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Short communication

Discordance rates between Trofile® test and short-term virological response to maraviroc

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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.

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 capacity to detect minor populations of CXCR4 (X4)-tropic virus is now available (Reeves et al., 2009). However, limitations such as the long waiting time and around 20% of "non-reportable" results make it necessary to explore additional methods to test HIV tropism in order to prescribe the CCR5 antagonist maraviroc (MRV). A clinical approach (Maraviroc Clinical Test, MCT) has recently been reported (Genebat et al., 2009), indicating that the virological response to a short-term MRV exposure could predict the indication for CCR5 (R5)-antagonists use. In this previous study, MCT and ES-Trofile® results were compared in 34 HIV-infected subjects with detectable viral load; a high concordance between both methods was observed (93.5%). Since then, the number of patients on MCT has increased. On the other hand, ES-Trofile® does not usually report results if the viral load is <1000 HIV-RNA copies/ml. Hence, for the comparison analysis, patients with detectable viral load of <1000 HIV-RNA copies/mL should not be considered. Thus, the aim of this study was to analyze discordance rates between the tropism result reported by ES-Trofile® and the MCT result, in a larger HIV-infected population with viral load ≥1000 HIV-RNA copies/ml.

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 eight-day monotherapy with MRV (MCT) as previously reported (Genebat et al., 2009). When compared with the previous study, only patients with viral load >1000 HIV-RNA copies/mL were considered: 33/47 patients (70.2%) were receiving no antiretroviral therapy (cART), as they had self-abandoned cART or remained under supervised treatment interruption (real MRV monotherapy: MRV without cART during eight days on MCT), while 14/47 (29.8%) subjects were on a failing cART and MRV was added for eight days to this therapy (functional MRV monotherapy: MRV plus previous failing cART during eight days on MCT). MCT was considered positive if a reduction ≥1 $\log_{10}$ HIV-RNA copies/mL or undetectability (<40 HIV-RNA copies/ml) was achieved after eight days of MRV exposure. An ES-Trofile® was performed from blood samples on the same day starting MCT (90%) or, if not possible, on 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 a previous 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-

Abbreviations: ES-Trofile®, enhanced sensitivity Trofile®; X4, CXCR4; MRV, maraviroc; MCT, Maraviroc Clinical Test; R5, CCR5; cART, combined antiretroviral therapy; D/M, dual/mixed.

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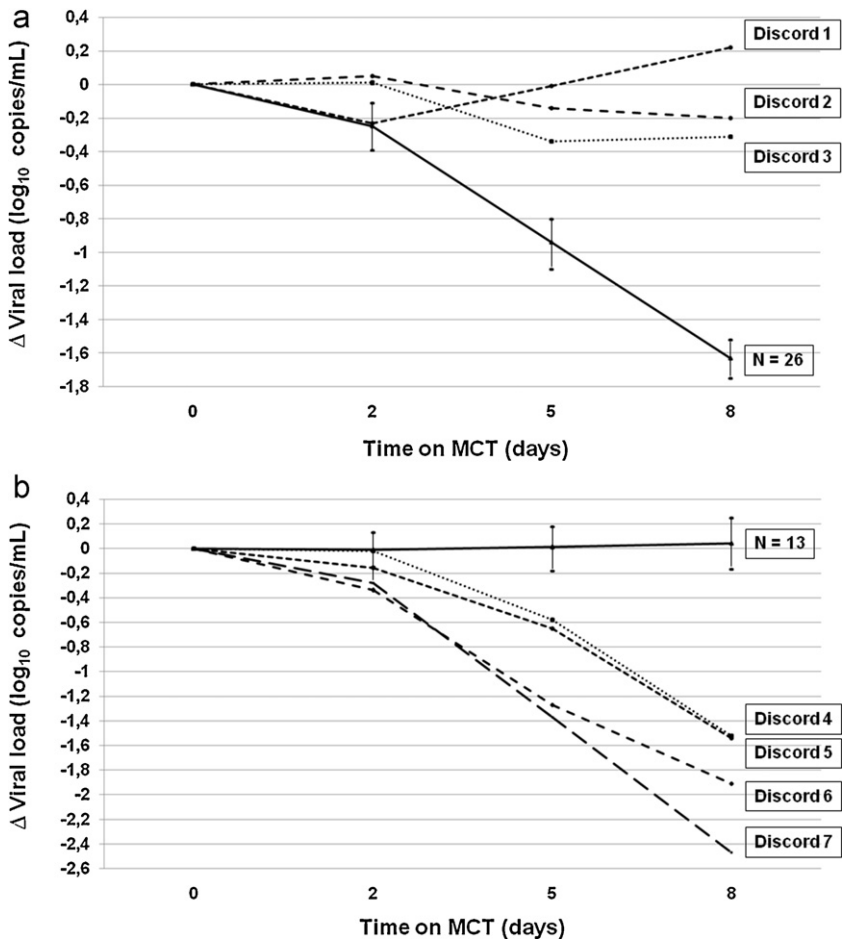

Fig. 1. (a) Viral load evolution in patients reported as R5 by ES-Trofile® ($N = 29$). Mean viral load evolution and confidence interval 95% (CI 95%) is shown in 26/29 patients who achieved a viral load reduction > 1 $\log_{10}$ HIV-RNA copies/mL during MCT. Three patients (Discord 1, Discord 2 and Discord 3) showed no viral load modification during MCT despite ES-Trofile® being reported as R5 (3/29 = 10.3%). (b) 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 who 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® being reported as D/M (4/17 = 23.5%).

reported by patients and estimated through Pharmacy registers, being 100% in all patients.

Results of ES-Trofile® were: 29/47 (61.7%) patients were reported to be infected by R5-tropic HIV variants (R5 patients) and 17/47 (36.2%) by dual/mixed (D/M) tropic virus (D/M patients); 1/47 (2.1%) was non-reportable and this patient achieved virus undetectability after MCT. As shown in Fig. 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 hand, as shown in Fig. 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 (Fig. 1a), while 4/17 (23.5%) patients with D/M virus experienced a viral load reduction > 1.5 $\log_{10}$ HIV-RNA copies/mL (Fig. 1b); thus, the global discordance rate between MCT and ES-Trofile® was 7/46 (15.2%). Results of MCT and ES-Trofile® are summarized in Table 1. The discordance rate was higher in patients reported as D/M compared to patients with tropism reported as R5, although not 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 the MCT result. Immunovirological evolution of these patients after MCT is shown in Fig. 2a; two of them remained 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, combined with lamivudine plus abacavir. Immunovirological evolution of these patients is shown in Fig. 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 to start cART after MCT.

The results presented herein show unexpected rates of discordance (around 15%) between the short-term virological response to MRV and the results reported by ES-Trofile®. The long periods of infection shown by our patients, that make the presence of X4-tropic variants easier, could be influencing the discordance rates. However, it should be noted that those are the MRV guidelines for clinical practice, as shown in the MOTIVATE studies (Gulick et al., 2008), and this is the context that should be considered when regarding discordance analysis. Hence, clinicians are currently taking decisions based on an assay that, according to our results, might not ensure virological success. Finally, discordance rates shown in this study should not be extended to HIV-infected cART naive subjects.

Discordance observed in R5 patients could be explained, despite maintaining the viral tropism as R5, because MRV could be non effective due to changes 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. If this is the case, MCT would be a useful tool to detect these particular patients. On the other hand, discordance observed

Table 1
Comparison between MCT and ES-Trofile® results ($n = 47$).

	R5 ES-Trofile® subjects (n)	D/M[a] ES-Trofile® subjects (n)	NR[b] ES-Trofile® subjects (n)
MCT positive subjects (n)	26	4	1
MCT negative subjects (n)	3	13	
Discordance MCT/ES-Trofile® n/n (%): 7/46 (15.2%)	3/29 (10.3%)	4/17 (23.5%)	Not applicable

[a] Dual/mixed.
[b] Non-reportable.

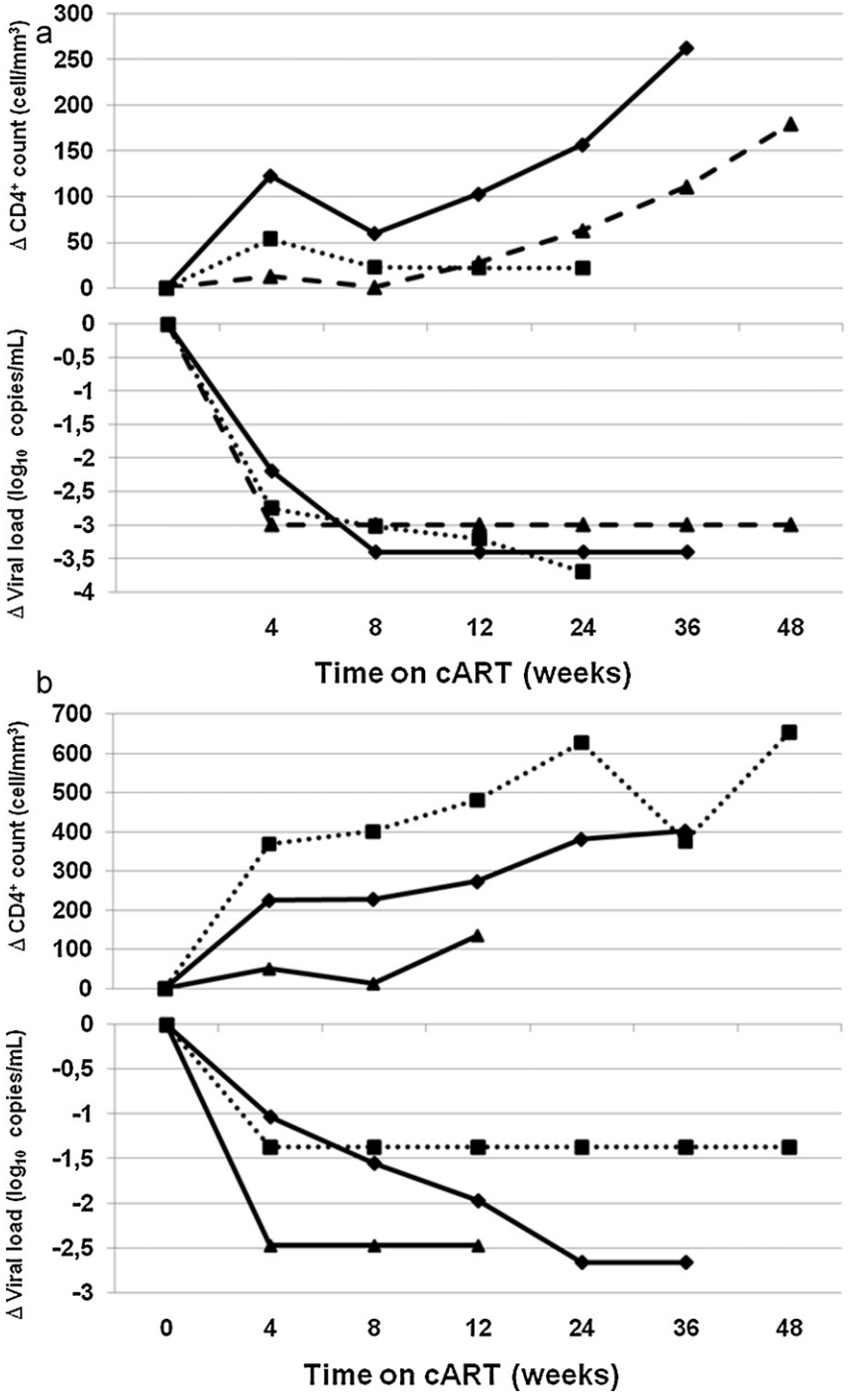

Fig. 2. (a) Immunovirological evolution of patients with R5 tropism according to ES-Trofile® and negative MCT, once the new cART was started after MCT. Total CD4⁺T-cell increase and viral load reduction once cART was started after MCT, in patients with negative MCT and R5-tropic virus according to ES-Trofile®. 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 remained 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. (b) 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.

in D/M patients was 23.5%, higher than in R5 patients; discordance in these patients could be explained due to the greater sensitivity of ES-Trofile® in detecting minor X4-tropic variants, that could lead to a D/M result in which R5-tropic variants are predominant enough to exert a virological response.

Higher sensitivity in detecting minor X4-tropic variants makes it 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 since clinicians only receive a categorical result (i.e. R5, X4, D/M), but the 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 as candidates to be treated with CCR5-antagonists.

Alternatives to ES-Trofile® have been suggested, such as 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 should be evaluated in future studies. The use of ultra-deep sequencing (Rozera et al., 2009) can also quantify minor variants, but this assay is not available in routine clinical practice and has not been clinically validated to consider a patient as a candidate to be treated with a CCR5 antagonist.

The main concern during MCT is the potential emergence of X4-tropic virus in patients with baseline R5 virus, which could lead to further immunovirological impairment. However, X4-tropic variants have been shown to be present in reservoirs before starting CCR5-antagonists treatment (Fätkenheuer et al., 2005). Besides, in our study patients with positive MCT but D/M tropism according to ES-Trofile® started a MRV-containing cART after MCT; all of them experienced an excellent immunovirological evolution, despite MRV being combined with low genetic barrier drugs (lamivudine and abacavir). Finally, we have recently shown the long-term efficacy of a MRV-containing regimen in routine clinical practice, in which the decision of including MRV as part of the cART in most patients was MCT-guided (Genebat et al., 2010).

In conclusion, our results show that ES-Trofile® does not completely correlate with the virological response to MRV after 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 treatment with CCR5 antagonists. According to our results, we recommend the use of MCT in order to decide on treatment with CCR5 antagonists.

Conflict of interest

None to declare.

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