objetivos:

Para llegar al objetivo final que da título a esta tesis, se plantean los 3 objetivos intermedios siguientes:

1. Describir y caracterizar la cohorte de pacientes coinfectados en seguimiento en el Servicio de Enfermedades Infecciosas del Hospital Carlos III, mediante un estudio epidemiológico descriptivo de cohortes.

    1.1. Describir las características demográficas de los pacientes coinfectados en seguimiento en el Hospital Carlos III durante el período 2000-2010.

    1.2. Describir la incidencia anual de pacientes coinfectados que entraron en la cohorte durante el período 2000-2010.

    1.3. Describir la mortalidad anual en la cohorte

    1.4. Describir la tasa anual de prescripción de Interferón pegylado y Ribavirina entre los pacientes coinfectados en el Hospital Carlos III durante el período 2000-2010

    1.5. Describir la tasa anual de respuesta virológica sostenida en esta cohorte durante el mismo período

    1.6. Describir la prevalencia anual de pacientes coinfectados con viremia positiva al VHC

    1.7. Estimar el impacto que tiene el tratamiento con Interferón pegylado y Ribavirina sobre la prevalencia de los genotipos en la cohorte de pacientes coinfectados por el VIH y el VHC en seguimiento en el Hospital Carlos III.

2. Determinar el valor predictivo de la infección por HIV mediante un estudio pronóstico.

    2.1. Comparar la tasa de recidiva del VHC entre pacientes con y sin coinfección por el VIH que completan un tratamiento completo con Interferón pegylado y Ribavirina

    2.2. *Determinar el valor predictivo del estatus HIV sobre la Respuesta virológica sostenida del virus de la hepatitis C.*

3. Desarrollar un índice pronóstico, mediante un estudio diagnóstico, para predecir la respuesta virológica sostenida.

    3.1. Desarrollar un índice predictivo para predecir la respuesta virológica sostenida en función de las características virales y del huésped basales

    3.2. Validar este modelo en una cohorte independiente de pacientes infectados por el VHC, con coinfección por el VIH.

C. Materiales y Métodos:

1. Población a estudio:

La población de pacientes coinfectados en seguimiento en el Servicio de Enfermedades Infecciosas del Hospital Carlos III ha permitido la realización de 3 grupos:

1.1. Cohortes Internas:

En adelante las denominaremos cohorte principal, cohorte recidivas y cohorte no-respondedores. La cohorte principal incluye todos los pacientes coinfectados en seguimiento desde el año 2000 en el HCIII. La cohorte recidivas incluye un subgrupo de pacientes coinfectados que alcanzaron viremia C indetectable al final del tratamiento. La cohorte no-respondedores incluye todos los pacientes tratados y que no suspendieron el tratamiento por efectos adversos o mala adherencia.

- Cohorte principal:

Todos los pacientes consecutivos coinfectados por el VIH y el VHC en seguimiento en el Hospital Carlos III fueron incluidos en la cohorte dinámica en cuanto el genotipo del VHC estuviera disponible. Los pacientes con seguimiento inferior a 1 año, incluyendo los que entraron en 2008, se excluyeron. La decisión de excluir los pacientes con seguimiento corto se tomó para descartar a los pacientes vistos esporádicamente (p ej, pacientes que consultaban para una segunda opinión) y que no estaban en seguimiento en el centro. Las principales características demográficas se recogieron retrospectivamente. La información clínica y la evolución se recogieron de la base de datos clínica y de la del servicio de farmacia hospitalaria.

Para establecer una cohorte dinámica de pacientes virémicos para el VHC, y analizar así la distribución anual de los genotipos, la fecha de la primera visita en el hospital se consideró como la fecha de entrada en la cohorte. Aquellos pacientes que alcanzaron una respuesta virológica sostenida después de un tratamiento con interferon pegylado y ribavirina, la fecha del final del tratamiento se consideró como la fecha de salida de la cohorte, pues estos pacientes ya no eran virémicos. El resto de los pacientes virémicos no tratados y aquellos pacientes que presentaron recidivas se consideraron como activos y presentes en la cohorte hasta la fecha de la última visita en el hospital. En

aquellos pacientes cuyo seguimiento se interrumpió, la mortalidad se comprobó en el instituto nacional de estadística.

Una vez establecida la cohorte de pacientes coinfectados en el Hospital Carlos III, se realizaron 2 subgrupos para los estudios pronósticos.

- Cohorte de recidivas:

Un primer subgrupo identificó a todos los pacientes coinfectados tratados que alcanzaron viremia indetectable tras finalizar un tratamiento completo y los comparó a una cohorte de pacientes monoinfectados con características similares. Esta cohorte permitió estudiar los factores pronósticos que determinan la recidiva del VHC, en pacientes con y sin coinfección por el VIH.

- Cohorte de no-respondedores:

El segundo subgrupo identificó a los pacientes coinfectados tratados que no suspendieron el tratamiento por efectos adversos o mala adherencia. Las variables desenlace en este grupo fueron RVS y no respuesta. Con esta definición, la no-respuesta incluyó los 3 eventos siguientes: recidiva, “breaktrought” y no-respuesta en semana 12. Esta población permitió estudiar los factores pronósticos asociados a la RVS y se utilizó para desarrollar el índice predictivo de respuesta virológica sostenida.

1.2. Cohortes externas:

La cohorte recidivas se comparó a una cohorte de pacientes monoinfectados del Servicio de Digestivo del HCIII. La cohorte de pacientes no-respondedores permitió desarrollar un índice predictor que se validó en una cohorte independiente de pacientes de similares características de la Unidad de enfermedades infecciosas del Hospital Universitario de Valme de Sevilla.

- Cohorte de pacientes monoinfectados del Servicio de Digestivo del HCIII:

Estos pacientes se identificaron a través de las dispensaciones del servicio de farmacia hospitalaria.

- Cohorte de pacientes coinfectados de la Unidad de Enfermedades Infecciosas del Hospital Universitario Valme de Sevilla:

La colaboración con este centro permitió unificar una cohorte de pacientes coinfectados tratados durante el mismo período.

Esta cohorte sirvió para validar el modelo desarrollado en la cohorte de no-respondedores. Los pacientes tenían los mismos criterios de inclusión. Se excluyeron aquellos pacientes que no tuvieron fibroscan.

2. Definiciones de los eventos desenlace:

En todos los casos, estas definiciones se basan en un seguimiento virológico (PCR cuantitativa del VHC) trimestral.

2.1. Respuesta virológica sostenida:

Se define como el mantenimiento de una viremia C indetectable durante un período de 24 semanas tras la finalización del tratamiento.

2.2. Recidivas:

Se define como la aparición de viremia C detectable, durante el período de 24 semanas tras la finalización de un tratamiento completo, en pacientes que habían alcanzado viremia indetectable antes del final de este tratamiento.

2.3. No respuesta:

Se define no respuesta como la ausencia de viremia C indetectable tras 12 semanas de tratamiento o como una respuesta suboptima durante el tratamiento.

2.4. Pérdida de seguimiento y Muerte

Aquellos pacientes que no tuvieron seguimiento durante el año 2008 fueron cotejados con la base de datos de defunciones del instituto nacional de estadística. Los pacientes ausentes en esta base de datos se consideraron perdidos en el seguimiento.

Variables explicativas:

2.5. Factores del Huésped:

- Comorbilidades

2.5..1. Estimación de la fibrosis hepática:

La fibrosis hepática se estimó a partir de elastometría hepática medida con FibroScan® siguiendo las instrucciones del fabricante (Echosens). Resumidamente, se realiza un mínimo de 10 medidas a través del espacio intercostal dirigido hacia el lóbulo hepático derecho. El paciente se coloca en decúbito supino con el brazo derecho en abducción. Es en este momento cuando se coloca la sonda del aparato entre las costillas. Se asume que la mediana de los valores obtenidos es representativa de la rigidez hepática. La unidad de medida se expresa en kilopascales (Kpa). Un grupo de medidas se considera válido cuando la tasa de éxito en las medidas es superior al 70% y cuando el rango intercuartil es inferior a un tercio de la mediana de los valores medidos. Las medidas no fiables se excluyen del análisis y todas las medidas se obtuvieron de operadores entrenados con la técnica. Los valores de elastometría hepática inferiores a 7, entre 7.1 y 9.4, entre 9.5 y 12.5 y superiores a 12.5 se corresponden con los estadíos Metavir F0-F1, F2, F3 y F4 respectivamente.

2.5..2. Coinfección VIH

Se anailzaron las variables habituales: carga viral, CD4, uso de TARGA, tiempo de infección, antecedentes de infecciones oportunistas. Se utilizó la base de datos local del servicio.

- Polimorfismo genético

Para los pacientes del HCIII, el genotipado se realizó en el “Duke Institute for Genome Sciences and Policy”. El genotipado se realizó de forma ciega en las muestras de ADN recogidas por los pacientes. Las muestras de los pacientes del Hospital de Valme fueron analizadas en el departamento de inmunología de la Facultad de ciencias de la Universidad de Jaen.

2.6. Factores del Virus C:

- Genotipo y Subtipo

El genotipado y subtipado del VHC se realizó utilizando un kit comercial de hibridización (Versant HCV Genotype v2.0 LiPA, Siemens, Barcelona) que reduce al máximo las probabilidades de mala clasificación del genotipo.

- Carga viral

La viremia C plasmática se midió utilizando una PCR en tiempo real (COBAS, TaqMan, Roche, Barcelona) que tiene un límite de detección inferior a 10 UI/ml.

2.7. Factores externos:

- Análisis de los tratamientos:

Se analizaron todos los tratamientos basados en interferón pegylado (alfa-2a o alfa-2b) dispensados en la farmacia hospitalaria desde el año 2000, fecha de introducción en el Hospital.

- Años calendario:

Los años calendario del período 2000-2008 se utilizaron como variable explicativa en el modelo de regresión lineal simple del estudio epidemiológico descriptivo.

3. Análisis estadísticos

De modo general, los resultados se presentan como medianas y percentiles (25 y 50) para las variables continuas y como frecuencias y porcentajes para las variables categóricas.

La información categórica y las proporciones se analizaron con el test de chi-cuadrado o el test de Fisher, según los casos. El test de T-student se utilizó para comparar medias de dos grupos con distribución normal. Todos los tests tenían 2 colas y se consideraron significativos con valores de $p < 0.05$. Los análisis estadísticos se realizaron con los programa SPSS en su versión 17.0 (Statistical package for social science, SPSS INC, Chicago, IL, USA) y Microsoft Excel 2007 (MS corp,Annex 3)

En el estudio epidemiológico de cohortes, las tendencias en la prevalencia y la incidencia se analizaron mediante un modelo de regresión lineal simple. La proporción de genotipos y subtipos representa la variable dependiente y el año calendario es la variable no-dependiente.

En el estudio pronóstico, se realizaron y compararon distintos modelos de regresión logística para identificar las variables explicativas asociadas a la variable desenlace principal (respuesta virológica sostenida). Los modelos incluyeron las variables explicativas que tenían significación estadística ($p<0.05$) en el análisis univariante. Se buscaron interacciones entre las variables “genotipo VHC” y “snpIL-28”, en busca de una modificación de efecto. El modelo obtenido permitió desarrollar un índice para predicir la RVS, a través de una función logística, que se denominó índice predictor de respuesta virológica sostenida (Predictive index of sustained virological response, PISVR).

En el estudio diagnósticos, el poder diagnóstico y los valores predictivos del PISVR se evaluaron calculando las áreas bajo la curvas ROC (areas under the receiver operating characterisitcs curves, AUC-ROCs) en las cohortes de desarrollo y de validación. Se evaluaron distintos puntos de corte para predecir RVS con el PISVR. Para obtener un alta sensibilidad (se) y alto valor predictivo negativo (VPN), se estableció un punto de corte bajo en 0,25. Para obtener la máxima especificidad (sp) y el máximo valor predictivo positivo (VPP), se estableció un punto de corte en 0,75. Finalmente, se analizó un punto de corte óptimo cercano a los valores máximos de sensibilidad y especificidad, en 0,5.

También calculamos los Likelihood ratios positivos y negativos (LR+ y LR-). Los LR describen cuantas veces una persona con el evento desenlace tiene una probabilidad de tener un resultado particular respecto a una persona que no tiene el evento. LRs contribute to change, after the test has been made, the probability that a target condition is present. Binary tests have two LRs, positive and negative (LR+, LR-). A LR of 1 indicates no diagnostic value. Los test

binarios (variable desenlace dicotómica) tienen 2 LRs, positivo y negativo. Un LR de 1 indica no poder diagnóstico.

Finalmente, también calculamos la odds ratio diagnóstica (Diagnostic odds ratio, DOR) que expresa la fuerza de la asociación entre el resultado de un test y la enfermedad. Es la razón entre las odds de un resultado positivo en una persona que tiene la enfermedad comparada a la de una persona que no tiene la enfermedad. Un DOR de 1 sugiere que el test no tiene poder diagnóstico. Un DOR superior a 10 tiene muy buen poder diagnóstico.

D. Resultados

1) Estudio epidemiológico de la cohorte de pacientes coinfectados en seguimiento en el HCIII:

   a) Características basales

Del total de 672 pacientes coinfectados por los VHC y VIH que entraron en la cohorte dinámica, 489 (73%) pacientes eran hombres. La edad media de entrada en la cohorte era de 36.6 (±5.8) años. La mayoría de los individuos (94.8%) eran españoles nativos y el principal modo de contagio fue el uso de drogas intravenosas (86.5%). (Table 6)

La distribución global de los genotipos en el estudio de cohortes durante la década fue la siguiente: HCV-1 57.1% (1a: 29.2%, 1b: 20.4%, subtipo desconocido: 7.6%), HCV-2 1.3%, HCV-3 25.4% y HCV-4 15.9%. Un sujeto se infectó con el subtipo 6 y ninguno con el subtipo 5.Figure 15

La estimación de la fibrosis hepática por elastometría estaba disponible para 545 (81.1%) pacientes del total de la cohorte a estudio. El total de las evaluaciones realizadas por primera vez a los pacientes, revelaba la distribución siguiente en los índices de rigidez hepática correlacionada con el índice Metavir: F0F1 en 311 (57.1%) pacientes, F2 en 83 (15.2%), F3 en 57 (10.5%) y F4 en 94 (17.2%). Entre los pacientes sometidos a la prueba, se puede considerar que el 27.8% de los pacientes tenían fibrosis avanzada (estimación por Metavir F3-F4).

Durante el período del estudio, 419 pacientes tuvieron un seguimiento longitudinal de la elastometría hepática. Tras un tiempo medio de 2.8 años (±0.98) desde el primer examen, la distribución de la fibrosis según la correlación con el score Metavir en la población fue la siguiente: F0F1 en 111 (26.5%), F2 en 94 (22.4%), F3 en 77 (18.4%) y F4 en 137 (327%).

En el último examen, 51.1% de los pacientes tenían fibrosis avanzada (Correlación con gradosmF3-F4 de Metavir)

   b) Genotipos que entran en la cohorte

Un total de 403 pacientes estaban presentes en la cohorte antes del año 2000 y 268 entraron en la cohorte dinámica durante el período del estudio. Hubo un ligero declive en el número de nuevos pacientes coinfectados que entraron en la cohorte durante el período del estudio (86 entraron en el año 2000 y 16 en el año 2007). Como se muestra en la Figure 11, la proporción the genotipos 4 incidentes, se incrementó un 3% anual (R^2: 0.67, b: 2.98, p=0.01).

c) Genotipos que salen de la cohorte

i) Tasas anuales de respuesta al tratamiento

Un total de 274 (40.8%) de los 672 pacientes coinfectados fueron tratados con pegIFN-RBV. De estos, 161 (58.8%) completaron el tratamiento previsto para la hepatitis C. En total, 116 de los pacientes alcanzaron RVS (intent-to-treat rate: 42.3%; on-treatment rate: 72%). Hay que reseñar que estas tasas de respuesta se alcanzaron tras un primer ciclo de tratamiento en la mayoría de los pacientes, mientras que para una minoría, la RVS se alcanzó tras varios ciclos de tratamiento (Figure 12).

i. Mortalidad y pérdidas de seguimiento

El seguimiento medio fue de 5.5 años, correspondientes a 4,108 patientes-año. Durante el periodo del estudio, 188 pacientes abandonaron la cohorte por otras razones que no fueron la RVS (116; 38.1%). Un total de 58 (19.1%) patientes falleció y 130 (42.7%) se perdieron de vista (Figure 14**)**.

La Table 8 muestra la distribución annual de los genotipos entre pacientes que abandonaron la cohorte tras alcanzar la RVS tras un tratamiento con IFN pegylado y ribavirina.

Es de reseñar que la erradicación del VHC tras tratamiento se alcanzó dos veces más frecuentemente en pacientes infectados con el HCV-2/3 (57/83; 68.7%) que con el HCV-1/4 (59/191; 30.9%) ($p<0.001$).

d) Prevalencia anual de los genotipos en la cohorte

Como resultado de las entradas y salidas en la cohorte dinámica (Figure 15 **y** Table 7),la prevalencia annual de los genotipos mostró variaciones significativas. La distribución final de los genotipos en 2008 fue la siguiente: 60.5% HCV-1 (1a: 31.3%, 1b: 20.4%, subtipo desconocido: 8.7%), 0.5% HCV-2, 21% HCV-3 and 18% HCV-4.

Se observó un incremento significativo en la prevalencia de los genotipos 1 y 4 desde el 72% en el año 2000 hacia el 78.5% en 2008 ($p=0.041$). Por lo contrario, se observó una caída en la prevalencia de los genotipos 2 y 3 desde el 28% en el año 2000 hacia el 21.5% en 2008 ($p=0.047$).Table 9

Finalmente, el análisis de las tendencias (Table 7) en las prevalencias mostró un incremento del 0.59% en la prevalencia anual del genotipo 1 (IC_{95} [0.43 to 0.74], R^2: 0.92, $p<0.001$), un incremento del 0.33% para el genotipo 4 (IC_{95} [0.17; 0.49], R^2: 0.77, $p=0.002$), y un incremento del 0.47% para el subtipo 1a (IC_{95} [0.28; 0.66], R^2: 0.83, $p=0.001$). Un decremento del 0.82% se observó en la prevalencia anual del genotipo 3 (IC_{95} [-1.00; -0.65], R^2: 0.94, $p<0.001$).

2) Estudio diagnóstico

a) Características basales:

La cohorte de pacientes tratados se comparó a una cohorte externa de pacientes con los mismos criterios de inclusión (Table 15). Los pacientes del hospital carlos III (159 casos; 64.9%)representaron la cohorte de desarrollo y los pacientes del hospital universitario de Valme de Sevilla (86 cases; 35.1%) representaron la cohorte de validación. Ambos grupos tenían características basales similares, auque el grupo de pacientes del Hopsital de Valme tenía mayores grados de fibrosis hepática y mayores niveles de carga viral basal del VHC.

b) Variables pronósticas y modelo predictivo:

En la cohorte de desarrollo, se identificaron las variables asociadas a la RVS mediante un análisis de regresión logística (Table 16). Estas variables fueron las que se incluyeron en el modelo y permitieron el desarrollo de la función logística (Figure 26).

La comparación de las curvas ROC demostró unas áreas bajo la curva similares (0,89 y 0,85) entre los modelos de desarrollo y de validación, quedando así validado el modelo (Figure 27 y Table 17).

8. Conclusiones

1) La población de pacientes coinfectados en el hospital Carlos III está compuesta mayoritariamente por varones de mediana edad que adquirieron la infección por vía intravenosa. Desde el año 2000, se prescribió tratamiento con interferón pegilado y ribavirina al 41% de los pacientes, produciendo unas tasas de respuesta del 31% en pacientes con genotipos 1-4 y del 69% en pacientes con genotipos 2-3. Esta diferencia en la respuesta terapéutica produjo un incremento de pacientes con genotipo 1 del 0,6% anual. Actualmente la mitad de los pacientes tiene un grado F3-F4 de fibrosis hepática.

2) En pacientes con infección crónica por el VHC que completan el tratamiento con interferón pegilado y ribavirina, la tasa de recidivas es significativamente mayor en pacientes coinfectados por el VIH (33%) que en pacientes monoinfectados (22%). Sin embargo, la infección por el VIH no es un factor independiente asociado a la recidiva del VHC. Más allá de la semana 24, los rebrotes de carga viral C son reinfecciones.

3) En pacientes con infección crónica por el VHC, los factores predictivos asociados a la recidiva son: la carga viral y el genotipo del VHC, la homogicosis CC del gen rs12979860 y la rigidez hepática. Un modelo pronóstico basado en estas 4 variables tiene un rendimiento diagnóstico del 0,89 en la cohorte de desarrollo y del 0,85 en la cohorte de validación.

4) Unos puntos de corte establecidos en 0,75 y 0,50 permiten establecer 3 intervalos que se asocian, respectivamente, a una probabilidad alta, media y baja de curación de la hepatitis crónica C.

9. Anexos

Annex 1: Approval of CEIC

Hospital Carlos III

SaludMadrid

Comunidad de Madrid

INFORME DEL COMITÉ ÉTICO Y DE INVESTIGACIÓN CLÍNICA

Dr. Carlos Lahoz Rallo, Secretario del Comité Ético y de Investigación Clínica del Hospital Carlos III .

CERTIFICA:

Que este Comité ha evaluado el día 25 de febrero de 2010 (Acta 176), el Proyecto de Investigación, titulado: "Validación de un índice predictor de respuesta al tratamiento en pacientes con infección crónica por el VHC, ponderado por el gen IL-28B, en pacientes con y sin coinfección por el VIH", con código interno P12-10 y cuya Investigadora Principal es la Dra. Luz Martín Carbonero.

Y considera que:

-Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y están justificados los riesgos y molestias previsibles para el sujeto.

-La capacidad de los investigadores y los medios disponibles son apropiados para llevar a cabo el estudio.

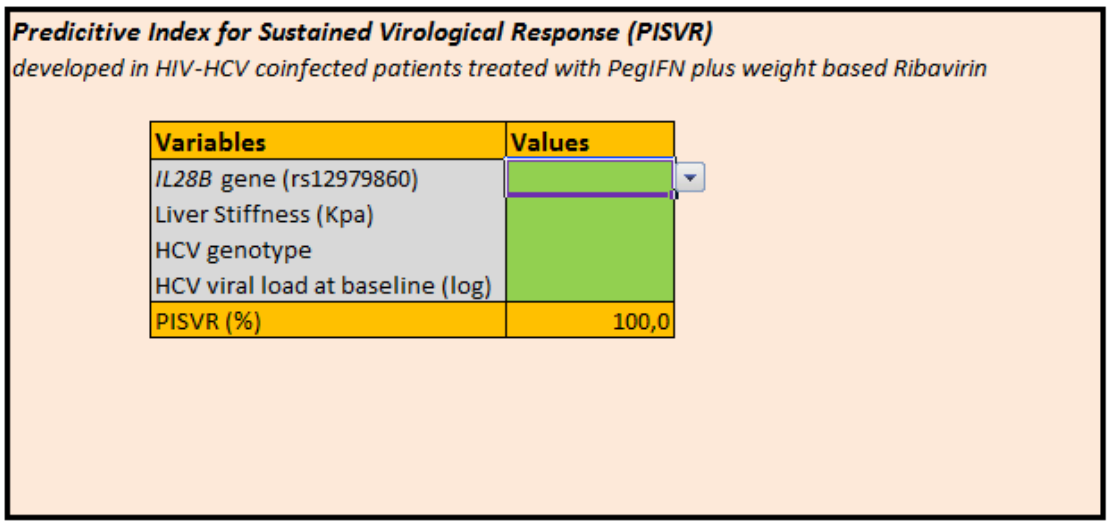
-El alcance de las compensaciones económicas previstas no interfiere con el respeto a los postulados éticos.

Lo que firmo en Madrid, a veinticinco de febrero de dos mil diez.

Fdo. Carlos Lahoz Rallo
Secretario del CEIC

CC/ Investigador Principal

Annex 2 On-line score



Predicitive Index for Sustained Virological Response (PISVR)

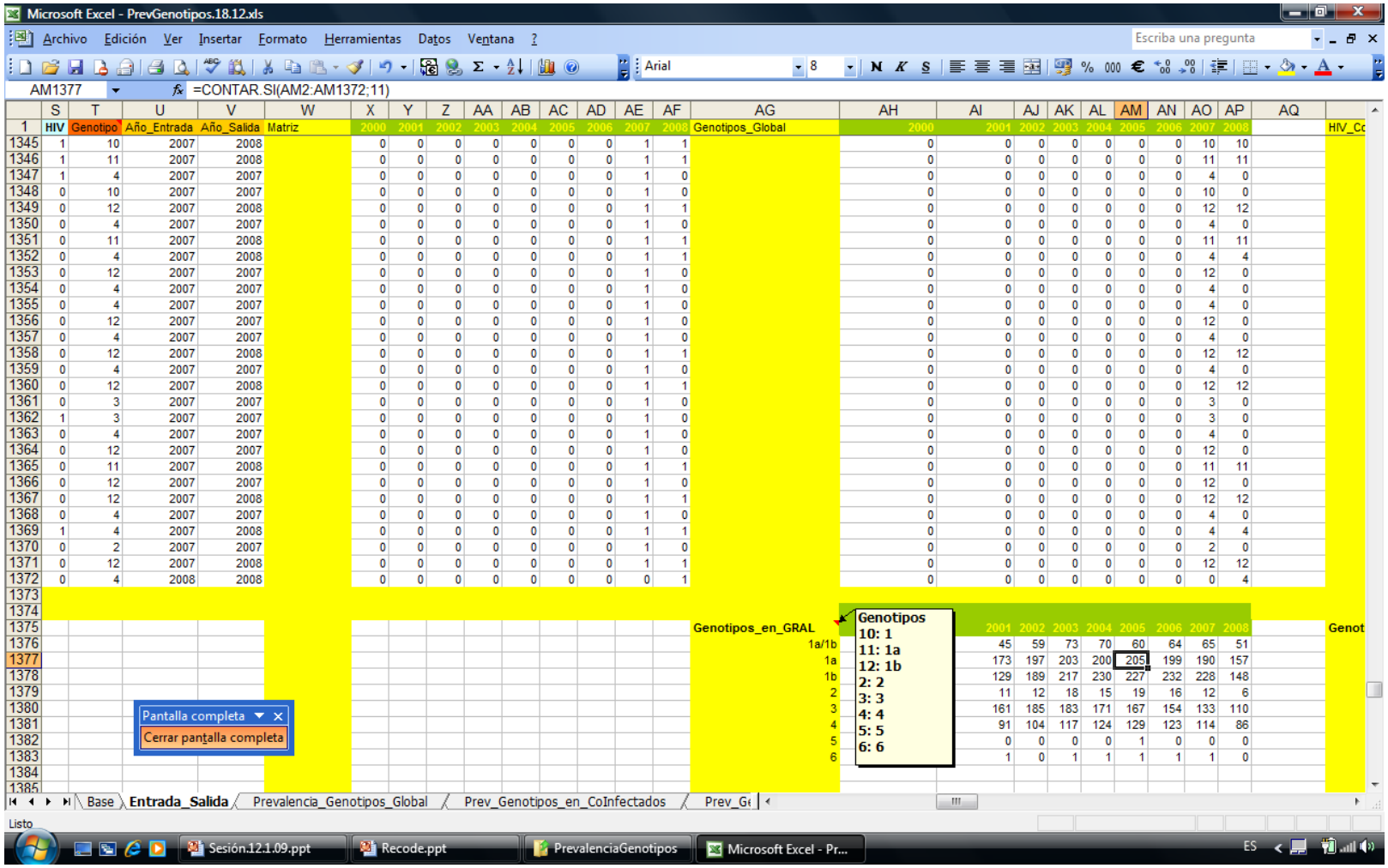
developed in HIV-HCV coinfected patients treated with PegIFN plus weight based Ribavirin

Variables	Values
IL28B gene (rs12979860)	
Liver Stiffness (Kpa)	
HCV genotype	
HCV viral load at baseline (log)	
PISVR (%)	100,0

Downloable at:

www.investigacion-clinica.org/pisvr.xls

Annex 3 ScreenShot of MS Excel file to analyse prevalence and incidence



Annex 4 informed consent

HOJA DE INFORMACIÓN AL PACIENTE

Proyecto de Investigación titulado: Validación de un índice predictor de respuesta al tratamiento en pacientes con infección crónica por el Virus de la Hepatitis C, ponderado por el gen IL-28B, en pacientes con y sin coinfección por el VIH.

Investigador principal: Dra. Luz Martín-Carbonero
Servicio de Enfermedades Infecciosas

Se solicita su participación en este Proyecto de Investigación, cuyo objetivo principal es analizar de forma prospectiva la asociación entre el polimorfismo rs12979860 y la respuesta virológica sostenida al tratamiento de la hepatitis crónica C.

Se estima que participen un total de 400 pacientes del Hospital Carlos III.

Es posible que de su participación en este estudio no obtenga un beneficio directo. Sin embargo, la identificación del polimorfismo rs12979860 en el gen de la IL28B y su asociación con la respuesta virológica sostenida al tratamiento de la hepatitis crónica C, podría beneficiar en un futuro a otros pacientes que padecen dicha enfermedad y podrá contribuir a un mejor conocimiento y tratamiento de esta enfermedad. Los resultados obtenidos en este estudio mejorarán el manejo clínico de estos pacientes redundando en una mejor calidad de vida para ellos.

Su participación en el estudio es totalmente voluntaria y si usted decide no participar recibirá todos los cuidados médicos que Ud. precise y la relación con el Equipo Médico que le atiende no va a verse afectada.

Si usted decide participar, se le realizará una historia clínica, una exploración física detallada y coincidiendo con un análisis rutinario de sangre, se le extraerán dos tubos adicionales (15cc) para obtener las muestras de células mononucleares de sangre periférica (CMSP) y plasma necesarias para la realización de los ensayos de este estudio.

La toma de muestras de sangre puede provocar una sensación de ardor en el punto en el que se introduce la aguja en la piel y le puede ocasionar un pequeño hematoma que desaparece en pocos días. Más raramente mareo en el momento de la extracción de sangre.

Se le pedirá su consentimiento para que con su sangre se hagan 2 cosas:

1.- Se separen el plasma y las CMSPs para que se caracterice el polimorfismo rs12979860 en el gen de la IL28B.

2.- Es probable que en un futuro se descubran más factores que puedan estar también involucrados en la respuesta al tratamiento anti-VHC. Por ello se le solicita que autorice al Investigador a almacenar su muestra para el estudio de otros factores que se puedan descubrir en el futuro. Si Ud acepta autorizar este almacenamiento, se eliminarán de la muestra todos los vínculos con su identidad, antes de guardarla, y no será posible llegar a conocer su identidad a partir de ella. Esta muestra sólo se utilizará para estudiar factores importantes implicados en la respuesta frente al tratamiento anti-VHC. No se realizarán otros estudios con ella.

Ud puede aceptar que sólo se estudien en su muestra de sangre el polimorfismo rs12979860 tal y cómo se describe en el punto 1.
Ud puede aceptar que sólo se guarde su muestra tal y cómo se describe en el punto 2.
Ud puede aceptar las dos propuestas
Ud puede decidir no aceptar ninguna

Si Ud acepta sólo los estudios descritos en el punto 1, su muestra se destruirá después completar la prueba.
Si Ud acepta que se guarde esa muestra para futuros estudios como se describe en el punto 2, el Investigador garantizará que guardará y utilizará la muestra hasta que ya no queden más células.

Ud debe otorgar su consentimiento informado por escrito, indicando que parte del estudio acepta y firmando este documento, antes de la obtención de la muestra.

Si cambia de opinión después de dar sangre para el estudio, Ud puede pedir que se destruya su sangre. No obstante, si ha aceptado que se guarde su muestra de plasma y CMSPs (punto 2), debe pedir que se destruya su muestra de sangre antes de que termine el estudio. Cuando finalice el estudio, se retirará el vínculo que liga a su muestra de plasma y CMSPs con su

identificación. Una vez se haya destruido este vínculo, no será posible encontrar su muestra y por tanto no podrá ser destruida.

Toda la información relacionada con el estudio es estrictamente confidencial. Todas las muestras de sangre recibirán un número y nunca el equipo investigador que lleve a cabo los análisis conocerá su identidad. Se le ha dicho a su médico que guarde esta Hoja de Información y la Hoja de su Consentimiento otorgado con su firma en un archivo especial seguro que no forma parte de su historia clínica. Representantes del Comité Etico de Investigación Clínica del Hospital y de las Autoridades Sanitarias Españolas podrán tener acceso a sus registros médicos con el fin de controlar y garantizar la correcta realización del estudio.

Los resultados del estudio podrán ser comunicados en reuniones científicas, Congresos Médicos o publicaciones científicas, sin embargo se mantendrá una estricta confidencialidad sobre la identidad de los pacientes.

Si Ud precisa mayor información sobre este estudio puede contactar con el Investigador principal, Dra Luz Martín Carbonero del Servicio de Enfermedades Infecciosas Tel: 91 453 2500, ext: 2531

MODELO DE CONSENTIMIENTO DEL PACIENTE POR ESCRITO

Título del estudio: Validación de un índice predictor de respuesta al tratamiento en pacientes con infección crónica por el Virus de la Hepatitis C, ponderado por el gen IL-28B en pacientes con y sin coinfección por el VIH.

Investigador responsable del estudio: Dra Luz Martín-Carbonero.

---

1. Yo …… declaro bajo mi responsabilidad que he leído la Hoja de Información sobre el estudio y acepto participar en dicho estudio.
2. Se me ha entregado una copia de la Hoja de Información al paciente y una copia de este consentimiento informado, fechado y firmado. Se me han explicado las características y el objetivo del presente estudio y los posibles beneficios y riesgos que puedo esperar. Se me ha dado tiempo y oportunidad para realizar preguntas. Todas las preguntas fueron respondidas a mi entera satisfacción.
3. Sé que se mantendrá en secreto mi identidad y que se identificará mi sangre y mis muestras de plasma y CMSPs con un número único.
4. Soy libre de retirarme del estudio en cualquier momento del estudio por cualquier razón y sin que tenga ningún efecto sobre mi tratamiento médico futuro.

Punto 1.- Yo DOY □ **/** No DOY □ mi consentimiento voluntariamente para que se pueda realizar en mi muestra de sangre el estudio del polimorfismo rs12979860 en el gen de la IL28B y su asociación con la respuesta al tratamiento anti-VHC

Punto 2.- Yo DOY □ **/** No DOY □ mi consentimiento voluntariamente para que se guarde mi muestra de sangre. Esto permitirá la realización de nuevas pruebas en el futuro cuando se tengan más conocimientos sobre los factores relacionados con la respuesta al tratamiento anti-VHC.

Consiento en participar voluntariamente en el apartado marcado de este estudio

Fecha: Firma del paciente:

Constato que he explicado las características y el objetivo del presente estudio y sus 2 apartados y los riesgos y beneficios potenciales al sujeto cuyo nombre aparece escrito más arriba. El sujeto consiente en participar por medio de su firma fechada en persona.

FechaFirma del Investigador o la persona que proporciona la información y el consentimiento

Nombre en letra impresa del Investigador o la persona designada de proporcionar la información

10. References

[1]. Danta M, Brown D, Bhagani S, Pybus OG, Sabin CA, Nelson M, et al. Recent epidemic of acute hepatitis C virus in HIV-positive men who have sex with men linked to high-risk sexual behaviours. *AIDS* 2007; **21**:983-991.

[2]. Low E, Vogel M, Rockstroh J, Nelson M. Acute hepatitis C in HIV-positive individuals. *AIDS Rev* 2008; **10**:245-253.

[3]. van de Laar TJ, van der Bij AK, Prins M, Bruisten SM, Brinkman K, Ruys TA, et al. Increase in HCV incidence among men who have sex with men in Amsterdam most likely caused by sexual transmission. *J Infect Dis* 2007; **196**:230-238.

[4]. Gonzalez-Lopez JJ, Guerrero ML, Lujan R, Tostado SF, de Gorgolas M, Requena L. Factors determining serologic response to treatment in patients with syphilis. *Clin Infect Dis* 2009; **49**:1505-1511.

[5]. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, et al. The natural history of hepatitis C virus infection: host, viral, and environmental factors. *JAMA* 2000; **284**:450-456.

[6]. Soriano V, Puoti M, Sulkowski M, Cargnel A, Benhamou Y, Peters M, et al. Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel. *AIDS* 2007; **21**:1073-1089.

[7]. Martin-Carbonero L, Benhamou Y, Puoti M, Berenguer J, Mallolas J, Quereda C, et al. Incidence and predictors of severe liver fibrosis in human immunodeficiency virus-infected patients with chronic hepatitis C: a European collaborative study. *Clin Infect Dis* 2004; **38**:128-133.

[8]. Hammer SM, Eron JJ,Jr, Reiss P, Schooley RT, Thompson MA, Walmsley S, et al. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA* 2008; **300**:555-570.

[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]. Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. Hepatotoxicity associated with antiretroviral therapy in adults infected with human

immunodeficiency virus and the role of hepatitis C or B virus infection. *JAMA* 2000; **283**:74-80.

[11]. Nunez M, Lana R, Mendoza JL, Martin-Carbonero L, Soriano V. Risk factors for severe hepatic injury after introduction of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2001; **27**:426-431.

[12]. Barreiro P, Labarga P, Martin-Carbonero L, Amor A, Ruiz-Sancho A, Castellares C, et al. Sustained virological response following HCV therapy is associated with non-progression of liver fibrosis in HCV/HIV-coinfected patients. *Antivir Ther* 2006; **11**:869-877.

[13]. Soriano V, Labarga P, Ruiz-Sancho A, Garcia-Samaniego J, Barreiro P. Regression of liver fibrosis in hepatitis C virus/HIV-co-infected patients after treatment with pegylated interferon plus ribavirin. *AIDS* 2006; **20**:2225-2227.

[14]. Labarga P, Soriano V, Vispo ME, Pinilla J, Martin-Carbonero L, Castellares C, et al. Hepatotoxicity of antiretroviral drugs is reduced after successful treatment of chronic hepatitis C in HIV-infected patients. *J Infect Dis* 2007; **196**:670-676.

[15]. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**:399-401.

[16]. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**:1105-1109.

[17]. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**:1100-1104.

[18]. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**:798-801.

[19]. de Ledinghen V, Douvin C, Kettaneh A, Ziol M, Roulot D, Marcellin P, et al. Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006; **41**:175-179.

[20]. Soriano V, Martin-Carbonero L, Garcia-Samaniego J. Treatment of chronic hepatitis C virus infection: we must target the virus or liver fibrosis? *AIDS* 2003; **17**:751-753.

[21]. Maida I, Soriano V, Barreiro P, Rivas P, Labarga P, Nunez M. Liver fibrosis stage and HCV genotype distribution in HIV-HCV coinfected patients with persistently normal transaminases. *AIDS Res Hum Retroviruses* 2007; **23**:801-804.

[22]. Sanchez-Conde M, Berenguer J, Miralles P, Alvarez F, Carlos Lopez J, Cosin J, et al. Liver biopsy findings for HIV-infected patients with chronic hepatitis C and persistently normal levels of alanine aminotransferase. *Clin Infect Dis* 2006; **43**:640-644.

[23]. Soriano V, Nunez M, Camino N, Maida I, Barreiro P, Romero M, et al. Hepatitis C virus-RNA clearance in HIV-coinfected patients with chronic hepatitis C treated with pegylated interferon plus ribavirin. *Antivir Ther* 2004; **9**:505-509.

[24]. Romero-Gomez M, Del Mar Viloria M, Andrade RJ, Salmeron J, Diago M, Fernandez-Rodriguez CM, et al. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128**:636-641.

[25]. Ramos B, Nunez M, Rendon A, Berdun MA, Losada E, Santos I, et al. Critical role of ribavirin for the achievement of early virological response to HCV therapy in HCV/HIV-coinfected patients. *J Viral Hepat* 2007; **14**:387-391.

[26]. Carrat F, Bani-Sadr F, Pol S, Rosenthal E, Lunel-Fabiani F, Benzekri A, et al. Pegylated interferon alfa-2b vs standard interferon alfa-2b, plus ribavirin, for chronic hepatitis C in HIV-infected patients: a randomized controlled trial. *JAMA* 2004; **292**:2839-2848.

[27]. Torriani FJ, Rodriguez-Torres M, Rockstroh JK, Lissen E, Gonzalez-Garcia J, Lazzarin A, et al. Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N Engl J Med* 2004; **351**:438-450.

[28]. Sherman KE, Shire NJ, Rouster SD, Peters MG, James Koziel M, Chung RT, et al. Viral kinetics in hepatitis C or hepatitis C/human immunodeficiency virus-infected patients. *Gastroenterology* 2005; **128**:313-327.

[29]. Lindahl K, Stahle L, Bruchfeld A, Schvarcz R. High-dose ribavirin in combination with standard dose peginterferon for treatment of patients with chronic hepatitis C. *Hepatology* 2005; **41**:275-279.

[30]. Dixit NM, Layden-Almer JE, Layden TJ, Perelson AS. Modelling how ribavirin improves interferon response rates in hepatitis C virus infection. *Nature* 2004; **432**:922-924.

[31]. Rendon AL, Nunez M, Romero M, Barreiro P, Martin-Carbonero L, Garcia-Samaniego J, et al. Early monitoring of ribavirin plasma concentrations may predict anemia and early virologic response in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2005; **39**:401-405.

[32]. Nunez M, Marino A, Miralles C, Berdun MA, Sola J, Hernandez-Burruezo JJ, et al. Baseline serum hepatitis C virus (HCV) RNA level and response at week 4 are the best predictors of relapse after treatment with pegylated interferon plus ribavirin in HIV/HCV-coinfected patients. *J Acquir Immune Defic Syndr* 2007; **45**:439-444.

[33]. Nunez M, Miralles C, Berdun MA, Losada E, Aguirrebengoa K, Ocampo A, et al. Role of weight-based ribavirin dosing and extended duration of therapy in chronic hepatitis C in HIV-infected patients: the PRESCO trial. *AIDS Res Hum Retroviruses* 2007; **23**:972-982.

[34]. Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, et al. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005; **352**:2609-2617.

[35]. von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, et al. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005; **129**:522-527.

[36]. Tural C, Sola R, Rubio R, Santin M, Planas R, Quereda C, et al. Safety and efficacy of an induction dose of pegylated interferon alpha-2a on early hepatitis C virus kinetics in HIV/HCV co-infected patients: the CORAL-1 multicentre pilot study. *J Viral Hepat* 2007; **14**:704-713.

[37]. Ferenci P, Laferl H, Scherzer TM, Gschwantler M, Maieron A, Brunner H, et al. Peginterferon alfa-2a and ribavirin for 24 weeks in hepatitis C type 1 and 4 patients with rapid virological response. *Gastroenterology* 2008; **135**:451-458.

[38]. Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, et al. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. *J Hepatol* 2006; **44**:97-103.

[39]. Martin-Carbonero L, Nunez M, Marino A, Alcocer F, Bonet L, Garcia-Samaniego J, et al. Undetectable hepatitis C virus RNA at week 4 as predictor

of sustained virological response in HIV patients with chronic hepatitis C. *AIDS* 2008; **22**:15-21.

[40]. Alberti A, Clumeck N, Collins S, Gerlich W, Lundgren J, Palu G, et al. Short statement of the first European Consensus Conference on the treatment of chronic hepatitis B and C in HIV co-infected patients. *J Hepatol* 2005; **42**:615-624.

[41]. Crespo M, Esteban JI, Ribera E, Falco V, Sauleda S, Buti M, et al. Utility of week-4 viral response to tailor treatment duration in hepatitis C virus genotype 3/HIV co-infected patients. *AIDS* 2007; **21**:477-481.

[42]. Buti M, Valdes A, Sanchez-Avila F, Esteban R, Lurie Y. Extending combination therapy with peginterferon alfa-2b plus ribavirin for genotype 1 chronic hepatitis C late responders: a report of 9 cases. *Hepatology* 2003; **37**:1226-1227.

[43]. Sanchez-Tapias JM, Diago M, Escartin P, Enriquez J, Romero-Gomez M, Barcena R, et al. Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; **131**:451-460.

[44]. Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, et al. Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006; **130**:1086-1097.

[45]. Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. *JAMA* 2000; **283**:74-80.

[46]. Rockstroh JK, Bhagani S, Benhamou Y, Bruno R, Mauss S, Peters L, et al. European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of chronic hepatitis B and C coinfection in HIV-infected adults. *HIV Med* 2008; **9**:82-88.

[47]. Bani-Sadr F, Denoeud L, Morand P, Lunel-Fabiani F, Pol S, Cacoub P, et al. Early virologic failure in HIV-coinfected hepatitis C patients treated with the peginterferon-ribavirin combination: does abacavir play a role? *J Acquir Immune Defic Syndr* 2007; **45**:123-125.

[48]. Mauss S, Valenti W, DePamphilis J, Duff F, Cupelli L, Passe S, et al. Risk factors for hepatic decompensation in patients with HIV/HCV coinfection and liver cirrhosis during interferon-based therapy. *AIDS* 2004; **18**:F21-5.

[49]. Garcia-Benayas T, Blanco F, Soriano V. Weight loss in HIV-infected patients. *N Engl J Med* 2002; **347**:1287-1288.

[50]. Vispo E, Barreiro P, Pineda JA, Mira JA, Maida I, Martin-Carbonero L, et al. Low response to pegylated interferon plus ribavirin in HIV-infected patients with chronic hepatitis C treated with abacavir. *Antivir Ther* 2008; **13**:429-437.

[51]. Sanchez-Conde M, Gil P, Sanchez-Somolinos M, Gonzalez-Lahoz J, Soriano V. Hepatic and renal safety profile of tenofovir in HIV-infected patients with hepatitis C, including patients on interferon plus ribavirin. *HIV Clin Trials* 2005; **6**:278-280.

[52]. Soriano V, Peters MG, Zeuzem S. New therapies for hepatitis C virus infection. *Clin Infect Dis* 2009; **48**:313-320.

[53]. Kuntzen T, Timm J, Berical A, Lennon N, Berlin AM, Young SK, et al. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naive patients. *Hepatology* 2008; **48**:1769-1778.

[54]. Nelson DR. Hepatitis C drug development at a crossroads. *Hepatology* 2009; **50**:997-999.

[55]. Mathurin P, Thibault V, Kadidja K, Ganne-Carrie N, Moussalli J, El Younsi M, et al. Replication status and histological features of patients with triple (B, C, D) and dual (B, C) hepatic infections. *J Viral Hepat* 2000; **7**:15-22.

[56]. Martin-Carbonero L, Barreiro P, Jimenez-Galan G, Garcia-Berriguete R, Nunez M, Rios P, et al. Clearance of hepatitis C virus in HIV-infected patients with multiple chronic viral hepatitis. *J Viral Hepat* 2007; **14**:392-395.

[57]. Nunez M, Maida I, Babudieri S, Fenu L, Camino N, Gonzalez-Lahoz J, et al. Hepatitis C viremia in HIV/HCV-coinfected patients: lower levels in presence of chronic hepatitis B. *HIV Clin Trials* 2005; **6**:103-106.

[58]. Sagnelli E, Pasquale G, Coppola N, Scarano F, Marrocco C, Scolastico C, et al. Influence of chronic coinfection with hepatitis B and C virus on liver histology. *Infection* 2004; **32**:144-148.

[59]. Puoti M, Bruno R, Soriano V, Donato F, Gaeta GB, Quinzan GP, et al. Hepatocellular carcinoma in HIV-infected patients: epidemiological features, clinical presentation and outcome. *AIDS* 2004; **18**:2285-2293.

[60]. Bonacini M, Louie S, Bzowej N, Wohl AR. Survival in patients with HIV infection and viral hepatitis B or C: a cohort study. *AIDS* 2004; **18**:2039-2045.

[61]. Soriano V, Barreiro P, Martin-Carbonero L, Castellares C, Ruiz-Sancho A, Labarga P, et al. Treatment of chronic hepatitis B or C in HIV-infected patients with dual viral hepatitis. *J Infect Dis* 2007; **195**:1181-1183.

[62]. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, et al. The natural history of hepatitis C virus infection: host, viral, and environmental factors. *JAMA* 2000; **284**:450-456.

[63]. Pawlotsky JM. Hepatitis C virus genetic variability: pathogenic and clinical implications. *Clin Liver Dis* 2003; **7**:45-66.

[64]. Soriano V, Mocroft A, Rockstroh J, Ledergerber B, Knysz B, Chaplinskas S, et al. Spontaneous viral clearance, viral load, and genotype distribution of hepatitis C virus (HCV) in HIV-infected patients with anti-HCV antibodies in Europe. *J Infect Dis* 2008; **198**:1337-1344.

[65]. Pizzillo P, Almasio PL, Ferraro D, Craxi A, Di Stefano R. HCV genotypes in Sicily: is there any evidence of a shift? *J Med Virol* 2009; **81**:1040-1046.

[66]. Martin-Carbonero L, Puoti M, Garcia-Samaniego J, De Luca A, Losada E, Quinzan G, et al. Response to pegylated interferon plus ribavirin in HIV-infected patients with chronic hepatitis C due to genotype 4. *J Viral Hepat* 2008; **15**:710-715.

[67]. Pol S, Soriano V. Management of chronic hepatitis C virus infection in HIV-infected patients. *Clin Infect Dis* 2008; **47**:94-101.

[68]. Soriano V, Peters MG, Zeuzem S. New therapies for hepatitis C virus infection. *Clin Infect Dis* 2009; **48**:313-320.

[69]. Castellares C, Barreiro P, Martin-Carbonero L, Labarga P, Vispo ME, Casado R, et al. Liver cirrhosis in HIV-infected patients: prevalence, aetiology and clinical outcome. *J Viral Hepat* 2008; **15**:165-172.

[70]. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**:41-52.

[71]. Graham CS, Baden LR, Yu E, Mrus JM, Carnie J, Heeren T, et al. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. *Clin Infect Dis* 2001; **33**:562-569.

[72]. Martin-Carbonero L, Benhamou Y, Puoti M, Berenguer J, Mallolas J, Quereda C, et al. Incidence and predictors of severe liver fibrosis in human immunodeficiency virus-infected patients with chronic hepatitis C: a European collaborative study. *Clin Infect Dis* 2004; **38**:128-133.

[73]. Weber R, Sabin CA, Friis-Moller N, Reiss P, El-Sadr WM, Kirk O, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. *Arch Intern Med* 2006; **166**:1632-1641.

[74]. Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**:1335-1374.

[75]. Medrano J, Barreiro P, Resino S, Tuma P, Rodriguez V, Vispo E, et al. Rate and timing of hepatitis C virus relapse after a successful course of pegylated interferon plus ribavirin in HIV-infected and HIV-uninfected patients. *Clin Infect Dis* 2009; **49**:1397-1401.

[76]. Mehta SH, Genberg BL, Astemborski J, Kavasery R, Kirk GD, Vlahov D, et al. Limited uptake of hepatitis C treatment among injection drug users. *J Community Health* 2008; **33**:126-133.

[77]. Grebely J, Raffa JD, Lai C, Krajden M, Kerr T, Fischer B, et al. Low uptake of treatment for hepatitis C virus infection in a large community-based study of inner city residents. *J Viral Hepat* 2009; **16**:352-358.

[78]. Rauch A, Egger M, Reichen J, Furrer H, Swiss HIV Cohort Study. Chronic hepatitis C in HIV-infected patients: low eligibility and applicability of therapy with pegylated interferon-alpha plus ribavirin. *J Acquir Immune Defic Syndr* 2005; **38**:238-240.

[79]. Kau A, Vermehren J, Sarrazin C. Treatment predictors of a sustained virologic response in hepatitis B and C. *J Hepatol* 2008; **49**:634-651.

[80]. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**:399-401.

[81]. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-

alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**:1105-1109.

[82]. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, et al. Genetic Variation in IL28B Is Associated With Chronic Hepatitis C and Treatment Failure: A Genome-wide Association Study. *Gastroenterology* 2010;.

[83]. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**:798-801.

[84]. Rallón n, Naggie s, Benito JM, Medrano J, Restrepo c, Goldstein d, et al. Strong Association of a Single Nucleotide Polymorphism Near the Interleukin-28B Gene with Response to Hepatitis C Therapy in HIV-HCV Coinfected Patients. *AIDS* 2010;.

[85]. Moons KG, Altman DG, Vergouwe Y, Royston P. Prognosis and prognostic research: application and impact of prognostic models in clinical practice. *BMJ* 2009; **338**:b606.

[86]. Royston P, Moons KG, Altman DG, Vergouwe Y. Prognosis and prognostic research: Developing a prognostic model. *BMJ* 2009; **338**:b604.

[87]. Moons KG, Royston P, Vergouwe Y, Grobbee DE, Altman DG. Prognosis and prognostic research: what, why, and how? *BMJ* 2009; **338**:b375.

[88]. Medrano J, Resino S, Vispo E, Madejon A, Labarga P, Tuma P. Hepatitis C virus (HCV) treatment uptake and changes in the prevalence of HCV genotypes in HIV-HCV coinfected patients. *J Viral Hepat* 2010;.

[89]. Ziol M, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**:48-54.

[90]. Castera L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, et al. Pitfalls of liver stiffness measurement: A 5-year prospective study of 13,369 examinations. *Hepatology* 2009; **51**:828-835.

[91]. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**:343-350.

[92]. Kirk GD, Astemborski J, Mehta SH, Spoler C, Fisher C, Allen D, et al. Assessment of liver fibrosis by transient elastography in persons with hepatitis C virus infection or HIV-hepatitis C virus coinfection. *Clin Infect Dis* 2009; **48**:963-972.

[93]. Weber R, Sabin CA, Friis-Moller N, Reiss P, El-Sadr WM, Kirk O, et al. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. *Arch Intern Med* 2006; **166**:1632-1641.

[94]. Larsen C, Pialoux G, Salmon D, Antona D, Le Strat Y, Piroth L, et al. Prevalence of hepatitis C and hepatitis B infection in the HIV-infected population of France, 2004. *Euro Surveill* 2008; **13**:18888.

[95]. Nunez-Fernandez C, Martin-Carbonero L, Valencia ME, Aguilera J, Garcia-Samaniego J, Gonzalez-Lahoz J, et al. Liver complications have reached a plateau as cause of hospital admission and death in HIV patients in Madrid. *AIDS Res Hum Retroviruses* 2009; **25**:383-385.

[96]. Mehta SH, Genberg BL, Astemborski J, Kavasery R, Kirk GD, Vlahov D, et al. Limited uptake of hepatitis C treatment among injection drug users. *J Community Health* 2008; **33**:126-133.

[97]. Grebely J, Raffa JD, Lai C, Krajden M, Kerr T, Fischer B, et al. Low uptake of treatment for hepatitis C virus infection in a large community-based study of inner city residents. *J Viral Hepat* 2009; **16**:352-358.

[98]. Cacoub P, Halfon P, Rosenthal E, Pialoux G, Benhamou Y, Perronne C, et al. Treatment of hepatitis C virus in human immunodeficiency virus infected patients in "real life": modifications in two large surveys between 2004 and 2006. *J Hepatol* 2008; **48**:35-42.

[99]. Laguno M, Murillas J, Blanco JL, Martinez E, Miquel R, Sanchez-Tapias JM, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for treatment of HIV/HCV co-infected patients. *AIDS* 2004; **18**:F27-36.

[100]. Perez Cachafeiro S, Del Amo J, Iribarren JA, Salavert Lleti M, Gutierrez F, Moreno A, et al. Decrease in serial prevalence of coinfection with hepatitis C virus among HIV-infected patients in Spain, 1997-2006. *Clin Infect Dis* 2009; **48**:1467-1470.

[101]. Altman DG, Vergouwe Y, Royston P, Moons KG. Prognosis and prognostic research: validating a prognostic model. *BMJ* 2009; **338**:b605.

[102]. Dahari H, Layden-Almer JE, Kallwitz E, Ribeiro RM, Cotler SJ, Layden TJ, et al. A mathematical model of hepatitis C virus dynamics in patients with high baseline viral loads or advanced liver disease. *Gastroenterology* 2009; **136**:1402-1409.

[103]. Labarga P, Vispo E, Barreiro P, Rodriguez-Novoa S, Pinilla J, Morello J, et al. Rate and Predictors of Success in the Retreatment of Chronic Hepatitis C Virus in HIV/HCV-Coinfected Patients With Prior Nonresponse or Relapse. *J Acquir Immune Defic Syndr* 2010;.

[104]. Tuma P, Jarrin I, Del Amo J, Vispo E, Medrano J, Martin-Carbonero L, et al. Survival of HIV-infected patients with compensated liver cirrhosis. *AIDS* 2010;.

[105]. Altman DG, Royston P. The cost of dichotomising continuous variables. *BMJ* 2006; **332**:1080.

[106]. Pepe M, Longton G, Janes H. Estimation and Comparison of Receiver Operating Characteristic Curves. *Stata J* 2009; **9**:1.

[107]. Pepe MS, Janes H, Longton G, Leisenring W, Newcomb P. Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. *Am J Epidemiol* 2004; **159**:882-890.

[108]. Grimes DA, Schulz KF. Refining clinical diagnosis with likelihood ratios. *Lancet* 2005; **365**:1500-1505.

[109]. Hanley JA. Receiver operating characteristic (ROC) methodology: the state of the art. *Crit Rev Diagn Imaging* 1989; **29**:307-335.

[110]. Soriano V, Puoti M, Sulkowski M, Cargnel A, Benhamou Y, Peters M, et al. Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV-HIV International Panel. *AIDS* 2007; **21**:1073-1089.