FACTORES PREDICTIVOS DE RESPUESTA AL TRATAMIENTO DEL VHC EN PACIENTES COINFECTADOS POR EL VIH

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TÍTULO DE LA TESIS: Factores Predictivos del tratamiento del VHC en pacientes co-infectados por el VIH

DOCTORANDO/A: Antonio Rivero Juárez

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El presente proyecto de Tesis Doctoral está constituido por siete trabajos realizados en el Instituto Maimonides de Investigación Biomédica de Córdoba (Hospital Universitario Reina Sofía. Universidad de Córdoba) sobre la predicción de la respuesta al tratamiento del Virus de la Hepatitis C (VHC) en pacientes co-infectados por el Virus de la Inmunodeficiencia Humana (VIH). Los resultados derivados del estudio del doctorando han sido comunicados en Congresos Nacionales e Internacionales de Enfermedades Infecciosas y publicados en revistas internacionales indexadas en Journal Citation Report en el Primer y Segundo Cuartil. Estas publicaciones tienen un factor de impacto acumulado de 32 puntos. Del mismo modo, algunas de dichas publicaciones han sido citadas en el Documento de Concenso de Gesida/Plan Nacional sobre el SIDA respecto al tratamiento antirretroviral en adultos infectados por el VIH (Enero 2013). Dichos trabajos han sido realizados por el doctorando desde el año 2011 hasta la actualidad. Por todo ello, consideramos que la presente Tesis Doctoral reúne los méritos suficientes para optar al Grado de Doctor Por la Universidad de Córdoba.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 13 de MARZO de 2013

Firma del/de los director/es

Fdo.: _____ Fdo.: _____

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A mis abuelos

Ana, Antonio, Eduardo y Juana

"*About 80 percent of chronic, post-transfusion Non-A Non-B Hepatitis (NANBH) patients from Italy and Japan had circulating HCV antibody; a much lower frequency (15 percent) was observed in acute, resolving infections. In addition, 58 percent of NANBH patients from the United States with no identifiable source of parenteral exposure to the virus were also positive for HCV antibody. These data indicate that HCV is a major cause of NANBH throughout the world*"

Kuo G *et al*. Science. 21 de Abril de 1989

INDICE

4. The IL28B effect on HCV kinetics of among HIV patients after the first weeks of Peg-IFN/RBV treatment varies according to HCV-1 subtype
5. Differences in HCV Viral Decline between Low and Standard-Dose Pegylated-Interferon-alpha-2a with Ribavirin in HIV/HCV Genotype 3 Patients
6. Baseline risk factors for relapse in HIV/HCV co-infected patients treated with PEG-IFN/RBV
7. Atazanavir-Based Therapy Is Associated with Higher Hepatitis C Viral Load in HIV Type 1-Infected Subjects with Untreated Hepatitis C

Discusión……..

1. Bloque I: Predicción de respuesta al tratamiento tras la finalización del mismo: evaluación del valor predictivo positivo sobre RVS de la semana 12 tras finalización del tratamiento.
2. Bloque II: Evaluación de la influencia de los factores genéticos IL28B (rs129679860) y LDLr (rs14158) en la respuesta al tratamiento mediante el estudio de la cinética viral durante las primeras semanas del mismo.

a. Efecto de IL28B (rs129679860)

b. Efecto de LDLr (rs14158)

c. Efecto de IL28B en los genotipos 1a y 1b del VHC

3. Bloque III: Evaluación de la respuesta al tratamiento del VHC genotipo 3 con distintas dosis de IFN-Peg/RBV mediante el estudio de la cinética viral durante las primeras semanas del mismo.
4. Bloque IV: Estudios sobre factores basales predictivos de respuesta al tratamiento.
   a. Factores basales asociados a VR del VHC en pacientes tratados con IFN-Peg/RBV coinfectados por el VIH
   b. Influencia de los fármacos integrantes del ART en la carga viral basal del VHC

Conclusiones……..

Anexo I: *Otras publicaciones derivadas del trabajo del doctorando*

Anexo II: *Estancias en centros internacionales derivadas del proyecto de tesis doctoral*

Anexo III: *Premios de investigacion conseguidos por el doctorando*

Anexo IV: *Proyectos financiados mediante convocatoria pública como investigador principal derivados del proyecto de tesis*

Anexo V: *Capítulos de libros derivados del proyecto de tesis doctoral*

Anexo VI: *Patente derivada del proyecto de tesis doctoral*

Abreviaturas:

AEMPS: Agencia Española de Medicamentos y Productos Sanitarios

ARN: Ácido ribonucleico

ATV: Atazanavir

BOC: Boceprevir

BOC+IFN-Peg/RBV: Boceprevir más interferón pegilado y ribavirina

BR: Bilirrubina

BV: Biliverdina

CD4: Linfocito T con cúmulo de diferenciación 4

CD81: cúmulo de diferenciación 81

CL: Cuerpos lipídicos

CLD-1: Claudina 1

dL: decilitro

FAD: Fármacos de acción directa frente al Virus de la Hepatitis C

g: gramo

GAGs: Glicosaminoglicanos

HLA-C: Antígeno Leucocitario Humano tipo C

IC: Intervalo de Confianza

IFN-Peg: Interferón pegilado

IFN-Peg/RBV: Interferón pegilado más ribavirina

IFN: Interferón

IL28B: Interleuquina 28B

IMC: Índice de Masa Corporal

IPs: Inhibidores de la proteasa del Virus de la Hepatitis C

IRES: Región de internación al ribosoma

Kb: Kilobases

Kg: Kilogramo

KIR: Killer immunoglobuline-like receptor

LDG: Grupo de dosis bajas de tratamiento

Lipoproteina de baja densidad: LDL

Lipoproteina de muy baja densidad: VLDL

Log_{10}: Logaritmo en base 10

mg: miligramo

mL: mililitro

NK: Células Natural Killer

NPC1L1: Niemann-Pick C1-like 1 cholesterol receptor

NR: No respuesta (Null-Responder)

OCL-1: Ocludina 1

PL: Partícula Lipoviral

RBV: Ribavirina

RE: Retículo endoplasmático

Receptor de la Lipoproteina de baja densidad: LDLr

RFT: Respuesta Fin de Tratamiento

RVPc: Respuesta Viral Precoz completa

RVPp: Respuesta Viral Precoz parcial

RVR: Respuesta Viral Rápida

RVS: Respuesta Viral Sostenida

SDG: Grupo de dosis estándar de tratamiento

SIDA: Síndrome de la Inmunodeficiencia Humana Adquirida

TAR: tratamiento antirretroviral

TPV: Telaprevir

TPV+IFN-Peg/RBV:Telaprevir más interferón pegilado y ribavirina

UGT1A1: UDP-glucuronosyltransferase 1 family 1

UI: Unidades Internacionales

VB: rebote viral (Viral Breakthrough)

VHC: Virus de la Hepatitis C

VIH: Virus de la Inmunodeficiencia Humana

VPP: Valor Predictivo Positivo

W12: semana 12 tras finalización del tratamiento con interferón pegilado más ribavirina.

µg: microgramo

Summary

The aim of this work was to study and identify factors associated with HCV treatment response using pegylated-interferon plus ribavirin (Peg-IFN/RBV) in HIV co-infected patients. The results of the present research have been published as seven different papers, which are summarized here.

The duration of HCV treatment varies according to HCV genotype. While the duration of treatment for patients infected with HCV genotypes 2 or 3 (higher responders) is 24-48 weeks, length of treatment for patients bearing HCV genotype 1 or 4 (lower responders) can be up to 72 weeks. Patients with undetectable serum HCV RNA achieve end-of-treatment response (ETR). If, after twenty-four weeks (W24), HCV viral load remains undetectable, the patient achieves sustained virological response (SVR), the gold standard of healing. Meanwhile, if HCV RNA is detected again during these 24 weeks, the patient shows a viral relapse (VR). In monoinfected HCV patients, a relapse generally occurs soon after successful therapy has been discontinued, and several studies have shown that testing HCV monoinfected patients for serum HCV RNA 12 weeks after completion of standard Peg-IFN/RBV therapy (W12) is predictive of SVR. Furthermore HIV-associated immune disorders could theoretically impair the definitive clearance of HCV infection after HCV therapy, so altering the timing of an

HCV relapse. For these reasons, there could be a difference between HCV and HIV/HCV co-infected patients. We performed two prospective studies to evaluate these points which included HIV/HCV co-infected patients who achieved ETR.

Firstly, we evaluated the positive predictive value (PPV) of the W12 assessment and whether it is as informative as one at W24. Of the 104 patients included, 83 (79.8%) had SVR at W24, and 21 (20.2%) had VR. At W12, HCV RNA was undetectable in 83 (79.8%) patients and all of these had SVR. An undetectable HCV RNA at both W12 and W24 had a 100% PPV [95% confidence interval (CI) 96.5%–100%] for SVR. We concluded that undetectable HCV RNA at W12 following treatment has a high PPV for SVR and that testing for HCV RNA at this moment may, therefore, be considered an appropriate point for identifying SVR or relapse in HIV/HCV co-infected patients receiving treatment with Peg-INF/RBV.

Secondly, we used the same population to analyze the factors associated with VR. Patients who relapsed showed the following: higher baseline HCV RNA levels (bivariate analysis: p = 0.012; multivariate OR [95%C]: 2.17 [1.2-7.9]); body mass index (BMI) >25 kg/m2 (bivariate analysis: p = 0.034; multivariate OR [95%C]: 1.6 [1.1-2.7]); significant liver fibrosis

(bivariate analysis: p= 0.001; multivariate OR [95%C]: 5.97 [2.1-11.9]); had been diagnosed with acquired immunodeficiency syndrome (AIDS)-defining criteria in the past (bivariate analysis: p = 0.001; multivariate OR [95% CI]: 3.86 [1.92-10.23]); and bore HCV genotypes 1/4 (bivariate analysis: p = 0.046 multivariate OR [95% CI]: 1.97 [1.01-5.91]) when compared with those who achieved SVR. We found that VR can be accurately predicted in HIV/HCV co-infected patients on the basis of the risk factors, which can be identified before treatment.

Additionally, it was evaluated the influence of Atazanavir based antiretroviral HIV treatment on HCV baseline viral load. We identified that HIV ATV-based therapy was associated with a higher HCV viral load than the therapy based in other dugs.

In recent years, the host's genetic factors have played an important role in predicting treatment response. Variations of the IL28B genotype are considered to be the most important baseline factor for treatment response among HIV co-infected patients and patients with the IL28B-CC genotype (SNP rs12979860) achieve SVR significantly more frequently than those bearing unfavorable genotypes (CT or TT). Several studies have suggested that the effect of the IL28B genotype on HCV viral kinetics can be seen in

the first few days after start of treatment. There was however limited information about the IL-28B effect once treatment has started and no data about the effect on HIV/HCV co-infected patients. For this reason, we designed a study to evaluate the effect of the IL28B genotype on HCV viral kinetics in the first 4 weeks after start of treatment with Peg-IFN/RBV in HIV/HCV co-infected patients. We found that bearing the IL28B-CC genotype was related to greater HCV viral decline during the first weeks of treatment with Peg-IFN/RBV among patients infected with HCV genotype 1 or 4, but not those infected with genotype 2 or 3. Because of the greater viral decline, the rate of rapid virological response (RVR)—defined as an undetectable HCV viral load at week 4—was higher among IL28B-CC patients than among IL28B non-CC patients. We concluded therefore that the higher SVR rate observed with IL28B in earlier studies might be due to the higher RVR rate, the most predictive on-treatment factor of SVR.

Similarly, we hypothesized that the effect of the IL28B genotype on treatment response could be influenced by various factors. It was necessary to identify potential modulators of the IL28B effect influencing HCV treatment response, which would be beneficial in clinical decision-making. So we designed three studies:

First, the IL28B genotype is associated with plasma LDL cholesterol levels and patients with IL28B-CC show significantly higher LDL cholesterol levels than those harboring the non-CC genotype. There is no explanation to date for this finding, although an interaction between IL28B and LDL receptor (LDLR) genes could be hypothesized. So, we tested variations of the LDLR genotype on HCV viral decline during the first weeks of treatment among patients coinfected with HIV/HCV genotype 1, according to their IL28B genotype. We found that patients carrying the LDLr-CC genotype showed greater HCV viral decline than those with the LDLr non-CC genotype during the first 4 weeks of treatment. Similarly, we observed that CC/CC patients had better rapid virological response (RVR) rates than CC/non-CC patients (41.2 versus 13.3%; $P<0.001$), as well as greater HCV viral decline than those with the CC/non-CC genotype during the first weeks of treatment. We concluded therefore that LDLr genotype is a pretreatment predictor of HCV kinetics in HIV/HCV co-infected patients bearing genotype 1 who start therapy with Peg-IFN/RBV, and that it modifies the effect of the IL28B genotype on HCV viral decline.

Second, the IL28B action mechanism has not been clearly explained. It has however been suggested that IL28B variations have different endogenous IFN activities (with IL28B-CC being lower than IL28B Non-CC). Thus,

patients with lower endogenous IFN activity would obtain a higher response when exogenous IFN was added, leading to higher viral clearance. As we reported for the first study in this phase, IL28B genotype has no impact on HCV genotype 2 or 3 treatment response. Various clinical trials have studied reduced doses in drug-based HCV therapy among monoinfected HCV genotype 3 patients. Reducing the Peg-IFNa2a dose from 180 mg/per week to 135 mg/per week was shown to give similar RVR and SVR rates, so that a reduced dose of HCV treatment drugs was appropriate for this population of patients. We hypothesized that a lower Peg-IFN dose might modify the impact of IL28B on treatment response in HIV/HCV genotype 3 co-infected patients, due to the mechanism of action related to IL28B IFN, set out above. We therefore evaluated the impact of IL28B on HCV viral decline during the first weeks of treatment in two different HCV genotype 3 populations. The first group received the standard dose of Peg-IFN/RBV, and the second, receiving lower doses of both treatment drugs, were enrolled in an open-label clinical trial. We found that the IL28B-CC genotype had greater HCV viral decline in the standard dose group compared to the low drug dose group. However, the greatest HCV viral decline was found among IL28B non-CC patients. These findings suggest that HCV viral decline among patients with HCV

genotype 3 is not due to the IL28B genotype, but to the Peg-IFN/RBV dose.

Thirdly, we evaluated the influence of IL28B genotype on HCV viral decline between HCV genotype 1 subtypes (1a and 1b). In our study, the IL28B-CC genotype had a positive effect on HCV viral clearance during the first weeks of treatment with Peg-IFN-alpha-2a/RBV and on RVR rates in HCV-1b genotype HIV co-infected patients, but not those with HCV-1a. Due to this effect HCV-1b infected patients could have a higher treatment response rate, as observed in elsewhere.

INTRODUCCIÓN:

1. Genoma y ciclo biológico del virus de la Hepatitis C (VHC).

1.1. Características Genómicas del VHC

El virus de la hepatitis C (VHC) es un virus con envoltura, ARN monocatenario positivo (de 9,6 Kb), perteneciente a la familia *Flaviviridae*, genero *Hepacivirus* [1]. Han sido descritos siete genotipos (1, 2, 3, 4, 5, 6 y 7) virales, diferentes en más del 25% de los nucleótidos de su genoma, a su vez divididos en subtipos (a, b, c, etc…) [2].

El genoma del VHC codifica 10 proteínas; 3 estructurales (core, E1 y E2), cinco no estructurales (NS3, NS4A, NS4B, NS5A y NS5b), y 2 situadas entre las proteínas estructurales y no estructurales (p7 y NS2) más similares a las proteínas no estructurales [3, 4].

1.2. Ciclo biológico

El ciclo biológico del VHC puede dividirse en tres bloques: 1) anclaje y fusión a la membrana del hepatocito, 2) translación y replicación viral, y 3) ensamblaje y liberación de los virones.

1.2.1. Anclaje y fusión a la membrana del hepatocito

El VHC está íntimamente ligado al metabolismo lipídico, usando el ciclo natural de las lipoproteinas para entrar en el hepatocito [5]. La forma infectiva del VHC es la partícula lipo-viral (PLV), consistente en la conjunción de la lipoproteína de muy baja densidad (VLDL) y el VHC [6].

La PLV se ancla a la superficie del hepatocito mediante su unión a dos receptores, los glicosaminoglicanos (GAGs) y el receptor de la lipoproteína de baja densidad (LDLr) (Figura 1A) [7, 8]. La fijación de la PLV por parte de estos receptores se produce mediante la interacción de dos componentes, uno viral (glicoproteínas de membrana E1 y E2) y otro de la VLDL (apo-E). Tras esta unión, la PLV se une al *scavenger-receptor class B type I* (SRB-1) mediante la región HVR1 de E2 [9]. La unión de E2 al SRB-1 produce una modificación conformacional de las glicoproteínas E1 y E2, aumentando la afinidad de E1 y E2 por otros correceptores de la superficie del hepatocito, concretamente al cumulo de diferenciación 81 (CD81). La unión de E2 a CD81 favorece la fusión y endocitosis del virus (reacción pH dependiente) [10]. Recientemente, se han descrito Claudina-1 y la Ocludina- 1, así como el receptor Niemann-Pick C1-like 1 cholesterol (NPC1L1)como nuevos factores de entrada al hepatocito [11-13].

1.2.2 Translación y replicación viral

Dentro del hepatocito, la PLV es degrada en el citoplasma celular, liberando el virus,produciéndose la decapsidación viral, liberando la cadena de ARN+ al citoplasma celular [14]. Esta cadena se une por el extremo 5´ mediante la región de internación al ribosoma (IRES) al ribosoma del retículo endoplasmático (RE) celular. El ribosoma codifica las 10 proteínas del virus (tanto estructurales como no estructurales) produciendo una cadena ARN- y una cadena de poliproteinas víricas [15]. En primer lugar, se produce la escisión proteica mediante la acción proteasa de NS3, conformándose estas 10 proteínas liberadas en la membrana del RE formándose el complejo replicativo (Figura 1B) [16].

1.2.3. Ensamblaje y liberación de los virones

El ensamblaje y liberación, al igual que la entrada del virus, está íntimamente relacionado con el metabolismo lipídico. El Core rodea los cuerpos lipídicos (CL), organelas celulares encargadas de almacenar triglicéridos y colesterol, produciendo su redistribución, haciendo que éstos se sitúen alrededor del complejo replicativo viral [17]. NS5A desencadena un mensaje de inicio del ensamblaje del virus. Una vez desencadenado el ensamblaje, la membrana del RE se escinde hacia el citoplasma celular

[18]. Esta escisión lipídica contiene el material genético del virus (ARN+), las proteínas víricas y componentes lipídicos conferidos por los CL [19]. De esta forma, está partícula viral entra en la VLDL donde madura. Una vez formada la PLV salé del hepatocito infectado mediante el ciclo normal de la lipoproteína VLDL, siendo la forma infectiva del VHC.

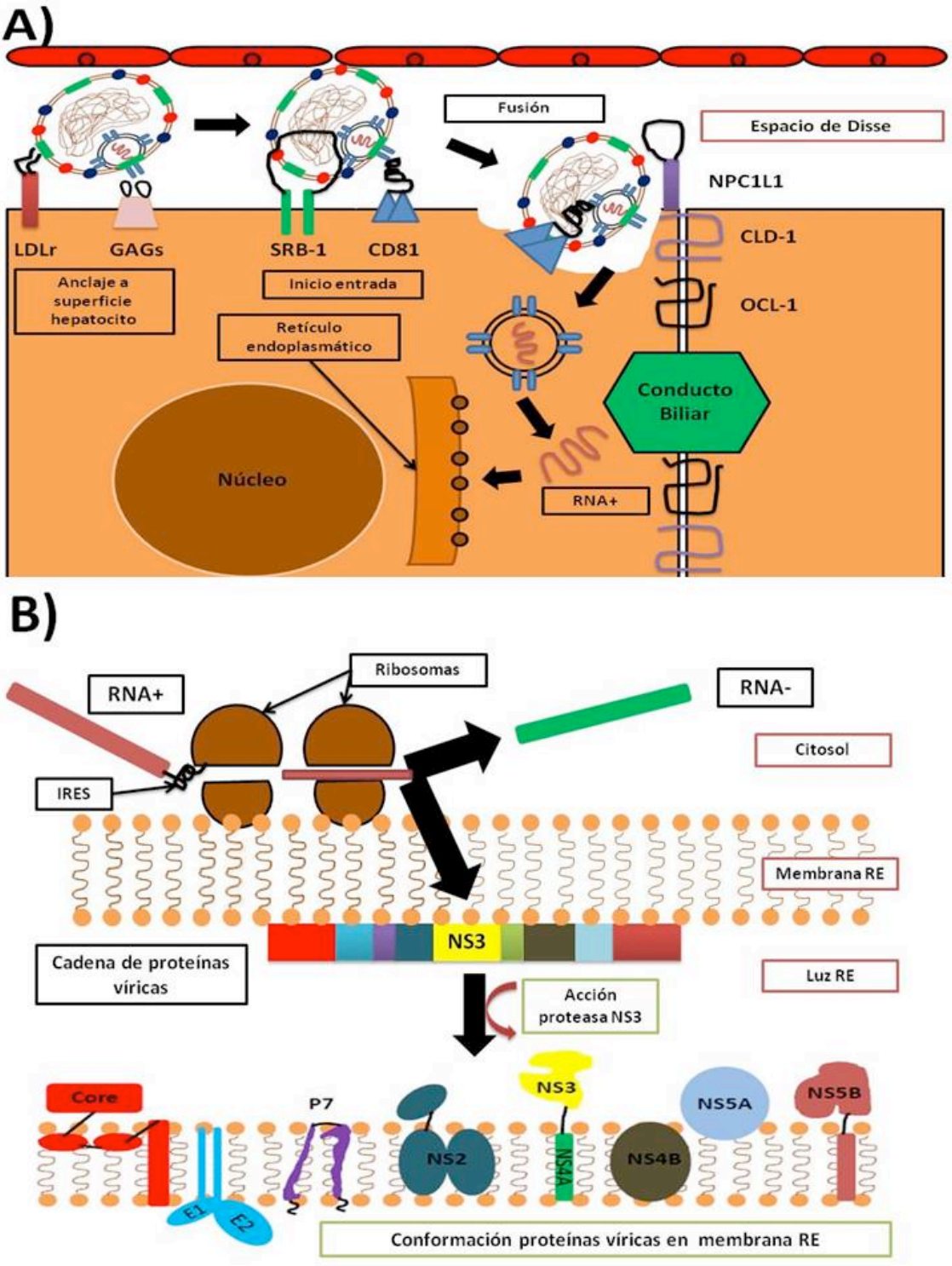

***Figura 1**: **A)** Anclaje y fusión en la membrana del hepatocito **B)** Formación del complejo replicatico y conformación proteínas virales en membrana del retículo*

endoplasmático (Rivero-Juarez A. Estructura Genómica y ciclo Biológico del VHC. En Rivero A, Pineda JA Eds. Coinfección VIH y VHC; Málaga 2012: 19-26).

2. Aspectos epidemiológicos de la infección por el VHC

La infección por este virus es un problema de salud mundial que afecta a más de 170 millones de personas, de las cuales se estima que alrededor de 5 millones está coinfectada por el virus de la inmunodeficiencia humana (VIH), esto supone el 20% del global de personas infectadas por el VIH [20]. Esta proporción es mayor en regiones en las que el consumo de drogas por vía parenteral ha constituido la principal vía de transmisión del VIH. De esta forma en España aproximadamente el 50% de los pacientes infectados por VIH se encuentran coinfectados por el VHC [21]. Tras una infección aguda, aproximadamente el 75% de los pacientes evolucionaran a infección crónica mediante el desarrollo de una fibrosis hepática progresiva [22]. El 4-24% de los casos evolucionarán a cirrosis hepática y el 0,7-23% lo harán hastacarcinoma hepatocelular [23]. La coinfección por VIH/VHC, incrementa la probabilidad de cronificación de la hepatitis por VHC, provoca unaprogresión mas acelerada de la fibrosis y, consecuentemente, una mayor probabilidad de desarrollo de cirrosis hepática y enfermedad

hepática terminal [24-25]. La alta prevalencia de infección crónica por VHC y la acelerada progresión de ésta enfermedad en pacientes infectados por el VIH, han conseguido situar a la enfermedad hepática terminal como una de las principales causa de muerte en estapoblación de pacientes [26, 27]. Por este motivo, el tratamiento de la infección crónica por VHC en pacientes coinfectados por el VIH reviste una especial importancia.

3. Tratamiento frente a la infección crónica por el VHC en el paciente coinfectado por el VIH

El tratamiento estándar frente al VHC ha cambiado de forma drástica durante los últimos 15 años. En primer lugar, el tratamiento con *interferón* (IFN) en monoterapia, posteriormente la incorporación de *ribavirina* (RBV) a la terapia con IFN, la sustitución del INF estándar por *interferón-pegilado* (IFN-Peg), y por último, recientemente, la incorporación de los *inhibidores de la proteasa NS3* (IPs), boceprevir (BOC) y Telaprevir (TPV), al tratamiento de los pacientes con infección por genotipo 1 del VHCen combinación con IFN-Peg/RBV [28]. Esta mejoría en el arsenal terapéutico de la infección por el VHC, ha conseguido que el porcentaje de pacientes que alcanzan *Respuesta Viral Sostenida* (RVS), gold estándar de curación, pase de un dramático 5% a un esperanzador 70% (Tabla 1) [29-

34]. Además, actualmente están en distintas fases de desarrollo más de 30 fármacos de acción directa (FAD) sobre el VHC, que mejorará aun más, el pronóstico de los pacientes infectados por el VHC [35].

Tabla 1. Porcentaje de RVS en pacientes con genotipo 1 en los diferentes ensayos en pacientes coinfectados por genotipo 1 del VHC y el VIH.

	APRICOT	**ACTG 5071**	**RIBAVIC**	**Barcelona**	**Study 110**	**Boceprevir**
N	868	133	412	95	60	64
Pacientes con genotipo 1 (%)	60	77	48	55	100	100
RVS pacientes con genotipo 1 (%)	29	14	17	38	70	60.7

3.1. Objetivos del tratamiento

El objetivo del tratamiento es conseguir la supresión sostenida de la replicación viral del VHC y que ésta se mantenga de forma indefinida una vez finalizado el tratamiento. Se considera que el tratamiento de un paciente ha conseguido erradicar su infección por VHC cuando la carga viral del VHC permanece indetectable24 semanas después de finalizar el mismo. Esta situación ha sido definida comoRVS, y su consecución se relaciona con curación de la enfermedad (Figura 2A).

Se han definido una serie de conceptos relacionados con la respuesta viral obtenida durante el tratamiento que guardan una estrecha relación con

la probabilidad de alcanzar RVS, que son de gran utilidad en la toma de decisiones respecto al mantenimiento o suspensión del tratamiento. A continuación definiremos cada uno de estos conceptos que serán utilizados a lo largo de la exposición [36]:

- *Respuesta Viral rápida* (RVR): definida como alcanzar una carga viral del VHC indetectable tras 4 semanas (28 días) de tratamiento (Figura 2B).
- *Respuesta viral precoz parcial*(RVPp): definida como la consecución de un descenso de la carga viral del VHC de más de 2 $\log_{10}$UI/mL tras 12 semanas de tratamiento (Figura 2C).
- *Respuesta viral precoz completa* (RVPc): definida como alcanzar una carga viral indetectable del VHC en la semanas 12 de tratamiento (Figura 2D).
- *No Respuesta-Null Responder*(NR): definida como conseguir un descenso de la carga viral del VHC de 2 log UI/mL tras 12 semanas de tratamiento (como ausencia de RVPp) (Figura 2E).
- *Rebote viral-Viral Breakthrough*(VB): definida como la reaparición del ARN-VHC durante el tratamiento tras haber alcanzado su indetectabilidad (Figura 2F).

- *Respuesta final de tratamiento* (RFT): definida como la indetectabilidad de la carga viral del VHC en el momento de finalización del tratamiento (Figura 2G).
- *Recidiva Viral-Viral Relapse (VR)*: definida como la reaparición del ARN-VHC tras la retirada del tratamiento del VHC (Figura 2H).

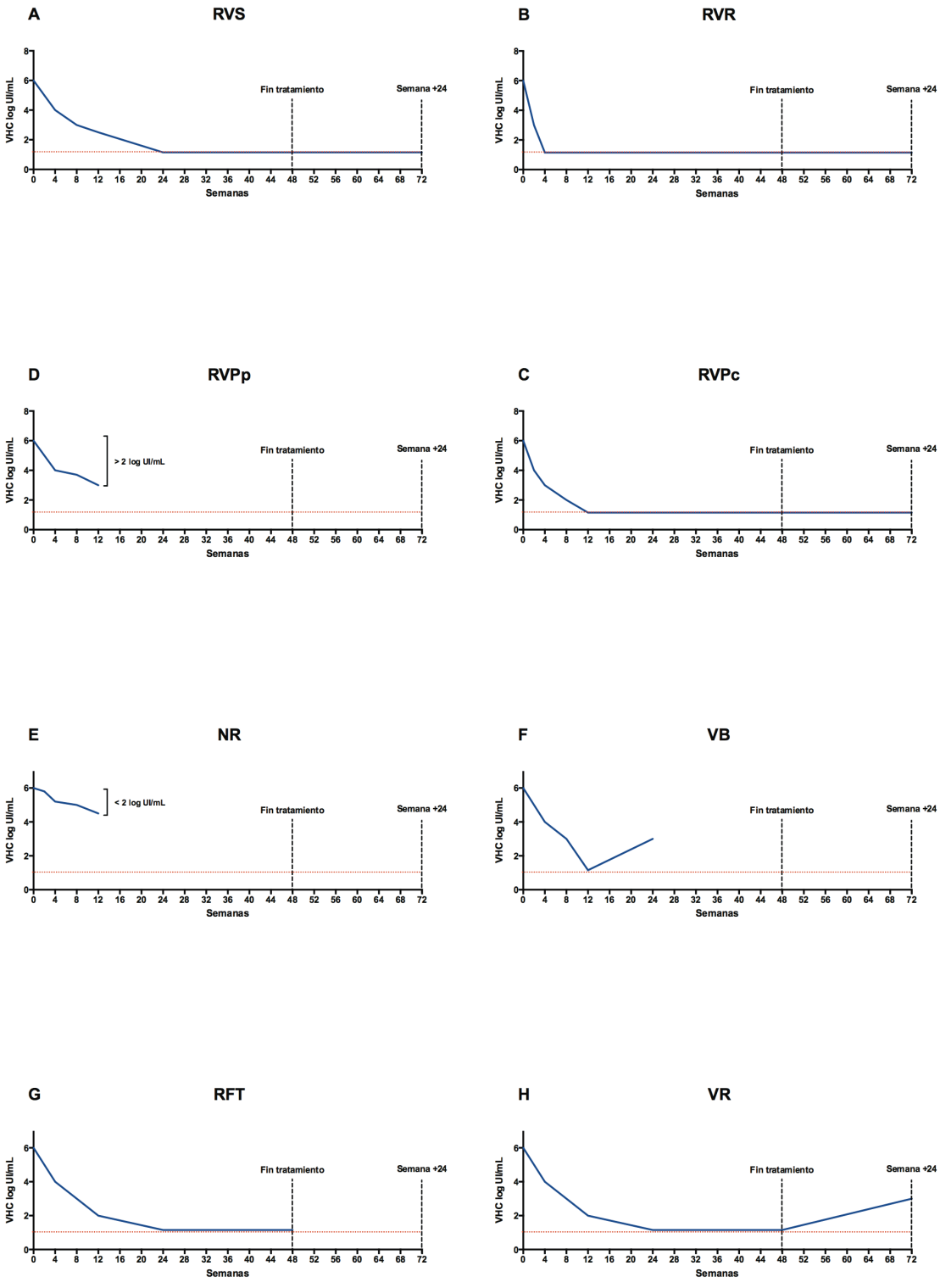
A
RVS
B
RVR
D
RVPp
C
RVPc
E
NR
F
VB
G
RFT
H
VR
VHC log UI/mL
Semanas
Fin tratamiento
Semana +24
> 2 log UI/mL
< 2 log UI/mL

Figura 2: *Representación gráfica de la respuesta viral durante el tratamiento con IFN-Peg/RBV (Rivero Juarez A). La línea roja discontinua marca el límite mínimo de detectabilidad (1.15 log IU/mL).*

3.2 Tratamiento con interferón pegilado más ribavirina:

La pauta estándar de tratamiento frente al VHC en pacientes coinfectados por VIH es la combinación de IFN-Peg (α-2a a dosis de 180 μg/semana y α-2b a dosis de 1,5 μg/kg/semana) mas RBV ajustada a peso (1.000 ó 1.200 mg/día respectivamente para pacientes con<75 kg ó ≥75 kg) durante 48 semanas [36]. Las tasas de RVS alcanzadas con esta pauta en los distintos ensayos clínicos depende del genotipo del VHC, oscilando entre el 17-46% en pacientes con genotipos 1 ó 4, y entre el 43-71% en pacientes con genotipo 2 ó 3 [29-34]. No se han encontrado diferencias en las tasas de RVS entre los dos tipos de IFN-Peg (α-2a oα-2b) [37].

En cuanto a la dosis óptima que debe usarse en los pacientes VIH infectados por genotipos 2 ó 3, la dosis recomendada es de 180 μg/semana, independientemente del peso, para IFN-Peg α-2a y de 1,5 μg/kg/semana para el α-2b [36]. No obstante, en pacientes monoinfectados por el VHC, se ha demostrado que dosis menores de ambos fármacos (135 μg/semana de IFN-Pegα-2a y 800mg/día de RBV) tienen similares tasas de RVS que las dosis establecidas como estándar [38]. Sin embargo, esta recomendación

podría no ser extrapolada a la población coinfectada por el VIH. Respecto a la dosis de RBV, en pacientes coinfectados la dosificación está menos firmemente establecida. En la mayoría de los ensayos clínicos iniciales en pacientes con infección por el VIH se utilizaron dosis de 800 mg/día de RBV, independientemente del genotipo del VHC por temor a una mayor incidencia de efectos adversos. En cambio, en pacientes coinfectados por VIH no existen ensayos clínicos comparativos de la eficacia de diferentes dosis de RBV en pacientes infectados por genotipo 2 ó 3, como pasa en pacientes monoinfectados [39-41]. Del mismo modo, no existen evidencias firmes que apoyen una dosificación estándar de RBV en pacientes VIH con genotipo 2 ó 3 del VHC, aunque dada la peor respuesta que presentan estos pacientes respecto a los pacientes monoinfectados la dosis de RBV debería ajustarse al peso. El estudio del efecto de diferentes dosis de IFN-Peg/RBV en la respuesta al tratamiento en pacientes coinfectados por el VIH y genotipo 3 del VHC es uno de los objetivos de nuestro estudio.

Por su parte, en pacientes VIH coinfectados por genotipo 1 ó 4 del VHC la dosis de IFN-Pegα-2a recomendada es de 180 μg/semana y de 1,5 μg/kg/semana para el α-2b [36]. En cambio, la dosis de RBV utilizada en los ensayos clínicos de pacientes coinfectados no ha sido uniforme. En algunos ha sido de 800 mg por temor a mayores tasas de efectos adversos

que en pacientes monoinfectados por el VHC y/o al desarrollo de interacciones medicamentosas. Sin embargo, en los ensayos clínicos en los que se ha usado RBV ajustada a peso, se han obtenido tasas de RVS más altas; por ello, es la dosis recomendada [29-34].

La duración de la terapia podría individualizarse en función del genotipo del VHC (1-4 ó 2-3) y de la cinética del VHC en respuesta al tratamiento. Así por ejemplo,se ha sugerido que en pacientes con genotipos 4 que no alcanzan respuesta RVR ó RVPc, el tratamiento debería prolongarse hasta 72 semanas, mientras que en pacientes con genotipo 3 que alcanzan RVR la terapia podría acortase hasta 24 semanas [36-42].

3.3. Uso de FAD en pacientes coinfectados por VIH y Genotipo 1 del VHC.

En los últimos años, se han desarrollado 2 FAD frente al VHC (TPV y BOC) con alta actividad frente al genotipo 1 del VHC [43]. Estos nuevos fármacos inhiben la acción como proteasa de la proteína NS3 del VHC. La adición de estos nuevos fármacos a un régimen basado en IFN-Peg/RBV consigue incrementar las tasas de RVS hastael 70% en pacientes sin tratamiento previo. No hay ensayos que comparen la eficacia y seguridad

entre ambos fármacos, no obstante un reciente metanálisis indirecto llevado a cabo en pacientes monoinfectados por VHC, no encontró diferencias en su tasas de RVS [44-45].

En la actualidad no está aprobada la indicación TPV y BOCen pacientes infectados por el VIH. En España, el acceso alos medicamentos en condiciones diferentes a las autorizadas está reguladopor el Real Decreto 1015/2009, de 19 de junio. Esta regulación, establece quela Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) «podráelaborar recomendaciones de uso de medicamentos en condiciones nocontempladas en la ficha técnica cuando el uso del medicamento en estascondiciones suponga un impacto asistencial relevante». De este modo laAEMPS ha elaborado unas recomendaciones que regula y permite el uso deBOC y TPV en pacientes coinfectados por VIH/VHC [46]. Las recomendaciones son:

Criterios dependientes del VHC

A.- Infección por VHC genotipo 1, independientemente de que elpaciente haya recibido o no tratamiento previo para el VHC

B.- Fibrosis F3 y F4 confirmada por biopsia hepática o rigidez hepáticamedida por Fibroscan >9.5 kPa. Independientemente del

grado defibrosis, se podrá considerar iniciar tratamiento con BOC o TPV en pacientescon manifestaciones extrahepáticas graves de la infección por VHC como porejemplo aquellas derivadas de la crioglobulinemia mixta policlonal.

C.- Hepatopatía crónica compensada (Child-Pugh grado A)

D.- Concentración de hemoglobina >11 g/dl en mujeres y >12 g/dl enhombres

Criterios dependientes del VIH

E.- Linfocitos CD4+ totales en sangre periférica >100 /ml o porcentaje delinfocitos CD4+ >12%

F.- Carga viral plasmática de VIH <1000 copias/ml (pacientes en tratamiento antirretroviral[TAR])

3.3.1. Uso de BOC

La estrategia de tratamiento con BOC consiste en un periodo de *lead-in*de 4 semanas con IFN-Peg/RBV, tras la cual se administrará conjuntamente BOC durante 44 semanas [47-48]. La posología de BOC es de 800mg (4 comprimidos) cada 8 horas administrados con comida [49]. Esta pauta ha demostrado su superioridad en las tasas de RVS frente a IFN-

Peg/RBV en dos ensayos clínicos aleatorizados doble ciego, tanto en pacientes que reciben un primer tratamiento como en previamente tratados (respondedores parciales y recidivantes) monoinfectados por el VHC [47-48]. Por otro lado, en población coinfectada los datos son escasos. Los datos parciales de un ensayo clínico llevado a cabo en población naïve al tratamiento con IFN-Peg/RBV, muestran un aumento significativo de las tasas de RVS cuando se co-administra BOC con la terapia estándar [33].

3.3.2.Uso de TPV

La estrategia de tratamiento con Telaprevir consiste en la administración de triple terapia con TPV+IFN-Peg/RBV durante 12 semanas, continuado de 36 semanas de tratamiento con IFN-Peg/RBV [50-52]. La estrategia de administrar 4 semanas con IFN-Peg/RBV previas a la administración de TPV, ha sido testada en pacientes monoinfectados por el VHC previamente tratados [51, 53]. Los resultados derivados de esta estrategia terapéutica mostraron no inferioridad en las tasas de RVS respecto a la estrategia terapéutica convencional. Por lo que, TPV podría ser usado siguiendo esta estrategia. La posología de TPV es de 750mg (2 comprimidos) cada 8 horas administrados con comida [49]. Esta estrategia, comprobada en tres ensayos clínicos, ha demostrado superioridad frente a

la terapia estándar con IFN-Peg/RBV tanto en pacientes naïve como pre-tratados (nulos y parciales respondedores y pacientes recidivantes) monoinfectados por el VHC [50-52]. Recientemente, se ha demostrado en un ensayo clínico aleatorizado doble ciego realizado en población monoinfectada por el VHC, que la administración de 1125 mg cada 12 de TPV tiene unas tasas de RVS similares a la administración de la dosis de 750mg cada 8 horas [54]. Al igual que ocurre con Boceprevir, solo se dispone de datosen población coinfectada por el VIH de un ensayo clínico en pacientes naïve, en el que se compruéba la superioridad de TPV sobre la terapia estándar en las tasas de RVS [34].

3.3.3. Limitaciones del tratamiento con Telaprevir y Boceprevir

El tratamiento del VHC con un régimen que incluya TPV o BOC adolece de diversas limitaciones. En primer lugar, estos nuevos fármacos no han mostrado actividad antiviral frente a los genotipos 2, 3 y 4, que provocan cerca de la mitad de los casos de hepatitis crónica por VHC en nuestro medio [42]. En segundo lugar, el uso de estos nuevos fármacos incrementa la incidencia de efectos adversos respecto al régimen IFN-Peg/RBV, siendo la anemia y el desarrollo de exantema los eventos más frecuentes [47, 48, 50-53]. En tercer lugar, la eficacia de estos fármacos en pacientes

infectados por VHC que no han respondido a un tratamiento previo con IFN-Peg/RBV es inferior a la observada en pacientes sin tratamiento previo [48-51]. Por último el uso de TPV o BOC supone un considerable aumento del coste del tratamiento [55].

En pacientes coinfectados por el VIH el uso de estos FAD presentaproblemas añadidos. La experiencia sobre la eficacia y seguridad de estos fármacos en pacientes infectados por el VIH es muy limitada [33, 34]. Además existen interacciones farmacológicas entre estos nuevos fármacos y los fármacos antirretrovirales que puede suponer una importante limitación para su uso conjunto [56, 57].

3.3.4. Importancia de la identificación de subgrupos de pacientes con alta probabilidad de alcanzar RVS con IFN-Peg/RBV.

En los ensayos clínicos de desarrollo de los IPs frente al VHC, las tasas de RVS en las ramas controles (pacientes tratados con IFN-Peg/RBV) fueron del 45% y el 29.5% respectivamente [33, 34]. Esto sugiere que una significativa proporción de pacientes tratados con IFN-Peg/RBV puede alcanzar RVS sin necesidad de usar FAD. Por ello, y dadas las limitaciones del uso de FAD comentadas anteriormente, tendría un alto interés estratégico identificar subgrupos de pacientes con hepatitis crónica por

VHC-genotipo 1 con alta probabilidad de alcanzar RVS con tratamiento con IFN-Peg/RBV. Ello permitiría individualizarel tratamiento en los pacientes infectados por genotipo 1 del VHCen función de la predicción de tolerancia, del beneficio clínico esperado, de las futuras opciones terapéuticas, y sobre de la predicción de la respuesta. Al mismo tiempo ello permitiría optimizar el tratamiento de la población coinfectada por VIH y genotipo 1 del VHC, al conseguir ahorrar recursos y efectos adversos innecesarios en el subgrupo de pacientes que no se beneficiarían del uso de FAD.

4.Predicción de la respuesta al tratamiento con IFN-Peg/RBV

En los últimos años se han descrito y estudiado múltiples factores, determinados tanto en el momento basal como durante el tratamiento, con un alto valor clínico y pronóstico de respuesta al tratamiento.

4.1. Factores basales de respuesta al tratamiento del VHC

El grado de fibrosis/esteatosis hepática, la carga viral basal del VHC, así como el propio genotipo del VHC y sus mutaciones tienen una relación lineal inversa con la obtención de RVS [29-32, 58-62]. Por otro lado, en los últimos 3 años, se han descrito factores genéticos, tanto del VHC como del hospedador, con alto valor predictivo de respuesta al tratamiento del VHC

con IFN-Peg/RBV, que pueden en un futuro próximo jugar un papel determinante en la toma de decisiones sobre el tipo y tiempo de tratamiento frente al VHC. Entre ellos, cabe destacar las variaciones genotípicas inmunológicas (HLA-C y *Killer Immunoglobulin-like Receptors* [KIRs] de las células NK) [63, 64], implicadas en el ciclo del virus (LDLr) [65], así como los relacionados con la sensibilidad del paciente al IFN (IL28B) [66-68]. El estudio de estos factores es uno de los objetivos de nuestro estudio.

4.2 Cinética viral del VHC

El estudio de la cinética viral del VHC en respuesta al tratamiento tiene una gran importancia en la predicción de la respuesta al mismo. Este hecho ha sido claramente demostrado tanto con el tratamiento IFN-Peg/RBV como con el tratamiento con los nuevos FAD.

La determinación de la cinética viral del VHC a las 4 semanas del tratamiento con IFN-Peg/RBV ha demostrado gran utilidad en la predicción de RVS. De este modo alcanzar negatividad de la carga viral del VHC a las 4 semanas de iniciado el tratamiento (RVR) se ha identificado como elfactor con mayor valor predictivo positivo (VPP) para RVS [69-71]. En este sentido, entre pacientes con genotipo 1 del VHC tiene especial importancia la observación obtenida en los ensayos clínicos de desarrollo

de TPV y BOC. En estos el grupo de pacientes que alcanzó RVR en la rama control (IFN-Peg/RBV) obtuvo unas tasas de RVS similares a aquellos pacientes que recibieron triple terapia (FAD+IFN-Peg/RBV) [47, 50, 52]. Esta observación sugiere que aquellos pacientes que alcanzan RVR no requerirían del uso de FAD para optimizar la probabilidad de respuesta al tratamiento.

Por el contrario, no conseguir alcanzar un descenso de la carga viral del VHC mayor de 1 $\log_{10}$ UI/mLa las 4 semanas de tratamiento conIFN-Peg/RBV tiene un alto valor predictivo negativo de RVS [47, 50, 52, 72].

La determinación de la cinética viral del VHC a las 12 semanas del tratamiento con IFN-Peg/RBV también ha demostrado gran utilidad en la predicción de RVS. Así, no conseguir una reducción dela carga viral basal del VHC enmas de 2 $\log_{10}$UI/mL a las 12 semanas de iniciado el tratamiento con IFN-Peg/RBV (ausencia de RVP) se ha identificado como el mejor factor predictivos negativo de RVS. De este modo la ausencia de RVP se utiliza en la práctica clínica como regla de parada del tratamiento del VHC con IFN-Peg/RBV [73].

Por último, la cinética viral a las 24 semanas de finalizado el tratamiento (RVS) es el criterio utilizado para definir la curación de la infección por VHC tras un curso de tratamiento [36].

El estudio de la cinética viral en respuesta al tratamiento con los nuevos FAD resulta también de gran utilidad. En pacientes que reciben tratamiento con BOC+IFN-Peg/RBV, no alcanzar una carga viral del VHC inferior a 100 kUI/mL a las 12 semanas o no negativizarla a las 24, tiene un alto valor predictivo negativo para RVS [74-76]. De tal modo que estos criterio se utilizan en la práctica clínica como regla de parada del tratamiento del VHC con BOC+IFN-Peg/RBV. Por otro lado, en pacientes que reciben tratamiento con TPV+IFN-Peg/RBV, no alcanzar una carga viral del VHC inferior a 1000 kUI/mL a las 4 semanas de tratamiento tiene también un alto valor predictivo negativo para RVS y es utilizado como regla de parada del tratamiento [75, 76].

No obstante, el valor pronóstico de la cinética viraldel VHC sobre la respuesta al tratamiento con IFN-Peg/RBV adolece de varias limitaciones. En primer lugar, la valoración de RVR permite clasificar tan soloa 1/3 de los pacientes que alcanzarán RVS al tratamiento con IFN-Peg/RBV [71]. Por lo tanto, una gran parte de los pacientes que alcanzarán RVS no

podrían ser clasificados con esta regla. Por otro lado, para poder clasificar a un paciente como no respondedor al tratamiento con IFN-Peg/RBV serían necesarias 12 semanas de tratamiento (ausencia de RVPp), exponiendo al paciente a efectos adversos. Por último, para establecer curación (RVS) es necesario esperar 24 semanas tras la retirada del tratamiento [36]. Por ello, la búsqueda de puntosmás precoces en la cinética viral y/o la implementación de nuevas criterios con alto valor pronóstico de respuesta que permitieran identificar un mayor numero de pacientes, tanto respondedores como no respondedores al tratamiento con IFN-Peg/RBV, tendría una alta relevancia clínica. Ello permitiría la implementación de alternativas terapéuticas precoces. El estudio de nuevos criterios de la cinética viral con alto valor pronóstico sobre RVS es uno de los objetivos de nuestro estudio.

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OBJETIVOS:

1. Evaluar el valor predictivo positivo sobre RVS de la determinación de la carga viral del VHC en la semana 12 tras finalización del tratamiento.
2. Evaluar la influencia del genotipo de IL28B (rs129679860) en la respuesta al tratamiento del VHC con IFN-Peg/RBV mediante el estudio de lacinética viral del VHCdurante las primeras semanas de inicio del tratamiento.
3. Evaluar la influencia del genotipo de LDLr (rs14158) en la respuesta al tratamiento del VHC con IFN-Peg/RBV mediante el estudio de la cinética viral del VHC durante las primeras semanas de inicio del tratamiento.
4. Evaluar el efecto de distintas dosis de IFN-Peg/RBV en la respuesta al tratamiento mediante el estudio de la cinética viral del VHC durante las primeras semanas de inicio del tratamiento en pacientes coinfectados por el VIH y genotipo 3 del VHC.
5. Identificar factores basales asociados a recidivas al tratamiento del VHC con IFN-Peg/RBV.
6. Evaluar la influencia del uso de distintos fármacos antirretrovirales en la carga viral basal del VHC.

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Twelve week post-treatment follow-up predicts sustained virological response to pegylated interferon and ribavirin therapy in HIV/hepatitis C virus co-infected patients

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Objectives: The aim of this study was to evaluate whether the assessment of hepatitis C virus (HCV) RNA serum at 12 weeks after the end of treatment (W12) was as informative as after 24 weeks (W24) for determining sustained virological response (SVR) in HIV/HCV co-infected patients who received a combination of pegylated interferon (PEG-INF) plus ribavirin (PEG-INF/RBV) and had a virological response at the end of treatment.

Methods: Treatment-naive HIV/HCV patients were included in this prospective study if they had completed a full course of therapy with PEG-INF/RBV, had an undetectable serum HCV RNA at the end of treatment and complied with the W12 and W24 schedule for determining HCV RNA. HCV RNA levels were measured using a quantitative PCR assay (detection limit = 15 IU/mL). Positive predictive value (PPV) was defined as the probability of an undetectable serum HCV RNA at W12 and W24 after the end of treatment.

Results: Of 186 patients treated during the study period, 104 (55.9%) were included in the study. At W24, 83 (79.8%) patients had an SVR and 21 (20.2%) had a virological relapse. At W12, HCV RNA was undetectable in 83 (79.8%) patients and all of these had SVR. Undetectable HCV RNA at W12 had a 100% PPV [95% confidence interval (CI) 96.5%–100%] for SVR.

Conclusions: Our results show that undetectable HCV RNA at W12 post-treatment has a high PPV for SVR. Testing for HCV RNA at this moment may therefore be considered an appropriate point in time for identifying SVR and relapse in HIV/HCV co-infected patients receiving treatment with PEG-INF/RBV.

Keywords: hepatitis C virus, human immunodeficiency virus, relapse, sustained viral response

Introduction

Pegylated interferon (PEG-INF) plus ribavirin (PEG-INF/RBV) is the standard treatment for chronic hepatitis C virus (HCV) infection. Achieving a sustained virological response (SVR), associated with durable eradication of infection, is the therapeutic goal for patients with chronic HCV.[1,2] The SVR for patients with chronic HCV infection is defined as an undetectable serum HCV RNA 24 weeks after discontinuation of treatment (W24). This definition is based on the results of many previous reports showing that the odds of HCV reappearing once SVR has been achieved are very low.[1–3] For this reason, testing for HCV RNA at W24 is the current gold standard for measuring the success of antiviral therapy in chronic HCV infection.[4]

However, in HCV mono-infected patients, relapses generally occur soon after a successful therapy has been discontinued, and thus several studies have shown that testing HCV mono-infected patients for serum HCV RNA 12 weeks following completion of standard PEG-INF/RBV therapy (W12) could predict SVR.[5,6]

In Western countries, a significant proportion of patients with chronic HCV infection are also co-infected with HIV.

HIV-associated immune disorders could in theory impair definitive clearance of HCV infection after HCV therapy and alter the timing of an HCV relapse. There is limited information about testing HCV RNA at W12 post-treatment to evaluate SVR in HIV/HCV co-infected patients and studies are needed to clarify this issue.[7] The objective of this study was to evaluate if measuring serum HCV RNA at W12 is as informative as at W24 to predict SVR in HIV/HCV co-infected patients with a virological end of treatment response (ETR).

Patients and methods

Patients

One hundred and eighty-six HIV-infected patients with chronic hepatitis receiving a combination therapy of PEG-INF/RBV were followed prospectively at two reference hospitals in Spain between January 2005 and June 2008. All patients were treatment naive. The criteria used to determine HCV therapy were in accordance with international guidelines.[7] Patients were included in this prospective study if they had completed a full course of therapy with ETR and complied with the 12 week and 24 week post-treatment follow-up schedule for determining serum HCV RNA. Patients who were positive for the hepatitis B surface antigen (HBsAg) were excluded.

Treatment regimens

All individuals were treated with either PEG-INF α2a or PEG-INF α2b at doses of 180 μg or 1.5 μg/kg/week, respectively, in combination with a weight-adjusted dose of oral ribavirin (1000 mg/day for $<$75 kg and 1200 mg/day for $\geq$75 kg). Following international guidelines,[7] 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, in accordance with the decision of the physician responsible for the patient. At weeks 12 and 24, PEG-INF/RBV was discontinued in non-responding individuals.

Definitions of virological responses

ETR and SVR were defined as an undetectable serum HCV RNA at the end of therapy and at 24 weeks following the end of treatment, respectively. A non-response was defined as a detectable serum HCV RNA at the end of treatment. Virological breakthrough was defined as detectable plasma HCV RNA after week 24 of therapy in patients with a previously undetectable HCV viral load. Virological relapse (VR) was defined as an undetectable serum HCV RNA at the end of treatment and a detectable serum HCV RNA at the 24 week post-treatment follow-up.

Virological evaluation

Plasma HCV RNA load measurements were performed using a quantitative PCR assay (Cobas TaqMan, Roche Diagnostic Systems Inc., Pleasanton, CA, USA), with a detection limit of 15 IU/mL.

Statistical analysis

The descriptive statistics of the patients are reported. Continuous variables are summarized as means $\pm$ SD and categorical variables as frequencies and percentages. The positive predictive value (PPV) was defined as the probability that an undetectable serum HCV RNA would occur at W12 and W24 following the end of treatment.

Ethical aspects

This study was designed and performed according to the Helsinki declaration and was approved by the Ethics Committees of both participating hospitals.

Results

A total of 186 HIV patients infected with chronic HCV were treated with PEG-INF/RBV during the study period. The baseline characteristics of all patients are shown in Table 1. Of the initial population treated, 82 patients (44.1%) experienced early virological failure or viral breakthrough during treatment, were discontinued prematurely because of adverse effects or were lost to follow-up before they achieved ETR. Overall, only 104 (55.9%) of the initial population completed a full course of therapy and achieved ETR. All of these complied with the W12 and W24 post-treatment follow-up schedules. At W24, 83 (79.8%) patients had an SVR and 21 (20.2%) had a VR.

At W12, serum HCV RNA was undetectable in 83 (79.8%) patients and all achieved SVR (Table 2). No relapse was observed after W12. Undetectable HCV RNA at W12 had a 100% (95% CI 96.5%–100%) PPV for SVR.

Discussion

This study, carried out on a cohort of HIV/HCV co-infected patients treated with PEG-INF/RBV, suggests that testing HCV

Table 1. Baseline characteristics of patients

Characteristics	ETR	SVR	VR
N	104	83	21
Male, *n* (%)	84 (80.8)	67 (80.7)	17 (81.0)
Age in years, mean (SD)	40.9 (5.5)	40.9 (5.6)	41.6 (5.1)
BVL $>$600 000, *n* (%)	56 (53.8)	41 (49.4)	15 (71.4)
Genotypes 1 and 4, *n* (%)	49 (47.1)	35 (42.2)	14 (66.7)
CD4 $<$250 cells/mm^3, *n* (%)	12 (11.5)	9 (10.8)	3 (14.3)
Category C (CDC), *n* (%)	34 (32.7)	30 (36.1)	4 (19.0)
HAART, *n* (%)	85 (81.7)	68 (81.9)	17 (81.0)
Liver fibrosis F3/F4, *n* (%)	41 (39.4)	26 (31.3)	15 (71.4)
Cirrhosis, *n* (%)	23 (22.1)	18 (21.7)	5 (23.8)
Interferon α2a, *n* (%)	75 (72.1)	59 (71.1)	16 (76.1)

BVL, baseline viral load; HAART, highly active antiretroviral treatment.

Table 2. Serum HCV RNA outcome during the 24 week post-treatment follow-up

Serum HCV RNA (follow-up)	Patients with HCV RNA (−)	Patients with SVR	PPV (95% CI)
End of treatment	104	83	79.8% (71.3%–86.7%)
W12	83	83	100% (96.5%–100%)
W24	83	83	100% (96.5%–100%)

RNA at W12 using a sensitive test with a lower detection limit of 15 IU/mL may be considered an appropriate timepoint for assessing virological response and identifying SVR and relapse in HIV/HCV co-infected patients.

As is the case with HCV mono-infected patients, achieving an undetectable serum HCV RNA at W24 is the current therapeutic objective when treating HIV-infected patients with chronic HCV.[8] However, studies in HCV mono-infected patients show that testing HCV RNA at W12 may be considered an appropriate timepoint for assessing virological response.[5,6,9] These studies have found that a more sensitive assay can detect residual serum HCV RNA in patients classified as having ETR with a less sensitive assay, reclassifying them as early relapses.[10] For this reason, a low-sensitivity HCV RNA assay (detection limit 15 UI/mL) may be an obstacle to early identification of patients with SVR. Martinot-Peignoux *et al.*,[6] evaluated 573 HCV mono-infected patients who received a combination of PEG-INF/RBV and had ETR to determine whether assessing serum HCV RNA at W12 using an assay with a detection limit of 5–10 IU/mL was as informative as at W24 for evaluating the SVR. At W12, serum HCV RNA was undetectable in 409 patients, and 408 patients had an SVR (PPV 99.7%, 95% CI 99.1%–100%). Aghemo *et al.*[9] obtained similar results (PPV 100%) using two HCV RNA assays with detection limits of 15 IU/mL and 50 IU/mL for 32 and 258 patients, respectively. In our study, using a HCV RNA commercially available assay with a detection limit of 15 IU/mL, no cases of relapse were observed between W12 and W24, and undetectable HCV RNA at W12 had a 100% PPV for SVR.

Information about the timing of HCV relapse in HIV/HCV co-infected patients is scarce. In one study, carried out on 143 HIV/HCV co-infected patients treated with PEG-INF/RBV who achieved ETR, all but 2 (45/47, 95.7%) relapses occurred before W12.[7] Phylogenetic analysis suggested that HCV re-infection occurred in one patient, and the possibility that the second patient might have been exposed again to the same source that was the cause of her original infection could not be discounted. Despite the special characteristics of HIV/HCV co-infected patients, our results suggest that HIV co-infection does not seem to increase the risk of HCV relapse beyond W12 after completion of HCV therapy.

Early knowledge of the post-treatment response status in patient therapy is likely to have a positive effect on the management of HCV patients. Reducing the post-treatment follow-up period to 12 weeks from the current standard of 24 could lead to a reduction in the costs associated with monitoring responses, improve patient care and enable relapsed patients to pursue alternative therapies earlier.

On the other hand, several studies have shown that the value of measuring serum HCV RNA at W12 to assess SVR is independent of whether the patient is treated with standard or pegylated interferon (α2a or α2b), the interferon dose or whether ribavirin is added to PEG-INF.[5,6,10] However, whether the predictive value of measuring serum HCV RNA at W12 to assess SVR also holds true for re-treating patients who have failed a previous course of interferon-based therapy remains to be established.

In summary, our results show that undetectable HCV RNA at W12 of the post-treatment follow-up has a 100% PPV (95% CI 96.5%–100%) for SVR, suggesting that testing for HCV RNA at this point using a sensitive test with a detection limit of 15 IU/mL may be considered an appropriate timepoint for assessing virological response and identification of SVR and relapse in HIV/HCV co-infected patients treated with PEG-INF/RBV for chronic HCV. Studies are needed to confirm these results.

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Transparency declarations

A. R. has received consulting fees from Bristol-Myers Squibb, Abbott, Gilead, Roche and Boehringer Ingelheim. J. A. P. has received consulting fees from GlaxoSmithKline, Bristol-Myers Squibb, Abbott, Gilead, Merck Sharp & Dohme, Janssen-Cilag and Boehringer Ingelheim. They have received research support from GlaxoSmithKline, Roche, Bristol-Myers Squibb, Schering-Plough, Abbott and Boehringer Ingelheim and have received lecture fees from GlaxoSmithKline, Roche, Abbott, Bristol-Myers Squibb, Boehringer Ingelheim and Schering-Plough. The remaining authors have no conflicts of interest to declare.

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Association between the IL28B genotype and hepatitis C viral kinetics in the early days of treatment with pegylated interferon plus ribavirin in HIV/HCV co-infected patients with genotype 1 or 4

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Objectives: To evaluate the effect of the interleukin 28B (IL-28B) genotype on hepatitis C virus (HCV) viral kinetics in the first 4 weeks from start of treatment with pegylated interferon plus ribavirin (PEG-IFN/RBV) in HIV/HCV co-infected patients.

Methods: HIV/HCV co-infected patients naive to PEG-IFN/RBV treatment were enrolled in a prospective study. HCV RNA plasma viral loads were measured at baseline and at weeks 1, 2 and 4 after commencement of treatment. Patients were grouped by HCV genotype (genotype 1/4 versus 3) and by IL-28B genotype (CC versus non-CC). Differences in viral load reduction were evaluated by IL-28B genotype between baseline, week 1, week 2 and week 4.

Results: One hundred and nineteen HIV/HCV patients were included in the study. HCV patients with genotype 1/4 and bearing the IL-28 CC genotype showed the greatest reductions in HCV RNA plasma levels between baseline and weeks 1 (B-1), 2 (B-2) and 4 (B-4) than did those with non-CC genotypes (B-1: 1.06 ± 0.89 versus 0.48 ± 0.48 log IU/mL, $P=0.009$; B-2: 1.36 ± 0.72 versus 0.77 ± 0.66 log IU/mL, $P=0.01$; and B-4: 1.91 ± 0.64 versus 1.38 ± 0.96 log IU/mL, $P=0.03$). However, differences between weeks 1 and 2 (W1-2) and between weeks 2 and 4 (W2-4) were not associated with the IL-28B genotype (W1-2: CC 0.48 ± 0.42 versus non-CC 0.38 ± 0.38 log IU/mL, $P=0.62$; W2-4: CC 0.32 ± 0.23 versus non-CC 0.39 ± 0.31 log IU/mL, $P=0.67$). No differences in decline of HCV RNA viral load were found in HCV genotype 3 patients.

Conclusions: The IL-28B genotype impacts on viral kinetics during the first week of treatment with PEG-IFN/RBV in patients with HCV genotype 1/4.

Keywords: interleukin 28B, HIV, HCV, pharmacogenetics, viral kinetics

Introduction

The hepatitis C virus (HCV) is the cause of one of the most common blood-borne infections worldwide.[1] It is considered to be the leading cause of cirrhosis and liver cancer in Europe and the USA.[1] In HIV co-infected patients, the current standard of care is pegylated interferon (PEG-IFN) plus ribavirin (PEG-IFN/RBV), although rates of sustained virological response (SVR) vary significantly according to virus, disease and a host of other related factors.[1]

Several studies have provided information about the clinical applicability of early viral kinetics in predicting SVR. Rapid virological response (RVR), defined as an undetectable serum HCV RNA level at week 4 of treatment with PEG-IFN/RBV, is a reliable predictor of SVR.[2] At the same time, it has been demonstrated that polymorphisms near the interleukin 28B (IL-28B) gene on chromosome 19 predict SVR and RVR in both HCV mono-infected and HIV/HCV co-infected patients carrying genotype 1/4 and treated with PEG-IFN/RBV.[3,4]

Several studies suggest that the effect of the IL-28B genotype on HCV viral kinetics can be seen in the first few days from start of treatment.[4,5] However, there is limited information about the IL-28B effect after treatment has started, and no data about its effect on HIV/HCV co-infected patients.[4,5] The objective of this study was to evaluate the effect of the IL-28B genotype on HCV viral kinetics in the first few weeks after starting treatment with PEG-IFN/RBV in HIV/HCV co-infected patients.

Methods

Patients

Caucasian HIV-infected patients with chronic hepatitis C, naive to HCV treatment and receiving a PEG-IFN/RBV combination therapy, were included in this prospective study. The criteria used to determine hepatitis C therapy were in accordance with international guidelines. Host, clinical and virological characteristics were collected.

Treatment regimens

All individuals were treated with either PEG-IFN-α2a or PEG-IFN-α2b, at doses of 180 μg or 1.5 μg/kg per week, respectively, in combination with a weight-adjusted dose of oral ribavirin (1000 mg/day for <75 kg, 1200 mg/day for ≥75 kg).

Virological evaluation

Plasma HCV RNA load measurements were taken at baseline and at weeks 1, 2 and 4 using a quantitative PCR assay (Cobas TaqMan, Roche Diagnostic Systems Inc., Pleasanton, CA, USA) with a detection limit of 15 IU/mL.

Determination of the IL-28B genotype

The rs129679860 single-nucleotide polymorphism (SNP) was genotyped using a custom TAQMAN genotyping assay (Applied Biosystems) on DNA isolated from whole blood samples. The DNA was genotyped according to manufacturer's instructions on an MX3005 thermocycler using MXpro software (Stratagene). Researchers responsible for genotyping procedures were unaware of other patient data. The IL-28B genotype was defined as CC or non-CC (TT/CT).

Statistical analysis

Continuous variables are expressed as mean ± standard deviations or medians (Q1–Q3), and were analysed by Student's *t*-test or the Mann–Whitney *U*-test. Categorical variables are expressed as the number of cases (percentage). Frequencies were compared using the χ^2 test or Fisher's exact test. Significance was defined as a P value <0.05. The subpopulation of patients with HCV genotype 4 was evaluated together with the HCV genotype 1 subpopulation. Reductions in plasma HCV RNA were evaluated by the IL-28B genotype between baseline, week 1, week 2 and week 4. Patients who presented an undetectable HCV viral load at any timepoint during the study were excluded when calculating HCV RNA reduction at any later point. Patients were classified as slow or fast responders, according to whether the reduction of HCV RNA viral load was above or below the median. The decline in HCV RNA was calculated according to the stage of liver fibrosis, HCV baseline viral load and PEG-IFN type. The analysis was performed using the SPSS statistical software package, version 15.0 (SPSS).

Ethical aspects

The study was designed and performed according to the Helsinki Declaration and approved by the ethics committee of the Reina Sofia University Hospital, Cordoba, Spain. All patients provided written informed consent before participating in this study.

Results

One hundred and nineteen HIV/HCV co-infected patients were included in the study. The baseline characteristics of the patients are summarized in Table 1.

Table 1. General population characteristics

Characteristics	
N	119
Male, *n* (%)	97 (81.5)
Age (years), mean ± SD	41.2 ± 2,6
HAART, *n* (%)	113 (94.9)
CD4 (cells/mm^3), mean ± SD	558 ± 45
Transmission, *n* (%)	
IDU	117 (98.3)
heterosexual	2 (1.7)
Log HCV RNA baseline, mean ± SD	5.98 ± 0.2
PEG-IFN, *n* (%)	
2a	93 (78.2)
2b	26 (21.8)
HCV genotype, *n* (%)	
1	65 (54.7)
4	16 (13.4)
3	38 (31.9)
IL-28B genotype, *n* (%)	
CC	49 (41.1)
CT	22 (18.6)
TT	48 (40.3)
Liver fibrosis stage, *n* (%)	
F0-F2	51 (42.9)
F3-F4	68 (57.1)
Log HCV RNA IU/mL baseline by HCV genotype, mean ± SD	
genotype 1/4 HCV	6.13 ± 0.85
genotype 3 HCV	5.76 ± 0.79
Log HCV RNA IU/mL baseline by IL28B genotype, mean ± SD	
IL-28B CC	6.15 ± 0.83
IL-28B non-CC	5.9 ± 0.9

HAART, highly active antiretroviral treatment; IDU, intravenous drug user.

HCV genotype 1/4 patients carrying the IL-28 CC genotype showed a greater reduction in plasma HCV RNA levels between baseline and weeks 1 (B-1), 2 (B-2) and 4 (B-4) than did non-CC genotype carriers (B-1: 1.06 ± 0.89 versus 0.48 ± 0.48 log IU/mL, $P=0.009$; B-2: 1.36 ± 0.72 versus 0.77 ± 0.66 log IU/mL, $P=0.01$; and B-4 1.91 ± 0.64 versus 1.38 ± 0.96 log IU/mL, $P=0.03$) (Figure 1a). However, no differences in HCV RNA reduction were found by the IL-28B genotype between weeks 1 and 2 (CC 0.48 ± 0.42 versus non-CC 0.38 ± 0.38 log IU/mL, $P=0.62$) or between weeks 2 and 4 (CC 0.32 ± 0.23 versus non-CC 0.39 ± 0.31 log IU/mL, $P=0.67$) in HCV genotype 1/4 patients.

In HCV genotype 3 patients, there were no observable differences of viral load reduction found between B-1, B-2 or B-4, irrespective of whether the IL-28B genotype was CC or non-CC (B-1: 1.81 ± 1.06 versus 1.65 ± 0.93 log IU/mL, $P=0.64$; B-2: 2.73 ± 0.88 versus 3.15 ± 1.4 log IU/mL, $P=0.28$; and B-4: 3.75 ± 0.88 versus 4.04 ± 1.06 log IU/mL, $P=0.45$) (Figure 1b).

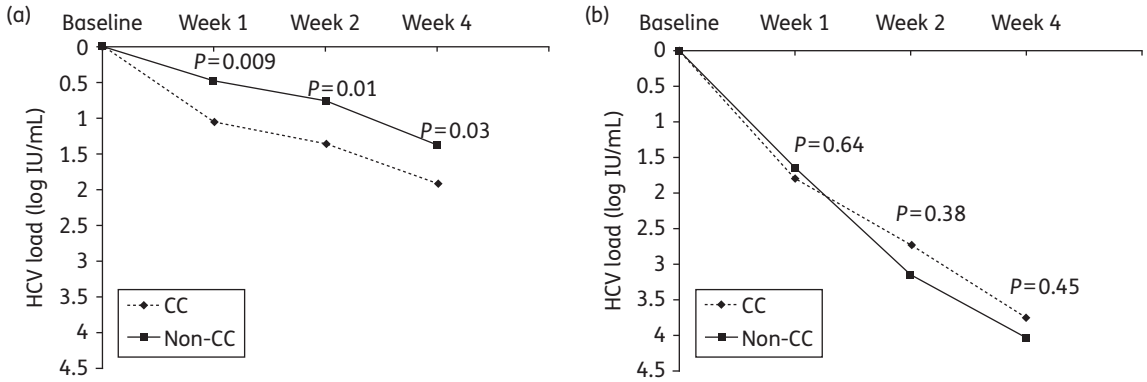

Figure 1. Mean reduction in viral load from baseline according to IL-28B genotype in HCV genotype 1/4 (a) and genotype 3 (b) patients.

Median HCV RNA reductions for HCV genotype 1/4 patients at B-1, B-2 and B-4 were 0.37 log IU/mL (0.11–0.44), 0.79 log IU/mL (0.38–0.91) and 1.28 log IU/mL (0.89–1.37), respectively. The IL-28B CC genotype was more common in patients who were fast responders than in slow responders at all timepoints analysed [B-1: 39/51 (76.4%) versus 10/58 (17.2%), $P<0.001$; B-2: 42/54 (77.7%) versus 7/55 (12.7%), $P<0.001$; and B-4: 42/54 (77.7%) versus 7/55 (12.7%), $P<0.001$].

A total of 37 (31.1%) patients attained RVR, and at significantly lower rates for genotype 1/4 than for genotype 3 [16/81 (19.7%) versus 21/38 (55.2%), $P=0.002$]. Among patients bearing genotype 1/4, the RVR rate was significantly higher for IL-28B CC genotypes than for non-CC [9/31 (29.03%) versus 7/50 (14%), $P=0.01$]. However, in patients bearing genotype 3, there were no observable differences in RVR rate between IL-28B CC and non-CC genotypes [10/18 (55%) versus 11/20 (55%), $P=0.985$].

No differences in HCV RNA reduction were found by liver fibrosis stage, baseline viral load or PEG-IFN type.

Discussion

In this study, the host IL-28B genotype was a pre-treatment predictor of HCV RNA kinetics in HIV/HCV co-infected patients carrying genotype 1/4 and who started therapy with PEG-IFN/RBV. The IL-28B CC genotype was associated with a greater decline in on-treatment viral load compared with patients who harboured the IL-28B non-CC type. This difference was observed as early as the first week from start of therapy and was maintained during the first 4 weeks of treatment.

As previously reported, a small but not clinically significant difference in median plasma HCV RNA load at baseline has been noted for the IL-28B genotype, with higher levels in CC patients.[6] It has been suggested that, as the product of the IL-28B gene is IFN-λ3, patients harbouring the IL-28B rs12979860 allele C might exhibit lower immune activity, permitting higher HCV replication.[6] Consequently, such patients might retain an enhanced susceptibility to exogenous PEG-IFN-based therapy.[6]

Viral decline during IFN therapy is biphasic. The first phase occurs during the first 72 h from start of therapy, with a slower second phase.[7] In one clinical trial, HCV viral load was measured at baseline and 24 h following a test dose of 9 MU IFN-α2a, and HCV RNA reduction after 24 h was used to randomly stratify patients carrying genotype 1.[5] The decline in plasma HCV RNA load after 24 h was much greater in IL-28B CC genotype than in non-CC genotype carriers.[5] Similar data were obtained for patients carrying genotype 4.[5] In another study, differences in HCV RNA load reduction between IL-28B CC, CT and TT genotypes were detectable at week 2, the earliest timepoint evaluated.[4] In our study we found differences in HCV RNA load reduction between IL-28B CC and non-CC genotypes from baseline at every timepoint analysed, although no difference in HCV RNA load reduction was found between week 1 and week 2, or week 2 and week 4. This is an important issue because it implies that the effect of the IL-28B genotype occurs during the first phase of viral decay, thought to be due to the process of free virion clearance and the inhibition of new viral production rather than the slower second phase, whose gradient is thought to mirror the process of infected cell clearance.

The fastest reductions in viral load correlated with increased rates of the appropriate on-treatment virological endpoint, such as RVR or complete early virological response.[5] At the same time, RVR has been demonstrated to be a critical predictor of SVR, independent of the host IL-28B genotype.[2] Our observations suggest that the major effect of this polymorphism is to increase the rate of early viral decline, leading to a higher RVR rate.

We found that the magnitude of viral decline for patients carrying genotype 1 or 4 was lower than for genotype 3 at all timepoints in the study. Differences in viral load reduction across the HCV genotypes were detectable as early as week 1, confirming that genotype is the most important factor for predicting an early response to antiviral therapy.[8]

Data concerning the relevance of the IL-28B genotype in patients carrying HCV genotype 3 are emerging. Mangia *et al.*[9] observed that genotype 2/3 patients with the IL-28B CC genotype who failed to achieve RVR were more likely to achieve SVR than their non-CC-type counterparts. In our study, the host IL-28B genotype in genotype 3 carriers was not found to have a significant impact on HCV viral kinetic response in the first few days of treatment. On-treatment viral kinetics provides a direct measurement of treatment response. The virological response to treatment for HCV genotype 3 is usually very strong and early, and this might limit the advantage conferred by a favourable IL-28B genotype.

A better-powered cohort is necessary to find statistically significant associations.

In summary, in HIV co-infected patients, SNP rs12979860 in the region of the IL-28B gene has an impact on early viral kinetics in response to PEG-IFN/RBV therapy for chronic hepatitis C caused by HCV genotypes 1 and 4. At present, as with viral genotype, information about IL-28B status is being used to inform patients about the likelihood of obtaining a response to PEG-IFN/RBV combination therapy. Whether determining viral kinetics during the first week from start of treatment will be a better predictor of response to HCV combination therapy, and whether this should be used for making decisions about treatment in such patients, remains to be studied.

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LDLr genotype modifies the impact of IL28B on HCV viral kinetics after the first weeks of treatment with PEG-IFN/RBV in HIV/HCV patients

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Objective: To evaluate the effect of low-density lipoprotein receptor (LDLr) and IL28B genotypes on hepatitis C virus (HCV) viral kinetics in the first 4 weeks of treatment with pegylated-interferon (PEG-IFN)/ribavirin (RBV) in HIV patients co-infected with HCV genotype 1.

Methods: HIV patients co-infected with HCV genotype 1 and naïve to PEG-IFN/RBV treatment were enrolled in a prospective study. HCV RNA viral loads were measured at baseline and at weeks 1, 2 and 4 after start of therapy. Differences in viral load decline were evaluated for IL28B (CC versus non-CC) and LDLr (CC versus non-CC) genotypes between baseline and weeks 1, 2 and 4. Additionally, the effect of LDLr genotype on HCV viral decline in IL28B CC genotype patients (CC/CC versus CC/non-CC) was analyzed.

Results: Eighty-seven HIV/HCV genotype 1 co-infected patients were included in the study. Patients carrying the LDLr-CC or IL28B-CC genotypes showed greater HCV viral decline than those with IL28B non-CC or LDLr non-CC genotypes at every time-point analyzed. CC/CC patients had higher rapid virological response (RVR) rates than CC/non-CC patients (41.2 versus 13.3%; $P < 0.001$). Moreover, at all time points, the CC/CC pattern was associated with greater HCV viral decline than the CC/non-CC genotype (week 1: 1.18 ± 0.51 versus 0.31 ± 0.29, $P = 0.041$; week 2: 1.55 ± 0.81 versus 0.93 ± 0.73, $P = 0.032$; week 4: 2.23 ± 1.1 versus 1.5 ± 0.94, $P = 0.039$).

Conclusion: The LDLr genotype impacts on viral kinetics during the first days of starting treatment with PEG-IFN/RBV in HIV/HCV genotype 1 co-infected patients, and modifies the impact of IL28B on HCV viral decay.

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Keywords: hepatitis C virus, HIV, IL28B, low-density lipoprotein-receptor, pegylated-interferon, ribavirin, viral kinetics

Introduction

The IL28B genotype is associated with greater viral decline in the hepatitis C virus (HCV) during the first weeks of treatment with pegylated-interferon (PEG-IFN) and ribavirin (PEG-IFN/RBV) in both HCV mono-infected and HIV/HCV co-infected patients bearing HCV genotype 1 [1–4]. As a result, a more rapid viral

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decline leads to a better response to treatment, with better rapid virological response (RVR) and sustained virological response (SVR) rates [5,6].

Hepatitis C virus may play a significant role in the serum lipid profile of patients with chronic HCV infection [7,8]. HCV viremia seems to be associated with lower serum cholesterol and triglyceride levels [9,10]. A high LDL cholesterol (LDL-C) level has been observed to be an independent predictive factor for response to current standards of care in HCV treatment, in both HCV-infected and HIV/HCV co-infected patients [11,12]. There is, however, no clear explanation for this.

Patients carrying the IL28B-CC genotype have been reported as having higher levels of LDL-C than those with the IL28B-non-CC genotype [5], suggesting that the beneficial effect of the IL28B genotype could be related to lipid metabolism, specifically LDL-C metabolism. In this respect, our group has reported that variations in the low-density lipoprotein receptor (LDLr) gene are associated with higher SVR rates in HIV/HCV genotype 1/4 co-infected patients and significantly increase the favorable impact of the IL28B CC genotype on SVR [13].

Here we analyze the effect of LDLr, and its association with the IL28B genotype, on HCV viral decline during the first weeks of treatment with PEG-IFN/RBV in HIV/HCV co-infected patients bearing genotype 1.

Methods

Patients

Caucasian HIV-infected patients with chronic hepatitis C, naive to HCV treatment and receiving a PEG-IFN/RBV combination therapy, were included in this prospective study. The criteria used to determine hepatitis C therapy followed international guidelines [14]. Host, clinical and virological characteristics were collected. Fibrosis stage was determined by biopsy or liver transient elastography (FibroScan, Echosen, Paris). Significant fibrosis was defined as a METAVIR fibrosis score of F3-F4 in liver biopsy or a liver stiffness value of at least 8.9 kPa.

Treatment regimens

All individuals were treated with either PEG-IFN α2a or PEG-IFN α2b, at doses of 180 μg or 1.5 μg/kg per week, respectively, in combination with a weight-adjusted dose of oral ribavirin (1000 mg/day for $<$75 kg, 1200 mg/day for $\geq$75 kg), in accordance with international guidelines [14].

Virological evaluation

Plasma HCV RNA load measurements were conducted at baseline and at weeks 1, 2 and 4, using a quantitative PCR assay (Cobas TaqMan; Roche Diagnostic Systems Inc., Pleasanton, California, USA), with detection limit of 15 IU/ml.

Single-nucleotide polymorphism genotyping

DNA was extracted using the automated MagNA Pure DNA extraction method (Roche Diagnostics Corporation, Indianapolis, Indiana, USA). Single-nucleotide polymorphisms (SNPs) rs14158 in the 3′UTR of the LDLR gene, and rs129679860, located 3 kilobases upstream of the IL28B, and in strong linkage disequilibrium with a nonsynonymous coding variant in the IL28B gene (213A $>$ G, K70R; rs81031142) (0), were genotyped. Genotyping was carried out using a custom TAQMAN assay (Applied Biosystems, Foster City, California, USA) on DNA isolated from whole blood samples, using a Stratagene MX3005 thermocycler with MXpro software (Stratagene, La Jolla, California, USA), according to manufacturer's instructions. The researchers responsible for genotyping were blinded to other patient data. The IL28B and LDLr genotypes were defined as CC or non-CC (TT/CT).

Statistical analysis

Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median (Q1–Q3) and were analyzed by Student's *t*-test, Mann–Whitney *U*-test or Kruskal–Wallis test. Categorical variables were expressed as number of cases (percentage). Frequencies were compared using the χ^2 test or Fisher's exact test. Significance was defined as a *P* value of less than 0.05. Plasma HCV RNA levels were analyzed by LDLr and IL28B genotypes between baseline and week 1, week 2 and week 4. Additionally, we analyzed the effect of the LDLr genotype on HCV viral decline in IL28B-CC patients (CC/CC versus CC/non-CC) and IL28B non-CC patients (non-CC/CC versus non-CC/non-CC).

Patients were classified as slow or fast responders according to whether HCV RNA viral load reduction was above or below the median HCV RNA load at the corresponding time-point. The relation between RNA HCV decline and liver fibrosis stage, baseline HCV viral load and PEG-IFN type was evaluated. The analysis was performed using the SPSS statistical software package, version 18.0 (IBM Corporation, Somers, New York, USA).

Ethical aspects

The study was designed and performed according to the Helsinki Declaration and approved by the ethics committee of the Reina Sofía University Hospital, Cordoba, Spain. All patients provided written informed consent before participating in this study.

Results

Baseline characteristics and IL28B and low-density lipoprotein receptor distributions

Eighty-seven HIV/HCV genotype 1 co-infected patients were included in this prospective study. Baseline patient characteristics, stratified according to IL28B and LDLr

Table 1. Baseline population characteristics stratified according to IL28B and LDLr genotypes.

Characteristics	IL28B CC/LDLR CC	IL28B CC/LDLR non-CC	IL28B non-CC/LDLR CC	IL28B non-CC/LDLR non-CC	*P*
N	17	15	32	23	
Age (years), mean ± SD	41.6 ± 3.2	40.8 ± 3.9	41.1 ± 4.3	42.7 ± 4.2	0.73
Male, *n* (%)	12 (70.5)	10 (66.6)	24 (75)	16 (69.5)	0.847
CD4 cell count (cells/μl), mean ± SD	549 ± 249	527 ± 298	541 ± 241	532 ± 284	0.81
Prior AIDS diagnosis, *n* (%)	6 (35.3)	2 (13.3)	6 18.7)	7 (30.4)	0.21
Undetectable HIV viral load, *n* (%)	16 (94.1)	14 (93.3)	30 (93.7)	20 (86.9)	0.87
Significant liver fibrosis, *n* (%)	12 (70.5)	12 (80)	24(75)	17 (73.9)	0.46
Liver cirrhosis, *n* (%)	5 (29.4)	5 (33.3)	8 (25)	7 (30.4)	0.61
PEG-IFN α2a, *n* (%)	11 (64.7)	13 (86.6)	22 (68.7)	16 (69.5)	0.76
Use of HAART, *n* (%)	17 (100)	14 (93.3)	30 (93.7)	21 (91.3)	0.94

HAART, highly active antiretroviral treatment; PEG-INF, pegylated interferon; SD, standard deviation.

genotypes, are shown in Table 1. The IL28B genotype distribution was: CC in 32 (36.8%) patients, CT in 46 (52.8%) patients and TT in nine (10.4%) patients. The distribution of LDLr was: CC in 52 (59.8%) patients, CT in 34 (39.1%) patients and TT in 1 (1.1%) patient. Among IL28B-CC patients, 17 (53.1%) carried the LDLr-CC genotype and 15 (46.9%) the LDLr non-CC genotype. Among IL28B non-CC patients, 32 (58.2%) carried the LDLr-CC and 23 (41.8%) the LDLr non-CC genotypes.

The IL28B-CC genotype was associated with a higher baseline HCV viral load than the non-CC genotype (6.17 ± 0.77 versus 5.89 ± 0.84; $P = 0.032$). In our study, the LDLr-CC genotype was not associated with lower baseline HCV viral loads (6.13 ± 0.919 versus 6.2 ± 0.73; $P = 0.349$).

Rapid virological response rate by IL28B and low-density lipoprotein receptor genotypes

Fourteen (16.1%) patients achieved RVR. Patients with the IL28B-CC genotype presented a higher RVR rate [9/32 (28.1%) versus 5/55 (9.1%); $P = 0.023$]. The LDLr-CC genotype, on the contrary, was not associated with achieving RVR [10/52 (19.2%) versus 4/35 (11.4%); $P = 0.265$]. The analysis of RVR rates according to IL28B/LDLr genotypes showed that CC/CC patients had higher RVR rates [CC/CC: 7/17 (41.2%); CC/non-CC: 2/15 (13.3%); non-CC/CC: 4/32 (12.5%); non-CC/non-CC: 1/23 (4.3%); $P < 0.001$]. No differences in RVR rate ($P = 0.423$) were found between non-CC/CC, CC/non-CC and non-CC/non-CC patients.

Rapid responders rate

Median (Q1-Q3) HCV viral decline from baseline to week 1, week 2 and week 4 was 0.37 (0.12–0.94), 0.75 (0.32–1.57) and 1.25 (0.75–2.51) log IU/ml, respectively. The LDLr-CC genotype was more frequently found in rapid responders than the non-CC genotype at week 1 [23/52 (44.2%) versus 7/35 (14.3); $P = 0.014$], week 2 [25/52 (48.1%) versus 9/35 (25.7%); $P = 0.028$] and week 4 [27/52 (51.9%) versus 9/35 (25.7%); $P = 0.015$]. Similarly, rapid responders were more common among patients with the IL28B-CC rather than the non-CC genotype, at week 1 [16/32 (50%) versus 13/55 (23.6%); $P = 0.01$], week 2 [19/32 (59.4%) versus 16/55 (29%); $P = 0.004$] and week 4 [20/32 (62.5%) versus 16/55 (29%); $P = 0.002$].

The IL28B/LDLr distribution of rapid responders at every time-point analyzed is shown in Table 2. The CC/CC genotype had higher rates of rapid responders than CC/non-CC, non-CC/CC and non-CC/non-CC genotypes at week 1 ($P = 0.003$), week 2 ($P = 0.001$) and week 4 ($P = 0.001$), and there were no differences in the rate of rapid responders between CC/non-CC, non-CC/CC and non-CC/non-CC patients at every time-point analyzed (week 1, $P = 0.49$; week 2, $P = 0.32$; week 4, $P = 0.32$).

Hepatitis C virus viral decline

There were 16 (18.4%) patients whose baseline HCV viral load was more than 600 000 IU/ml, and the degree of HCV viral decline in these patients was less, although

Table 2. Relation between IL28B/LDLr genotypes and rapid responders' rate (compared by χ^2 test).

	Rapid responders					
Genotype	Week 1	*P*	Week 2	*P*	Week 4	*P*
CC/CC	12/17 (70.6%)	0.002	14/17 (82.3%)	0.001	14/17 (82.3%)	0.001
CC/non-CC	3/15 (20%)		4/15 (26.3%)		4/15 (26.3%)	
Non-CC/CC	7/29 (24.1%)		9/32 (28.1%)		9/32 (28.1%)	
Non-CC/non-CC	4/20 (20%)		5/23 (21.7%)		5/23 (21.7%)	

not statistically significant, than in those with a low HCV baseline viral load at week 1 (0.56 ± 0.79 versus 0.59 ± 0.39; $P = 0.871$), week 2 (0.86 ± 0.64 versus 1.1 ± 0.87; $P = 0.529$) and week 4 (1.32 ± 1.05 versus 1.68 ± 1.11; $P = 0.233$). Likewise, there were no differences in HCV viral decline between patients without or with significant liver fibrosis (week 1: 0.61 ± 0.81 versus 0.59 ± 0.58, $P = 0.877$; week 2: 1.16 ± 0.89 versus 0.92 ± 0.99, $P = 0.305$; week 4: 1.87 ± 1.24 versus 1.56 ± 1.25, $P = 0.318$) or between PEG-IFN α2a and PEG-IFN α2b (week 1: 0.67 ± 0.8 versus 0.63 ± 0.68, $P = 0.577$; week 2: 1.1 ± 0.97 versus 0.89 ± 0.76, $P = 0.118$; week 4: 1.96 ± 1.25 versus 1.81 ± 1.01, $P = 0.175$).

Patients carrying the IL28B-CC genotype showed greater HCV viral decline from baseline than those with the IL28B non-CC genotype at week 1 (0.88 ± 0.6 versus 0.35 ± 0.54; $P = 0.006$), week 2 (1.37 ± 0.87 versus 0.54 ± 0.62; $P = 0.017$) and week 4 (2.1 ± 0.75 versus 1.14 ± 0.91; $P = 0.026$). However, we did not find any differences of HCV viral decline among IL28B genotypes between weeks 1 and 2 (CC 0.45 ± 0.4 versus non-CC 0.33 ± 0.37; $P = 0.295$) or weeks 2 and 4 (CC 0.69 ± 0.61 versus non-CC 0.58 ± 0.68; $P = 0.571$).

Patients carrying the LDLr-CC genotype showed greater HCV viral decline than those with the non-CC genotype at week 1 (0.73 ± 0.836 versus 0.37 ± 0.54 log IU/ml; $P = 0.042$), week 2 (1.16 ± 0.97 versus 0.68 ± 0.81; $P = 0.027$) and week 4 (1.81 ± 1.27 versus 1.3 ± 1.14; $P = 0.06$) (Fig. 1). There was no association between LDLr genotypes and HCV viral decline between weeks 1 and 2 (CC 0.38 ± 0.39 versus non-CC 0.35 ± 0.38; $P = 0.796$) or weeks 2 and 4 (CC 0.64 ± 0.7 versus non-CC 0.57 ± 0.59; $P = 0.743$).

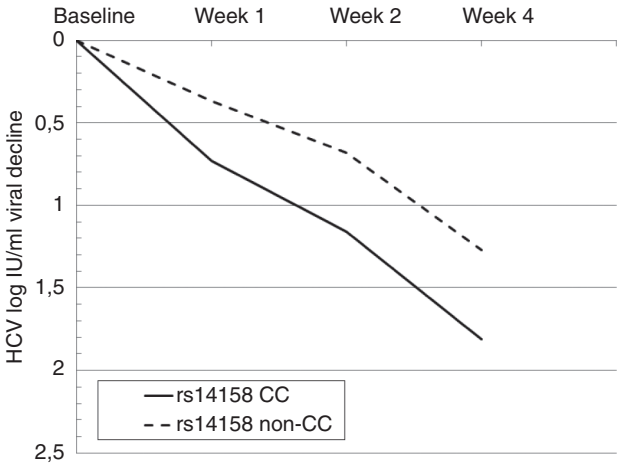

Fig. 1. HCV viral decline between the LDLr genotype (rs14158) during the first weeks of treatment with PEG-IFN/RBV (compared using Student's *t* test).

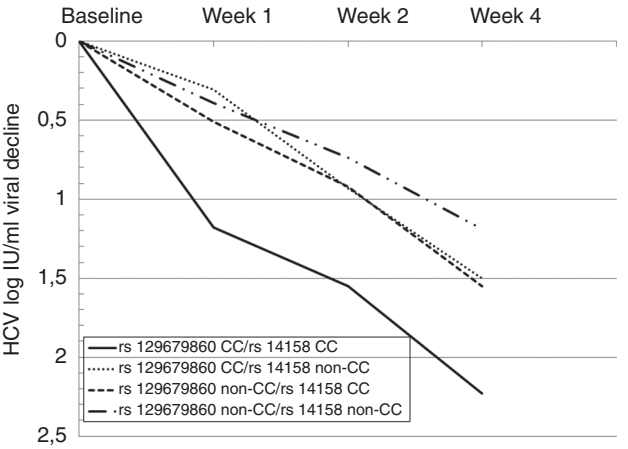

Fig. 2. HCV viral decline between IL28B and LDLr genotypes (rs12967860/rs14158) during the first weeks of treatment (compared using the Kruskal–Wallis test).

Patients carrying the CC/CC genotype showed greater viral decline at week 1 (CC: 1.18 ± 0.51; CC/non-CC: 0.31 ± 0.29; non-CC/CC: 0.51 ± 0.53; non-CC/non-CC: 0.39 ± 0.41, $P = 0.041$), week 2 (CC: 1.55 ± 0.81; CC/non-CC: 0.93 ± 0.73; non-CC/CC: 0.94 ± 0.69; non-CC/non-CC: 0.74 ± 0.56, $P = 0.032$) and week 4 (CC: 2.23 ± 1.1; CC/non-CC: 1.5 ± 0.94; non-CC/CC: 1.57 ± 1.12; non-CC/non-CC: 1.19 ± 0.92, $P = 0.039$) (Fig. 2). No differences in HCV RNA viral decline were found between CC/non-CC, non-CC/CC and non-CC/non-CC patients at week 1 ($P = 0.42$), week 2 ($P = 0.317$) or week 4 ($P = 0.18$). No differences in HCV viral decline were observed by IL28B/LDLr genotypes between weeks 1 and 2 (CC/CC 0.46 ± 0.38; CC/non-CC 0.4 ± 0.37; non-CC/CC 0.36 ± 0.29; non-CC/non-CC 0.29 ± 0.33 $P = 0.55$) or weeks 2 and 4 (CC/CC 0.52 ± 0.39; CC/non-CC 0.47 ± 0.58; non-CC/CC 0.42 ± 0.34; non-CC/non-CC 0.39 ± 0.45 $P = 0.69$).

Discussion

The study found that the LDLr genotype was a pretreatment predictor of HCV kinetics in HIV/HCV co-infected patients bearing genotype 1 starting therapy with PEG-IFN/RBV, and that it modified the effect of the IL28B genotype on HCV viral decline.

Low-density lipoprotein receptor seems to play an important role in HCV cell entry [15]. Recent data show that HCV entry is a complex multistep process involving the presence of several factors [16,17]. HCV enters the hepatocyte in the form of HCV lipo-viral particles (HCV-LVP), using cell surface LDLr, glycosaminoglycans, such as heparan sulfate, and scavenger receptors class B type I (SR-BI) as initial attachment

factors [15–17]. LDLr and SR-BI recognize the LDL and HDL, respectively, of the LVP [16,17]. The virus attached to the hepatocyte subsequently interacts with cellular protein to provide the final key to HCV entry [18]. In this way, variations in the LDLr gene determine HCV attachment and entry.

It has been observed that patients carrying the LDLr-CC genotype have lower HCV viral loads than non-CC patients [13], so that the beneficial impact of LDLr gene variations on treatment response could be the reduction in baseline HCV viral load, an independent predictor of treatment response [19]. Our study did not find this relation, although results showed that the LDLr-CC genotype was associated with a greater decline in on-treatment viral load when compared with patients harboring LDLr non-CC. This difference was observed as early as the first week from start of therapy and was maintained during the first 4 weeks of treatment. We also found differences in reduction of HCV RNA load from baseline between LDLr-CC and non-CC genotypes at every time-point analyzed, although none between week 1 and week 2, or between week 2 and week 4. This is an important issue because it suggests that the beneficial effect of the LDLr genotype is due, at least in part, to its initial effect on viral kinetics and that it occurs during the first phase of viral decay.

At the same time, our study confirms the beneficial impact of IL28B on HCV viral decline during the first week of treatment with PEG-IFN/RBV. Our results match those for HCV monoinfected patients, as well as those previously demonstrated by our group for HCV/HIV co-infected patients [1–3]. Our findings suggest that the IL28B genotype has an impact on HCV/HIV co-infected patients only during the first phase of HCV viral kinetics, although it has recently been reported that the IL28B genotype was associated with greater viral decline in HCV monoinfected patients during the first and second phases of HCV viral kinetics [4]. Studies using a more powerful cohort of HIV/HCV co-infected patients are needed to clarify this point. The exact mechanism by which IL28B exerts an influence on early HCV viral kinetics is unclear. One suggestion is that viral infection induces the synthesis of this cytokine with antiviral activity. Like IFN-a, IFN-l stimulates the Janus kinase/signal transducers and activators of transcription pathway that prompts the expression of interferon-stimulated genes (ISGs), which results in antiviral activity against HCV [20]. Abnormal expression of the IL28B (IFN-λ3) gene could, in theory, enhance or reduce antiviral activity [21]. However, ISGs (associated with a lower response to INF-α) are strongly linked to IL28B alleles [21], so that patients with reduced ISG expression would have lower endogenous interferon activity but a correspondingly better response to exogenous interferon [21,22]. Our study was consistent with this hypothetical mechanism; patients with the IL28B-CC genotype had higher baseline HCV viral loads than non-CC patients (lower endogenous IFN-λ3 activity), and greater HCV viral decline in the first weeks of starting treatment than non-CC patients (providing the best response to exogenous IFN- α).

A previous study carried out by our group revealed that IL28B and LDLr genotypes had a synergistic effect on SVR rates in HIV/HCV genotype 1/4 co-infected patients [13]. The results of the present study show that the LDLr genotype modifies the influence of the IL28B genotype on HCV viral kinetics in response to PEG-IFN/RBV treatment. This enables us to hypothesize that the potential synergistic effect between LDLr and IL28B genotypes could be due to enhanced HCV RNA viral decline, leading to a higher RVR rate [13]. In our study, we found differences of HCV RNA load reduction between IL28B-CC /LDLr-CC and IL28B-CC /LDLr non-CC genotypes from baseline at every time-point analyzed, but not between weeks 1 and 2, nor between weeks 2 and 4. This is an important issue because it implies that the synergistic effect of the IL28B/LDLr genotype occurs during the first phase of viral decay, thought to be due to the clearance of free virions and the inhibition of new viral production, rather than in the slower second phase, the gradient of which is thought to mirror the process of infected cell clearance.

In our study, patients carrying the IL28B-CC but not the LDLr-CC genotype showed similar declines in HCV viral load to the IL28B non-CC genotype at every time-point analyzed (Fig. 2). This is significant because it could suggest that the increased early viral decline rate associated with IL28B-CC is only be demonstrated in the presence of an LDLr genotype. If this was confirmed, it would have considerable repercussions on clinical practice, since a determination of IL28B on its own without the LDLr genotype would have limited power for clinical decision-making.

Our study has several limitations. Firstly, the number of patients included was small, and did not have the statistical power to detect differences among CC/non-CC and IL28B non-CC patients. A powerful cohort of HIV/HCV genotype 1 co-infected patients is needed to analyze the synergistic effect of IL28B and LDLr genotypes. Secondly, our study looked at the impact of LDLr and IL28B by determining only SNPs rs14158 and rs12979860, respectively. Studies analyzing the synergistic effect of other known LDL SNPs (rs3826810, rs2738464, rs2738465, rs1423099, rs2738466) and IL28B (rs8099917) are needed. A third limitation is that this study included only HIV/HCV co-infected patients, which represents a unique population. HIV/HCV co-infected patients, in fact, attain SVR less frequently than HCV mono-infected individuals and their HCV viral decline is slower [23,24]. Hence, further studies on the predictive yield of LDLr gene variations are needed that include HCV monoinfected

patients. However, it is not expected that rs14158 behavior and its synergistic effect with rs12979860 will differ in the HCV monoinfected subset.

In conclusion, our results show a positive association between LDLr (rs14158) and IL28B (rs12979860) on HCV viral decline during the first week after start of treatment with PEG-IFN/RBV in HIV-infected patients carrying HCV genotype 1. Currently, information about their IL28B status is being used to inform patients about the likelihood of obtaining a response from PEG-IFN/RBV combination therapy. Whether the additional determination of LDLr status will be a better predictor of response to HCV combination therapy, and whether it should be used for making decisions about treatment in these patients, remains to be studied.

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Conflicts of interest

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The IL28B effect on HCV kinetics of among HIV patients after the first weeks of Peg-IFN/RBV treatment varies according to HCV-1 subtype

Running head: IL28B impact on HCV-1 subtypes

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The IL28B effect on HCV kinetics of among HIV patients after the first weeks of Peg-IFN/RBV treatment varies according to HCV-1 subtype

Introduction

Human immunodeficiency virus (HIV) and Hepatitis C virus (HCV) co-infection is a worldwide health problem [1]. Until recently, the standard of care for HCV genotype 1 treatment in HIV/HCV patients was a combination of pegylated-interferon (Peg-IFN) plus ribavirin (Peg-IFN/RBV). This combination leads to a sustained virological response (SVR) rate of 14–38% [2-5]. This rate is considerably lower than that obtained in HCV monoinfected populations [6]. Due to this, and to the more rapid progression to HCV liver disease and HIV infection [7], a more effective HCV-genotype 1 treatment is a priority among HIV/HCV co-infected patients. In this respect, the incorporation of a new family of drugs acting directly on the HCV has caused the SVR rate to soar in this population [8, 9]. However, there are several limitations to the use of these drugs, which could hinder its application in this subset of patients. These limitations include drug-drug interactions between HCV and antiretroviral drugs, a higher rate of adverse events, higher costs and limited experience with the use of the drugs [10]. On the other hand, the results of clinical trials performed with HCV-1 monoinfected patients have shown that there are some patients who do not benefit, in terms of response, from the addition of an HCV protease inhibitor to Peg-IFN/RBV [11, 12]. For these reasons, a key aspect, particularly among HIV/HCV co-infected patients, is the selection of the most appropriate treatment regimen, based on cost, safety and, mainly, prediction of response.

Several factors have been identified as predictive of treatment response to the Peg-IFN/RBV combination in HIV/HCV co-infected patients [13]. In recent years, the host's genetic factors have played an important role in this [14, 15]. The variations of the IL28B genotype is considered to be the most important baseline factor for treatment response among HIV/HCV co-infected patients, so that patients with the IL28B-CC genotype (SNP rs12979860) achieved SVR significantly more frequently than those bearing the unfavorable genotypes (CT or TT) [15]. This improved SVR rate is due to the higher HCV viral decline during the first weeks of starting treatment with Peg-IFN/RBV [16]. Even though the precise action mechanism of the IL28B genotype has not been clarified, it has been reported that it might be related to endogenous IFN production [17]. However, the effect of IL28B genotype on treatment response could be influenced by several factors [18, 19].

Among patients infected by genotype 1, it appears that those with the HCV-1b genotype have higher response rates than those infected by the HCV-1a genotype [20]. The reason for this is unknown, although it could be related to the differences of IFN sensitivity between the two subtypes [21]. As a result of this, it is thought that the possible differences in IFN sensitivity could be influenced by the IL28B genotype. Thus, the aim of our study is to consider the influence of IL28B on treatment response of HIV/HCV co-infected patients infected with the HCV-1a or HCV-1b genotypes, as reflected in HCV viral decline during the first weeks of treatment.

Methods

Patients included

Caucasian HIV-infected patients with chronic hepatitis C and naïve to HCV Peg-IFN/RBV combination therapy were included. Patients who were positive for the

hepatitis B surface antigen (HBsAg) were excluded. Variables related to the patient were collected (age [years], body mass index [Kg/m^2] and gender), as well as clinical variables related to the liver (fasting LDL cholesterol [mg/dL], ALT [IU/L] and liver fibrosis staging); HIV characteristics (use of HAART, HIV viral load [IU/mL], AIDS clinical condition, baseline CD4 cell count [cells/mL] and risky practices for HIV infection); and HCV variables (HCV genotype 1 subtype, HCV viral load [IU/mL] and IL28B genotype). Fibrosis stage was determined by liver biopsy, liver fibrosis staging on the basis of the METAVIR fibrosis score. Liver transient elastography (FibroScan®, Echosens, Paris) was used to measure the liver stiffness values of those patients who had not undergone a liver biopsy. In such cases, a liver stiffness value ≥14.6 kPa was defined as indicating liver cirrhosis [22].

Treatment Regimens

All individuals were treated with Peg-IFN-alpha-2a, at doses of 180 μg per week, in combination with a weight-adjusted dose of oral ribavirin (1000 mg/day for <75 kg and 1200 mg/day for ≥75 kg, respectively) for 48 or 72 weeks, following international guidelines [23].

Virological evaluation and response

Plasma HCV RNA loads were measured at baseline and at weeks 1, 2 and 4 using a quantitative PCR assay (CobasTaqMan, Roche Diagnostic Systems Inc., Pleasanton, CA, USA), with a detection limit of 15 IU/mL. The HCV genotype was determined using a hybridization assay (INNO-LiPa HCV, Bayer Corp., Tarrytown, NY, USA) and HCV subtypes were defined as 1a or 1b. Rapid virological response (RVR) was defined as an undetectable viral load at week 4 after starting treatment.

Single Nucleotide Polymorphism (SNP) genotyping

DNA was extracted using the automated MagNA Pure DNA extraction method (Roche Diagnostics Corporation. Indianapolis, IN 46250, USA). SNP rs129679860, located 3 kilobases upstream of the IL28B, and in strong linkage disequilibrium with a non-synonymous coding variant in the IL28B gene (213A>G, K70R; rs81031142), was genotyped. Genotyping was carried out using a custom TAQMAN assay (Applied Biosystems, Foster City, California, USA) on DNA isolated from whole blood samples, using a Stratagene MX3005 thermocycler with MXpro software (Stratagene, La Jolla, California, USA), according to manufacturer's instructions. The researchers responsible for genotyping were blinded to other patient data. The IL28B genotype was defined as CC or non-CC (TT/CT).

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation and were analyzed by the Student's *t* test, Mann-Whitney *U*-test or Kruskal-Wallis test. Categorical variables were expressed as number of cases (percentage). Frequencies were compared using the χ^2 test or Fisher's exact test. Significance was defined as a *p* value of less than 0.05. Plasma HCV-RNA kinetic throughout the first 4 weeks of therapy was analyzed according to HCV-1 subtype (1a *versus* 1b). We also analyzed the effect of the IL28B genotype on HCV viral decline in HCV-1a and HCV-1b genotype patients (CC *versus* non-CC). Three linear regression models were performed to identify independent predictors of HCV viral decline during the first weeks of treatment. The first model developed included the total population, and HCV-1 subtype (1a or 1b) was entered into the model as a variable; the second model developed included only patients infected by the HCV-1a genotype; and the third model used patients infected by the HCV-1b genotype. The coefficient (B) of the models was shown as an adjusted coefficient. The Durbin-Watson statistic was used to detect the presence of autocorrelation in variables

included in the models. The analysis was performed using the SPSS statistical software package, version 18.0 (IBM Corporation, Somers, NY, USA).

Ethical aspects

The study was designed and performed according to the Helsinki Declaration and approved by the ethics committee of the Reina Sofía University Hospital, Cordoba, Spain.

Results

Patients included

Two hundred and six patients in follow-up at two reference hospitals in Spain were included in the study. Of these, 113 (54.8%) and 93 (45.2%) were infected with the HCV-1a and 1b genotypes, respectively. The baseline characteristics of population are shown in Table 1. Thirty-two (15.5%) patients achieved RVR.

Viral decline according to HCV-1 subtypes

Patients infected with the HCV-1b genotype had higher, although not statistically significant, HCV viral declines than those infected by HCV-1a, at week 1 (1b: 0.77 ± 0.38; 1a: 0.51 ± 0.41, p = 0.14), week 2 (1b: 1.12 ± 0.44; 1a: 0.96 ± 0.58, p = 0.358) and week 4 (1b: 1.89 ± 0.46; 1a: 1.66 ± 0.4, p = 0.279) (Figure 1).

The linear regression models of HCV viral decline at the different time points analyzed identified only baseline HCV viral load and IL28B genotype as independent predictive factors (Table 2). This analysis did not identify the HCV-1b genotype predictive of higher HCV viral decline compared to the HCV-1a genotype.

Impact of the IL28B genotype by the two HCV-1 subtypes

The IL28B-CC genotype had a weak impact on HCV viral decline among patients infected with the HCV-1a genotype, at week 1 (CC: 0.46 ± 0.34; non-CC: 0.51 ± 0.38, p = 0.804), week 2 (CC: 1.04 ± 0.58; non-CC: 0.88 ± 0.51, p = 0.52) and week 4 (CC: 1.73 ± 0.35; non-CC: 1.58 ± 0.41, p = 0.63) (Figure 2A). However, among patients infected with HCV-1b, those with IL28B-CC showed greater reductions in HCV viral load than those with the IL28B non-CC genotype, at weeks 1 (CC: 1.53 ± 0.33; non-CC: 0.27 ± 0.24, p < 0.001), 2 (CC: 1.81 ± 0.39; non-CC: 0.74 ± 0.39, p = 0.002) and 4 (CC: 2.97 ± 0.53; non-CC: 1.2 ± 0.61, p < 0.001) (Figure 2B). Furthermore, when HCV viral decline among patients carrying the IL28B-CC genotype was compared on the basis of HCV-1 subtype, those infected with HCV-1b showed greater reductions than those with the HCV-1a genotype, at every time point analyzed (week 1: p = 0.003; week 2: p = 0.026; week 4: p < 0.001).

The two linear regression models of the different subsets of patients (HCV-1a and HCV-1b genotypes) identified the IL28B genotype as an independent predictor of HCV viral decline only among patients infected with the HCV-1b genotype, at every time point analyzed (Table 2).

RVR rate

Figure 3 shows RVR rates. HCV-1a and HCV-1b subtypes had similar rates of RVR and there were no differences of RVR rate found among patients infected by HCV-1a, on the basis of IL28B genotype. However, for HCV-1b, patients bearing IL28B-CC had higher RVR rates than IL28B non-CC patients.

Discussion

In our study, we found no differences of HCV viral decline during the first weeks of treatment or of RVR rate between HCV genotypes 1a and 1b in HIV co-infected

patients naïve to Peg-IFN/RBV treatment. At the same time, the positive effect on viral clearance that is associated with IL28B-CC was observed in HCV-1b infected patients, but not those with the HCV-1a subtype. The higher response rate to treatment of HCV-1b over HCV-1a has been reported in several studies [20, 24], although the reason for it has not been clearly explained. On the basis of our results, the better rate of response associated with the HCV-1b genotype could be due to the IL28B-CC genotype exerting a stronger effect on HCV viral clearance than it does among HCV-1a patients. The reason for this is unknown. It has been reported that mutations in HCV proteins (both structural and nonstructural) have a modulatory impact on treatment response [25, 26]. The mechanism could be associated with the sensitivity of the HCV virus to IFN [27, 28]. So, patients bearing HCV strains with mutations at core amino acid 70R showed higher SVR rates than those with mutations at 70Q [29, 30]. In the same way, various mutations of the HCV NS5A protein are able to enhance or decrease HCV sensitivity to IFN, so affecting the response to treatment [27]. The HCV-1b genotype tends to have more "favourable" mutations associated with IFN sensitivity, so that the better Peg-IFN-alpha-2a -based response to therapy among HCV-1b patients could be the result of this mechanism [21]. The different variations of the IL28B genotype have been related to higher (IL28B non-CC genotype) or lower (IL28B-CC genotype) endogenous IFN-lambda activity, so that it would be expected for lower endogenous IFN-lambda activity to trigger a higher antiviral response when exogenous Peg-IFN-alpha-2a was added [31]. For this reason, patients with a more IFN-sensitive virus and the IL28B-CC genotype should experience higher viral decline when exogenous Peg-IFN-alpha-2a is added. Other studies have reported a similar association between HCV viral protein mutations and the IL28B genotype [32-34]. However, we did not carry out assays to investigate IFN sensitivity mutations of the HCV genome in our patients. It would be

extremely useful to clarify whether the different impact of HCV-1 subtypes of the IL28B genotype in HIV co-infected patients correlates with other HCV amino acid mutations.

We currently have direct-acting antiviral drugs available for HCV which increase the SVR of both HCV-1 infected and HIV co-infected patients [8, 9]; although even as the SVR increases, so does the rate of adverse events; furthermore, even though the therapeutic arsenal continues to expand, Peg-IFN will remain a part of HCV treatment in the coming years. In our study, 33.3% of patients infected with the HCV-1b genotype and carrying ILB28-CC achieved RVR, the best predictor of SVR. This suggests that this subset of patients could be considered as potential responders to Peg-IFN-alpha-2a/RBV therapy. This observation could help clinicians decide on the proper treatment regimen for each patient, chiefly on the basis of its clinical benefit.

In conclusion, in our study, the IL28B-CC genotype had a positive effect on HCV viral clearance during the first weeks of treatment with Peg-IFN-alpha-2a/RBV and on RVR rates in HCV-1b genotype HIV co-infected patients, but not those with HCV-1a. Both clinical and experimental studies are needed to clarify the possible association between IL28B and the HCV-1b subtype.

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Table 1 Baseline characteristics of population.

Characteristics	Total	HCV-1a	HCV-1b	*p**
N	206	113	93	
Male. No. (%)	177 (85.9)	93 (82.3)	80 (86.02)	0.509
Age (Years). Mean ± SD	42.1 ± 5.6	41.6 ±5.2	42.6 ± 6.2	0.223
BMI (Kg/m^2). Mean ± SD	23.9 ± 3.97	24.3 ± 3.6	23.6 ± 4.3	0.258
AIDS clinical condition. No. (%)[†]	55 (26.7)	22 (19.4)	23 (24.7)	0.355
IDU. No. (%)	181 (87.8)	102 (90.2)	88 (94.6)	0.247
Undetectable HIV viral load. No. (%)[‡]	181 (87.8)	97 (85.8)	84 (90.3)	0.34
Use of HAART. No. (%)	194 (94.1)	107 (94.6)	86 (92.4)	0.482
CD4 cells count (cells/mL). Mean ± SD	562 ± 271	560 ± 295	548 ± 236	0.744

Baseline HCV viral load (log_{10} IU/mL). Mean ± SD	6.2 ± 0.79	6.2 ± 0.79	6.18 ± 0.79	0.665
Liver cirrhosis stage. No. (%)	70 (33.9)	41 (36.3)	29 (31.1)	0.419
Fasting LDL cholesterol (mg/dL). Mean ± SD	86.2 ± 29.4	84.3 ± 28.6	87.7 ± 31.7	0.445
ALT (IU/L). Mean ± SD	80 ± 57	82.1 ± 63.2	77.9 ± 51.2	0.613
IL28B-CC genotype. N (%)	84 (40.7)	45 (39.8)	39 (41.9)	0.723

Legend: Hepatitis C virus genotype 1a (HCV-1a); hepatitis C virus genotype 1b (HCV-1b); number of patients (No.); standard deviation (SD); Body Mass Index (BMI): acquired immunodeficiency syndrome (AIDS): injecting drug user (IDU); human immunodeficiency virus (HIV); highly active antiretroviral treatment (HAART); low-density-lipoprotein (LDL); alanine transaminase (ALT); interleukin 28B (IL28B).

**p* value compared value of HCV-1a and HCV-1b genotypes.

[†]Classified on the basis of Center for Disease Control and Prevention (CDC) recommendations *(Revised surveillance case definitions for HIV infection among adults, adolescents, and children aged <18 years and for HIV infection and AIDS among children aged 18 months to <13 years-United States, 2008. MMWR 2008; 57 (No RR-10): 1-14).*

[‡]HIV viral load was measured by PCR (CobasTaqMan, Roche Diagnostic Systems Inc., Pleasanton, CA, USA), detection limit set at 20 IU/mL.

Table 2 Linear regression models for HCV viral decline at weeks 1, 2 and 4 after starting treatment.

Week 1*						
Condition	**Total population**		**Genotype 1a**		**Genotype 1b**	
	B	***p***	**B**	***p***	**B**	***p***
BMI	-0.021	0.851	-0.131	0.454	0.187	0.252
Baseline HCV viral load	-0.253	0.025	-0.206	0.254	-0.106	0.545
Liver fibrosis F3-F4	-0.122	0.264	-0.183	0.269	-0.095	0.577
LDL cholesterol	0.116	0.276	0.068	0.691	-0.077	0.663
IL28B Non-CC	**-0.287**	**0.01**	-0.025	0.889	**-0.62**	**0.001**
HCV genotype 1b	0.179	0.106				
Week 2[†]						
Condition	**Total population**		**Genotype 1a**		**Genotype 1b**	
	B	***p***	**B**	***p***	**B**	***p***
BMI	-0.038	0.665	-0.15	0.232	0.14	0.273

Baseline HCV viral load	-0.133	0.134	-0.084	0.505	-0.166	0.228
Liver fibrosis F3-F4	-0.142	0.114	-0.236	0.062	-0.145	0.287
LDL cholesterol	0.036	0.685	-0.014	0.98	-0.16	0.397
IL28B Non-CC	**-0.223**	**0.012**	0.074	0.553	**-0.52**	**<0.001**
HCV genotype 1b	0.006	0.942				
Week 4‡						
Condition	**Total population**		**Genotype 1a**		**Genotype 1b**	
	B	***p***	**B**	***p***	**B**	***p***
BMI	-0.067	0.415	-0.138	0.251	0.092	0.395
Baseline HCV viral load	-0.098	0.225	-0.078	0.516	-0.04	0.702
Liver fibrosis F3-F4	0.011	0.892	0.025	0.836	-0.058	0.579
LDL cholesterol	0.116	0.164	0.111	0.356	-0.043	0.710
IL28B No-CC	**-0.319**	**<0.001**	-0.033	0.786	**-0.698**	**<0.001**
HCV genotype 1b	0.022	0.79				

Legend: coefficient (B), body mass index (BMI), hepatitis C virus (HCV), low-density-lipoprotein (LDL), interleukin 28B (IL28B). Coefficients show the difference of HCV viral decline of a condition, with respect to its opposite, at different time points. BMI, HCV viral decline per unit increase of BMI; Baseline viral load, HCV viral decline per unit increase (log/IU) of HCV viral load; Liver fibrosis F3-F4, HCV viral decline compared with absence of F3-F4 liver fibrosis; LDL cholesterol, HCV viral decline per unit increase in LDL cholesterol; IL28B non-CC, HCV viral decline compared with IL28B-CC genotype; HCV genotype 1b, HCV viral decline compared with HCV genotype 1a.

*Week 1 model adjusted R^2 values for Total Population (R^2= 0.235), Genotype 1a (R^2= 0.058) and Genotype 1b (R^2 = 0.432) models.

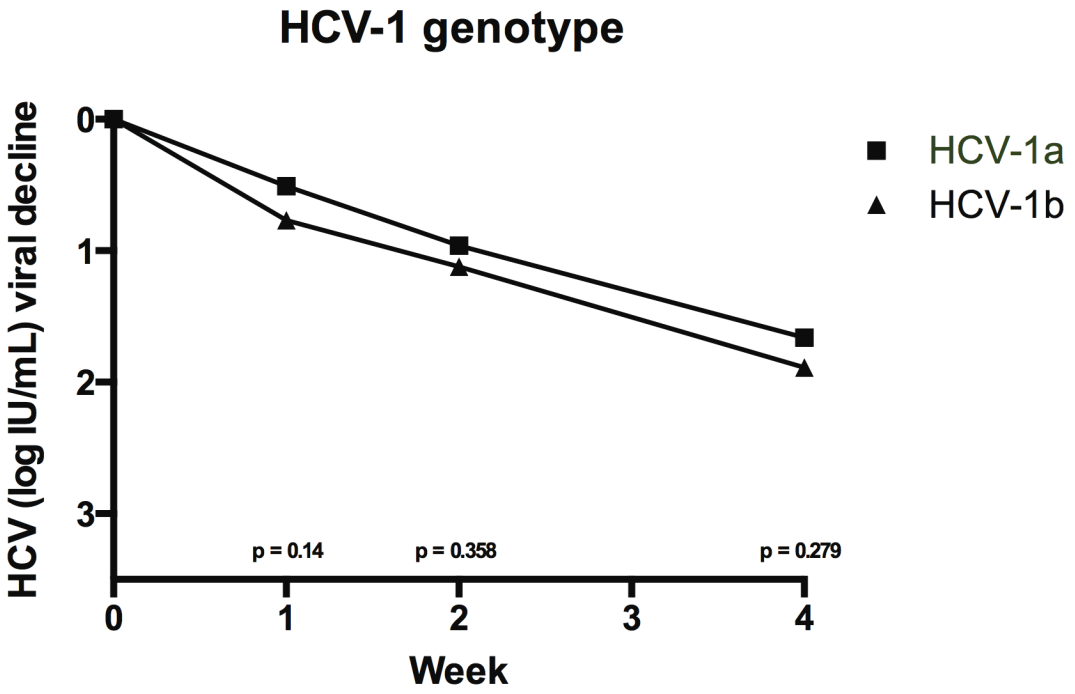
†Week 1 model adjusted R^2 values for Total Population (R^2= 0.087), Genotype 1a (R^2= 0.081) and Genotype 1b (R^2 = 0.270) models.

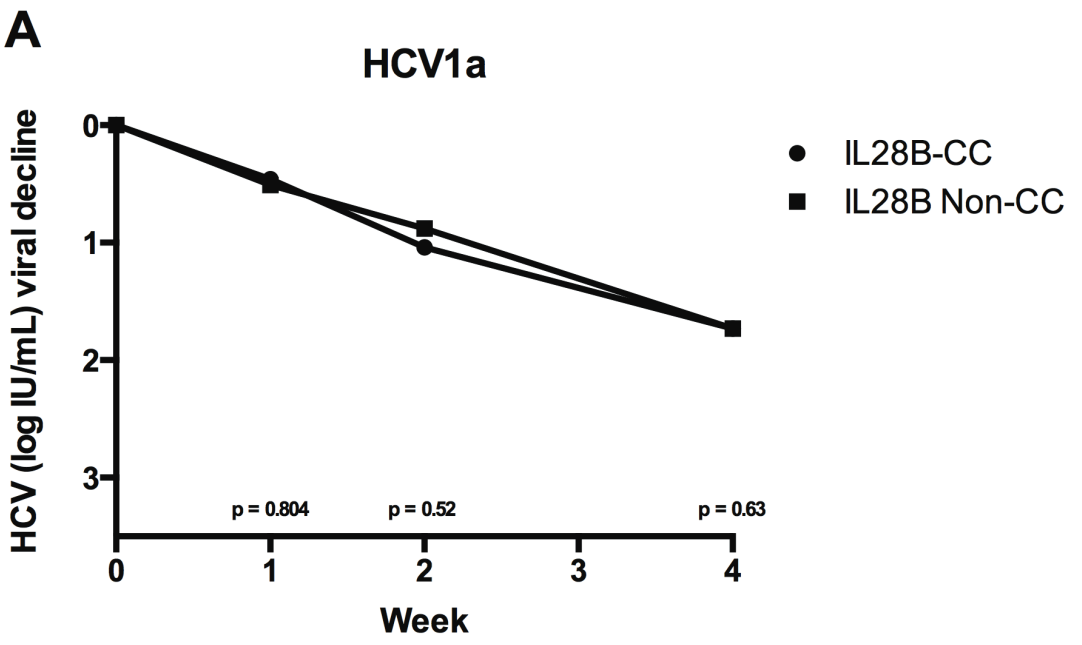
‡Week 1 model adjusted R^2 values for Total Population (R^2= 0.145), Genotype 1a (R^2= 0.033) and Genotype 1b (R^2 = 0.468) models.

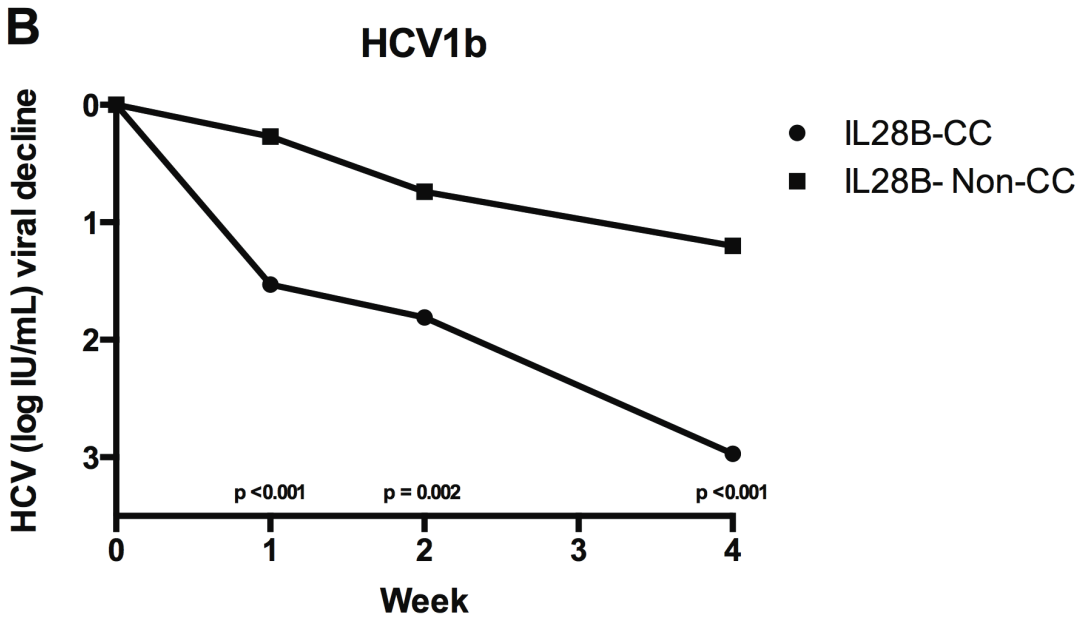
Figure 1 HCV viral decline (log_{10} IU/mL) of patients infected with HCV-1a and HCV-1b genotypes at weeks 1, 2 and 4 after starting treatment with Peg-IFN/RBV.

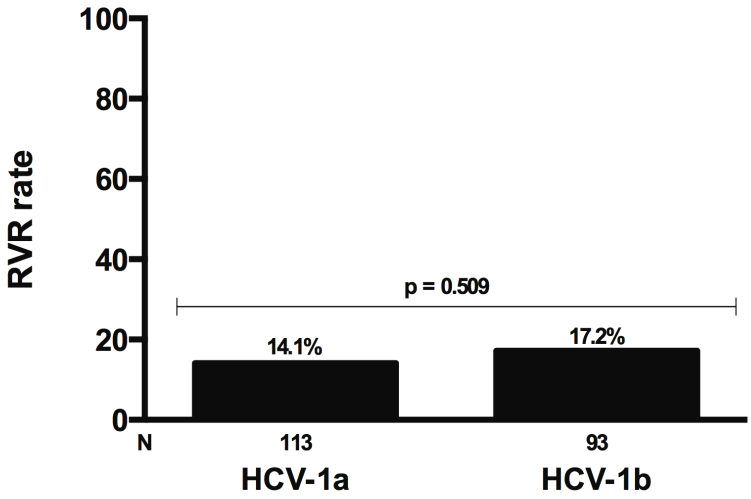
Figure 2 HCV viral decline (log_{10} IU/mL) of patients infected with the HCV-1a (**A**) and HCV-1b (**B**) genotypes at weeks 1, 2 and 4 after starting treatment with Peg-IFN/RBV, by IL28B genotype (CC *versus* Non-CC).

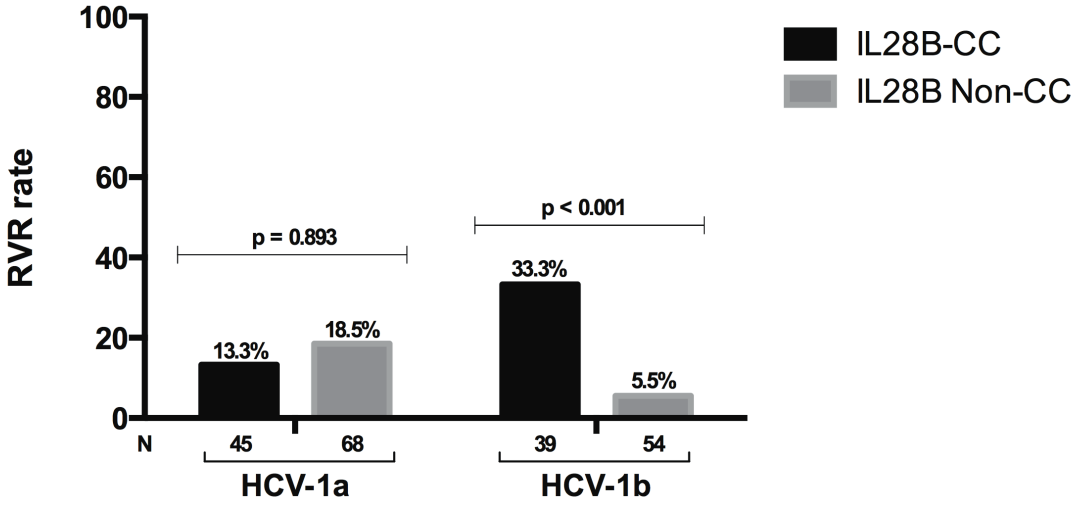
Figure 3 RVR rates according to HCV-1 subtype and IL28B genotype.


HCV-1 genotype
HCV (log IU/mL) viral decline
0
1
2
3
p = 0.14
p = 0.358
p = 0.279
0
1
2
3
4
Week
HCV-1a
HCV-1b


A
HCV1a
HCV (log IU/mL) viral decline
0
1
2
3
p = 0.804
p = 0.52
p = 0.63
0
1
2
3
4
Week
IL28B-CC
IL28B Non-CC


B
HCV1b
HCV (log IU/mL) viral decline
0
1
2
3
p <0.001
p = 0.002
p <0.001
0
1
2
3
4
Week
IL28B-CC
IL28B- Non-CC


RVR rate
100
80
60
40
20
0
p = 0.509
14.1%
17.2%
N
113
93
HCV-1a
HCV-1b


RVR rate
100
80
60
40
20
0
p = 0.893
p < 0.001
13.3%
18.5%
33.3%
5.5%
N
45
68
39
54
HCV-1a
HCV-1b
IL28B-CC
IL28B Non-CC

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Differences in HCV Viral Decline between Low and Standard-Dose Pegylated-Interferon-Alpha-2a with Ribavirin in HIV/HCV Genotype 3 Patients

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Abstract

Background: The aim of the study was to analyze the different impact of standard and low-dose Peg-IFN-α2a/RBV therapies on HCV viral decline in HIV/HCV genotype 3 co-infected patients during the first weeks of treatment.

Methods: Plasma HCV viral decline was analyzed between baseline and weeks 1, 2 and 4 in two groups of treatment-naïve HCV genotype 3 patients with HIV co-infection. The Standard Dose Group (SDG) included patients who received Peg-IFN at 180 μg/per week with a weight-adjusted dose of ribavirin; Low-Dose Group (LDG) patients received Peg-IFN at 135 μg/per week with 800 mg/day ribavirin. The effect of IL28B genotype on HCV viral decline was evaluated in both groups. HCV viral decline was analyzed using a multivariate linear regression model.

Results: One hundred and six patients were included: 48 patients in the SDG and 58 in the LDG. HCV viral decline for patients in the LDG was less than for those in the SDG (week 1:1.72±0.74 $\log_{10}$ IU/mL *versus* 1.78±0.67 $\log_{10}$ IU/mL, p = 0.827; week 2:2.3±0.89 $\log_{10}$ IU/mL *versus* 3.01±1.02 $\log_{10}$ IU/mL, p = 0.013; week 4:3.52±1.2 $\log_{10}$ IU/mL *versus* 4.09±1.1 $\log_{10}$ IU/mL, p = 0.005). The linear regression model identified the Peg-IFN/RBV dose as an independent factor for HCV viral decline at week 4.

Conclusions: Our results showed that HCV viral decline was less for patients in the low-dose group compared to those receiving the standard dose. Until a randomized clinical trial is conducted, clinicians should be cautious about using lower doses of Peg-IFN/RBV in HIV/HCV genotype 3 co-infected patients.

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Introduction

The best determinant of response to hepatitis C virus (HCV) treatment using pegylated-interferon (Peg-IFN) in combination with ribavirin (Peg-IFN/RBV) is the HCV genotype itself. Rates of sustained virological response (SVR) in genotypes 1 or 4 HCV patients co-infected with HIV vary between 17 and 46% in different studies, while for genotype 3 HCV/HIV co-infected patients these values range between 43 and 71% [1,2]. The higher treatment response rate for genotype 3 HCV patients is due the higher rate of HCV viral clearance in the first weeks of therapy [3]. On the other hand, it has also been observed that HCV genotype 3 patients progress more rapidly to advanced stages of liver fibrosis and hepatic steatosis and are at a significantly higher risk of developing hepatocellular carcinoma [4–6]. For these reasons, patients infected with HCV genotype 3 constitute a population which requires the early implementation of treatment.

The current recommended treatment for genotype 3 HCV/HIV co-infected patients is 48 weeks with Peg-IFN-α2a or Peg-IFN-α2b (180 μg/kg and 1.5 μg/kg per week, respectively) combined with ribavirin (weight-adjusted) [7,8]. However, neither duration of treatment nor drug dose have been clearly optimized. For genotype 3 HCV monoinfected patients, the recommended length of treatment is 24 weeks, and even 12–16 weeks for patients achieving rapid virological response (RVR), and lower doses of Peg-IFN/RBV have been shown to achieve similar SVR rates to the standard dose [9–11]. However, because of the different responses to treatment, more rapid progression to liver fibrosis, interaction with antiretroviral treatment drugs and immunological characteristics, the results cannot be extrapolated to HIV/HCV co-infected patients [12,13].

Early viral kinetics gives clinicians valuable information about the outcome of HCV treatment. Several studies have shown that HCV viral decay in the first weeks after start of treatment can help identify which patients will respond to treatment and which will not [14–19]. HCV viral decline during the first weeks of treatment is also useful for analyzing the effects of different drug doses, including the impact on HCV clearance.

The aim of this study was to analyze the different impacts of standard and lower-than- standard dose Peg-IFN-α2a/RBV therapy on HCV viral decline in HIV/HCV genotype 3 co-infected patients during the first weeks after start of treatment.

Methods

Patients, Study Design and Treatment Regimen

Two groups of HIV/HCV genotype 3 co-infected patients who were naïve to HCV treatment were included in the study.

The Standard Dose Group (SDG) included patients enrolled in a prospective study designed to evaluate the efficacy of a treatment strategy for chronic hepatitis C genotype 3, who were administered Peg-IFN-α2a at 180 μg/per week combined with a weight-adjusted dose of ribavirin (1000 mg/day for <75 kg, 1200 mg/day for ≥75 kg); length of treatment (24 or 48 weeks) was determined according to whether or not RVR was achieved.

The Low Dose Group (LDG) included patients enrolled in an open-label, single-arm clinical trial (Reference: NCT00553930) evaluating the efficacy of Peg-IFN-α2a at a dose of 135 μg/per week combined with a daily ribavirin dose of 800 mg.

Ethical Aspects

The SDG study was designed and carried out according to the Helsinki declaration and was approved by the Ethics Committee of the Hospital Reina Sofia of Cordoba. (Reference: NCT00553930). All patients were informed and signed an informed consent form before participating in the study. The protocol of the LDG trial and supporting CONSORT checklist are available as supporting information; see [20]. The study protocol was approved by the Agencia Española del Medicamento and a central ethics committee (Comité Autonómico de Ensayos Clínicos, Consejería de Salud, Junta de Andalucía). The study was conducted according to the Declaration of Helsinki and current guidelines on Good Clinical Practices. This trial is registered at NIH register (ClinicalTrials.gov: NCT00553930) and EMEA (N°EudraCT: 2007-000814-35).

Data Collection

Host, clinical and virological characteristics were collected. Fibrosis stage was determined by biopsy or liver transient elastography (FibroScan®, Echosen. Paris). Significant fibrosis was defined as a METAVIR fibrosis score of F2–F4 in liver biopsy or a liver stiffness value of ≥8.9 kPa [21].

Virological Evaluation

Plasma HCV RNA load measurements were conducted at baseline and weeks 1, 2 and 4, using a quantitative PCR assay (Cobas TaqMan, Roche Diagnostic Systems Inc., Pleasanton, CA, USA) and using a detection limit of 15 IU/mL. Viral load was expressed as $\log_{10}$IU/mL.

IL28B Genotyping

DNA was extracted using the automated MagNA Pure DNA extraction method (Roche Diagnostics Corporation. Indianapolis, IN 46250, USA). Single nucleotide polymorphism (SNP) rs129679860, located 3 kilobases upstream of the IL28B, and in strong linkage disequilibrium with a non-synonymous coding variant in the IL28B gene (213A>G, K70R; rs81031142), was genotyped. Genotyping was carried out using a custom TAQMAN assay (Applied Biosystems, Foster City, California, USA) on DNA isolated from whole blood samples, on a Stratagene MX3005 thermocycler with MXpro software (Stratagene, La Jolla, California, USA), following the manufacturer's instructions. The researchers responsible for genotyping were blinded to other patient data. The IL28B genotype was defined as CC or non-CC (TT/CT).

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation or median (Q1–Q3) and were analyzed by the Student's *t* test, Mann-Whitney *U*-test or Kruskal-Wallis test. Categorical variables were expressed as numbers of cases (percentage). Frequencies were compared using the χ^2 test or Fisher's exact test. Significance was defined as a p value of less than 0.05. Plasma HCV RNA decline, according to SDG and LDG dose, was analyzed from baseline to weeks 1, 2 and 4. The effect of IL28B genotype on HCV viral decline was also analyzed in both treatment groups. Patients presenting an undetectable HCV viral load at any time point during the study were excluded when calculating reduction of HCV RNA levels at a later time. A multivariate linear regression model was used to analyze HCV viral decline between baseline and the various time points. In addition, two linear regression models of HCV viral decline from baseline to the different time points were analyzed according to treatment group. The analysis was performed using the SPSS statistical software package, version 18.0 (IBM Corporation, Somers, NY, USA).

Results

One hundred and six HIV/HCV genotype 3 co-infected patients were included in the study. Forty-eight (45.3%) patients were included in the SDG and 58 (54.7%) in the LDG. The baseline population characteristics of the two groups are shown in Table 1.

HCV Viral Decline According to Treatment Group

HCV viral decline of patients given the lower dose treatment was less than for those in the SDG, at weeks 2 and 4 after start of treatment, although not at week 1 (week 1:1.72±0.74 $\log_{10}$ IU/mL *versus* 1.78±0.67 $\log_{10}$ IU/mL, p = 0.827; week 2:2.3±0.89 $\log_{10}$ IU/mL *versus* 3.01±1.02 $\log_{10}$ IU/mL, p = 0.013; week 4:3.52±1.2 $\log_{10}$ IU/mL *versus* 4.09±1.1 $\log_{10}$ IU/mL, p = 0.005) (Figure 1). The multivariate linear regression models of factors associated with HCV viral decline at weeks 1, 2 and 4 showed that

Table 1. Baseline Population Characteristics.

Characteristic	SDG	LDG	P
N	48	58	
Male. N (%)	39 (81.2)	51 (87.9)	0.379
Age (years). Mean (SD)	40.4 (8.86)	43.6 (5.37)	0.612
Use of HAART, n (%)	45 (93.7)	52 (89.6)	0.588
AIDS diagnosis in the past, n (%)	13 (27.1)	10 (17.2)	0.213
Baseline CD4 count (cells/mm^3). mean (SD)	479.9 (234.7)	496.5 (266.4)	0.743
PIDU, n (%)	43 (89.5)	48 (82.7)	0.454
HCV baseline viral load (log_{10} IU/mL). Mean (SD)	5.72 (0.75)	5.51 (1)	0.247
Liver fibrosis stage F2–F4. n (%)	32 (66.6)	33 (56.9)	0.277
Liver Cirrhosis. n (%)	12 (25)	21 (36.2)	0.253
ALT (IU/L). Mean (SD)	80.3 (47.7)	91 (57)	0.409
AST (IU/L). Mean (SD)	65.5 (35.2)	79.9 (33)	0.541
Total fasting cholesterol (mg/dL). Mean (SD)	156.1 (40.4)	147 (37)	0.325
LDL cholesterol (mg/dL). Mean (SD)	86.8 (33.6)	76 (29)	0.147
Platelet count (10^3/μL). Mean (SD)	183 (65)	176 (68)	0.654
IL28B-CC genotype. N (%)	13 (43.4)†	24 (53.3)‡	0.409

Standard drug dose group (SDG); low drug dose group (LDG); human immunodeficiency virus (HIV); highly active antiretroviral treatment (HAART); acquired immunodeficiency syndrome criteria in the past (AIDS); previous intravenous drug user (PIDU); hepatitis C virus (HCV); interleukin 28B (IL28B).
†Available for 30 patients. ‡Available for 45 patients.
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the steeper and sustained HCV viral decline from week 2 to week 4 was associated with a lower baseline HCV RNA viral load and with patients in the SDG (Table 2). IL28B-CC and HCV viral decline were not associated.

Table 2. Multivariate linear regression model of HCV viral decline between baseline and weeks 1, 2 and 4 after start of treatment.

HCV decline at week 1

Factor	Condition	B	P
Treatment Group	SDG	−0.175	0.327
Baseline HCV viral load (log_{10} IU/mL)		0.059	0.637
Significant liver fibrosis stage	Yes	−0.247	0.676
IL28B genotype	Non-CC	0.296	0.113

HCV decline at week 2

Factor	Condition	B	P
Treatment Group	SDG	0.274	**0.037**
Baseline HCV viral load		0.329	**0.026**
Significant liver fibrosis stage	Yes	−0.113	0.340
IL28B genotype	Non-CC	−0.103	0.551

HCV decline at week 4

Factor	Condition	B	P
Treatment Group	SDG	0.335	**0.025**
Baseline HCV viral load		0.339	**0.002**
Significant liver fibrosis stage	Yes	−0.180	0.092
IL28B genotype	Non-CC	−0.041	0.791

adjusted coefficient (B), hepatitis C virus (HCV), interleukin 28B (IL28B), standard drug dose group (SDG). $R^2 = 0.327$.
doi:10.1371/journal.pone.0048959.t002

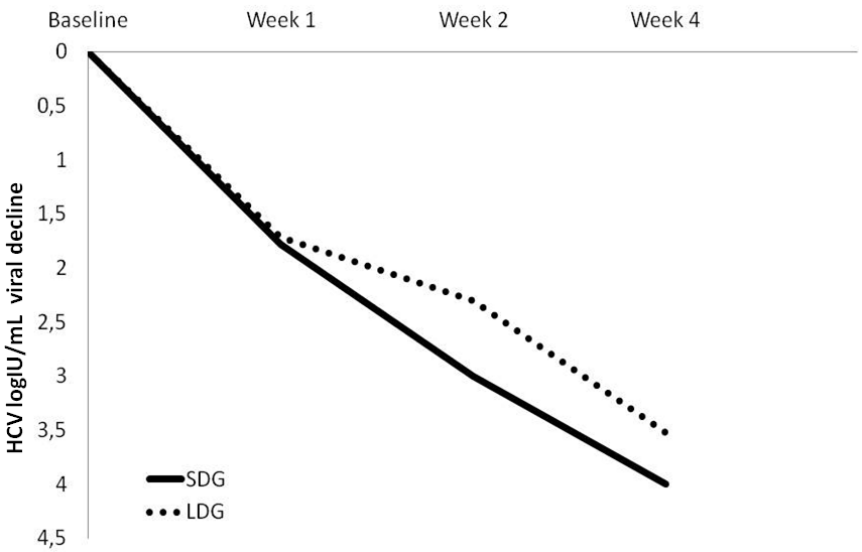

Figure 1. Mean HCV viral decline between baseline and weeks 1, 2 and 4 for the standard drug dose group (SDG) and the low drug dose group (LDG).
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HCV Viral Decline According to Treatment Group and IL28B Genotype

Among SDG patients, there were no differences of HCV viral decline by IL28B genotype at week 1 (1.82 ± 0.91 $\log_{10}$ IU/mL *versus* 1.74 ± 0.87 $\log_{10}$ IU/mL, $p = 0.852$), week 2 (3 ± 1.1 $\log_{10}$ IU/mL *versus* 3.08 ± 1.09 $\log_{10}$ IU/mL, $p = 0.807$) or week 4 (4.14 ± 0.84 $\log_{10}$ IU/mL *versus* 4.17 ± 1.06 $\log_{10}$ IU/mL, $p = 0.938$) (Figure 2A). Similarly, no differences of HCV viral decline by IL28B genotype were found among LDG patients at week 1 (2.07 ± 0.47 $\log_{10}$ IU/mL *versus* 1.42 ± 1.08 $\log_{10}$ IU/mL, $p = 0.071$), week 2 (2.46 ± 0.86 $\log_{10}$ IU/mL *versus* 2.03 ± 1.35 $\log_{10}$ IU/mL, $p = 0.257$) or week 4 (3.44 ± 0.94 $\log_{10}$ IU/mL *versus* 3.26 ± 1.01 $\log_{10}$ IU/mL, $p = 0.573$) (Figure 2B).

Among IL28B-CC genotype patients, HCV viral decline was greater in the SDG than in the LDG at weeks 2 and 4, but not at week 1 (week 1: $p = 0.362$; week 2: $p = 0.051$; week 4: $p = 0.033$). Likewise, HCV viral decline was greater among SDG patients carrying the IL28B non-CC genotype than among their LDG non-CC counterparts, at weeks 2 and 4 (week 1: $p = 0.343$; week 2: $p = 0.034$; week 4: $p = 0.037$).

Rapid Virological Response Rate

HCV viral load for 6 (5.6%) patients could not be evaluated at week 4. Of the remaining 100 patients, 66 (66%) achieved RVR: thirty-five (72.9%) in the SDG, and 31 (59.6%) in the LDG ($p = 0.174$). RVR rates by treatment group and IL28B genotype are shown in Table 3.

Table 3. Rapid virological response (RVR) rate by treatment group and IL28B genotype.

Treatment group	IL28B genotype	RVR. N (%)	P
SDG	CC	9 (40.9)	0.748
	Non-CC	12 (44.4)	
LDG	CC	13 (38.2)	0.642
	Non-CC	12 (35.3)	
IL28B genotype	**Treatment group**	**RVR. N (%)**	**P**
CC	SDG	9 (40.9)	0.642
	LDG	13 (38.2)	
Non-CC	SDG	12 (44.4)	0.221
	LDG	12 (35.3)	

Interleukin 28B (IL28B), rapid virological response (RVR), standard drug dose group (SDG), low drug dose group (LDG).
doi:10.1371/journal.pone.0048959.t003

Discussion

In our study, HCV viral decay of patients who received low-dose Peg-IFN/RBV treatment was less during the first weeks of treatment than for those receiving the standard Peg-IFN/RBV dose. This finding suggests that a lower Peg-IFN/RBV dose has less antiviral activity than the standard dose.

High viral decline during the first weeks of treatment leads to a high RVR rate [3]. Several factors have been identified as determining HCV viral decay [3,21]. Our study found that HCV viral decay correlated with Peg-IFN dose in HIV/HCV genotype 3 co-infected patients, with steeper viral decline from week 2 to week 4 in the SDG compared to the LDG. Our study also found that the dose of Peg-IFN administered during treatment was the most important factor affecting HCV viral decline in these patients. RVR rates in our study were also higher in the SDG compared to the LDG (72.9% *versus* 59.6%), although the differences between the two groups were not statistically significant. This point should be interpreted with caution, since a better powered cohort might be required for statistically significant associations, due to the high RVR rate among HCV genotype 3 patients.

Reducing the dose in drug-based HCV therapy for mono-infected HCV genotype 3 patients has been studied in various clinical trials. Firstly, reducing the dose of Peg-IFNα2a from 180 µg/per week to 135 µg/per week was shown to give similar RVR and SVR rates [9,10]. Secondly, SVR rates did not differ significantly according to whether a lower daily dose or a weight-adjusted dose of ribavirin was used [22–24]. A reduced dose of both drugs is, therefore, applicable to this patient population.

However, in HCV genotype 3 patients co-infected with HIV, a low-dose Peg-IFN/RBV combination would have a considerable impact, in terms of a high SVR, greater cost savings and fewer adverse events than the standard dose. A previous open-label,

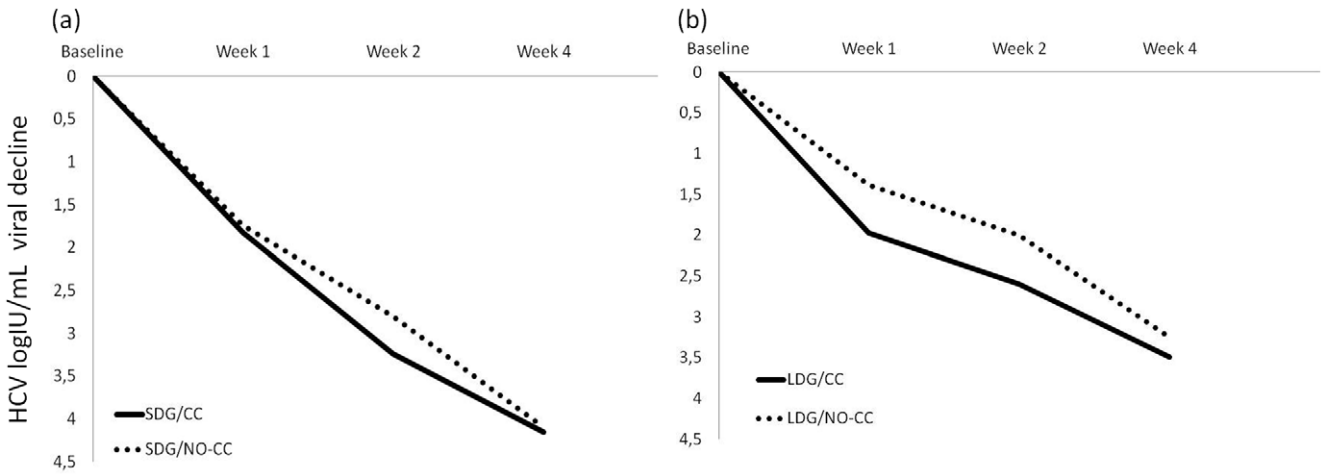

Figure 2. Mean HCV viral decline by IL28B genotype for the standard dose group (SDG) (Figure 2A) and the low-dose group (LDG) (Figure 2B).
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single-arm pilot clinical trial involving 58 HCV/HIV co-infected patients receiving low doses of Peg-IFN/RBV found that SVR rates were 58.3% based on intention-to-treat [20]. The main limitation of this study was the fact that it was not randomized but a single-arm study whose results were compared with those observed in earlier clinical trials The results of the pilot study suggested that a lower Peg-IFN/RBV dose might be as effective as the standard dose, so supporting the design of a randomized controlled trial. There are no data however about the efficacy or safety of standard Peg-IFN/RBV dose compared to a lower-than standard dose.

To the best of our knowledge, this is the first study to compare the efficacy of HCV viral clearance using low-dose and standard-dose drug therapy in HIV/HCV co-infected patients in the first weeks after treatment starts. Our findings suggest that the antiviral activity of the lower Peg-IFN/RBV dose is weaker than with the standard dose, which does not support equating the two for HIV/HCV co-infected patients. Our results also suggest that HCV viral decline during the first weeks of treatment would be dose-dependent, although the mechanism responsible for the difference is unknown.

In our study, we found no relation between IL28B genotype and viral decline in either of the regimens evaluated. The positive effect on treatment response associated with the IL28B-CC genotype has only been observed in patients bearing genotype 1/4 [25,26]. In fact, a previous study developed by our group reported that variations in IL28B do not have a positive impact on HCV viral decline in the first weeks after start of therapy using a standard drug dose [3]. Nor did IL28B-CC have a positive effect on RVR or SVR at standard or low drug doses in patients bearing genotype 3 [20,25].

Our study has several limitations. Firstly, our study is not a randomized clinical trial, and the presence of significant bias cannot therefore be ruled out. Secondly, due to the higher RVR rate in HCV genotype 2/3 patients, our study did not have the statistical power to detect differences in RVR rate by drug treatment dose. Thirdly, our study looked at the impact of IL28B by determining only SNP rs12979860, although the impact observed for this is not expected to be different from the other known IL28B SNP (rs8099917).

In conclusion, our results show that patients who received 135 μg/per week with a 800 mg/day ribavirin dose had less HCV viral decline in the first weeks after treatment started than those who received 180 μg/per week with a weight-adjusted ribavirin dose. The implications of weaker HCV viral decline in terms of treatment outcome are unknown, although it would be expected for a weaker HCV decline to lead to a lower RVR and consequently to a lower SVR. In order to resolve this point, our findings provide justification for the design of a randomized clinical trial to compare the specific efficacy endpoints of the two Peg-IFN/RBV doses in HIV/HCV co-infected patients. Until such a randomized clinical trial with these specific endpoints is conducted, therefore, clinicians should be cautious about using lower-than-standard Peg-IFN/RBV doses in HIV/HCV genotype 3 co-infected patients.

Author Contributions

Conceived and designed the experiments: ARJ LFLC AR. Performed the experiments: ARJ LFLC JAP A. Camacho MMS AR. Analyzed the data: ARJ AR. Contributed reagents/materials/analysis tools: AR ARJ LFLC A. Camacho ATC JAP MMS A. Caruz RRV JTC AGV. Wrote the paper: ARJ AR. Critical discussion of the manuscript: AR ARJ LFLC A. Camacho ATC JAP MMS A. Caruz RRV JTC AGV.

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CLINICAL AND EPIDEMIOLOGICAL STUDY

Baseline risk factors for relapse in HIV/HCV co-infected patients treated with PEG-IFN/RBV

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Abstract
Purpose Hepatitis C virus (HCV) viral relapse (VR) after end-of-treatment response (ETR) in human immunodeficiency virus (HIV) co-infected patients is observed in as many as one in three co-infected patients. The aim of the study was to identify baseline risk factors for VR in HIV/HCV co-infected patients treated with pegylated interferon plus ribavirin (PEG-INF/RBV).
Methods A total of 212 Caucasian HIV-infected patients with chronic hepatitis C naïve for PEG-INF/RBV were followed prospectively. Patients were included in this prospective study if they had completed a full course of therapy with an ETR. We assessed the relationship between VR rate and potential predictors of relapse.
Results Of the patients followed, 130 (61.3 %) attained ETR and 103 (79.2 %) achieved sustained virological response (SVR). Consequently, 27 (20.8 %) showed VR. Patients who relapsed were more often male ($p = 0.036$), carried the non-CC rs14158 genotype in the low-density lipoprotein receptor (LDLr) gene ($p = 0.039$), had higher baseline HCV RNA levels ($p = 0.012$), body mass index (BMI) $\geq$25 kg/m^2 ($p = 0.034$), significant liver fibrosis ($p < 0.001$), had been diagnosed with acquired immunodeficiency syndrome (AIDS)-defining criteria in the past ($p = 0.001$) and bore the HCV genotypes 1/4 ($p = 0.046$) when compared with SVR patients. The IL28B genotype was not associated with relapse. Multivariate binary logistic regression showed that high baseline HCV RNA, significant liver fibrosis, HCV genotypes 1/4, being overweight and being diagnosed with AIDS-defining criteria in the past were independently associated with relapse.
Conclusions Our study shows that VR can be accurately predicted in HIV/HCV co-infected patients on the basis of risk factors which can be identified before treatment.

Keywords HCV · HIV · End-of-treatment response · Viral relapse · Risk factors

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Introduction

Chronic hepatitis C virus (HCV) infection is a worldwide health problem that affects over 170 million people and induces significant morbidity and mortality [1]. Approximately 4–5 million of these patients are co-infected with human immunodeficiency virus (HIV). Treating HCV in HIV co-infected patients is a priority, since these patients progress more rapidly to end-stage liver disease, have poor tolerance to antiretroviral treatment and are at greater risk of hepatotoxicity [2, 3].

Pegylated interferon plus ribavirin (PEG-IFN/RBV) is currently the standard treatment for HCV infection. HCV treatment is less effective in HIV/HCV co-infected patients

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than in HCV monoinfected patients [4]. The reasons for these differences are unclear, although a higher relapse rate following completion of a course of therapy may contribute to lower sustained virological response (SVR) rates [5]. Indeed, a high proportion of HIV/HCV co-infected patients with an end-of-treatment response (ETR) experience viral relapse (VR) [5, 6]. In some studies, VR is observed in as many as one in three co-infected patients, even among those infected with HCV genotype 3 [7].

The aim of this study was to identify baseline risk factors for HCV relapse in HIV/HCV co-infected patients treated with PEG-IFN/RBV.

Methods

Patients

Caucasian HIV-infected patients with chronic hepatitis C bearing a single genotype and receiving a combination therapy of PEG-IFN/RBV were followed prospectively at two reference hospitals in Spain, from January 2007 to June 2010. All patients were treatment-naïve for HCV. The criteria used to manage hepatitis C therapy were in accordance with international guidelines [8]. Patients were included in this study if they had completed a full course of therapy with ETR and the 24-week post-treatment evaluation of serum HCV RNA. Patients who tested positive for hepatitis B surface antigen (HBsAg) were excluded.

Data analysis

Baseline host characteristics [age, body mass index (BMI), sex, low-density lipoprotein receptor (LDLr) and interleukin 28B (IL28B) genotype], baseline virological characteristics (viral load and HCV genotyping) and baseline characteristics concerning HIV infection [use of highly active antiretroviral therapy (HAART), CD4 cell count and acquired immunodeficiency syndrome (AIDS)-defining criteria in the past] were recorded.

Liver fibrosis evaluation

Fibrosis stage was determined by biopsy or liver transient elastography (FibroScan®, Echosens, Paris, France). Significant fibrosis was defined as a METAVIR fibrosis score of F2–F4 in liver biopsy or a liver stiffness (LS) value of $\geq$8.9 kPa.

Treatment regimens

All individuals were treated with either PEG-IFN α2a or PEG-IFN α2b, at doses of 180 or 1.5 μg/kg per week, respectively, in combination with a body weight-adjusted dose of oral ribavirin (1,000 mg per day for <75 kg, 1,200 mg per day for $\geq$75 kg). Patients with HCV genotypes 1/4 received either 48 or 72 weeks of treatment, while patients with HCV genotype 3 received 24 or 48 weeks of treatment, depending on the decision of the physician responsible for the patient [8]. At weeks 12 and 24, PEG-IFN/RBV was discontinued in non-responders.

Definitions of virological responses

ETR and SVR were defined as an undetectable serum HCV RNA level at completion of treatment and 24 weeks after the end of treatment, respectively. VR was defined as an undetectable serum HCV RNA level at the end of treatment, but detectable at the 24-weeks post-treatment date.

Virological evaluation

Plasma HCV RNA load measurements were performed using a quantitative polymerase chain reaction (PCR) assay (COBAS TaqMan, Roche Diagnostic Systems, Inc., Pleasanton, CA, USA), with a detection limit of 15 IU/mL. The HCV genotype was determined using a hybridisation assay (INNO-LiPa HCV, Bayer Corp., Tarrytown, NY, USA).

Single nucleotide polymorphism genotyping

DNA was extracted using the automated MagNA Pure DNA extraction method (Roche Diagnostics Corp., Indianapolis, IN, USA). Single nucleotide polymorphism (SNP) rs14158 in the 3′ UTR of the LDLr gene and 129679860, located 3 kb upstream of the IL28B genotype, and with a strong linkage disequilibrium with a non-synonymous coding variant in the IL28B gene (213A>G, K70R; rs81031142) were genotyped. Genotyping was carried out using a custom TAQMan assay (Applied Biosystems, Foster City, CA, USA) on DNA isolated from whole-blood samples. A Stratagene MX3005 thermocycler was used with MXpro software (Stratagene, La Jolla, CA, USA), following the manufacturer's instructions.

Statistical analysis

We assessed the relationship between VR rate and the following parameters: sex (male vs. female); BMI (<25 vs. $\geq$25 kg/m^2); liver fibrosis stage (F0–F1 or LS < 8.9 kPa vs. F2–F4 or LS $\geq$ 8.9 kPa); IL28B genotype (CC vs. non-CC); LDLr genotype (CC vs. non-CC); age; AIDS-defining condition in the past (no vs. yes) [9]; HAART (use vs. non-use); CD4 cell count ($\leq$250 vs. >250 cells/mL); PEG-IFN (α2a vs. α2b); HCV genotype (1/4 vs. 2/3); HCV genotype 1 subtypes (1a vs. 1b); and baseline HCV RNA level ($\geq$600,000 vs. <600,000 IU/mL).

The descriptive statistics for patient characteristics were reported. Continuous variables were summarised as mean ± standard deviation and categorical variables as frequencies and percentages. Statistical significance for continuous variables was analysed by the Student's *t*-test or the Mann–Whitney *U*-test. The Chi-square or Fisher's exact test were used for categorical variables. A stepwise logistic regression analysis was conducted as the multivariate analysis. Statistical analyses were carried out using SPSS software (version 18, SPSS Inc., Chicago, IL, USA).

Ethical aspects

This study was designed and performed according to the Helsinki declaration and was approved by the Ethical Committees of both participating hospitals.

Results

A total of 212 treatment-naïve HIV/HCV co-infected patients were included in the study, with 137 (64.6 %) and 75 (35.4 %) bearing genotypes 1/4 and 3, respectively. Of these individuals, 130 patients (61.3 %) had ETR; 103 (79.2 %) of them achieved SVR and 27 (20.8 %) presented VR, and were included in the analysis. The baseline and relapse HCV genotype was the same in all patients that experienced VR. HCV genotype 3 patients had a higher rate of ETR than genotypes 1/4 patients ($p < 0.001$).

The baseline characteristics of the 130 individuals who achieved ETR and were included in the analysis are presented in Table 1.

Risk factors identified by the univariate analysis were included in the multivariate binary logistic regression. The following factors were significantly associated with relapse in the multivariate analysis: a high baseline HCV RNA (≥600,000 IU/mL) level; significant liver fibrosis (F2–F4); HCV genotypes 1/4; BMI ≥25 kg/m^2; and AIDS-defining criteria in the past (Table 2).

Discussion

This study identifies factors that contribute to VR in HIV/HCV co-infected patients treated with PEG-IFN/RBV. Factors that were significantly associated with relapse included: significant liver fibrosis, high baseline serum HCV RNA, AIDS-defining criteria in the past, BMI ≥25 kg/m^2 and HCV genotypes 1/4. The IL28B non-CC genotype, however, was not a predictor of relapse.

Advanced liver fibrosis has been identified as a risk factor for non-response in HCV infected patients, while the association between significant liver fibrosis and relapse has not been firmly established [10, 11]. Thus, Cheng et al. [12] demonstrated that relapse rates for HCV monoinfected patients markedly increased with advanced fibrosis (F0, 16 %; F1, 23 %; F2, 26 %; F3, 50 %; F4, 80 %, $p < 0.001$). However, Shin et al., in a cohort of 242 treatment-naïve HCV monoinfected patients achieving

Table 1 Comparison of the characteristics of patients achieving sustained virological response (SVR) against patients who experienced viral relapse (VR) (*p*-value compares SVR and VR in each category)

	ETR ($n = 130$)	SVR ($n = 103$)	VR ($n = 27$)	*p*-value
Sex, *n* (%)				
Male	106 (81.5)	80 (75.5)	26 (24.5)	0.036
Female	24 (18.5)	23 (95.8)	1 (4.1)	
BMI, mean ± SD	25.7 ± 3.8	25.6 ± 3.7	25.9 ± 4.2	0.478
BMI, *n* (%)				
<25 kg/m^2	101 (77.7)	83 (82.2)	18 (17.8)	0.034
≥25 kg/m^2	29 (22.3)	20 (69)	9 (31)	
Significant liver fibrosis, *n* (%)				
Yes	80 (61.5)	58 (72.5)	22 (27.5)	<0.001
No	50 (38.5)	45 (90)	5 (10)	
Use of HAART, *n* (%)				
Yes	116 (89.2)	92 (81.1)	24 (20.69)	0.915
No	14 (10.8)	11 (78.5)	3 (21.5)	
Age (years), mean ± SD	40.9 ± 5.4	40.9 ± 5.6	41.2 ± 5.1	0.79
AIDS-defining condition in the past, *n* (%)				
No	73 (56.2)	67 (91.8)	6 (8.2)	0.001
Yes	57 (43.8)	36 (63.2)	21 (36.8)	
CD4 cell count, *n* (%)				
<250 cells/mL	16 (12.4)	12 (75)	4 (25)	0.632
≥250 cells/mL	114 (87.6)	91 (79.8)	23 (20.2)	
PEG-IFN, *n* (%)				
α2a	90 (69.2)	70 (77.8)	20 (22.2)	0.156
α2b	40 (30.7)	34 (85)	6 (15)	
HCV genotype				
1/4	64 (49.2)	46 (71.9)	18 (28.1)	0.046
3	66 (50.8)	57 (86.3)	9 (13.7)	
HCV genotype 1				
1a	26 (61.9)	15 (57.7)	11 (42.3)	0.279
1b	16 (38.1)	12 (75)	4 (25)	
Baseline HCV RNA level				
<600,000 IU/mL	62 (47.7)	55 (88.7)	7 (12.3)	0.012
≥600,000 IU/mL	68 (52.3)	48 (70.6)	20 (29.4)	
IL28B genotype, *n* (%)[a]				
CC	62 (59)	51 (82.2)	11 (17.8)	0.498
Non-CC	43 (41)	33 (76.7)	10 (23.2)	
LDLr genotype, *n* (%)[b]				
CC	64 (64)	54 (84.4)	10 (15.6)	0.039
Non-CC	36 (36)	26 (72.2)	10 (27.8)	

SD standard deviation (SD); *BMI* body mass index; *HAART* highly active antiretroviral therapy; *PEG-INF* pegylated interferon; *IL28B* interleukin 28B; *LDLr* low-density lipoprotein receptor

[a] Available in 105 patients

[b] Available in 100 patients

Table 2 Multivariate analysis of risk factors for relapse

Factors	Condition	OR (95 % CI)	Multivariate *p*-value
Baseline serum HCV RNA level	≥600,000 IU/mL	2.17 (1.2–7.9)	0.023
Liver fibrosis stage	Significant	5.97 (2.1–11.9)	0.002
HCV genotype	1 or 4	1.97 (1.01–5.91)	0.04
LDLr	Non-CC	1.78 (0.39–3.61)	0.143
Sex	Male	1.1 (0.31–1.98)	0.24
BMI	≥25 kg/m^2	1.6 (1.1–2.7)	0.047
Previous AIDS-defining condition	Yes	3.86 (1.92–10.23)	0.01

OR odds ratio, *CI* confidence interva, *BMI* body mass index, *LDLr* low-density lipoprotein receptor

ETR, did not identify advanced liver fibrosis as a risk factor for relapse [13]. Nevertheless, the latter study is limited in this respect, since the liver fibrosis stage was only determined in 35.9 % of patients. An association between significant liver fibrosis and a higher rate of relapse has not been established for HIV/HCV co-infected patients. In the PRESCO study, significant liver fibrosis was not identified as a risk factor for relapse, although the stage of liver fibrosis in this study could only be determined in 32.8 % of patients [7]. In our study, HIV/HCV co-infected patients with a liver fibrosis score of F2–F4 had a higher VR rate than those with a score of F0–F1; moreover, significant liver fibrosis was the strongest risk factor for VR.

High baseline serum HCV RNA level is a known risk factor for relapse in both HIV/HCV co-infected and HCV monoinfected patients, despite the variable thresholds used in different studies for considering high serum HCV RNA. Using a relatively high threshold for high serum HCV RNA (2,000,000 IU/mL), Shin et al. [13] identified high HCV RNA as a risk factor for relapse in monoinfected Korean patients with HCV genotypes 1/4. On the other hand, Deschênes et al. [14] used a relatively low threshold (400,000 IU/mL) for a cohort of 432 treatment-naïve HCV genotype 1 monoinfected patients with ETR, and found that patients with high HCV baseline viral loads had higher relapse rates (86 vs. 66 %, $p = 0.001$). The same relationship between high serum HCV RNA level and relapse rate has been demonstrated for HIV/HCV co-infected patients. Thus, Núñez et al. [7] identified high serum HCV RNA as the highest risk factor for relapse in Caucasian HIV/HCV co-infected patients [relative risk (RR) 4.81 (95 % CI; 1.52–15.22)], using a threshold of 500,000 IU/mL. Our study, using a cut-off point of 600,000 IU/mL, confirms these results and identifies high serum HCV RNA as a risk factor for relapse in HIV/HCV co-infected patients.

Several clinical trials conducted on HIV/HCV co-infected patients have shown that patients with HCV genotypes 1/4 have higher rates of relapse than those bearing HCV genotype 3. In the Adult AIDS Clinical Trials Group (ACTG) protocol 5071, Chung et al. [15] observed that patients with HCV genotype 1 who received PEG-INF/RBV had higher relapse rates than those with a non-1 genotype (33.3 vs. 4.3 %, $p = 0.031$). In the RIBAVIC trial, there was a higher relapse rate for HIV/HCV co-infected patients with genotypes 1/4 who received PEG-INF/RBV than for patients bearing genotypes 2/3 (33.3 vs. 12.5 %, $p = 0.04$) [16]. Similar results were obtained (23.8 vs. 3.2 %, $p = 0.001$) in the APRICOT trial [17]. Our study supports these findings and shows that, for the co-infected HIV/HCV patient, the HCV genotypes 1/4 are risk factors for relapse.

Our study shows that being overweight might be associated with a higher relapse rate. When Rodríguez-Torres et al. [18] studied a cohort of 35 Hispanic origin patients bearing HCV genotypes 2/3, they found a difference—albeit not statistically significant—in the VR rate between patients weighing >75 kg and those weighing less (28.6 vs. 14.3 %, $p = 0.088$). Previous studies have shown that a suboptimal dose of ribavirin is an important cause of VR, so weight-based ribavirin is recommended in order to reduce the virologic relapse rate [19]. A possible explanation for this finding might be that inadequate therapeutic ribavirin dosing is more likely in overweight patients. However, in our study, all patients were treated with weight-adjusted doses of oral ribavirin. On the other hand, suboptimal PEG-IFN dosing is more likely in overweight patients using standard doses of PEG-IFN α2a, which might favour VR, although there are also studies showing that BMI predicts relapse with weight-based doses of PEG-IFN α2b [20–22]. These findings point to explanations other than the ribavirin or PEG-IFN dose.

In our study, there were higher relapse rates for patients with AIDS-defining criteria in the past. Information about the relationship between VR and more advanced HIV disease is scarce. Núñez et al. [7] pointed out that relapse rates in HIV/HCV co-infected patients using antiretroviral therapy were higher than in those who did not use it (80.6 and 67.4 %, respectively, $p = 0.046$), although the authors hypothesised that the influence was not directly associated with the use of HAART but, rather, with more advanced stages of HIV disease, in line with the criteria used in the

past for beginning antiretroviral therapy. Our study supports this hypothesis, because patients with AIDS-defining criteria in the past, but not those on HAART, had a higher risk of relapse. This finding, if confirmed in further studies, suggests that an advanced stage of HIV disease may be a risk factor for relapse. The cause of high VR rates among patients with more advanced HIV disease remains uncertain. A possible explanation for this finding could be that patients with previous symptomatic HIV disease may have an associated loss of immune function involved in HCV clearance, which may determine the higher rate of relapse. Studies are needed in order to confirm this point.

The IL28B genotype has been identified as a strong predictor of SVR in both HCV monoinfected and HIV co-infected patients [22, 23]. However, information about the relationship between the IL28B genotype and VR is scarce. It has recently been reported that variations in the IL28B genotype are not associated with viral relapse in HCV monoinfected and co-infected patients treated with PEG-IFN/RBV [22, 24]. Our study corroborates this finding, showing that patients who carry the IL28B non-CC genotype did not have higher relapse rates than those with the IL28B-CC genotype.

According to a previous study carried out by our group, patients carrying the non-CC LDLr genotype were more likely to suffer a relapse (13 vs. 30 %, $p = 0.023$) [25]. However, the present study shows that, although the non-CC LDLr genotype was associated with a higher percentage of relapses, the relationship was not confirmed in the logistic regression model. For this reason, our study cannot be used for demonstrating a correlation between LDLr genotype and percentage of relapses. In this study, one of the groups derived as the result of categorising the presence or not of relapse as a dependent variable contained only 27 patients. Consequently, our logistic regression model might fail to identify variables which could, in fact, be associated with relapse, due to its lack of power. A better powered study could help clarify the possible relationship.

Our study has several limitations. This study confirmed that the baseline and relapse HCV genotype was the same in all patients, although we did not perform a comparative genome study of the virus at both these time-points. Therefore, reinfection and superinfection reactivation could not be dismissed entirely [26, 27]. Second, the standard of care for HCV treatment is due to change for HIV/HCV genotype 1 patients in the coming years. The incorporation of the new protease inhibitors, boceprevir and telaprevir, to PEG-IFN/RBV will improve the rate of treatment response in this group of patients, as has been the case with HCV monoinfected patients [28–31]. Our study shows the risk factors for relapse using the current HIV/HCV standard of care, so that those risk factors identified in our study may have limited power with those patients who add the new protease inhibitors to the standard of care. Studies are needed in order to analyse the baseline risk factors for relapse in patients receiving boceprevir or telaprevir.

In conclusion, our study shows that VR in HIV/HCV co-infected patients can be predicted on the basis of risk factors (significant liver fibrosis, HCV baseline viral load $\geq$600,000 IU/mL, BMI $\geq$25 kg/m^2, HCV genotypes 1/4 and AIDS-defining criteria in the past) which can be identified prior to treatment. This may allow physicians to evaluate alternative treatment strategies for patients at high risk of relapse.

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Atazanavir-Based Therapy Is Associated with Higher Hepatitis C Viral Load in HIV Type 1-Infected Subjects with Untreated Hepatitis C

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Abstract

We assessed the relationship between atazanavir (ATV)-based antiretroviral treatment (ART) and plasma hepatitis C virus (HCV) viral load in a population of HIV/HCV-coinfected patients. HIV/HCV-coinfected patients who received ART based on a protease inhibitor (PI) or nonnucleoside reverse transcriptase inhibitor (NNRTI) were included. Patients were stratified by ART drug [ATV/rtv, lopinavir (LPV/rtv), efavirenz (EFV), nevirapine (NVP), and other PIs], HCV genotype (1/4 and 2/3), and IL28B genotype (CC and non-CC). The Kruskal–Wallis test and chi-squared test were used to compare continuous and categorical variables, respectively. Multivariate analysis consisted of a stepwise linear regression analysis. Six hundred and forty-nine HIV/HCV-coinfected patients were included. HCV genotype 1/4 patients who received ATV had higher HCV RNA levels [6.57 (5.9–6.8) log IU/ml] than those who received LPV [6.1 (5.5–6.5) log IU/ml], EFV [6.1 (5.6–6.4) log IU/ml], NVP [5.8 (5.5–5.9) log IU/ml], or other PIs [6.1 (5.7–6.4) log IU/ml] ($p=0.014$). This association held for the IL28B genotype (CC versus non-CC). The association was not found in patients carrying HCV genotypes 2/3. The linear regression model identified the IL28B genotype and ATV use as independent factors associated with HCV RNA levels. ATV-based therapy may be associated with a higher HCV RNA viral load in HIV/HCV-coinfected patients.

Hemoglobin catabolism is closely related to the replication of the hepatitis C virus (HCV).[1] Heme oxygenase-1 (HO-1) catalyzes the breakdown of the heme molecule to yield equimolar quantities of biliverdin (BV), iron, and carbon monoxide. The oxidation of heme by HO-1 releases at least two antiviral agents, iron and BV.[2] Lehman *et al*. reported that BV has antiviral activity in replicon cells, noting that antiviral activity was accompanied by a rise in specific interferon-stimulated gene (ISG) products.[2] Likewise, Zhu *et al*. recently demonstrated that BV has potent antiviral activity against HCV, with inhibitory action over HCV NS3/4A protease.[3] Iron has also been shown to inhibit HCV replication by preventing divalent caption binding to RdRp.[4]

Atazanavir (ATV) is a protease inhibitor used in HIV therapy. ATV interferes with hemoglobin catabolism because of its competing inhibitory activity against the uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) enzyme, responsible for bilirubin (BR) conjugation.[5] This inhibition is directly

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associated with hyperbilirubinemia, the most common side effect of patients treated with ATV boosted with ritonavir (ATV/rtv). We hypothesize that this inhibition could modify the modulatory effect of the products of hemoglobin catabolism on HCV replication.

The aim of this study therefore was to assess the relationship between the use of ATV-based antiretroviral treatment (ART) and plasma HCV viral load in a population of HIV/HCV-coinfected patients.

The study population was 829 HCV treatment-naive patients who had been consecutively enrolled from October 2004 to December 2011 in the Spanish HEPAVIR prospective cohort of HIV/HCV-coinfected patients. Further details of these cohorts have been reported elsewhere.[6] Patients who received ART based on two ITIAN plus a protease inhibitor (PI) or nonnucleoside reverse transcriptase inhibitors (NNRTI) were included in the study. Patients who received estavudine, didanosine, or zidovudine were excluded from the study. Only patients maintained for a minimum of time of 12 months on an unchanged regimen were included. HCV RNA levels in plasma were measured using a commercial PCR assay (Cobas Taqman; Roche Diagnostic Systems Inc., Pleasanton, CA: detection limit of 50 IU/ml).

Liver fibrosis stage was determined by biopsy or liver transient elastography (FibroScan, Echosen, Paris). Significant fibrosis was defined as a METAVIR fibrosis score of F3–F4 in liver biopsy or a liver stiffness (LS) value of ≥8.9. SNP rs129679860, located 3 kilobases upstream of the IL28B gene, was genotyped using a custom Taqman assay (Applied Biosystems, Foster City, CA) on DNA isolated from whole blood samples. The bilirubin data of those patients on ATV/rtv treatment were also collected. Patients were stratified by ART drug [ATV/rtv, lopinavir (LPV), efavirenz (EFV), nevirapine (NVP), or other PI (saquinavir, nelfinavir, darunavir, or indinavir)], HCV genotype (1/4 and 2/3), and IL28B genotype (CC and non-CC). The Kruskal–Wallis test and chi-squared test were used to compare continuous and categorical variables, respectively. Post hoc comparisons between groups were carried out using the Mann–Whitney *U* test and the chi-squared test. To test our hypothesis, we used the Pearson or the bivariate Spearman's rank correlation coefficient to analyze the relationship between HCV viral load and bilirubin levels among patients treated with ATV/rtv. Associations with p values of <0.05 were considered significant for comparisons between groups. Multivariate analysis consisted of a stepwise linear regression analysis.

Six hundred and forty-nine HIV/HCV-coinfected patients were included in the analysis. Forty patients (7.3%) were treated with ATV/rtv, 128 (23.4%) with LPV/rtv, 225 (41.2%) with EFV, 41 (7.5%) with NVP, and 112 (20.5%) received some other PI. Only nine patients who might have been included in the analysis and fulfilled the criteria for inclusion in the study were receiving darunavir/r. Given the low number of patients, we decided not to include them as an independent group for analysis The most significant characteristics are shown in Table 1. T1 ▶

Median HCV RNA levels of HCV genotypes 1/4 and 2/3 were 6.13 (5.6–6.7) log IU/ml and 5.7 (5.3–6.2) log IU/ml, respectively ($p<0.001$). HCV genotype 1/4 patients who received ATV/rtv had higher HCV RNA levels [6.57 (6.2–6.8) log IU/ml] than those receiving LPV/rtv [6.1 (5.5–6.5) log IU/ml], EFV [6.1 (5.6–6.4) log IU/ml], NVP [5.8 (5.5–5.9) log IU/ml], or another PI drug [6.1 (5.7–6.4) log IU/ml ($p=0.014$)]. However, this association was not found in patients bearing HCV genotype 2/3 [ATV/rtv: 5.74 (5.4–5.9) log IU/ml], LPV/rtv [5.7 (5.2–5.8) log IU/ml], EFV [5.8 (5.6–6) log IU/ml], NVP [5.3 (4.9–5.5) log IU/ml], and other PI drug [5.6 (5.4–5.9) log IU/ml, $p=0.34$].

Table 1. Baseline Population Characteristics

Characteristics	
N	649
Age (years), mean (SD)	40.8 (5.56)
Male gender, *n* (%)	537 (82.7)
AIDS criteria, *n* (%)	192 (29.6)
HbsAg positive, *n* (%)	8 (1.2)
Undetectable HIV viral load, *n* (%)	472 (72.7)
CD4 cell count (cells/mm^3), mean (SD)	518 (266)
HCV genotype 1/4, *n* (%)	452 (69.6)
Liver cirrhosis stage, *n* (%)	154 (23.7)
IL28B-CC genotype, *n* (%)[a]	115 (38.1)

[a]Available for 302 patients.
SD, standard deviation; AIDS, acquired immunodeficiency syndrome criteria in the past; HbsAg, hepatitis B surface antigen; IL28B, interleukin 28B.

The IL28B-CC genotype was associated with a higher HCV viral load than the non-CC genotype [6.3 (6.2–6.6) log IU/ml versus 6 (5.9–6.3) log IU/ml, $p=0.001$]. There were no differences in HCV viral load found on the basis of detectable HIV viral load [detectable, 6.1 (5.8–6.4) versus undetectable 6 (5.4–6.2), $p=0.247$] or liver fibrosis stage [advanced liver fibrosis: 6.2 (5.8–6.5) versus absence of liver fibrosis stage: 6.1 (5.9–6.3), $p=0.472$]. When HCV RNA viral loads were analyzed in terms of the drug used for ART, patients with IL28B-CC receiving ATV/rtv had higher viral loads than those receiving another drug [ATV/rtv: 7.3 (6.9–7.4) log IU/ml; LPV/rtv: 6.6 (6.2–6.8) log IU/ml; EFV: 6.6 (6.3–6.8) log IU/ml; other PI: 6.4 (6.2–6.9) log IU/ml, $p=0.038$]. Patients with IL28B non-CC receiving ATV had higher baseline HCV viral loads than those receiving another ART regimen [ATV/rtv: 6.15 (6–6.4) log IU/ml; LPV/rtv: 5.9 (5.8–6.2) log IU/ml; EFV: 5.8 (5.6–6) log IU/ml; other PIs: 5.9 (5.8–6.1) log IU/ml, $p=0.032$]. The mean (±SD) bilirubin level among those patients who received ATV/rtv was 2.31±0.87. In these patients, HCV viral load correlated positively with bilirubin levels ($r=0.39$; $p=0.0129$).

Table 2 shows the linear regression model. Independent factors associated with HCV RNA levels were IL28B genotype ◀ T2

Table 2. Linear Regression Model for Hepatitis C Virus Baseline Viral Load in Patients Bearing the Hepatitis C Virus Genotype 1

Variable	*Condition*	*B*	p
IL28B	CC	0.7	0.033
ATV/rtv	Use	0.8	0.017
Liver fibrosis stage	Significant	0.075	0.884
HIV viral load	Undetectable	0.18	0.321

$R^2=0.22$.
B, adjusted coefficient; IL28B, interleukin 28B; ATV/rtv, atazanavir boosted with ritonavir; HIV, human immunodeficiency virus.

($B=2.7$; $p=0.033$) and use of ATV ($B=2.8$; $p=0.017$). R^2 was 0.22.

This is the first study to show the association between ATV/rtv use and HCV RNA viral load. Since a high HCV RNA viral load is a risk factor for nonresponse to treatment, this may be an important issue in conditioning the response to pegylated-interferon (PEG-IFN) with ribavirin (PEG-IFN/RBV) or to therapy based on NS3 protease inhibitors in HIV/HCV genotype 1-coinfected patients.[7–9] However, the relationship between ATV/rtv and treatment response has not yet been analyzed.

The reason ATV use might be associated with higher HCV RNA viral loads is unknown. *In vitro* studies have reported that BR and BV show antiviral activity against HIV, the herpes virus, and HCV.[3] More specifically, the antiviral action against HCV is due to both recombinant and endogenous NS3/4A protease from replicon microsomes being inhibited by BR and BV.[3] HO-1, on the other hand, potentiates interferon (IFN)-α, and so enhances antiviral activity.[2] Hyperbilirubinemia and ATV use are directly associated because of ATV's competing inhibitory activity against UGT1A1, responsible for BR conjugation in the liver. Inhibition may modify hemoglobin catabolism by suppressing various enzymes with antiviral activity against HCV (BR-R, BV-S, and HO-1) due to the enhancement of the resulting metabolite (BR) or reduced ISG activity (from the inhibition or downregulation of HO-1). There is no evidence that ATV blocks the activity of HO-1, in addition to inhibiting UGT1A1. However, a clinical trial is currently in progress whose secondary objective is to determine the effect of atazanavir-induced hyperbilirubinemia on HO-1 induction (NCT00916448), which may clarify this question. On the other hand, our study showed that NVP use was associated with lower HCV viral load, as has previously been reported by our group.[10]

Our study has several limitations. First, the number of patients with ATV-based therapy was relatively low. Second, the design was not a randomized study, which may have led to bias not being recognized. Third, no concomitant drugs were considered in the analysis. Fourth, although BR is closely related to ATV/rtv use, BR levels were not collected. Fifth, no concomitant diseases were analyzed in our study, and it is known that several liver pathologies may condition BR levels, as Gilbert's syndrome.

In conclusion, on the basis of our results, ATV-based therapy may be associated with higher HCV RNA viral loads among HIV/HCV-coinfected patients, a finding that could have an important impact on the outcome of HCV therapy. Studies are needed to confirm the relationship found in our study, as well to analyze this association in HCV treatment response.

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DISCUSIÓN:

Los trabajos científicos presentados en esta memoria se diseñaron para mejorar el conocimiento y la práctica clínica habitual en el tratamiento del paciente coinfectado por el VIH/VHC. Los resultados obtenidos en los trabajos presentados nos permiten realizar algunas consideraciones generales y específicas sobre la predicción de la respuesta al tratamiento con IFN-Peg/RBV en el paciente coinfectado por el VIH/VHC. Los estudios realizados se agrupan en:

1. Bloque I: Predicción de respuesta al tratamiento tras la finalización del mismo: evaluación del valor predictivo positivo sobre RVS de la semana 12 tras finalización del tratamiento.
2. Bloque II: Evaluación de la influencia de los factores genéticos IL28B (rs129679860) y LDLr (rs14158) en la respuesta al tratamiento mediante el estudio de la cinética viral durante las primeras semanas del mismo.
3. Bloque III: Evaluación de la respuesta al tratamiento del VHC genotipo 3 con distintas dosis de IFN-Peg/RBV mediante el estudio de la cinética viral durante las primeras semanas del mismo.
4. Bloque IV: Estudios sobre factores basales predictivos de respuesta al tratamiento.

Nota: las referencias bibliográficas citadas en cada uno de los bloques se corresponde a las referenciadas en la sección References del final de cada trabajo presentado.

Bloque I: Predicción de respuesta al tratamiento tras la finalización del mismo: evaluación del valor predictivo positivo sobre RVS de la semana 12 tras finalización del tratamiento.

Los resultados presentados en este estudio muestran que la determinación de la carga viral del VHC en la semana 12 tras finalización del tratamiento (W12), mediante una técnica sensible con un límite mínimo de detección de 15 UI/mL, puede ser considerada como un punto apropiado para evaluar RVS y recidivas en pacientes coinfectados por el VIH/VHC.

Como ocurre en el caso de los pacientes monoinfectados, alcanzar carga viral indetectable en semana 24 tras finalización del tratamiento, es el principal objetivo del tratamiento frente al VHC en pacientes coinfectados por el VIH [8]. Sin embargo, estudios realizados en población monoinfectada por el VHC han identificado la W12 tras finalización del tratamiento como un punto apropiado para valorar RVS [5, 6, 9]. Martinot-Peignoux *et al*, evaluaron en una población de 573 pacientes monoinfectados por el VHC tratados con IFN-Peg/RBV y que alcanzaron RFT el valor predictivo positivo de la determinación de la W12 sobre RVS usando un límite mínimo de detección de 5-10 UI/mL [6]. En la W12, 409 pacientes permanecieron con carga viral indetectable, de los que 408

alcanzaron RVS (VPP: 99,7%; IC 95%: 99,1-100%), concluyendo que la determinación de este punto es igual de significativa que la determinación en la semana 24 para valorar RVS. Por otro lado, Aghemo *et al*, obtuvieron similares resultados (VPP= 100%) usando dos técnicas diferentes con unos límites mínimos de detección de 15 UI/mL y 50 UI/mL, en 32 y 258 pacientes respectivamente [9]. En nuestro estudio, usando un límite mínimo de detección de 15 kUI/mL, ninguna recidiva se produjo después de la W12, teniendo una VPP para RVS del 100%.

La cadencia de la recidiva del VHC en el paciente coinfectado por el VIH ha sido poco estudiada. En un estudio llevado a cabo en 143 pacientes coinfectados por el VIH tratados con IFN-Peg/RBV que alcanzaron RFT, todas las recidivas menos 2 (45/47; 95,7%) se produjeron antes de la W12 [7]. Análisis filogenéticos del virus en estos pacientes sugieren que en uno de ellos se produjo una reinfección por otro virus (diferente genotipo), y que el segundo paciente tuvo contacto con el mismo virus que le produjo la primoinfección. Nuestro estudio sugiere que la propia infección por el VIH no aumenta el riesgo de VR del VHC después de la W12.

El conocimiento precoz del estatus post-tratamiento del paciente puede tener un efecto beneficioso sobre el manejo del paciente tratado

frente al VHC. De manera que, reducir el periodo de seguimiento en 12 semanas respecto a las 24 semanas estándares, podría reducir los costes asociados a la monitorización de la respuesta al tratamiento, mejorar el cuidado del paciente y detectar de forma precoz las recidivas,y así, implementar de forma precoz alternativas terapéuticas. No obstante, el valor predictivo de la W12 sobre RVS solo se ha evaluado en pacientes que inician un primer tratamiento con IFN-Peg/RBV. Por ello, sería necesario evaluar este marcador de respuesta en pacientes que han fracasado a una terapia anterior.

Bloque II: Evaluación de la influencia de los factores genéticos IL28B (rs129679860) y LDLr (rs14158) en la respuesta al tratamiento mediante el estudio de la cinética viral durante las primeras semanas del mismo.

Efecto de IL28B (rs129679860)

Se analizó el efecto de las variaciones genotípicas de IL28B en una cohorte de 119 pacientes coinfectados por el VIH/VHC sin restricciones de genotipos. En este estudio se observó que las variaciones en el genotipo de IL28B son un potente marcador predictivo pre-tratamiento del descenso viral del VHC durante el tratamiento con IFN-Peg/RBV en pacientes coinfectados por el VIH y genotipos 1/4 del VHC. De forma que los pacientes con genotipo CC de IL28B (definido como favorable) tienen un descenso en la carga viral del VHC mayor que los pacientes con genotipo CT oTT (No-CC) de IL28B. Este efecto se observa tan precozmente como en la semana 1 tras inicio de la terapia, y se mantiene hasta la semana 4, aumentando las tasas de RVR.

En nuestro estudio no se observaron diferencias entre los diferentes genotipos de IL28B en el descenso viral del VHC, ni en las tasas de RVR, entre pacientes infectados por genotipo 3. No obstante, debido a que los

pacientes con genotipo 3 tienen un alto decline viral y tasas de RVR, sería necesario una cohorte mayor para poder encontrar estas diferencias.

La cinética viral del VHC durante el tratamiento con IFN es bifásica. La primera fase, en la que se produce un aclaramiento rápido del virus, tiene lugar durante las primeras 72h de tratamiento y está relacionada con la eliminación de los virones libres y la inhibición de la producción de nuevos virus [7]. La segunda fase es más lenta y prolongada y está relacionada con la eliminación de las células infectada por VHC. Stättermayer *et al* evaluaron el descenso viral del VHC durante las primeras 24h de tratamiento, observando que los pacientes con genotipo CC de IL28B tuvieron un mayor descenso que los pacientes con genotipo no-CC de IL28B [5]. En otro estudio, Thompson *et al*, detectaron diferencias entre los genotipos de IL28B desde la 2 semana de tratamiento, punto más precoz analizado [4]. En nuestro estudio encontramos diferencias significativas entre los genotipos de IL28B entre el momento basal y semana 1 en pacientes coinfectados por VHC genotipo 1. Sin embargo no encontramos diferencias en la cinética viral entre las semanas 1-2 ni entre la 2-4. Este es un hallazgo importante, ya que implica que el efecto de IL28B se produciría durante la primera fase de la cinética viral.

Esta aclaramiento mas rápido del VHC incrementaría a su vez las tasas de RVR, que ha demostrado ser el mayor factor predictivo de RVS.

Efecto de LDLr (rs14158)

El VHC tiene una estrecha relación con el metabolismo lipídico del hospedador [15]. LDLr es un receptor fundamental en la entrada del virus en el hepatocito, por lo tanto, las variaciones genéticas de este receptor pueden condicionar la ciclo replicativo del virus. Este hecho queda patente en otros estudios publicados por nuestro grupo de investigación en el que se observa que los pacientes con genotipo CC de LDLr (favorable) tienen una carga viral basal menor que los que pacientes con genotipo TT o CT (no-CC) [13]. En nuestro estudio encontramos diferencias significativas en el descenso viral del VHC durante las primeras semanas de tratamiento con IFN-Peg/RBV entre genotipos de LDLr. Esta diferencia solo se encontró entre el momento basal y la semana 1, aspecto que sugiere que este marcador, al igual que IL28B, influye en la primera fase de la cinética viral del VHC.

Por otro lado, nuestro estudio muestra que los pacientes con genotipo CC de IL28B, solo presentan un impacto positivo en la cinética viral del VHC durante las primeras de tratamiento si son portadores del genotipo CC

de LDLr. Este efecto sinérgico entre IL28B/LDLr provoca un mayor aclaramiento del VHC durante las primeras semans del tratamiento y aumento del porcentaje de pacientes que alcanzan RVR. De esta forma los pacientes con genotipo CC de IL28B que no portaban genotipo CC de LDLr mostraron un descenso viral similar a los pacientes con genotipo no-CC de IL28B. Este hallazgo es importante, ya que sugiere que el efecto de IL28B en la cinética viral precoz solo se produce en presencia de un genotipo concreto de LDLr. Si esto fuese confirmado por otros estudios sería de gran importancia clínica, ya que la simple determinación de IL28B, sin la determinación del genotipo de LDLr, en la toma de decisiones clínicas en el tratamiento del VHC, tendría un papel limitado.

Efecto de IL28B (rs129679860) en los genotipos 1a y 1b del VHC

Se ha descrito que los pacientes infectados por genotipo 1b del VHC tienen unas tasas de respuesta al tratamiento con IFN-Peg/RBV superiores a los pacientes infectados por genotipo 1a [20, 24]. La causa sin embargo es desconocida. En base a nuestros resultados la mayor tasa de respuesta observada en pacientes infectados por genotipo 1b puede ser debida a que el genotipo CC de IL28B tiene un mayor efecto en este genotipo que en el genotipo 1a. La razón es desconocida. Se ha descrito que ciertas

mutaciones tanto en las proteínas estructurales como no estructurales del VHC tienen la capacidad de modular la respuesta al tratamiento [25, 26]. El mecanismo por el cual se asocian estas mutaciones con una mayor o menor tasa de respuesta al tratamiento está relacionado con la sensibilidad del virus a IFN [27, 28]. De manera que, aquellos pacientes con la mutación del core 70R muestran unas mayores tasas de RVS que aquellos pacientes con la mutación 70Q [29, 30]. Del mismo modo, se han identificado varias mutaciones en la proteína NS5A relacionadas con unas mayores o menores tasas de RVS [27]. Estas mutaciones favorables son más frecuentes en el genotipo 1b del VHC [21], por lo tanto la mayor tasa de respuesta tratamiento con IFN-Peg/RBV observada en pacientes con genotipo 1b puede ser debido a este mecanismo. Por otro lado, las variaciones en el genotipo de IL28B han sido relacionadas con una mayor (IL28B no-CC) o menor (IL28B-CC) actividad endógena de IFN-lambda [31]; por ello es de esperar que en pacientes con una menor actividad de IFN endógeno, se produzca un mayor aclaramiento viral cuando IFN exógeno es administrado. Por ello, se podría especular que los pacientes mas sensibles al IFN (IL28B-CC) infectados por un virus más sensible al IFN (genotipo 1b) tendrían unas tasas mayores de respuesta al tratamiento. No obstante, este punto no deja de ser una hipótesis, debido a que en nuestro estudio no

se han determinado las mutaciones virales asociadas con la sensibilidad al IFN.

Bloque III: Evaluación de la respuesta al tratamiento de distintas dosis de IFN-Peg/RBV mediante el estudio de la cinética viral del VHC genotipo 3 durante las primeras semanas del mismo.

En nuestro estudio la dosis administrada de IFN-Peg/RBV resultó ser el factor más influyente en el descenso viral del VHC durante las primeras semanas de tratamiento en pacientes coinfectados por el VIH y genotipo 3 del VHC. Este diferencia en el descenso viral durante las primeras semanas de tratamiento provocó a su vez que las tasas de RVR entre los pacientes que recibieron dosis estándar de tratamiento (SDG; 72,9%) fuera superior a la de los que recibieron dosis baja de tratamiento (LDG; 59,6%). Estas diferencias no alcanzaron significación estadística (p =0,174), probablemente porque las altas tasas de RVR alcanzadas por los pacientes infectados por genotipo 3 del VHC, podrían hacer necesaria una población de estudio mayor.

El empleo de dosis bajas de tratamiento es una práctica habitual en pacientes monoinfectados por el VHC genotipo 3, debido a que ha sido demostrada su no inferioridad en las tasas de RVR y RVS frente a dosis estándar de tratamiento en varios ensayos clínicos [9, 10, 22-24]. En pacientes coinfectados por el VIH, el empleo de dosis más bajas de

tratamiento podría ser de utilidad ya que podría condicionar un menor coste del tratamiento y una menor incidencia de efectos adversos. No obstante, al contrario de lo ocurre en población monoinfectada por el VHC, no existen ensayos clínicos aleatorizados que avalen el empleo de dosis más bajas de tratamiento en la población coinfectada por el VIH. Un ensayo clínico piloto de una sola rama de tratamiento en el que se incluyeron 58 pacientes coinfectados por el VIH y genotipo 3 del VHC tratados con dosis bajas de IFN-Peg/RBV obtuvo unas tasas de RVS del 58.3% (análisis por intención de tratar) [20]. En base a esta tasa de respuesta, se ha sugerido que dosis bajas de tratamiento podrían tener una efectividad similar a la obtenida con el uso de dosis estándar. Sin embargo, nuestros resultados muestran diferencias en la cinética viral y en las tasas de RVR con el empleo de dosis bajas y estándar de tratamiento. Por ello, hasta que se lleve a cabo un ensayo clínico aleatorizado que compare la eficacia y seguridad de ambas dosis de tratamiento, debe tenerse precaución en el uso de dosis bajas de tratamiento en pacientes coinfectados por el VIH y genotipo 3 del VHC.

Por otro lado, nuestro estudio no encontró asociación entre el genotipo de IL28B y el descenso viral del VHC durante las primeras semanas de inicio de la terapia en ambos grupos de tratamiento. Esta

observación confirma los resultados obtenidos en el primer estudio presentado en el bloque II.

Bloque IV: Estudios sobre factores basales predictivos de respuesta al tratamiento.

Factores basales asociados a VR del VHC en pacientes tratados con IFN-Peg/RBV coinfectados por el VIH

Nuestro estudio identifica factores basales asociados a VR del VHC en una cohorte de 212 pacientes tratados con IFN-Peg/RBV. Los factores identificados fueron el presentar una carga viral alta (> 600.000 UI/mL), un índice de masa corporal (IMC) $\geq$25 kg/m^2, estar infectado por genotipos 1 ó 4, presentar un grado F3-F4 de fibrosis hepática (en base a la escala METAVIR) y el haber desarrollado previamente un evento definitorio de *Síndrome de Inmunodeficiencia Humana Adquirida* (SIDA).

Tanto el grado fibrosis hepática, la carga viral basal alta como el índice de masa corporal han sido identificados previamente en estudios realizados en pacientes monoinfectados por el VHC [7, 13-24]. Sin embargo la identificación del desarrollo de criterio de SIDA como factor asociado a VR no había sido observada previamente. La causa de esta asociación es desconocida. Una hipótesis que podría explicar este hallazgo sería la posible pérdida de la inmunidad asociada al aclaramiento del VHC en los pacientes con criterio de SIDA. Esta hipótesis permitiría también

explicar las mayores tasas de VR observadas en pacientes coinfectados por el VIH en relación a los pacientes monoinfectados por el VHC.

Por otro lado, nuestro estudio no identificó las variaciones en los genotipos de IL28B y de LDLr como factores asociados a VR en pacientes coinfectados por el VIH.

Influencia de los fármacos integrantes del ART en la carga viral basal del VHC

En nuestro estudio el uso de ATV como parte del TAR del paciente coinfectado por el VIH y genotipo 1 ó 4 del VHC se asoció a unamayor carga viral del basal VHC respecto a otros ART.

La causa de este efecto es desconocida. ATV es un inhibidor de la proteasa del VIH que interfiere en el catabolismo de la hemoglobina mediante la inhibición de la enzima UGT1A1, responsable de la conjugación de la bilirrubina (BR) [5]. Esta inhibición se asocia a hiperbilirrubinemia; el efecto mas común asociado a ATV. Estudios in-vitro han comprobado que tanto BR como Biliverdina (BV), un metabolito del grupo hemo, tienen efecto antiviral directo frente al VIH y VHC [3]. Específicamente este efecto antiviral se debe a una inhibición de la proteína NS3/4a del VHC. Por otra parte, la hemo-oxigenasa, enzima encargada de

la oxidación del grupo hemo, potencia la producción de IFN, por lo que aumenta la actividad antiviral [2]. Por ello, la interferencia en el catabolismo del grupo hemo, por parte de ATV podría disminuir el efecto antiviral asociado a los catabolitos resultantes y por lo tanto asociarse a una mayor carga viral.

El presentar una carga viral alta es un factor asociado tanto a baja respuesta al tratamiento, como a un mayor riesgo de VR del VHC [7-9]. Por ello, este hallazgo podría tener una importante repercusión clínica en la práctica clínica del paciente coinfectado por el VIH y VHC. Serían necesarios nuevos estudios que evaluasen la posible influencia de esta asociación en la respuesta al tratamiento.

CONSLUSIONES

1. Undetectable HCV RNA at W12 post-treatment has a high PPV for SVR. Testing for HCV RNA at this moment may therefore be considered an appropriate point in time for identifying SVR and relapse in HIV/HCV co-infected patients receiving treatment with Peg-INF/RBV.
2. The IL28B (rs12129679860) genotype impacts on viral kinetics during the first week of treatment with Peg-IFN/RBV in patients with HCV genotype 1 or 4 co-infected with HIV.
3. The IL28B (rs12129679860) genotype has not impact on viral kinetics during the first week of treatment with Peg-IFN/RBV in patients with HCV genotype 3 co-infected with HIV.
4. The fastest reductions in viral load observed in IL28B-CC patients correlated with increased rates of RVR in HIV/HCV genotype 1 or 4 co-infected patients.
5. The LDLr (rs14158) genotype impacts on HCV viral kinetics during the first days of starting treatment with Peg-IFN/RBV in HIV/HCV genotype 1 co-infected patients
6. Both IL28B and LDLr CC genotype have a synergistic effect on HCV viral decline during first weeks after starting treatment with

Peg-IFN/RBV and RVR rate in HIV/HCV genotype 1 co-infected patients.

7. The IL28B-CC genotype had a positive effect on HCV viral clearance during the first weeks of treatment with Peg-IFN/RBV and on RVR rates in HCV-1b genotype HIV co-infected patients, but not those with HCV-1a
8. HCV viral decline was less for patients in the low-dose group compared to those receiving the standard dose. Until a randomized clinical trial is conducted, clinicians should be cautious about using lower doses of Peg-IFN/RBV in HIV/HCV genotype 3 co-infected patients.
9. The IL28B-CC genotype (rs12129679860) does not have a positive impact on HCV viral decline in the first weeks after start of therapy using a lower drug doses in HIV/HCV co-infected patients.
10. Significant liver fibrosis, high baseline serum HCV RNA, AIDS-defining criteria in the past, BMI >25 kg/m2 and HCV genotypes 1 or 4 are factors associated with VR.
11. ATV-based therapy is associated with a higher HCV RNA viral load in HIV/HCV-genotype 1 co-infected patients.

ANEXO I: OTRAS PUBLICACIONES DERIVADAS DEL TRABAJO DEL DOCTORANDO

1.- Macias J, del Valle J, Rivero A, Mira JA, Camacho A, Merchante N, Perez-Camacho I, Neukam K, **Rivero-Juarez A**, Mata R, Torre-Cisneros J, Pineda JA. Changes in liver Stiffness in patients with Chronic hepatitis C with and without HIV co-infection treated with pegylated interferon plus ribavirin. Journal of Antimicrobial Chemotherapy. 2010; 65: 2204-11.**IF (JCR) 4,659**

2.- Perez-Camacho I, **Rivero-Juarez A**, Kindelan JM, Rivero A. Present-day treatment of tuberculosis and latent tuberculosis infection (review). Enfermedades Infecciosas y Microbiología Clínica. 2011; Supple 1: 41-6. **IF (JCR): 1,656.**

3.- Pineda JA, Caruz A, Di Lello FA, Camacho A, Mesa P, Neukam K, **Rivero-Juarez A**, Macias J, Gomez-Mateo J, Rivero A. Low-density lipoprotein receptor genotyping enchances the predictive value of IL28B genotype in HIV/hepatitis C virus coinfected patients. AIDS. 2011; 25: 1415-20. **IF (JCR) 6.348.**

4.- Neukam K. **Rivero-Juarez A**, Caruz A, DiLello FA, Torre-Cisneros J, Lopez-Biedma A, Cifuentes C, Camacho A, Garcia-Rey S, Rivero A,

Pineda JA. Influence of the combination of low-density liporpotein receptor and interleukin 28B genotypes on lipid plasma levels in HIV/hepatitis C-coinfected patients. Journal of Accquire Immune Defic Syndr. 2011; 58: 115-7. **IF (JCR) 4,262**.

5.- Merchante N. **Rivero-Juarez A**. Tellez F. Merino D. Rios Villegas MJ. Marquez-Solero M. Omar M. Macias J. Camacho A. Perez-perez M. Gomez-Mateos J. Rivero A. Pineda JA. Liver stiffness predicts clinical outcome in HIV/HCV-coinfected patients with compensated liver cirrosis. Hepatology. 2012; 56: 228-238. **IF (JCR) 11,665.**

6.- Recio E. Macias J. **Rivero-Juarez A**. Tellez F. Merino D. Rios M. Marquez M. Omar M. Rivero A. Lorenzo S. Merchante N. Pineda JA. Liver stiffness correlates with Child-Pugh-Turcotte and MELD scores in HIV/hepatitis C virus coinfected patients with cirrhosis. Liver International. 2012; 32: 1031-2. **IF (JCR) 3.824**

7.- Mira JA. Rivero A. de los Santos-Gil I. Lopez-Cortes LF. Giron-Gonzalez JA. Marquez-Solero M. Merino D. Del mar Viloria M. Tellez F. Rios-Villegas MJ. Omar M. **Rivero-Juarez A**. Pineda JA. Hepatitis C virus genotype 4 responds better to pegylated inteferon plus ribavirin tan

genotype 1 in hiv-infected patients. AIDS. 2012; 26:1721-4. **IF (JCR) 6,348.**

8.- **Rivero-Juarez A**. Morgaz J. Camacho A. Muñoz-Rascon P. Dominguez JM. Sanchez-Cespedes R. Torre-Cisneros J. Rivero A. Liver stiffness using transient elastography is applicable to canines for hepatic disease models. Plos ONE. 2012; 7: e41557. **IF (JCR) 4.441**

9.-Mira JA, García-Rey S, Rivero A, de Los Santos-Gil I, López-Cortés LF, Girón-González JA, Téllez F, Márquez M, Merino D, Ríos-Villegas MJ, Macías J, **Rivero-Juárez A**, Pineda JA. Response to Pegylated Interferon Plus Ribavirin Among HIV/Hepatitis C Virus-Coinfected Patients With Compensated Liver Cirrhosis. Clinical Infectious Diseases. 2012; 55: 1719-26. **IF (JCR) 9.154**

10.- Neukam K, Almeida C, Caruz A, **Rivero-Juárez A**, Rallón N, Di Lello F, Herrero R, Camacho A, Benito J, Macias J, Rivero A, Soriano V, Pineda JA. A model to predict the response to therapy against hepatitis C virus (HCV) including low-density lipoprotein receptor genotype in HIV/HCV-coinfected patients. Journal of Antimicrobial Chemotherapy. 2012; In press. **IF (JCR): 5,068**

11.- Mira JA, **Rivero-Juarez A**, Lopez-Cortes LF, Giron-Gonzalez JA, Tellez F, Santos-Gil I, Macias J, Merino D, Marquez M, Rios-Villegas MJ, Gea I, Merchante N, Rivero A, Torres-Cornejo A, Pineda JA. Benefits from sustained virological response to pegylated interferón plus ribavirin in HIV/HCV-coinfected patients with compensated cirrosis. Clinical Infectious Diseases. 2013; In press. **IF (JCR): 9.154**

ANEXO II: ESTANCIAS EN CENTROS INTERNACIONALES DERIVADAS DEL PROYECTO DE TESIS DOCTORAL

Centro: Food and Drugs Administration (FDA). National Institutes of Health (NIH). Laboratory of Molecular and Developmental Immunology. Division of mononuclear antibodies. Bethesda. Maryland. USA.

Duración: 3 meses. (Octubre-Diciembre 2012)

Responsable:M.D./PhD. Francisco Borrego

Contacto:Francisco.Borrego@fda.hhs.gov

Dirección: 29 Lincoln Drive, Building 29B, Room 3NN04. Bethesda, MD, 20892.

ANEXO III: PREMIOS DE INVESTIGACION CONSEGUIDOS POR EL DOCTORANDO

1.- Premio Salud Investiga 2010 en la modalidad *Alianzas y Cooperación* de la Consejería de Salud de la Junta de Andalucía, como integrante del Grupo para el estudio de las hepatitis víricas (HEPAVIR) de la sociedad andaluza de enfermedades infecciosas (SAEI).

2.- "Young investigator award" en el 19th Conference on Retroviruses and Opportunistics infections (CROI). 5-8 March, 2012 Seattle (USA).

3.- Premio a la mejor comunicación en tema *Tratamiento* en el XIII Congreso Nacional Sobre SIDA (SEISIDA), Santiago de Compostela. 16-18 de Julio 2010.

4.- Premio a la mejor comunicación en tema *Ciencias Básicas* en el XIV Congreso Nacional Sobre SIDA (SEISIDA) Zaragoza, 15-17 de Junio 2011.

5.- Premio a la mejor comunicación en tema *Clínica* en el XIV Congreso Nacional Sobre SIDA (SEISIDA) Zaragoza, 15-17 de Junio 2011.

6.- Premio a la mejor comunicación en tema *Tratamiento* en el XIV Congreso Nacional Sobre SIDA (SEISIDA) Zaragoza, 15-17 de Junio 2011.

ANEXO IV: PROYECTOS FINANCIADOS MEDIANTE CONVOCATORIA PÚBLICA COMO INVESTIGADOR PRINCIPAL DERIVADOS DEL PROYECTO DE TESIS

Título: Influencia de los receptores *killer immunoglobulin-like receptors* (KIRs) de las células *Natural Killer* en la respuesta al tratamiento del VHC en pacientes coinfectados por el VIH/VHC.

Investigadores Colaboradores: Antonio Rivero Román, José Peña Martínez, Ángela Camacho Espejo, Inés Pérez Camacho.

Entidad Financiadora: Consejería de Salud de la Junta de Andalucía.

Anualidades: 3 (2013-2015)

Código: 0430-2012

ANEXO V: CAPITULOS DE LIBROS DERIVADOS DEL PROYECTO DE TESIS DOCTORAL

1.- Coinfección por VIH y VHC. Capitulo 1: Estructura genómica y ciclo biológico del VHC. (Rivero A, Pineda JA Eds. Málaga 2012: 19-26.ISBN: 978-84-695-4272-9)

2.- Coinfección por VIH y VHC. Capítulo 5: Trastornos metabólicos asociados a la hepatitis C: esteatosis, dislipidemias, alteraciones glucídicas y alteraciones metabólicas. (Rivero A, Pineda JA Eds. Málaga 2012: 53-62. ISBN: 978-84-695-4272-9)

3.- Coinfección por VIH y VHC. Capítulo 13: Tratamiento de la hepatitis C por genotipo 1: fármacos, pautas y reglas de parada. (Rivero A, Pineda JA Eds. Málaga 2012: 137-146. ISBN: 978-84-695-4272-9)

ANEXO VI: PATENTE DERIVADA DEL PROYECTO DE TESIS DOCTORAL

Título: Método de obtención de datos útiles para predecir la respuesta al tratamiento de la Hepatitis C.

Autores: José Peña Martínez. Rafael González Fernández. Antonio Rivero Román. Bárbara Manzanares Martín. **Antonio Rivero Juárez**. Ángela Camacho Espejo. Julián Torre Cisneros. (Propiedad intelectual a partes iguales).

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País: España