UNIVERSIDAD DE SEVILLA

Uso clínico de Maraviroc (MRV) en pacientes con infección por VIH-1: diseño de una nueva estrategia para la determinación de la sensibilidad clínica a MRV mediante una exposición a corto plazo al fármaco. Evolución inmunovirológica y seguridad a largo plazo de un régimen antirretroviral conteniendo MRV.

Departamento de Medicina, Universidad de Sevilla

Doctorando: Miguel Genebat González

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El Dr. Manuel Leal Noval, Profesor Asociado del Departamento de Medicina de la Universidad de Sevilla, y el Dr. Ezequiel Ruiz-Mateos Carmona, Doctor por la Universidad de Sevilla,

CERTIFICAN QUE: Don Miguel Genebat González, con DNI 28.618.912-N y Licenciado en Medicina por la Universidad de Sevilla, ha realizado bajo su dirección el trabajo

"Uso clínico de Maraviroc (MRV) en pacientes con infección por VIH-1: diseño de una nueva estrategia para la determinación de la sensibilidad clínica a MRV mediante una exposición a corto plazo al fármaco. Evolución inmunovirológica y seguridad a largo plazo de un régimen antirretroviral conteniendo MRV", para optar al grado de doctor en formato compendio de publicaciones.

En Sevilla a 15 de septiembre de 2010,

Directores de Tesis Doctorando

Dr. Manuel Leal Noval Miguel Genebat González

Dr. Ezequiel Ruiz-Mateos Carmona

“Si no conozco una cosa,

la investigaré”

Louis Pasteur

“Cuando Dios borra,

es que va a escribir algo ”

Jacques Benigne Bousset

(clérigo francés, siglo XVII)

…a mis padres e hijos

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INTRODUCCIÓN

La introducción de la terapia antirretroviral de gran actividad (TARGA) ha supuesto una reducción en la morbimortalidad asociada a la infección por el virus de la inmunodeficiencia humana (VIH)[1], al lograr un control más efectivo de la viremia y un aumento significativo en el número de linfocitos T-CD4^{+}. Sin embargo, un porcentaje variable de pacientes se encuentra en situación de fracaso virológico a pesar de recibir TARGA. Como consecuencia de la exposición previa a regímenes subóptimos, estos pacientes han desarrollado múltiples mutaciones asociadas a resistencia a las tres familias clásicas de antirretrovirales: inhibidores de la transcriptasa inversa análogos de nucleótidos (ITIAN), inhibidores de la transcriptasa inversa no análogos de nucleótidos (ITINAN) e inhibidores de la proteasa (IP). Estos sujetos suponen en la actualidad un reto para el clínico a la hora de ofertarles un TARGA que logre una supresión de la viremia.

La primera era del TARGA surgió con la disponibilidad en clínica de los IP. Sin embargo, muchos pacientes ya habían desarrollado mutaciones asociadas a resistencia y terminaron fracasando a estos regímenes. En 2003 se comercializó el primer fármaco con un mecanismo de acción diferente: enfuvirtide (T-20), un inhibidor de la fusión. Este fármaco demostró la eficacia en el rescate de pacientes en situación de fracaso virológico en primera instancia[2], pero al ser el único fármaco completamente activo del nuevo TARGA gran parte de estos pacientes desarrollaron finalmente fracaso virológico. En 2008 nació la que se conoce como segunda era del TARGA, al comercializarse nuevos antirretrovirales. Por un lado, nuevas generaciones de las familias clásicas, con mayor barrera genética y mayor actividad en pacientes infectados por virus con multirresistencia: Etravirina en la familia de los ITINAN y Darunavir en la familia de los IP. Por otro lado, fármacos pertenecientes a nuevas familias con

novedosos mecanismos de acción ya que actúan en diferentes puntos del ciclo vital del VIH: Raltegravir, un inhibidor de la integrasa, y Maraviroc (MRV), un inhibidor de la entrada. Todos ellos han demostrado por separado su eficacia inmunovirológica en el rescate de pacientes en situación de fracaso virológico[3-5]; su aparición conjunta en el escenario de la infección por VIH ha permitido ofrecer una alternativa terapéutica a estos pacientes, al asociar más de un fármaco completamente activo.

El único fármaco comercializado de la familia de los antagonistas del correceptor de quimiocinas CCR5 es MRV. La entrada del VIH en la célula requiere, además de la unión al receptor $CD4^+$ de la célula T, la unión a determinados receptores de quimiocinas que actúan como correceptores virales. Se han descritos dos correceptores de quimiocinas (CXCR4 y CCR5) que son utilizados por el VIH para completar el proceso de entrada, distinguiéndose cepas virales R5, X4 o de tropismo dual/mixto (D/M) según utilicen el correceptor CCR5, el CXCR4 o puedan emplear ambos, respectivamente[6].

El uso clínico de MRV está actualmente indicado en pacientes virémicos, pretratados e infectados por VIH con fenotipo viral R5[7]. Por tanto, antes de iniciar un tratamiento antirretroviral que pretende incluir MRV es necesario determinar el tropismo viral. El único ensayo validado clínicamente para la determinación del fenotipo viral es Trofile®, un método fenotípico basado en la generación de virus recombinantes de ciclo único que ha sido recientemente modificado[8,9]. Trofile® tiene, sin embargo, varias limitaciones: 1) Disponibilidad: sólo se realiza en Estados Unidos (Monogram Biosciences Inc., South San Francisco, CA); 2) Tiempo: suele tardar entre 4-6 semanas desde la extracción de la muestra hasta la recepción del resultado; 3)

Elevado coste, lo que encarece el tratamiento con los antagonistas de correceptores y a la vez supone una limitación para su uso en países con escasos recursos económicos; 4) Se requiere que la viremia del paciente sea superior a 1000 copias ARN VIH-1/mL, lo que limita el uso de antagonistas de correceptores en pacientes con viremias más bajas pero que se encuentran en situación de fracaso virológico; 5) En torno al 20% de los resultados son indeterminados; 6) Alrededor del 10% de los pacientes presentan un "cambio" de resultado de tropismo (de R5 a dual/mixto o X4) entre dos muestras separadas un mes[10,11], reflejando la escasa reproductibilidad del Trofile® en algunos pacientes; 7) Hasta en un 7% de pacientes infectados por virus R5 en base a Trofile® se han descrito *in vitro* mutaciones en la región V3 que podrían estar implicadas en resistencia a MRV, por lo que pacientes con potencial resistencia a MRV podrían recibir tratamiento con este fármaco si sólo se considera el test de tropismo[12].

Estas limitaciones hace que sean necesarias alternativas más rápidas, económicas y sencillas. Otros métodos fenotípicos han sido explorados[13,14], pero siguen siendo complejos, costosos y con baja reproductibilidad. Igualmente, métodos genotípicos que se basan en la secuencia de la región V3 para predecir el tropismo viral[15,16], han demostrado tener una alta especificidad pero baja sensibilidad para clasificar las cepas X4. Hasta ahora, ninguna alternativa clínica ha sido explorada. En este contexto, el primer objetivo de la presente Tesis Doctoral fue desarrollar un procedimiento clínica sencilla que permita determinar la sensibilidad clínica a MRV de un paciente en poco tiempo, el cual fue abordado en el trabajo "***Correlation between Trofile® test and virological response to a short-term Maraviroc exposure in HIV-infected patients"*** **(J Antimicrob Chemother 2009)**.

Una vez desarrollada dicha herramienta clínica (Maraviroc Clinical Test; MCT) y establecida una buena correlación con el estándar de oro (Trofile®) con los primeros pacientes, en nuestra práctica clínica diaria se estableció MCT como criterio para considerar a un paciente candidato a recibir tratamiento con MRV. Adicionalmente, en estos pacientes se practicaba un test fenotípico de tropismo (Trofile®) para comparar su resultado con el de MCT. Establecer las tasas de discordancia entre MCT y Trofile® constituye el segundo objetivo de la presente Tesis Doctoral, que se abordó en el trabajo "***Discordance rates between Trofile® test and short-term virological response to maraviroc"*** **(Antivir Res 2010).**

Por otro lado, MRV ha demostrado eficacia inmunovirológica y seguridad a largo plazo asociado a un régimen optimizado en pacientes con fracaso virológico persistente y con múltiples mutaciones asociadas a resistencia en los estudios MOTIVATE[4]. Sin embargo, no existen datos acerca de la eficacia y seguridad de MRV en una población de pacientes con una elevada tasa de coinfección por el virus de la hepatitis C (VHC) y empleados en la rutina asistencial habitual. Por tanto, el tercer objetivo de la presente Tesis Doctoral fue evaluar la eficacia inmunovirológica y tolerabilidad de un régimen antirretroviral que incluyese MRV en nuestra práctica clínica habitual, en la que un 50% de los pacientes presenta coinfección por VHC; este objetivo se desarrolló en el trabajo ***"Long-term immunovirological effect and tolerability of a maraviroc-containing regimen in routine clinical practice"*** **(Curr HIV Res 2010)**.

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OBJETIVOS

Los objetivos de esta Tesis Doctoral, abordados en las diferentes publicaciones, fueron los siguientes:

1. Desarrollar una herramienta clínica (MCT) rápida y sencilla que permita determinar la sensibilidad clínica a MRV de un paciente. **Correlation between Trofile® test and virological response to a short-term Maraviroc exposure in HIV-infected patients (J Antimicrob Chemother 2009).**

2. Establecer las tasas de discordancia entre MCT y Trofile® y la posterior evolución inmunovirológica de los pacientes con discordancia entre ambos métodos. **Discordance rates between Trofile® test and short-term virological response to maraviroc (Antivir Res 2010, en revisión).**

3. Estudiar la eficacia inmunovirológica y la seguridad a largo plazo (48 semanas) de un régimen TARGA de rescate que contenga MRV asociado a otros antirretrovirales en una población con elevada tasa de coinfección por VHC. **Long-term immunovirological effect and tolerability of a maraviroc-containing regimen in routine clinical practice (Curr HIV Res 2010)**.

MATERIAL Y MÉTODO,

RESULTADOS, DISCUSIÓN Y

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Correlation between the Trofile® test and virological response to a short-term maraviroc exposure in HIV-infected patients

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***Objectives*: The current validated assay to determine tropism of HIV variants is Trofile®, which has some limitations. The aim of this work was to correlate the virological response to a short-term maraviroc exposure with Trofile®.**

***Methods*: From 1 July 2008 to 1 March 2009, 34 consecutive HIV-infected patients with detectable viral load during the last 6 months began an 8 day exposure to maraviroc (MCT group); six HIV-infected patients without antiretroviral therapy received no treatment (control group). Plasma viral load was evaluated on days 0, 2, 5 and 8. Baseline Trofile® was performed in MCT group patients. The maraviroc clinical test (MCT) was considered positive if viral load was undetectable (<40 HIV-RNA copies/mL) or a reduction $\geq$1 $\log_{10}$ HIV-RNA copies/mL was achieved after 8 days of maraviroc exposure.**

***Results*: Global concordance between MCT and Trofile® was 93.5%. In patients with R5 virus according to Trofile®, MCT was positive in 19/20 (concordance 95%); in patients with dual/mixed virus, MCT was negative in 10/11 (concordance 90.9%). An additional phenotypic tropism assay was performed in patients with discordance between MCT and Trofile®, being concordant with MCT in both cases. Three patients showed a non-reportable Trofile® result, and all of them achieved undetectability after MCT.**

***Conclusions*: A clinical approach like short-term maraviroc exposure could be an additional resource to genetic and phenotypic HIV tropism assays. This clinical approach shows high concordance with Trofile®, and could allow patients with non-reportable results by Trofile® to benefit from maraviroc therapy.**

Keywords: CCR5 antagonists, tropism assays, antivirals

Introduction

Maraviroc is currently the only commercialized drug of a new antiretroviral family: the CCR5 receptor antagonists. According to actual guidelines, maraviroc is indicated in pretreated and viraemic patients who have been shown to be infected with R5 virus.[1] In order to determine the tropism of HIV variants in HIV-infected patients, the current validated assay is Trofile® (Monogram BioSciences, San Francisco, CA, USA).[2] However, this assay has some limitations, such as high cost, prolonged time to obtain a confirmed result, availability (samples need to be sent to San Francisco), a variable proportion of 'non-reportable' results and limited access in developing countries. Besides, discordance in two consecutive Trofile® results before being exposed to CCR5 inhibitors has been observed in ~10% of patients.[3,4] Moreover, it has been recently described that 7% of patients with R5 virus according to the tropism assay showed primary mutations in the V3 loop that could be involved with maraviroc resistance;[5] hence, patients with potential resistance to maraviroc could receive treatment with this CCR5 antagonist if the tropism assay alone is considered.

These limitations may exclude some patients with virological failure from using maraviroc as part of a rescue regimen.

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Therefore, additional resources different from Trofile® to assay HIV tropism, such as other phenotypic and genotypic assays, are being explored, but no clinical approaches have been developed. Various genotypic analyses have been examined, but caveats, such as high specificity but low sensitivity to classify X4 virus, are being observed.[6,7] More recently, correlation between genotypic and phenotypic assays has been described, but the combination of these methods is still expensive, time consuming and requires sophisticated laboratories.[8,9]

We propose a clinical approach [maraviroc clinical test (MCT)] in which the virological response to short-term exposure to maraviroc may predict the indication for maraviroc use. Hence, the objective of our study was to correlate the result of MCT with the result of Trofile®.

Methods

Patients

From 1 July 2008, a prospective study was started in the Infectious Diseases Department at Virgen del Rocio University Hospital (Seville, Spain). To participate in this study, inclusion criteria were: (i) persistently detectable viral load (>50 HIV-RNA copies/mL) during the last 6 months; (ii) no highly active antiretroviral therapy (HAART) modification in the last 6 months; (iii) no HAART reintroduction in the last 6 months in patients under previous supervised treatment interruption (STI); (iv) no previous treatment with co-receptor antagonists; and (v) available future therapeutic options apart from maraviroc.

Up to 1 May 2009, 34 consecutive HIV-infected patients were included (MCT group). Nineteen of these patients were under STI and treatment was required due to low CD4+ cell count; the other 15 patients were under HAART, despite which viral load was persistently detectable at least during the last 6 months. In order to determine the intra-patient variability of the viral load, six HIV-infected patients without any antiretroviral therapy (three of them were naive and the other three were under STI) were used as controls (the control group).

Patients, or legal guardians for those patients under 18 years old, had given written informed consent and the Ethical Committee of the Hospital approved the study.

Intervention

Patients in the MCT group began an 8 day exposure to maraviroc at a dose of 300 mg twice daily, adjusted if necessary for associated antiretrovirals. In the MCT group, patients under STI ($n=19$) were exposed only to maraviroc during MCT, while patients not under STI ($n=15$) received maraviroc with the previous failing regimen. Control group patients ($n=6$) received no treatment.

Patients in both groups were evaluated prospectively on days 0, 2, 5 and 8, analysing plasma viral load at each timepoint. Trofile® was performed for MCT group patients from blood samples obtained not more than 12 weeks before starting MCT, and in 24/34 (70.6%) the date of starting MCT was the same as Trofile®.

MCT was considered positive if a significant viral load reduction, defined as a reduction of $\geq 1 \log_{10}$ HIV-RNA copies/mL or an undetectable viral load (<40 HIV-RNA copies/mL), was achieved on day 8 after the addition of maraviroc.

Once the result of MCT was obtained, a new HAART regimen was started according to the following criteria: (i) previous genotype resistance testing results; (ii) previous antiretroviral exposure; and (iii) response to MCT, in order to include maraviroc or not in the new HAART.

Laboratory tests

Plasma HIV-1 RNA was measured in fresh samples by quantitative PCR (COBAS Ampliprep/COBAS Taqman HIV-1 test; Roche Molecular Systems, Basel, Switzerland) according to the manufacturer's instructions.

Determination of HIV-1 co-receptor usage

(i) Trofile®: circulating virus was tested from the plasma of patients using the PhenoSense HIV Entry assay for co-receptor tropism (Monogram Biosciences Inc., South San Francisco, CA, USA). Enhanced sensitivity Trofile® was employed for all samples.

(ii) Tropism of virus isolates from patients: frozen peripheral blood mononuclear cells (PBMCs) of the patients from HIV BioBank integrated in the Spanish AIDS Research Network (RIS) were co-cultured with phytohaemagglutinin (PHA; Boehringer Ingelheim)-stimulated HIV–hepatitis C virus (HCV)-uninfected donor PBMCs, as previously described.[10] Briefly, cells were cultured in medium supplemented with interleukin-2 (IL-2; R&D Systems) and cell-free supernatants were harvested and used for further infection assays. Co-receptor usage was determined modifying the technique used previously,[10] using U87 cell lines (U87-CD4+CCR5+ and U87-CD4+CXCR4+) that were infected with cell-free virus supernatant overnight and maintained for 6 days and tested for HIV-1 RNA copies/mL as described above. We used the X4-tropic NL4.3 strain and the R5-tropic BaL strain as positive controls. In this way, the measurement of HIV-1 RNA copies/mL in the supernatant of the cultures of X4 and R5 cell lines showed the tropism of the virus isolates of the patients.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS 16.0; SPSS, Inc., Chicago, IL, USA). Differences between groups were analysed using the Mann–Whitney *U*-test. Means and minimum–maximum ranges were used to describe continuous variables or means and 95% confidence intervals (CI) when stated. All differences between groups with $P<0.05$ were considered statistically significant.

Results

Correlation between MCT and Trofile®

The baseline characteristics of MCT group patients are shown in Table 1. Mean viral load evolution during MCT in control group patients, patients with an R5 Trofile® result (R5 patients) and patients with a dual/mixed Trofile® result (D/M patients) is shown in Figure 1. No significant viral load modification was observed in control group patients during MCT, similar to viral load evolution in D/M patients; however, a mean viral load reduction of 0.18, 0.76 and 1.41 $\log_{10}$ HIV-RNA copies/mL was observed in R5 patients on days 2, 5 and 8 of MCT, respectively.

Response to MCT and the result of the Trofile® test in every patient is shown in Table 2. A global concordance of 93.5%

between MCT and Trofile® was observed. In R5 patients the MCT was positive in 19/20 (4 patients achieved undetectability and 15 achieved a viral load reduction of $>1\ \log_{10}$ HIV-RNA copies/mL); so, concordance between Trofile® and MCT in R5 patients was 95%. Another phenotypic method to detect HIV tropism[10] was performed in the 'non-concordant' patient and the result was D/M (97.63% of the virus was R5 and 2.37% was X4), concordant with MCT but not with Trofile®. In D/M patients, no significant viral load reduction was observed in 10/11 (concordance 90.9% between MCT and Trofile®). In this 'non-concordant' case, when HIV tropism was assayed by the other phenotypic method the result was R5 (100% of the viruses were R5), again concordant with MCT. Finally, three patients showed a non-reportable Trofile® result, and all of them achieved undetectability after MCT therapy. Every patient completed maraviroc exposure with no significant increase in liver enzymes during MCT (data not shown) and the drug was well tolerated, except for one patient who showed dizziness during the first 2 days after starting MCT.

Table 1. Baseline characteristics of MCT group patients ($n=34$)

Age, years	37 (8–50)
Male sex, *n* (%)	24 (70.6)
HCV co-infection,[a] *n* (%)	13 (38.2)
Viral load, $\log_{10}$ copies/mL	4.13 (1.94–6.03)
CD4+, cells/mm^3	264 (2–871)
Sexual transmission, *n* (%)	8 (23.5)
IDU transmission, *n* (%)	19 (55.9)
Vertical transmission, *n* (%)	5 (14.7)
Blood transfusion, *n* (%)	2 (5.9)
Stage C, CDC, *n* (%)	9 (26.5)
Patients with CD4 <200 cell/mm^3, *n* (%)	15 (44.1)
Previous STI, *n* (%)	19 (55.9)
Malignancies, *n* (%)	4 (11.8)

IDU, intravenous drug user.
Values other than *n* (%) are expressed as mean (minimum–maximum).
[a]Positive PCR for HCV.

Immunovirological evolution with rescue therapy after MCT

Those patients with a positive MCT started rescue therapy containing maraviroc plus an optimized HAART, while those patients with a negative MCT started a rescue therapy without maraviroc, independent of the Trofile® result. Up to now, no virological rebound has been observed in any patient once optimized therapy was started. Mean CD4+ gain and HIV-RNA viral load reduction after a mean follow-up of 15 weeks once the rescue therapy was started (with or without maraviroc) is shown in Figure 2; a progressive CD4+ increase and a mean viral load reduction of $>2\ \log_{10}$ HIV-RNA copies/mL was observed. No significant differences were observed between patients on maraviroc and patients not including maraviroc as part of the new rescue HAART (data not shown). Seven patients started the rescue therapy having achieved undetectability after MCT (7/34, 20.59%); the percentage of patients on rescue therapy with undetectable viral load during follow-up is shown in Figure 3; 15/16 patients (93.75%) achieved undetectability (limit of detection: 40 HIV-RNA copies/mL) and 100% showed <200 copies/mL at week 24 of follow-up.

Discussion

The results of this study show that a clinical approach analysing the virological response to short-term maraviroc exposure could

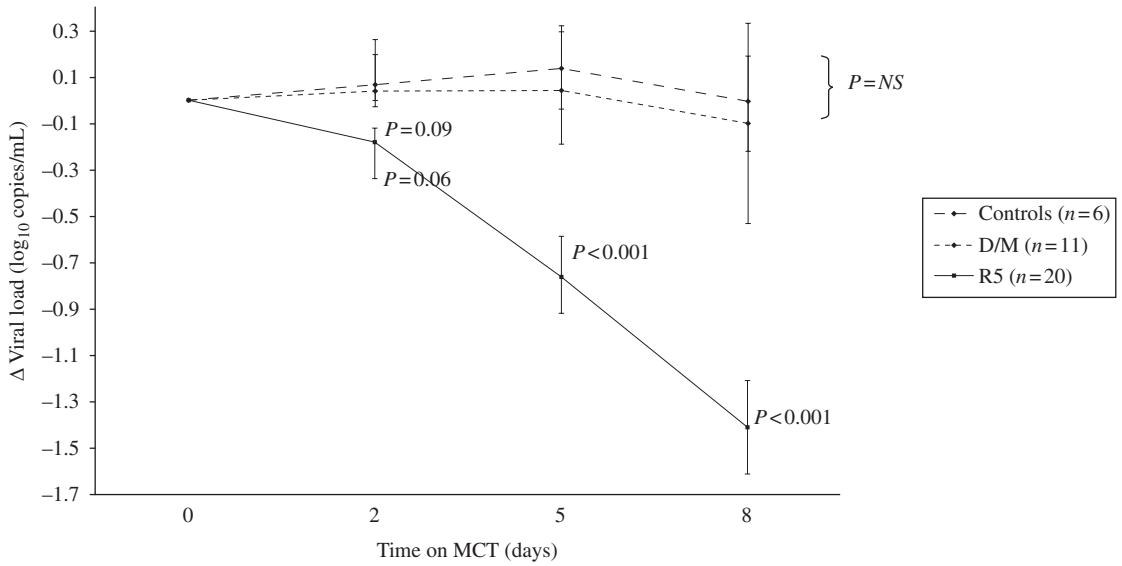

Figure 1. Mean viral load evolution in the control group and MCT group patients during MCT according to the Trofile® result, not including patients with a non-reportable result of Trofile®. Viral load evolution during MCT is expressed as mean and 95% CI. No significant viral load reduction was observed in control group patients during MCT; similar viral load evolution was observed in patients with a dual/mixed (D/M) Trofile® result. However, a progressive viral load reduction during MCT was observed in patients with an R5 Trofile® result. On day 2 of MCT, differences in viral load reduction between R5 and D/M patients were almost significant ($P=0.06$; Mann–Whitney *U*-test) and differences in viral load reduction between R5 and the control group were also almost significant ($P=0.09$; Mann–Whitney *U*-test). Significant differences in viral load reduction on days 5 and 8 during MCT were achieved between R5 patients and the other groups (control and D/M, $P<0.001$; Mann–Whitney *U*-test).

Table 2. Correlation between Trofile® and MCT

Result of Trofile®/MCT	Number of patients	Baseline mean viral load (log_{10} copies/mL)
R5/MCT+	19	4.19 (2.47–5.62)
D/M/MCT−	10	4.48 (2.43–6.03)
R5/MCT−	1	4.60
D/M/MCT+	1	4.19
Non-reportable/MCT+	3	2.49 (1.94, 2.63, 2.92)
Non-reportable/MCT−	0	

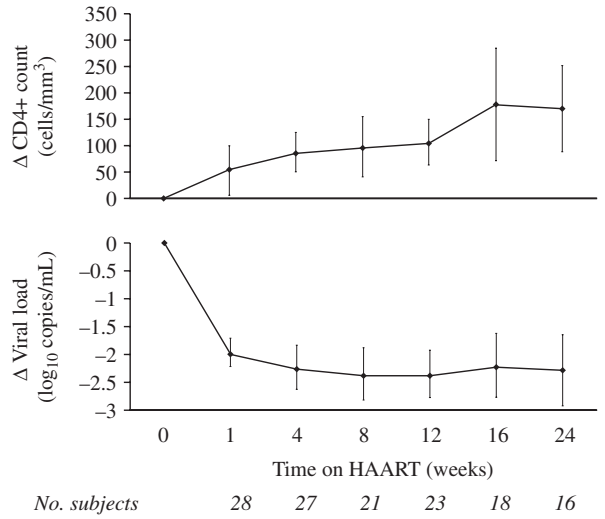

Figure 2. Mean CD4+ gain and viral load reduction in MCT group patients once rescue therapy with or without maraviroc was started after MCT, up to week 24. Values at each timepoint are expressed as mean and 95% CI. A progressive CD4+ increase and a viral load reduction of >2 log_{10} HIV-RNA copies/mL was observed during the follow-up.

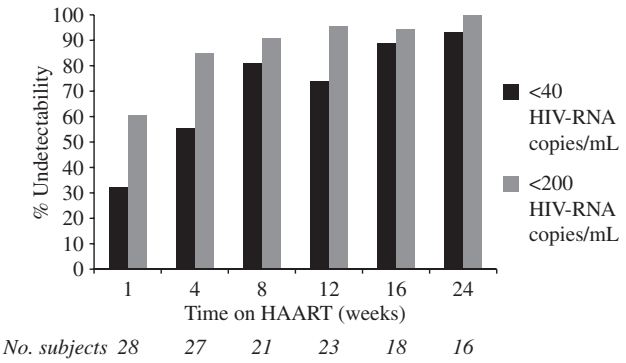

Figure 3. Percentage of patients in the MCT group with an undetectable viral load (black, <40 HIV-RNA copies/mL; grey, <200 HIV-RNA copies/mL) at each timepoint, once rescue therapy was started after MCT. After 12 weeks on rescue therapy ($n=23$), nearly 75% of patients achieved undetectability and >95% had a viral load of <200 HIV-RNA copies/mL. Up to week 24, 93.8% of patients achieved a viral load of <40 HIV-RNA copies/mL and 100% of patients achieved a viral load of <200 HIV-RNA copies/mL ($n=16$).

be an effective, low-cost and fast strategy to decide the potential indication for this CCR5 antagonist.

In our study, global concordance between MCT and Trofile® was 29/31 (93.5%). Four patients showed a significant viral load reduction despite being reported as D/M (one case) or non-reportable (three cases) by Trofile®. Thus, from our group of patients, 20/34 (58.8%) fulfilled criteria to receive maraviroc according to the Trofile® result; compared with 23/34 (67.6%) who received treatment with maraviroc based on the positive result of the MCT.

The significant virological response observed in patients with non-reportable Trofile® results could be explained because the tropism of HIV variants in these patients was probably R5, but due to the low viral load the tropism could not be assayed. Despite a global concordance of >90% between MCT and Trofile®, discordance between the two methods was observed in two patients; when tropism was assayed by another modified phenotypic method[10] in these patients, the result was coincident with MCT in both of them.

One potential limitation of our study is the unknown long-term consequences of this functional or true monotherapy with maraviroc. However, in our study, once rescue therapy was started no virological rebound was observed, mean CD4+ gain was >100 cells/mm^3, mean viral load reduction was >2 log_{10} HIV-RNA copies/mL and 100% of patients achieved a viral load <200 HIV-RNA copies/mL, at least after 24 weeks of follow-up. Emergence of detectable X4 virus is a concern related to this maraviroc exposure in patients with R5 virus; however, Fätkenheuer *et al.*[11] showed that when a monotherapy with maraviroc was explored in 63 naive HIV-infected patients over 10 days, this phenomenon was observed in only 2/63 patients and the X4 virus was not a mutated variant from an R5 virus but an X4 virus present in reservoirs before being exposed to maraviroc. Westby *et al.*[12] have reported similar results. In addition, new drugs active against the X4 virus were administered after MCT. The use of maraviroc in D/M patients during MCT could be related to worse immunovirological evolution once rescue therapy was started. However, our results suggest that MCT is a safe approach and MCT patients are not at a higher risk of virological failure or immunological impairment, although this should be confirmed with long-term follow-up (ongoing). Moreover, although in a different scenario, a recent study by Saag *et al.*[13] showed that maraviroc treatment in non-R5 HIV-infected patients is related to a significant CD4+ increase despite absence of virological benefit.

It remains unclear whether an 8 day follow-up and a decrease of 1 log_{10} HIV-RNA copies/mL is enough to consider the MCT 'positive', but in the study by Fätkenheuer *et al.*[11] a median decrease of 1.6 log_{10} HIV-RNA copies/mL was achieved after 10 days of monotherapy in naive patients; in that study, the efficacy of maraviroc in R5-tropic virus was assessed through a clinical test, as we did in this approach. Moreover, previous 14 day monotherapy with vicriviroc, another CCR5 antagonist, has been described and a median viral load decrease of $\sim$1 log_{10} HIV-RNA copies/mL was achieved.[3,4]

In conclusion, a clinical approach such as MCT evaluating the virological response to short-term exposure to maraviroc could be an additional strategy to decide the potential indication for the use of CCR5 antagonists.

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Transparency declarations

None to declare.

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Keywords: Tropism assay; maraviroc; clinical approach.

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Abstract: Enhanced sensitivity Trofile® (ES-Trofile®) is the most frequently used technique to assay HIV tropism. A clinical approach to predict CCR5-antagonists efficacy, based on the virological response to a short-term maraviroc exposure (Maraviroc Clinical Test, MCT), has been recently reported. We compared the results of ES-Trofile® with MCT in 47 HIV-infected patients, and a global discordance around 15% was observed between the phenotypic method and the clinical approach. Discordance results were mainly found in patients with an ES-Trofile® reported as dual/mixed. These provocative results might have important clinical implications and should be considered in order to accurately prescribe treatment with CCR5-antagonists.

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DISCORDANCE RATES BETWEEN TROFILE® TEST AND SHORT-TERM VIROLOGICAL RESPONSE TO MARAVIROC

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Abbreviations: enhanced sensitivity Trofile® (ES-Trofile®), CXCR4 (X4), maraviroc (MRV), maraviroc clinical test (MCT), CCR5 (R5), combined antiretroviral therapy (cART), dual/mixed (D/M)

ABSTRACT

Enhanced sensitivity Trofile® (ES-Trofile®) is the most frequently used technique to assay HIV tropism. A clinical approach to predict CCR5-antagonists efficacy, based on the virological response to a short-term maraviroc exposure (Maraviroc Clinical Test, MCT), has been recently reported. We compared the results of ES-Trofile® with MCT in 47 HIV-infected patients, and a global discordance around 15% was observed between the phenotypic method and the clinical approach. Discordance results were mainly found in patients with an ES-Trofile® reported as dual/mixed. These provocative results might have important clinical implications and should be considered in order to accurately prescribe treatment with CCR5-antagonists.

Keywords: tropism assay, maraviroc, clinical approach.

Current validated assay to test HIV tropism is Trofile®, by Monogram BioSciences (Whitcomb et al, 2007). An enhanced sensitivity Trofile® (ES-Trofile®) with a greater capability to detect minor populations of CXCR4 (X4)-tropic virus is now available (Reeves et al, 2009). However, limitations related to this technique make necessary to explore additional methods to test HIV tropism. A clinical approach (Maraviroc Clinical Test, MCT) has been recently reported (Genebat et al, 2009), showing that the virological response to a short-term maraviroc (MRV) exposure could predict the indication for CCR5 (R5)-antagonists use. Although in this previous study a high correlation between MCT and ES-Trofile® was observed, the percentage of discordant results has increased when the number of patients has been increased. Thus, the aim of this study was to analyze discordance rates between the tropism result reported by ES-Trofile® and the result of MCT.

Between July 2008 and March 2010, 47 asymptomatic and treatment experienced HIV-infected patients with persistently detectable viral load attended at the Infectious Diseases Service, Virgen del Rocio University Hospital (Seville, Spain), started an 8 days monotherapy with MRV (MCT) as previously reported (Genebat et al, 2009). Compared with the previous study, only patients with viral load above 1000 HIV-RNA copies/mL were considered: 33/47 patients (70.2%) were receiving no antiretroviral therapy (cART), because they had self-abandoned cART or remained under supervised treatment interruption (real MRV monotherapy during MCT), while 14/47 (29.8%) subjects were on a failing cART and MRV was added for 8 days to this therapy (functional MRV monotherapy during MCT). MCT was considered positive if a reduction $\geq$ 1 $\log_{10}$ HIV-RNA copies/mL was achieved or viral load was undetectable (< 40 HIV-RNA copies/mL) after 8 days of MRV

exposure. An ES-Trofile® was performed from blood samples the same day starting MCT (90%) or, if not possible, in samples obtained not more than 12 weeks before starting MCT. The result of MCT was compared with the tropism assay reported by ES-Trofile®.

Baseline characteristics of the patients were: 36 (76.6%) were males, 18 (38.3%) showed hepatitis C virus coinfection and 13 (27.7%) had developed an AIDS-defining event (CDC stage C). Baseline median [interquartile range (IQR)] age was 43 [37 – 47] years, time since HIV diagnosis was 17 [12 – 20] years, viral load 4.71 $\log_{10}$ HIV-RNA copies/mL [4.19 – 5.07] and $CD4^+$ cell count 221 [92 – 431] cell/mm^3. Adherence to therapy during MCT was self-reported by patients and estimated through Pharmacy registers, being 100% in all the patients.

Results of ES-Trofile® were: 29/47 (61.7%) patients were reported to be infected by R5-tropic HIV variants and 17/47 (36.2%) by dual/mixed (D/M) tropic virus; non-reportable result was reported in 1/47 patients (2.1%) and this patient achieved undetectability after MCT. As shown in figure 1a, mean viral load reduction > 1.6 $\log_{10}$ HIV-RNA copies/mL was observed during MCT in 26/29 patients with R5-tropic virus according to ES-Trofile®. On the other side, as shown in figure 1b, no viral load modification was observed in 13/17 patients with an ES-Trofile® reported as D/M. However, 3/29 (10.3%) patients with R5-tropic virus showed no viral load modification after MCT (figure 1a), while 4/17 (23.5%) patients with D/M virus experienced a viral reduction > 1.5 $\log_{10}$ HIV-RNA copies/mL (figure 1b); hence, global discordance rate between MCT and ES-Trofile® was 7/46 (15.2%). Discordance rate was higher in patients reported as D/M compared with patients with tropism reported as R5, although no statistically

significant (23.5% vs 10.3%, respectively; p = 0.22, Chi-square test). Three patients with R5-tropic virus and no viral load reduction after MCT started a MRV-sparing cART, according to MCT result. Immunovirological evolution of these patients after MCT is shown in figure 2a; two of them remain with undetectable viral load after 36 and 48 weeks, respectively, while the other patient achieved a viral load reduction > 3 $\log_{10}$ HIV-RNA copies/mL after 24 weeks. Three patients with D/M tropic virus and positive MCT started a MRV-containing regimen after MCT, associated to lamivudine plus abacavir. Immunovirological evolution of these patients is shown in figure 2b; viral load was undetectable after 12, 36 and 48 weeks, respectively. The other patient with positive MCT and an ES-Trofile® reported as D/M refused starting cART after MCT.

Results presented herein show unexpected rates of discordance between the short-term virological response to MRV and the results reported by ES-Trofile®. Although a high global concordance was achieved (nearly 85%), rates of discordance around 15% was observed. Hence, clinicians are currently taking decisions based on an assay that might not ensure the virological success, attending to our results.

Discordance observed in R5 patients could be explained because, despite maintaining the viral tropism as R5, MRV could be no effective due to changes mainly in the V3 loop that could be related with CCR5-antagonist resistance (Soulié et al, 2008); hence, despite an adequate tropism reported by ES-Trofile®, virological efficacy could be impaired. On the other side, discordance observed in patients with D/M tropic virus attending to ES-Trofile® was 23.5%, higher than in R5 patients; discordance in D/M patients could be explained due to the greater

sensitivity of ES-Trofile® to detect minor X4-tropic variants, that could lead to a D/M result when R5-tropic variants are predominant enough to exert a virological response.

Higher sensitivity to detect minor X4-tropic variants makes less likely to offer treatment with a CCR5-antagonist to patients with a minor representation of X4 virus. Moreover, ES-Trofile® is a qualitative test and clinicians only receive a categorical result (i.e. R5, X4, D/M), but percentage of X4 variants is not reported. Thus, our results show that establishing the clinically significant cut-off of X4-tropic variants is required to accurately consider some patients candidate to be treated with CCR5-antagonists.

Alternatives to ES-Trofile® have been suggested, like other phenotypic or genotypic assays (Chueca et al, 2009; Poveda et al, 2009; Trouplin et al, 2001). Genotypic methods are being used to assay HIV tropism with a greater frequency due to their availability, good correlation with phenotypic methods and simplicity (Recordon-Pinson et al, 2010). Discordance between MCT and genotypic approaches is unknown and should be evaluated in future studies. The use of the ultra-deep sequencing (Rozera et al, 2009) could quantify minor variants, but this assay is not available in routine clinical practice and has not been clinically validated to consider a patient candidate to be treated with a CCR5-antagonist.

In conclusion, our results show that ES-Trofile® does not completely correlate with the virological response to MRV after a short-term exposure, especially when D/M results are reported. These provocative results may have important clinical implications in routine clinical practice in order to accurately

prescribe CCR5-antagonists treatment. Potential discordance between MCT and genotypic methods should be determined in future studies.

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determine human immunodeficiency virus type 1 co-receptor tropism. Antimicrob. Agents Chemother. 51:556-575.

FIGURE CAPTIONS

Figure 1a. Viral load evolution in patients reported as R5 by ES-Trofile® (N = 29). Mean viral load evolution and confidence interval 95% (CI 95%) is shown in 26/29 patients that achieved a viral load reduction > 1 $\log_{10}$ HIV-RNA copies/mL during MCT. Three patients (Discord 1, Discord 2 and Discord 3) showed no viral load modification during MCT despite ES-Trofile® was reported as R5 (3/29 = 10.3%).

Figure 1b. Viral load evolution in patients reported as D/M by ES-Trofile® (N = 17). Mean viral load evolution and confidence interval 95% (CI 95%) is shown in 13/17 patients that showed no viral load modification during MCT. Four patients (Discord 4, Discord 5, Discord 6 and Discord 7) experienced a viral load reduction > 1.5 $\log_{10}$ HIV-RNA copies/mL despite ES-Trofile® was reported as D/M (4/17 = 23.5%).

Figure 2a. Immunovirological evolution of patients with R5 tropism according to ES-Trofile® and negative MCT, once the new cART was started after MCT. Total $CD4^+$T-cell increase and viral load reduction once cART was started after MCT, in patients with negative MCT and R5-tropic virus according to ES-Trofile®. A progressive $CD4^+$T-cell increase and viral load reduction is observed. Two of them achieved undetectability (< 40 HIV-RNA copies/ml) after 36 and 48 weeks under cART after MCT, respectively; the other patient remain with low-level detectable viral load after 12 weeks under cART, and a viral load reduction > 3 HIV-RNA copies/ml was achieved at this timepoint.

Figure 2b. Immunovirological evolution of patients with D/M tropism according to ES-Trofile® and positive MCT, once the new cART was started after MCT. Total $CD4^+$T-cell increase and viral load reduction once cART was started after MCT, in patients with positive MCT and D/M-tropic virus according to ES-Trofile®. A progressive $CD4^+$T-cell increase and viral load reduction is observed. All of them achieved undetectability (< 40 HIV-RNA copies/ml) after 12, 36 and 48 weeks under cART after MCT, respectively.

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Fig. 1a. Mean (CI 95%) viral load evolution in patients with ES-Trofile® reported as R5 (n = 29)

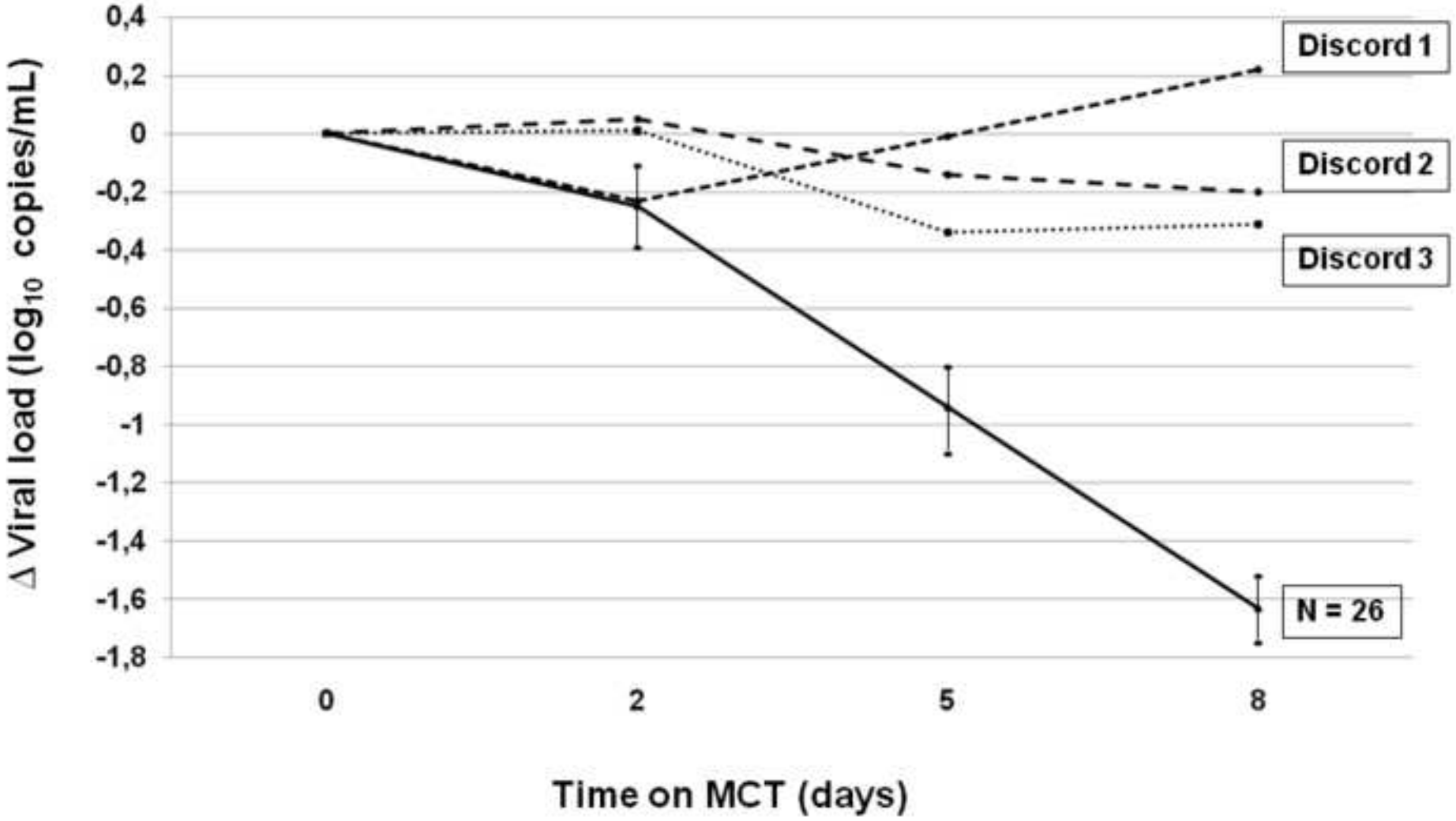

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Fig. 1b. Mean (CI 95%) viral load evolution in patients with ES-Trofile® reported as D/M (n = 17)

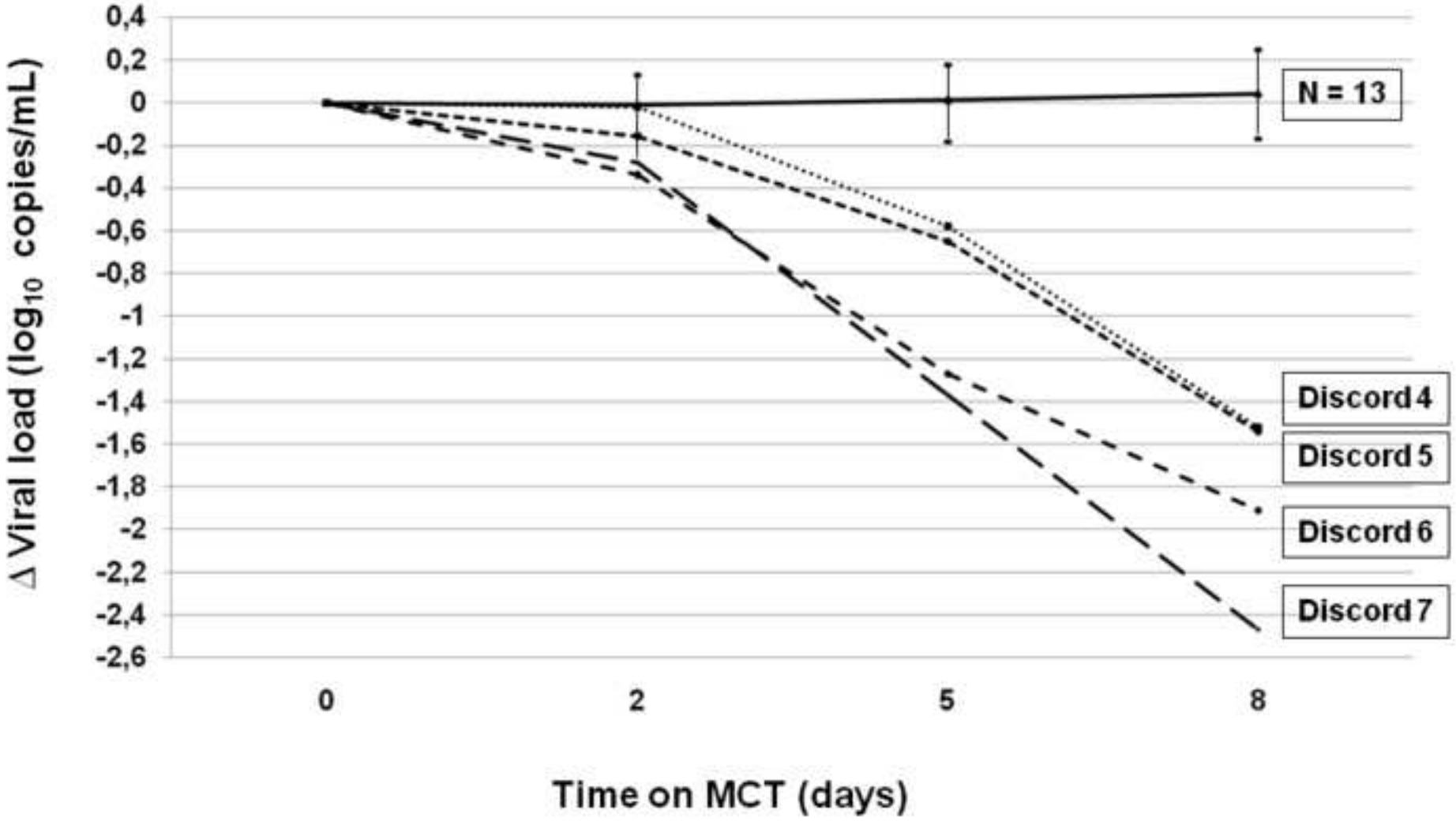

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Fig. 2a. Immunovirological evolution of patients with R5 tropism according to ES-Trofile® and negative MCT, once the new cART was started after MCT

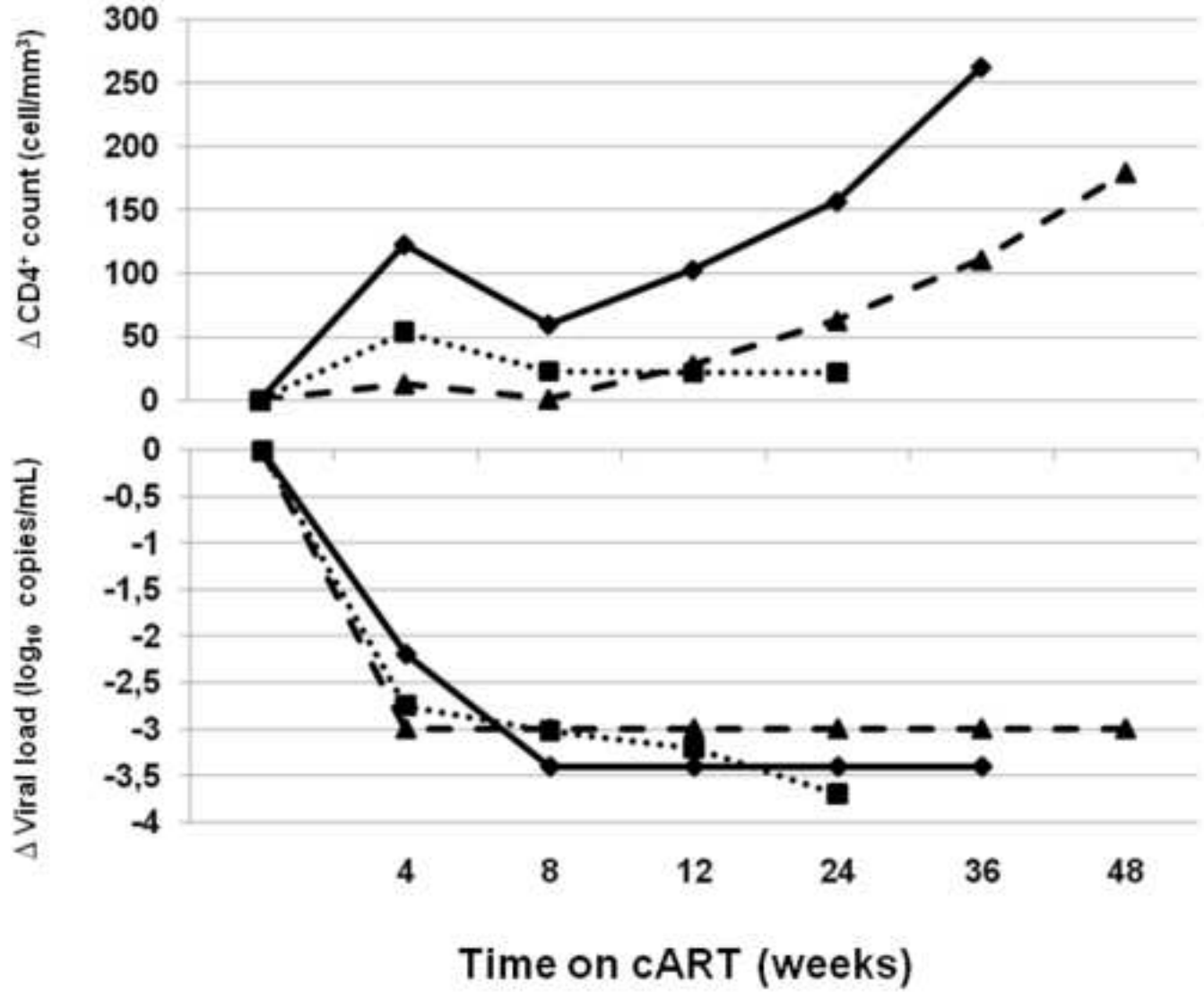

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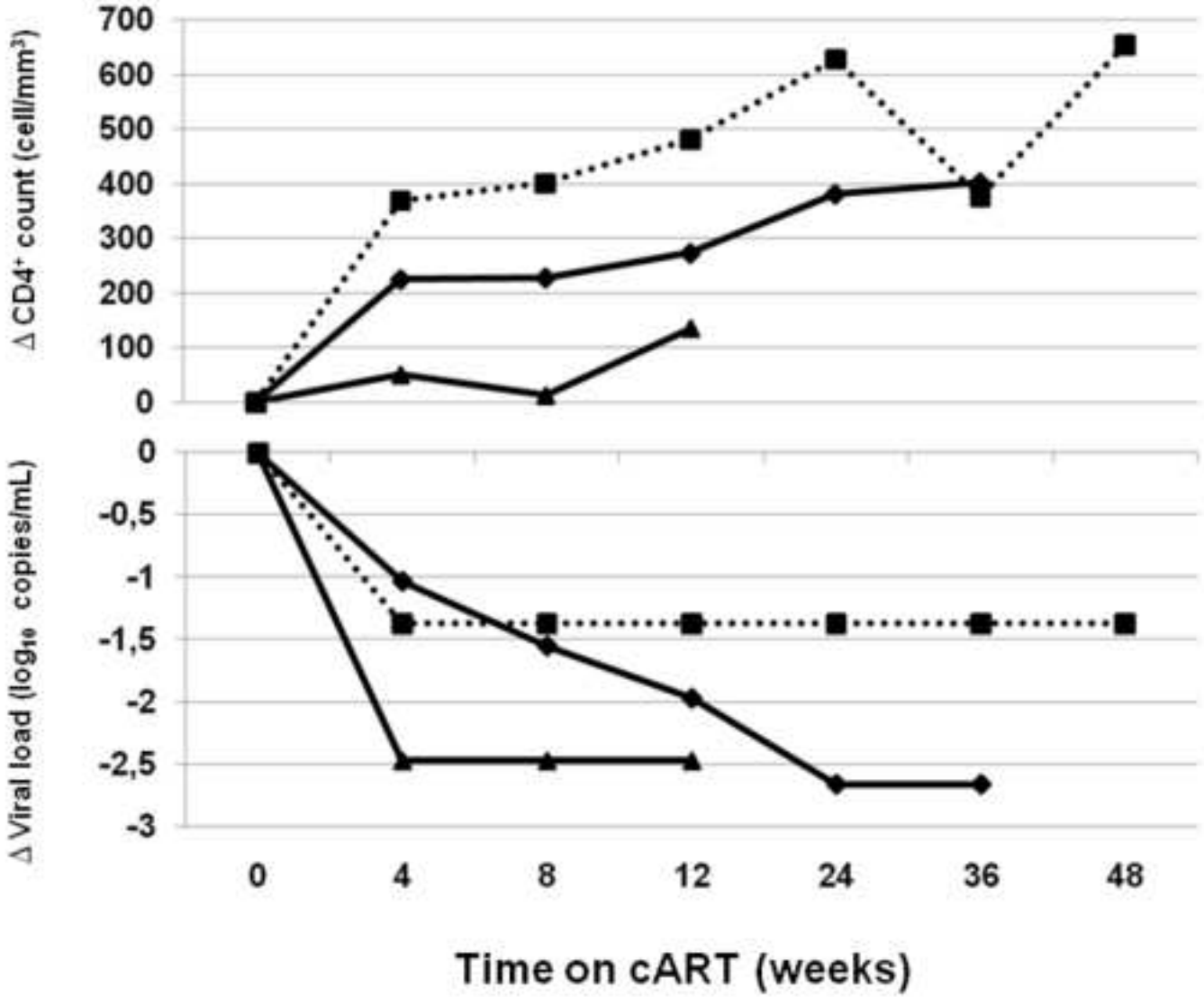
Fig. 2b. Immunovirological evolution of patients with D/M tropism according to ES-Trofile® and positive MCT, once the new cART was started after MCT



Long-Term Immunovirogical Effect and Tolerability of a Maraviroc-Containing Regimen in Routine Clinical Practice

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Abstract: *Objectives*: to analyze the long-term immunovirological effect and tolerability of a maraviroc-containing antiretroviral therapy in viraemic and pretreated HIV-infected patients with a high prevalence of hepatitis C virus (HCV) coinfection.

Methods: forty-six R5 HIV-infected patients (48% HCV-coinfected) started a maraviroc-containing antiretroviral regimen, including patients with multidrug resistant virus and patients after first virologic failure. A retrospective study was performed, analysing percentage of patients with undetectable viral load, mean $CD4^+$ gain, liver enzymes, clinical events and treatment modification up to week 48.

Results: Raltegravir plus a boosted protease inhibitor was combined with maraviroc in 65.2% of the patients (mainly patients with multidrug resistant virus), while the coformulation lamivudine/abacavir was combined with maraviroc in 26.1% (all of them patients after first virologic failure). After 48 weeks on maraviroc-containing regimen, 96.3% of the patients had achieved undetectability and a mean $CD4^+$ count increase of 151 cells/mm^3 was observed. Liver enzymes did not increase along the follow up. One patient died after 24 weeks follow up due to heroin overdose. One patient developed a non-Hodgkin lymphoma after 36 weeks follow up, despite undetectable viral load and significant $CD4^+$ increase was achieved (the only AIDS-defining event observed). Treatment modification was performed in 19.6% of the patients: 77.7% of them experienced a treatment simplification and only 1/46 suspended maraviroc.

Conclusions: maraviroc-containing regimen is long-term effective and well tolerated in HIV-infected patients in routine clinical practice and in different clinical scenarios.

Keywords: Hepatitis C virus, HIV, maraviroc, routine clinical practice.

INTRODUCTION

In the last two years new antiretrovirals (ARV) have been commercialized: two of them have a novel mechanism of action, maraviroc (MRV) and raltegravir (RGV); another two, darunavir (DRV) and etravirine (ETV), are new generation drugs of the previous families (from now on, novel ARV will be considered MRV, RGV, DRV and ETV). All of them have shown an independent immunovirological benefit in rescue therapy in different studies [1-4].

MOTIVATE studies [1] showed the efficacy of MRV in CCR5 (R5) HIV-infected patients. In MOTIVATE studies, patients were classified as R5 attending to the only tropism assay available at that time -first generation Trofile® [5]-, rates of hepatitis C virus (HCV) coinfection and malignancies at baseline were unknown and the use of other novel ARV was not allowed. A recent study showed the efficacy of MRV plus RGV and ETV in patients after multiple failing regimens, but only 8 patients were HCV-coinfected [6]. Hence, potential increased hepatotoxicity after MRV administration in HCV-coinfected population and potential interactions when combining MRV with other ARV in different clinical scenarios apart from rescue therapy are unknown.

HIV-infected population attended in our Service is characterized by high rates of HCV-coinfection. Thus, the aim of this study was to analyze the immunovirological effect and tolerability of a MRV-containing combined antiretroviral therapy (cART) in routine clinical practice.

MATERIAL AND METHODS

Patients

In this observational retrospective study, 46 consecutive HIV-infected patients attended at the Infectious Diseases Service at Virgen del Rocio University Hospital began a MRV-containing cART between January 2008 and January 2010. All of them had been previously exposed to ARV, HIV tropism was reported as R5 (see below) and plasma viral load had been persistently detectable (above 50 HIV-RNA copies/mL) at least during the last 3 months. Patients were clinically evaluated at baseline and at weeks 1, 4, 8, 12, 16, 24 and every 12 weeks since then, analysing $CD4^+$ count,

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HIV-RNA plasma levels and liver enzymes. To be included in this analysis, patients should have received at least one dose of the new MRV-containing cART. The study was censored on April 2010 and 48 weeks intention to treat analysis was performed.

This population starting a MRV-containing cART included patients on persistent virologic failure (exposed to multiple ARV, including enfuvirtide, and with major resistance associated mutations to the three classical ARV families) and patients previously exposed to ARV but not on persistent virologic failure (patients on supervised treatment interruption and patients after first virologic failure).

Determination of HIV-1 Co-Receptor Usage

To determine the coreceptor usage, two different methods were used: 1) First generation Trofile® [5] was used between January and July 2008: circulating virus was tested from the plasma of patients using the PhenoSense HIV Entry assay for coreceptor tropism (Monogram Biosciences Inc., South San Francisco, CA). 2) Maraviroc Clinical Test (MCT) was used between July 2008 and January 2010 to consider patients candidate to receive MRV therapy, as previously reported [7].

Efficacy and Tolerability

Efficacy was evaluated in every time point analysing the proportion of patients with virological response, i.e. viral load below detectable threshold (< 50 HIV-RNA copies/mL). Mean evolution of $CD4^+$ T-cell was also estimated. Tolerability to this new regimen was evaluated by the rate of abandon due to adverse effects and by the liver enzymes evolution (aspartate aminotransferase and alanine aminotransferase, AST and ALT from now on).

Laboratory Analysis

Plasma HIV-1 RNA was measured in fresh samples by a quantitative PCR with a detection limit of 50 HIV-RNA copies/mL (COBAS Ampliprep/COBAS Taqman HIV-1 test, Roche molecular systems, Basel, Switzerland) according to the manufacturers' instructions. Absolute number of $CD4^+$ T-cell was determined in fresh samples using the Epics XL-MCL (Beckman-Coulter Inc., California) flow cytometer, according to the manufacturer´s instructions. A qualitative PCR amplification was performed for plasma HCV RNA amplification (COBAS Amplicor, Roche Diagnosis, Barcelona, Spain), with a detection limit of 15 IU/mL. Plasma samples were tested for hepatitis B virus (HBV) surface antigen (HBsAg) using HBV-ELISA (Siemens Healthcare Diagnosis, USA).

Statistical Analysis

Continuous variables are expressed as median [interquartile range (IQR)] or mean [confidence interval 95% (CI 95%)], when appropriated. Categorical ones are expressed as total number of cases (percentage). Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS 17.0, Chicago, Illinois, USA).

RESULTS

Baseline characteristics of the patients are summarized in Table **1**. A boosted protease inhibitor (PI), mainly DRV, plus RGV were combined with MRV in 30/46 patients (65.2%); this cART based on novel ARV was mainly prescribed in patients on persistent virologic failure, because of the limited therapeutic options available for these patients. Lamivudine/abacavir (3TC/ABV) was combined with MRV in 12/46 patients (26.1%); finally, 4/46 patients (8.7%) received MRV combined with RGV alone due to severe lipodystrophy. Six patients were lost on follow up (6/46, 13%), all of them after 12 weeks on MRV-containing cART.

Table 1. Baseline Characteristics of the Patients (n=46)

Age (years)	43 [40-48]
Male sex (%)	33 (71.7)
Sexual transmission (%)	19 (41.3)
Non-sexual transmission (%)	27 (58.7)
CH-coinfection[a] (%)	23 (50)
$CD4^+$ count (cells/mm^3)	265 [172-374]
Viral load (log_{10} copies/mL)	4.3 [3.2 - 4.9]
Time HIV diagnosis (years)	17 [12-20]
Stage C, CDC[b] (%)	12 (26.1)
MCT[c] (%)	30 (65.2)
Malignancies (%)[d]	6 (13)
Resistance associated mutations	2 (1-7)
Persistent virologic failure (%)[e]	15 (32.6)

Values expressed as median [interquartile range (IQR)] and number of cases (%).
[a]Chronic hepatitis coinfection: Positive PCR for hepatitis C virus (22/46, 47.8%) or positive hepatitis B surface antigen (1/46, 2.2%).
[b]Centre for Diseases Control.
[c]Patients selected to receive MRV therapy based on MRV Clinical Test.
[d]Malignancies: systemic mastocytosis, breast cancer, cutaneous basal cell carcinoma, cervix carcinoma, non-Hodgkin lymphoma, cervix plus nasopharyngeal carcinoma in the same patient.
[e]Patients exposed to multiple ARV, failure to enfuvirtide-based cART and with at least one major resistance associated mutation to nucleoside retrotranscriptase inhibitors, non-nucleoside retrotranscriptase inhibitors and protease inhibitors.

Percentage of patients who reached undetectability at every time-point is shown in Fig. (**1**). After 48 weeks follow up 26/27 (96.3%) of the patients achieved a viral load below detectable limit, and 100% showed a viral load below 200 HIV-RNA copies/ml. $CD4^+$ count evolution is shown in Fig. (**2**). A mean $CD4^+$ increase of 151 cell/mm^3 was achieved after 48 weeks follow up. Liver enzyme levels (AST and ALT) did not increase during the follow up, as shown in Fig. (**3**).

Treatment modification was performed in 9/46 patients (19.6%). Seven of them (7/9, 77.8%) experienced a treatment simplification, being suspended the boosted PI and starting a PI-sparing regimen together with MRV. The other 2/9 (22.2%), both exposed to MRV plus 3TC/ABV, developed an adverse event in the first week with the new

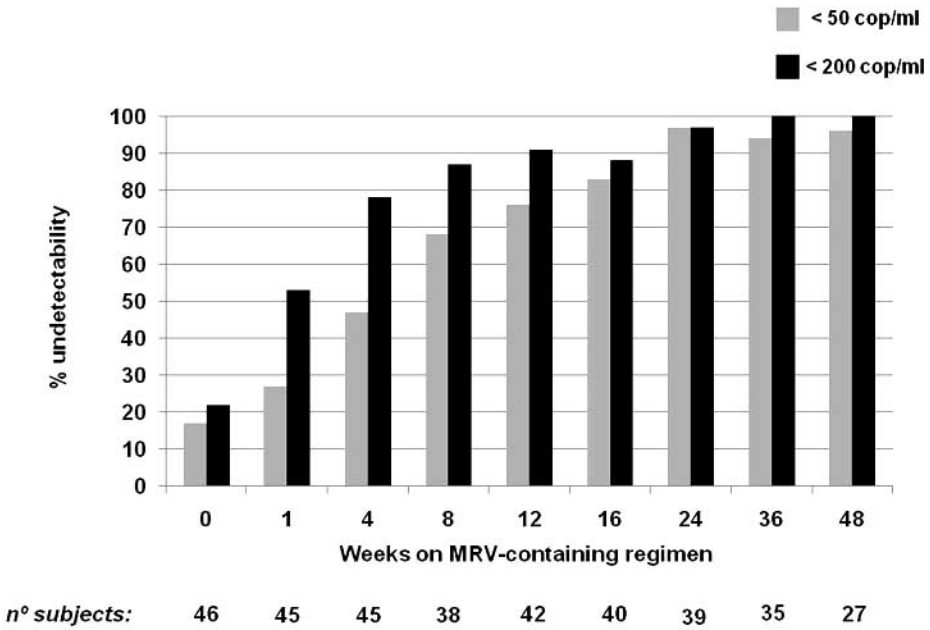

Fig. (1). Virological response: percentage of patients with an undetectable viral load (grey, < 50 HIV-RNA copies/mL, black <200 HIV-RNA copies/mL) in every timepoint, once MRV-containing cART was started. Notice that at baseline 17% of the patients had an undetectable viral load; these patients achieved undetectability during MCT and viral load was < 50 HIV-RNA copies/ml once cART was started. After 24 weeks of follow up (n=39), 38/39 patients (97%) had achieved undetectability and only 1/39 patients (3%) had a viral > 200 HIV-RNA copies/mL. Up to week 48 (n=27), 96.3% of patients had achieved < 50 HIV-RNA copies/mL and 100% a viral load < 200 HIV-RNA copies/mL.

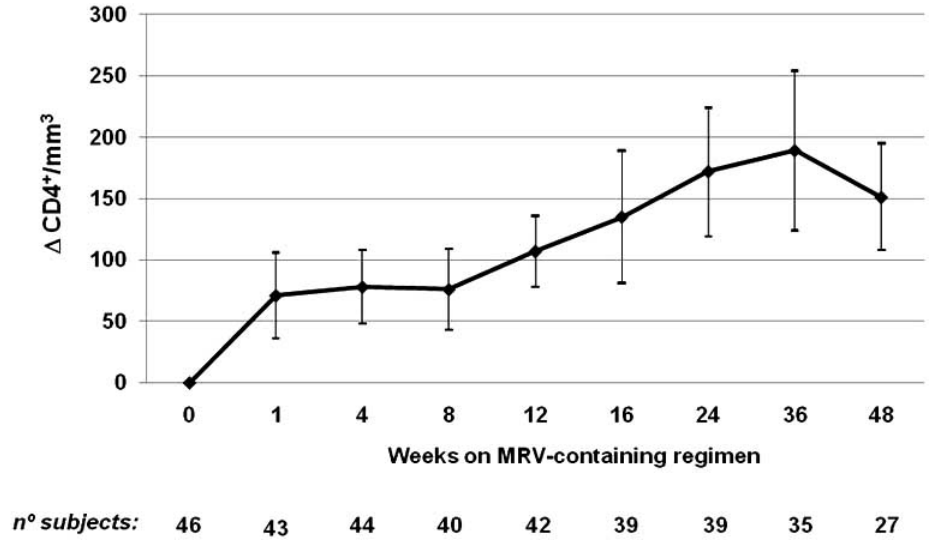

Fig. (2). Immunological response: mean [CI 95%] $CD4^+$ gain once MRV-containing cART was started, up to week 48. A progressive $CD4^+$ increase is observed along the follow up.

cART. The first one developed fever and rash probably related to ABV, despite HLAB*5701 was negative, and treatment was modified to tenofovir/emtricitabine (TDF/FTC) plus MRV. The second one developed an acute hepatitis and cART was suspended; this patient recognized a huge alcohol intake, but the cause of the hepatitis could not be established. Once transaminases returned to baseline values, a new cART not including MRV was started. In summary, only 1/46 patient (2.2%) suspended MRV along the observational period due to an acute hepatitis probably not related to MRV.

Clinical events were developed in 4/46 patients (8.7%). One patient died after 24 weeks follow up due to heroin overdose. One HCV-coinfected patient with previous Child C stage liver cirrhosis developed liver-related encephalopathy after 4 weeks with the new regimen, experiencing a good evolution with standard treatment. One patient developed a monometameric herpes-zoster after 24 weeks, with an excellent clinical response to acyclovir. Finally, one patient developed a non-Hodgkin lymphoma after 36 weeks of follow up; this patient had been in persistent virologic failure for many years and experienced a $CD4^+$ gain above 200 cell/mm^3 from baseline once MRV-

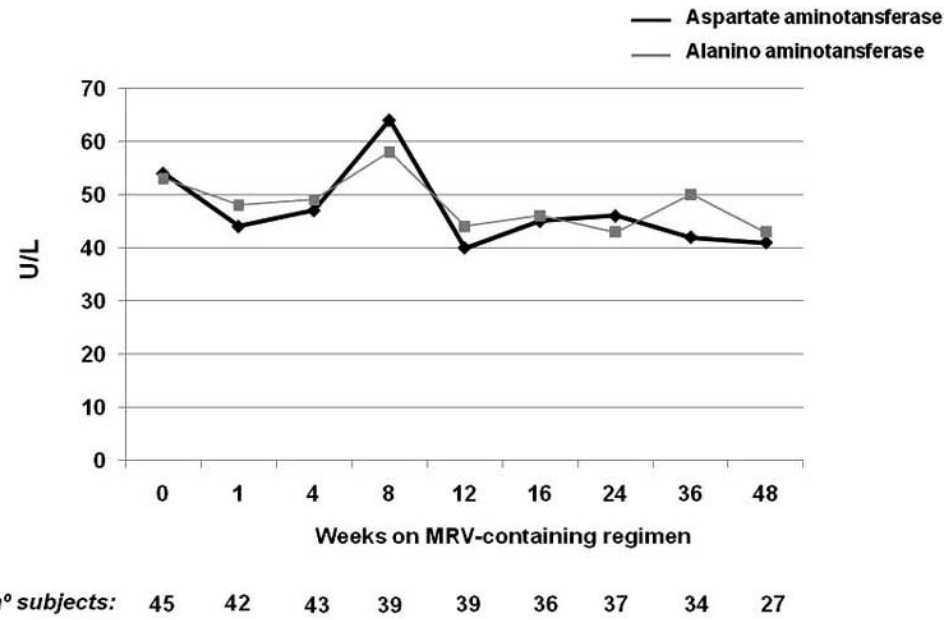

Fig. (3). Hepatotoxicity: mean liver enzymes (AST and ALT) evolution was analyzed to assess potential increased hepatotoxicity of MRV-containing cART in a population with a high prevalence of HCV-coinfection. No significant modification in liver enzymes was observed during the observational period.

containing cART was started. Viral load was undetectable when the clinical event occurred in all of them and MRV-containing cART was maintained in every patient.

Concomitant drugs were used together with MRV-containing cART in 5/46 patients (10.9%): two patients were taking tuberculostatic drugs, two other patients were on chemotherapy and one patient was taking antipsychotic drugs. No increased toxicity or clinically relevant drug-drug interactions were observed.

DISCUSSION

Our results show that a MRV-containing cART combined with different ARV (including novel ARV) and in different clinical scenarios is long-term effective and well tolerated in an HIV-infected cohort with a high prevalence of HCV-coinfection.

Despite limitations related to its observational design, our study adds relevant novelties: different clinical scenarios were considered and different ARV were added to MRV (conventional *vs* novel ARV-based cART); 50% of the patients showed chronic HCV or HBV coinfection and nearly 15% had developed malignancies at baseline; finally, results presented herein show the routine clinical practice avoiding Clinical Trials restrictions.

As previously mentioned, MOTIVATE studies showed the immunovirological benefit of MRV in the context of a salvage therapy. In addition, in these studies the simultaneous use of other novel ARV was not permitted [1]. However, attending to current guidelines MRV should be considered from the first virologic failure [8] and not only once multidrug resistant virus have emerged, a context with a greater probability of X4-tropic virus emergence [9] in which MRV will not be useful. In Spain, it has been reported that CCR5-tropic HIV-1 virus is prevalent in nearly 70% of treatment-experienced patients [10], showing that MRV is active in most of these patients. Besides, novel cART combinations need to be explored in routine clinical practice, as shown when TDF plus didanosine were used together and unexpected high toxicity and immunovirological impairment were observed [11, 12]. Efficacy and tolerability of MRV in routine clinical practice and in a population with a high prevalence of HCV-coinfection has not been previously explored, as we do in the present study.

Nozza *et al.* have recently shown the efficacy of MRV plus RGV and ETV as a salvage therapy in 28 HIV-infected patients, but only 8 patients showed HCV-coinfection [6]. In contrast, our study shows different scenarios in which a MRV-containing cART was prescribed: patients on persistent virologic failure, in whom MRV was combined with novel ARV (RGV plus a boosted PI, mainly DRV, instead of ETV) because therapeutic options were limited; and patients not on persistent virologic failure, in whom MRV was combined with more simplified regimens, an scenario not considered by Nozza *et al.* Global long-term immunovirological outcome obtained in our study is excellent, achieving nearly 97% of undetectability after 48 weeks and a progressive $CD4^+$ gain.

The mechanism of action of MRV, not acting against cellular enzymes, could reduce the possibility of developing serious adverse events and drug-drug interactions. On the other hand, blocking the CCR5 coreceptor has been reported to be associated with an increased susceptibility to certain infections [13]. Besides, other CCR5-antagonists have not been commercialized due to serious adverse events: vicriviroc was associated with the development of malignancies [14], while aplaviroc was related to severe hepatotoxicity [15]. In our study, only one patient suspended MRV and most treatment modifications were due to treatment simplifications. The only infectious event was a

monometameric herpes zoster and despite 15% of the patients had developed malignancies before starting MRV-containing cART and 50% of patients showed chronic hepatitis coinfection, no increased hepatotoxicity or malignancies relapse were observed. The only AIDS-defining event observed in our study was a non-Hodgkin lymphoma; this patient had been on persistent virologic failure for many years before starting MRV-containing cART, and viral load was undetectable at the moment in which the neoplasm was developed.

The excellent immunovirological data obtained with this MRV-based cART could be explained because: 1) patients were strictly followed because of the novelty of the new cART; hence, standard of care was improved in these patients; 2) apart from MRV, other novel ARV with a great antiviral activity were added in patients with multidrug resistant virus.

Most of the patients included in this study started MRV therapy being classified as R5 through MCT, an attractive method recently reported [7]. Although it was not an objective of this study, it is worthy to note that the immunovirological evolution of patients classified as R5 by MCT was similar than patients classified as R5 by first generation Trofile® (data not shown).

CONCLUSION

We conclude that a MRV-containing cART is long-term effective and well tolerated in an HIV-infected population with a high prevalence of HCV-coinfection and using different combinations of ARV.

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TRANSPARENCY DECLARATION

Conflict of interests: none to declare.

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DISCUSIÓN

La comercialización simultánea de cuatro nuevos fármacos antirretrovirales, unos con alta barrera genética y otros con novedosos mecanismos de acción, ha permitido que desde principios de 2008 estemos disfrutando de lo que se ha llamado una segunda era de TARGA, facilitando por un lado el rescate de pacientes en fracaso virológico y por otro la simplificación de regímenes antirretrovirales previos con importantes efectos secundarios.

Especialmente interesante por su mecanismo de acción extracelular es MRV, el único fármaco comercializado de la familia de los inhibidores de la entrada. En concreto, este fármaco ejerce su acción antirretroviral bloqueando el correceptor CCR5, un correceptor de quimiocinas fundamental para que se complete el proceso de entrada y fusión entre la proteína gp120 del VIH y el receptor CD4 del linfocito T, lo que permitirá finalmente que el material genómico del VIH penetre en la célula hospedadora. Sin embargo, además del CCR5, este proceso de entrada puede llevarse a cabo también a través del correceptor CXCR4, caso en el que MRV no sería eficaz. El uso de uno u otro correceptor varía a lo largo de la historia natural de la infección por VIH, siendo habitualmente cepas virales con tropismo R5 las que predominan en la infección aguda y durante los primeros años de infección crónica, para posteriormente emerger las cepas con tropismo X4. Por tanto, antes de indicar tratamiento con MRV es necesario conocer cuál es el correceptor empleado para completar este proceso de entrada, es decir, qué tropismo tienen las cepas virales de nuestro paciente. Para ello existen diferentes ensayos de tropismo: los métodos fenotípicos (Trofile® es el estándar de oro en la actualidad) son costosos, complejos y lentos, mientras que los genotípicos tienen alta sensibilidad para detectar cepas X4 pero tienen baja especificidad. Todo ello limita el uso de MRV en la práctica clínica.

Como alternativa a estos ensayos ya conocidos, en el primer objetivo de la presente tesis se planteaba una herramienta clínica (MCT) que fuese sencilla y rápida. Los resultados de esta aproximación clínica se correlacionaron con el ensayo fenotípico clínicamente validado y de referencia (Trofile®), observándose una concordancia global del 93.5%. Además, MCT permitió determinar la sensibilidad clínica a MRV de pacientes en fracaso virológico pero con cargas virales por debajo de las 1000 copias/ml, una situación en la que Trofile® no puede determinar el tropismo viral. Por tanto, en este estudio se demostró que la respuesta virológica a una exposición a corto plazo a MRV podría ser una alternativa poco costosa y rápida para seleccionar a pacientes candidatos a tratamiento con antagonistas del CCR5.

Desde entonces, la indicación clínica de MRV en nuestros pacientes se ha basado en el resultado de MCT, si bien antes de iniciar la misma se solicitaba el ensayo fenotípico. A tenor de nuestros resultados, y pese a una concordancia alta entre MCT y Trofile® en una aproximación inicial, las tasas de discordancia se vieron incrementadas a medida que el número de pacientes fue aumentando. La relevancia clínica de estas discrepancias es evidente, ya que podríamos estar indicando MRV en pacientes que no deberían recibirlo y viceversa. Por ello, nos planteamos analizar las tasas de discordancia entre ambos métodos, observando una discrepancia global mayor al 15%. Una posible explicación a esta elevada discrepancia es que los métodos fenotípicos tienden a aumentar la sensibilidad para detectar cepas con tropismo X4, de tal manera que Trofile® informa un resultado de tropismo como D/M si detecta al menos un 0.3% de cepas con tropismo X4. De esta manera, pacientes potencialmente candidatos a recibir MRV, no lo recibirían por la presencia de cepas minoritarias con tropismo X4, cuya relevancia clínica es desconocida hasta ahora.

Por último, la indicación de MRV está basada en los resultados de los estudios MOTIVATE, es decir, en el contexto de un ensayo clínico y en los que se desconocía la tasa de coinfección por VHC de los pacientes, además de tener limitado el uso de otros antirretrovirales de reciente comercialización. El tercer estudio abordado en esta tesis demuestra que el uso de MRV en la rutina clínica diaria, en un grupo de pacientes con elevada tasa de coinfección por VHC y asociado a diferentes antirretrovirales es eficaz desde un punto de vista inmunovirológico y seguro.

En resumen, los resultados presentados en esta tesis, de inmediata aplicación clínica, abren una puerta más para la determinación de la sensibilidad clínica a MRV, más allá de un mero resultado categórico de tropismo, y demuestran la eficacia y seguridad de MRV en su uso en la rutina clínica asistencial. Como consecuencia de los resultados aquí presentados, recomendamos el uso de MCT antes de indicar tratamiento con antagonistas de CCR5.

CONCLUSIONES

Como resultado de las publicaciones presentadas en la presente tesis doctoral, se desprenden las siguientes conclusiones:

- Una aproximación clínica que analiza la respuesta virológica a una exposición a corto plazo a MRV es una herramienta sencilla y efectiva para determinar la indicación de antagonistas de CCR5.

- Las tasas de discrepancia observadas entre MCT y Trofile® pueden tener relevancia clínica a la hora de prescribir con seguridad un TARGA que incluya antagonistas de CCR5.

- Un régimen antirretroviral con MRV es eficaz y seguro a largo plazo asociado a diferentes regímenes antirretrovirales, en diferentes escenarios clínicos y en una población con elevada tasa de coinfección por VHC.

ANEXO: OTRAS PUBLICACIONES GENERADAS DURANTE EL PROGRAMA MIR Y DESARROLLO DE LA TESIS

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A CD4$^+$ Cell Count <200 Cells per Cubic millimeter at 2 years After Initiation of Combination Antiretroviral Therapy Is Associated With Increased Mortality in HIV-Infected Individuals With Viral Suppression

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Objective: To determine the long-term impact of immunologic discordance (viral load <50 copies/mL and CD4$^+$ count ≤200 cells/mm^3) in antiretroviral-naive patients initiating combination antiretroviral therapy (cART).

Methods: Our analysis included antiretroviral-naive individuals from a population-based Canadian Observational Cohort that initiated cART after January 1, 2000, and achieved virologic suppression. Multivariable Cox proportional hazards regression was used to examine the association between 1-year and 2-year immunologic discordance and time to death from all-causes. Correlates of immunologic discordance were assessed with logistic regression.

Results: Immunologic discordance was observed in 19.9% (404 of 2028) and 10.2% (176 of 1721) of individuals at 1 and 2 years after cART initiation, respectively. Two-year immunologic discordance was associated with an increased risk of death [adjusted hazard ratio = 2.69; 95% confidence interval (CI): 1.26 to 5.78]. One-year immunologic discordance was not associated with death (adjusted hazard ratio = 1.12; 95% CI: 0.54 to 2.30). Two-year immunologic discordance was associated with older age (aOR per decade = 1.23; 95% CI: 1.03 to 1.48), male gender (aOR = 1.86; 95% CI: 1.09 to 3.16), injection drug use (aOR = 2.75; 95% CI: 1.81 to 4.17), and lower baseline CD4$^+$ count (aOR per 100 cells = 0.24; 95% CI: 0.19 to 0.31) and viral load (aOR per $\log_{10}$ copies/mL = 0.46; 95% CI: 0.33 to 0.65).

Conclusions: Immunologic discordance after 2 years of cART in antiretroviral-naive individuals was significantly associated with an increased risk of mortality.

Key Words: antiretroviral therapy, discordance, HIV, immunologic nonresponders, mortality, virologic suppression

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All additional research team members are listed at the end of the article in Appendix I.

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INTRODUCTION

Advances in the pharmacotherapy of HIV infection have resulted in important reductions in disease-related morbidity and mortality, mostly attributable to viral suppression and resultant immune reconstitution.[1,2] However, immunologic discordance, in which an increase in CD4$^+$ lymphocyte count does not accompany virologic suppression after the start of combination antiretroviral therapy (cART), has been reported

High Levels of CD57+CD28- T-Cells, Low T-Cell Proliferation and Preferential Expansion of Terminally Differentiated CD4+ T-Cells in HIV-Elite Controllers

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Abstract: The study of clinical and demographic characteristics related to virus control and disease progression in patients who spontaneously control HIV vireamia (HIV-controllers) is of major interest. A particular cause of HIV control has not been found and the scenario could be partially explained by special homeostatic and immunological features. In this study, CD57+CD28- phenotype, T-cell activation and levels of proliferating T-cells in elite-controllers were studied in relation to spontaneous virus control. In HIV-controllers, 9% were AIDS-diagnosed and there was a high proportion of women. In elite-controllers, high T-cell CD57+CD28- phenotype and activation levels were found and, interestingly, there was a low proliferation of total and naïve T-cells and a high proliferation of the CD4+ T_{EM}RA subset. Low T-cell proliferation and preferential expansion of terminally differentiated effector T-cell subsets could be an important factor for virus control in elite-controllers.

Keywords: HIV, elite-controllers, immunosenescence, activation, CD57+, proliferation, T_{EM}RA.

INTRODUCTION

In HIV infection, an unsual group of patients (1-5%) exists who are able to maintain very low levels of plasma viraemia for long periods of time in the absence of antiretroviral treatment. The patients who maintain plasmatic viraemia below detection limits are known as HIV-controllers or elite-controllers [1-4]. Understanding the mechanisms of virus control in these special patients will help in the design of immunotherapies to deal with HIV infection better. Due to this, it is very important to increase the available information from this particular group of patients for which a number of questions remain to be answered. The natural history of HIV-controllers is not fully understood; for instance, it is not well known whether at some point they will lose the capability to control the virus, or what proportion of these patients will experience some AIDS defining illnesses.

Several immunological mechanisms have been postulated to explain the spontaneous control of viraemia exhibited by these patients. These include a strong HIV-specific CD8 T-cell response associated with the presence of different HLAs [5-9] and polyfunctional CD4-T cells [10-12]. Independent from the host, the presence of a defective virus was anticipated as an important mechanism of control [13]. Nevertheless, fully competent viruses isolated from elite-controllers have been reported [14, 15]. Hence, in this scenario, an exclusive cause of HIV control has not been found [16]. Besides, this "preserved" immune status is in contrast with the high immune activation levels found in elite-controllers [17]. The effects of long-term immune activation are one of the main causes of HIV disease progression independent of viral load [18-20], although these effects are unknown in controllers. Generally, in HIV-infected patients, the permanent immune activation has been associated with increased proliferation and T-cell differentiation [21, 22]. This immune deregulation is characterized by elevated levels of T-cells with a highly differentiated phenotype, unable to proliferate. These cells express CD57, a marker that has been associated with replicative senescence [23, 24]. This immune exhaustion eventually leads to a state of disease progression [22]. As opposed to these beliefs, recent studies have shown how CD57+ cells express high levels of cytolytic enzymes and are able to proliferate after stimulation, while arguing against the role of this advance differentiation status marker in apoptosis and immune exhaustion [25, 26]. In any case, the CD57+CD28- phenotype has not been studied in controllers. These patients have been infected for long periods of time, even decades, with a low viral load in the absence of antiretroviral treatment. This situation could be explained, at least in part, by special homeostatic and immunological features at present unknown.

The first aim of this work was to study clinical and demographic characteristics of HIV-controllers in relation to virus control and disease progression. The second aim consisted of analyzing different cellular phenotypes to identify a possible immune deregulation and special features associated with the long-term HIV control, such as T-cell hyperactivation, terminally differentiated phenotypes and proliferation.

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The TLR4 ASP299GLY Polymorphism is a Risk Factor for Active Tuberculosis in Caucasian HIV-Infected Patients

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Abstract: *Introduction*: Tuberculosis (TB) is a pandemic infectious disease especially frequent in HIV-infected patients. Toll-like receptor (TLR) 4 has been described to play a main role in the innate immunity against TB. In fact, single nucleotide polymorphisms (SNPs) in TLRs may influence AIDS disease progression. The association between two particular SNPs in human TLR4 (Asp299Gly and Thr399Ile) and active TB has been studied in non-HIV Africans with contradictory results. However, studies focusing on the effect of these TLR4 SNPs in active TB within a Caucasian HIV population are lacking.

Objectives: To analyze the association between TLR4 Asp299Gly and Thr399Ile SNPs and active TB, in Caucasian Mediterranean HIV-infected individuals.

Methods: 468 HIV-infected patients were analyzed. TLR4 genotyping was performed by real-time PCR and melting curve technology.

Results: TB was diagnosed in 59 (12,6%) patients. In a bivariate analysis several variables resulted significantly associated with active TB; intravenous drugs use (OR= 2.2; 95% CI [1.2-3.8]), hepatitis C virus (HCV) co-infection (OR= 3.4; 95% CI [1.6-7.1]), CD4 count ($p<0.001$), HIV viral load ($p=0.003$), latent TB prophylaxis (OR= 0.3; 95% CI [0.1-0.5]), and TLR4 Asp299Gly (OR= 2.0; 95% CI [1.1-4.2]). No statistical association was found for the TLR4 Thr399Ile. After a multivariate analysis, HCV co-infection (OR= 3.8; 95% CI [2.2-6.5]), baseline CD4 count (OR= 0.996; 95% CI [0.994-0.998]), TLR4 Asp299Gly (OR= 2.57; 95% CI [1.18-5.61]) were independently associated with active TB and inversely with latent TB prophylaxis (OR= 0.24; 95% CI [0.01-0.60]).

Conclusions: We describe an independent association between TLR4 Asp299Gly SNP and active TB in Caucasian Mediterranean HIV-infected patients.

Keywords: HIV, Toll like receptor 4, single nucleotide polymorphism, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a pandemic infectious disease with high morbidity and mortality worldwide [1]. Even more dramatic is the situation of HIV-infected patients. In fact, TB is the leading cause of death among this group of patients in low-developed countries [2]. The knowledge of the innate immune response against *Mycobacterium tuberculosis* is still partial. However, the role of macrophages phagocytosis, subsequent CD4 cells activation, IFNγ production and granulomas formation are decisive to control the infection [3]. *M. tuberculosis* recognition by macropaghes is mainly mediated *via* Toll-like receptors (TLRs), an important family of receptors involved in the innate immune response. Specifically, TLR2, 4 and 9 have been reported to be implicated in the recognition of mycobacteria [3, 4].

Increasing evidence about the importance of genetic determinants in susceptibility to TB has been reported [5]. In this respect, single nucleotide polymorphisms (SNPs) in different TLRs have been previously associated with the susceptibility and the clinical outcome of severe infections [6-8], including AIDS progression [9, 10]. The presence of two SNPs in human TLR4 (Asp299Gly and Thr399Ile) have shown lower response to inhaled lipopolysaccharide (LPS), a potent stimulus for TLR4 activation [11]. The first study analyzing the association between TLR4 Asp299Gly SNP and human TB susceptibility found no association in a group of HIV-negative Gambian subjects [12]. Similar results have been reported later in Mexicans [13].

HIV infection represents a different clinical scenario characterized by a marked inmunodepression that could be relevant to reveal TLRs SNPs effects, as previously reported for other immunosupressive conditions like hematopoietic precursors transplantation [8]. In the context of HIV infection, only one previous study has recently reported a tendency, not statistically significant, towards a higher susceptibility to develop active TB, in Tanzanian patients bearing the TLR4 Asp299Gly SNP [14]. Previous studies mentioned above are mainly focused on African populations but not on Caucasian race. Moreover, TLR4 Thr399Ile variant has not been previously studied due to the practically absence among Africans [15].

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Original article

Non-steroidal anti-inflammatory drugs increase the antiretroviral activity of nucleoside reverse transcriptase inhibitors in HIV type-1-infected T-lymphocytes: role of multidrug resistance protein 4

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Background: The multidrug resistance proteins (MRPs) form a subfamily within the ATP binding cassette transporters that confer resistance to a variety of structurally unrelated compounds. MRP4 has been reported to transport antiretroviral drugs out of cells in an active process. Although the main therapeutic effects of non-steroidal anti-inflammatory drugs (NSAIDs) are their ability to inhibit cyclooxygenase activity, in recent years, some pharmacological effects independent of this action have been described, such as inhibition of the activity of MRP4.

Methods: Detection of MRP4 expression was carried out by Western blot analysis, immunofluorescence and flow cytometry in peripheral blood lymphocytes (PBLs). Cells were infected with HIV type-$1_{NL4.3}$ isolate, and treated with antiretroviral drugs plus different NSAIDs. Agp24 was measured by ELISA 3 days post-infection. Intracellular [^{3}H] zidovudine (AZT) was quantified by a scintiller counter. Expression of different cell markers was assessed by flow cytometry.

Results: NSAIDs, as well as probenecid, were able to potentiate the antiretroviral effect of several nucleoside reverse transcriptase inhibitors (NRTIs). PBLs expressed MRP4 and treatment with ibuprofen did not affect this expression. However, MRP4 expression increased following phytohaemaglutinin and AZT treatment. This decrease of Agp24 was correlated with an increase in the intracellular AZT concentration. This effect was unrelated to changes on expression of CD4, CXCR4, cell viability or activation. Interestingly, patients treated with highly active antiretroviral therapy, who had a detectable viral load, presented a higher expression of MRP4 than those with an undetectable viral load.

Conclusions: NSAIDs can improve the antiretroviral activity of NRTIs, increasing their intracellular concentration by blocking MRP4. This finding could have implications for success of antiviral therapy.

Introduction

Treatment with highly active antiretroviral therapy (HAART) leads to emergence of drug-resistant virus [1]. Although therapy failure is primarily the result of viral mutations, some infected individuals present resistance symptoms without resistant virus. This fact is related to the possibility of existence of cellular mechanisms that contribute to HIV treatment failure [2]. For an effective therapy, it is necessary to reach an adequate intracellular concentration of antiretroviral drugs, which is controlled by influx and efflux processes [3], their balance being pivotal in overall therapeutic efficacy. Specifically, the increased efflux of phosphorylated drugs has been proposed to explain decreased drug accumulation and resistance to retroviral inhibitors [4].

The ATP binding cassette (ABC) transporter superfamily contains membrane proteins that translocate a wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids and sterols, and drugs. Although many are uncharacterized, some of them have been shown to transport anionic substances against a concentration gradient with the energy supplied by ATP hydrolysis [5].

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ORIGINAL RESEARCH

Non-medically supervised treatment interruptions among participants in a universally accessible antiretroviral therapy programme

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Background

We examined clinical outcomes, patient characteristics and trends over time of non-medically supervised treatment interruptions (TIs) from a free-of-charge antiretroviral therapy (ART) programme in British Columbia (BC), Canada.

Methods

Data from ART-naïve individuals ≥18 years old who initiated triple combination highly active antiretroviral therapy (HAART) between January 2000 and June 2006 were analysed. Participants having ≥3 month gap in HAART coverage were defined as having a TI. Cox proportional hazards modelling was used to examine factors associated with TIs and to examine factors associated with resumption of treatment.

Results

A total of 1707 participants were study eligible and 643 (37.7%) experienced TIs. TIs within 1 year of ART initiation decreased from 29% of individuals in 2000 to 19% in 2006 ($P<0.001$). TIs were independently associated with a history of injection drug use (IDU) ($P=0.02$), higher baseline CD4 cell counts ($P<0.001$), hepatitis C co-infection ($P<0.001$) and the use of nelfinavir (NFV) ($P=0.04$) or zidovudine (ZDV)/lamivudine (3TC) ($P=0.009$) in the primary HAART regimen. Male gender ($P<0.001$), older age ($P<0.001$), AIDS at baseline ($P=0.008$) and having a physician who had prescribed HAART to fewer patients ($P=0.03$) were protective against TIs. Four hundred and eighty-eight (71.9%) participants eventually restarted ART with male patients and those who developed an AIDS-defining illness prior to their TI more likely to restart therapy. Higher CD4 cell counts at the time of TI and unknown hepatitis C status were associated with a reduced likelihood of restarting ART.

Conclusion

Treatment interruptions were associated with younger, less ill, female and IDU participants. Most participants with interruptions eventually restarted therapy. Interruptions occurred less frequently in recent years.

Keywords: access to therapy, adherence, antiretroviral therapy, treatment interruptions

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Introduction

Improving access to highly active antiretroviral therapy (HAART) is an important public health objective in all regions of the globe. Not only is HAART associated with markedly improved survival among HIV-infected individuals [1,2], but it can also contribute to reducing the number of new HIV infections at the population level [3,4]. Continued access to HAART is often limited by patient-incurred costs, especially in low- or middle-income countries [5] or in industrialized countries without universal health care insurance programmes [6]. However, other factors associated with poor access or continuation

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Effect of the Substitution of One Nucleoside Analogue by One Non-nucleoside Reverse Transcriptase Inhibitor over Mitochondrial DNA Levels

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Abstract

Background: Long-term antiretroviral therapy is associated with several side effects, like mitochondrial toxicity related to nucleoside reverse transcriptase inhibitors (NRTIs). Our objective was to analyze the effect of the substitution of one NRTI by one non-nucleoside reverse transcriptase inhibitor (NNRTI) in the antiretroviral regime of HIV-1-infected patients who were on a regime containing either two NRTIs and one NNRTI, or one NRTI, one NNRTI and one protease inhibitor (PI), over mtDNA level. Decreasing NRTIs could increase mtDNA level.

Methods: Fifteen HIV-1-infected patients were included in the study. As controls, 17 healthy individuals and 15 HIV-1-infected patients naïve for antiretroviral treatment were also analyzed. mtDNA level was quantified at baseline and after 48 weeks of treatment.

Results: Control groups showed higher levels of mtDNA than the study group ($p < 0.001$). Among this latter group, no statistical differences between baseline and after 48 weeks were found. Naïve HIV-infected patients had lower mtDNA than healthy volunteers ($p < 0.001$). Two patients had two consecutive blips (low viral load increases) but they did not show NNRTI-related resistance mutations.

Conclusions: This study shows that although this treatment was immunovirologically effective, mtDNA level did not increase at least after 48 weeks.

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Introduction

The introduction of highly active antiretroviral therapy (HAART) based on the combination of two nucleoside reverse transcriptase inhibitors (NRTIs) plus one protease inhibitor (PI) has led to a dramatic reduction in mortality and morbidity among HIV-infected patients [1, 2]. However, long-term use of PIs is associated with several side effects like lipodystrophy syndrome, severe hepatic damage and other metabolic disturbances, such as dyslipidemia or diabetes mellitus [3–6]. An effective alternative to reduce these side effects has been the substitution of PIs by non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the antiretroviral regimen [7].

Nevertheless, NRTIs may inhibit human mitochondrial DNA (mtDNA) polymerase γ driving to mitochondrial toxicity and depletion [8, 9]. This inhibition leads to the development of different adverse effects [10, 11]. Changes in mitochondrial DNA in the peripheral blood cells have been reported to be a good marker of treatment toxicity [12]. Reported data have associated mitochondrial toxicity with the NRTIs included in the treatment [13], long-term antiretroviral treatment [14], different combinations among them [15], and/or genetic host factors. Others reported no association between mitochondrial toxicity and the type of NRTI used [16]. Furthermore, HIV infection with no antiretroviral treatment induces mtDNA depletion [17].

We hypothesized that mtDNA level could increase among those patients whose HAART regimen (either two NRTIs and one NNRTI, or one NRTIs, one PI and one NNRTI) is replaced by one NRTI and two NNRTIs. Hence, the objective of our study was to analyze the effect

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Short communication

Control of HIV-1 RNA load after HAART interruption: Relationship with CCR5 co-receptor density and proviral DNA load in HIV-infected patients

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Abstract

Background: CCR5 co-receptor density has been reported to play a role in the level of HIV production. In addition, reports about the relationship between proviral DNA load and plasma HIV load are controversial.
Objectives: To analyse the role of CCR5 co-receptor density and proviral DNA load in the control of plasma HIV-viral load after HAART interruption, comparing patients whose plasma HIV load was persistently below 4 $\log_{10}$ RNA copies/mL, defined as "HIV controllers", with patients who showed a viral load higher than 4 $\log_{10}$ RNA copies/mL, defined as "non-controllers".
Study design: Proviral DNA load quantification ($N = 55$) and CCR5 co-receptor density ($N = 29$) were determined in HIV-infected patients on prolonged HAART interruption.
Results: Twenty-three percent of our HAART interruption cohort were classified as HIV controllers, while 77% were classified as non-controllers. CCR5 co-receptor density was statistically higher in HIV controllers than in non-controllers, while proviral DNA load was not different between them. CCR5 co-receptor density in activated CD4 cells was independently associated with HIV plasma load after interruption.
Conclusions: The observation of a higher CCR5 co-receptor expression in HIV controllers suggests that HIV infection leads to the selection of CD4 cells with low CCR5 co-receptor density after HAART interruption.

Keywords: HIV controllers; CCR5 density; Proviral DNA load; HAART interruption

1. Introduction

In the absence of antiretroviral treatment some HIV-infected patients are able to maintain low to moderate plasma HIV-viral load (i.e., 75–10,000 RNA copies/mL) (Deeks et al., 2004) or even persistently undetectable, named as HIV controllers (Lambotte et al., 2005; Madeca et al., 2005). On the other hand, in our prolonged HAART interruption cohort we described a group of patients whose viral load was persistently below 4 $\log_{10}$ RNA copies/mL (Vallejo et al., 2005).

Understanding the mechanisms involved in HIV-viral load control is critical for the knowledge of HIV pathogenesis, i.e. the involvement of HIV-specific response has already been well documented (Betts et al., 1999; Emu et al., 2005; Pantaleo and Koup, 2004). However, other potential factors have not been deeply studied or reports are controversial. CCR5 co-receptor density has been reported to play a role in HIV production (Reynes et al., 2000). In addition, some studies suggest that proviral load may be an indicator of spread

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HIV–hepatitis C virus co-infection is associated with decreased plasmatic IL-7 levels

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We analysed the potential influence of hepatitis C virus (HCV) co-infection over IL-7 levels and thymic function in naive HIV-infected patients and after effective HAART. HIV–HCV-co-infected patients had lower plasmatic IL-7 levels compared with HIV-monoinfected patients. This effect may not be associated either with HCV monoinfection or with the rate of liver injury. These lower levels may explain, at least partly, the lower CD4 cell repopulation of HIV–HCV-co-infected patients after HAART.

HIV-infected patients co-infected by hepatitis C virus (HCV) may have an increased risk of progression to AIDS and lower CD4 cell repopulation after HAART [1]. Although these results have not been confirmed by other authors [2], a recent meta-analysis supported these findings [3]. This lower CD4 cell repopulation may be explained partly by the impairment of at least one of the main mechanisms involved in T-cell homeostasis, thymic function and plasmatic IL-7 levels [4,5]. A recent work has reported lower T-cell rearrangement excision circle (TREC) levels in HCV-monoinfected patients compared with non-infected controls, suggesting an immune impairment [6].

Whether HCV co-infection influences this CD4 cell homeostatic system has not been studied. In this work, we performed a cross-sectional study to analyse the potential influence of HCV co-infection over IL-7 levels and thymic function in both naive HIV-infected patients and after effective HAART.

The CD4 cell count and IL-7 levels (Quantikine HS IL-7 immunoassay kit; R&D Systems, Minneapolis, Minnesota, USA) were measured in 97 naive HIV-infected patients, including 40 HCV-co-infected patients. Among these patients, 25 HIV-monoinfected and 20 HIV–HCV-co-infected patients had previously participated in other studies and had their thymic volume measurements recorded [7]. In addition, 92 HIV-infected patients on HAART, with undetectable HIV plasma viraemia, including 49 HCV-co-infected patients, were also analysed. Among them, 29 HIV-monoinfected and 24 HIV–HCV-co-infected patients had their thymic volume recorded [8]. A comparison of thymic volume between these two study populations was not possible because of technical changes. However, the analysis of the effect of HCV co-infection could be performed within each single group (naive patients and HAART-treated patients). The only limitation for the selection of the patients was the availability of frozen plasma samples. None of the HCV-infected patients had received treatment for HCV infection.

Control populations for IL-7 level quantification included 28 healthy volunteers and 31 HCV-monoinfected patients who had never received treatment. In addition, in order to analyse the potential influence of liver injury on IL-7 levels, an additional group of 34 HIV–HCV-co-infected HAART-treated patients were studied. Subjects' written informed consent had been obtained and the ethical committee approved the study.

Real-time polymerase chain reaction (LightCycler; Roche Diagnostics, Branchburg, New Jersey, USA) was used for the quantification of both the characteristic signal-joint sequences harboured in the generated TREC, and β-globin gene [7]. TREC levels were measured in peripheral blood mononuclear cells, yielding the number of TREC per 10^6 peripheral blood mononuclear cells in the same patients whose thymic volume had previously been measured.

As shown in Fig. 1, HIV–HCV-co-infected patients, both naive and HAART-treated patients, showed statistically lower IL-7 levels than HIV-monoinfected patients. In addition, healthy volunteers showed statistically lower levels than naive HIV-infected patients, although no statistical differences with either HAART-treated patients or HCV-monoinfected patients were found.

In order to determine which factors were associated with IL-7 levels, HIV-infected populations under study were analysed together (97 naive plus 92 HAART-treated patients). The CD4 cell count, HCV co-infection, TREC levels and thymic volume had $P < 0.1$ in the univariate analysis and were introduced in the stepwise

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Disseminate and fatal cytomegalovirus disease with thymitis in a naive HIV-patient after early initiation of HAART: Immune restoration disease?

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Abstract

We describe a naïve HIV-infected patient who developed a *Pneumocystis carinii* pneumonia and disseminate and fatal cytomegalovirus disease within 3 months after initiation of HAART, suggesting due to coincidence in time, an immune restoration disease. We propose an alternative hypothesis.

Keywords: Immune restoration disease; Thymitis cytomegalovirus; AIDS

1. Introduction

HIV-patients under HAART may experience severe systemic inflammatory reactions that have been defined as immune restoration disease (IRD). It has been communicated that IRD has two different patterns; an earlier pattern during the first 3 months of HAART as an immune response against viable opportunistic pathogens, and a later pattern as an immune response against non-viable opportunistic pathogens months to years after HAART (French et al., 2004). During the IRD, a baseline CD4 cell count below 100 cells/mm^3 has been reported among patients, while after HAART an increase above 200 cells/mm^3 is reached (Price et al., 2001). Outcomes range from minimal morbidity to fatal progression (French et al., 2004; Hirsch et al., 2004). In this way, atypical presentations of mycobacterial, cytomegalovirus (CMV), hepatitis B virus, hepatitis C virus and JC virus have been described after initiating HAART (French et al., 2004; Safdar et al., 2002).

We here report the case of a naïve HIV-infected patient who developed *Pneumocystis carinii* pneumonia as well as disseminated and fatal CMV infection coinciding with the initiation of HAART.

2. Case report

In 1993, a 32-year-old woman was diagnosed of HIV infection in our unit. Then, her CD4 T cell count was 840 cells/mm^3, and HIV plasma viral load (pVL) was above 75,000 copies/mL. The moment that primoinfection occurred in the past was unknown because it was asymptomatic. Since she declined to receive antiretroviral therapy, a progressive CD4$^+$ T cell count decrease was taking place during the following 9 years. In July 2002, she began HAART with zidovudine, lamivudine, and abacavir, having HIV pVL above 75,000 copies/mL, CD4$^+$ T cell count of 90 cells/mm^3, and a thymic volume (measured by mediastinic computed

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Long-term virological outcome and resistance mutations at virological rebound in HIV-infected adults on protease inhibitor-sparing highly active antiretroviral therapy

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***Objective*: To assess the durability of the undetectability of HIV plasma viraemia (pV) and to determine the factors associated with virological rebound (VR) in HIV-infected adults on protease inhibitor (PI)-sparing highly active antiretroviral therapy (HAART). The development of resistance mutations during virologically successful therapy and VR was also analysed.**

***Materials and methods*: One hundred and twenty-six HIV-infected adults on PI-sparing HAART were prospectively followed from April 1998 to December 2002: *Group 1*, naive for antiretroviral drugs (n = 26); *Group 2,* previously PI-HAART-exposed patients (n = 19); *Group 3*, previously exposed to suboptimal therapy (n = 81). Genotypic resistance tests on peripheral blood mononuclear cells or on plasma RNA (when feasible) were carried out when undetectable HIV pV was demonstrated for at least 48 weeks. Additionally, patients showing a therapy adherence >95% developing VR were also tested at rebound, at simplification and during previous suboptimal therapy exposure.**

***Results*: The median follow-up time was 630 [329–903] days. VR was considered as two consecutive pV levels >50 copies/mL. Twenty-two (17.5%) patients developed VR. Only therapy adherence <95% was independently associated with VR (adjusted hazard ratio: 8.42; 95% CI: 3.33–21.27). Twenty (40%) of the 50 patients with pV < 50 copies/mL for at least 48 weeks showed at least one thymidine-associated mutation (TAM) but none had NNRTI-resistance mutations. Ten (83.3%) of 12 available adherent patients showing VR harboured NNRTI-resistance-associated mutations; 50% of them were considered as wild-type strains at simplification time. However, the TAM number and resistance mutations profile found on suboptimal exposure were very similar to those found at VR on simplification therapy.**

***Conclusions*: PI-sparing HAART allows maintenance of successful long-term control of HIV replication, adherence to therapy being the main factor associated with VR. However, a small proportion of patients on simplification regimen may develop VR regardless of therapy compliance. VR on PI-sparing HAART is characterized by the emergence of NNRTI cross-resistance mutations. Finally, TAMs 'archived' during previous suboptimal exposures are partially involved in subsequent VR on simplification HAART.**

Keywords: PI-sparing HAART, simplification therapy, virological rebound, resistance mutations

Introduction

The introduction of highly active antiretroviral therapy (HAART) including protease inhibitor (PI) drugs and nucleoside reverse transcriptase inhibitors (NRTIs) has dramatically changed the course of human immunodeficiency virus (HIV) infection, reducing mortality and morbidity events associated with this disease.[1,2] These regimens have allowed successful control of HIV replication. However, the burden of toxicity resulting from the use of PI drugs is of concern as it constitutes a threat to the sustained success of HIV treatment.[3] Thus,

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“En las adversidades

sale a la luz la virtud”

Aristóteles

…a Elena, virtuosa en las adversidades