Dynamic of Mixed HCV Infection in Plasma and PBMC of HIV/HCV Patients Under Treatment With Peg-IFN/Ribavirin

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Abstract: The extent of mixed hepatitis C virus (HCV) genotype in different compartments (plasma and peripheral blood mononuclear cell, PBMC) and possible association with treatment efficacy in HIV/HCV coinfected patients remains to be unknown.

The objective of this study was to elucidate the frequency of mixed genotype infection (MG), its profile in different compartments during anti-HCV treatment, and the possible influence of different genotypes on the response rate.

The compartmentalization of HCV population was investigated by next-generation sequencing in 19 HIV/HCV coinfected patients under anti-HCV treatment with peginterferon/ribavirin (P–R). Ten individuals were nonresponder (NR) or relapser (RE) to P–R treatment and 9 had a sustained virological response (SVR).

Eleven/nineteen (58%) patients had MG in plasma compartment. Ten or 12 patients infected by a difficult to treat genotype (DTG) 1 or 4 as dominant strain, had an MG, whereas only 1/7 individuals infected by easy to treat genotype (ETG) harbored a mixed genotype, P = 0.006. HCV–RNA was more frequently detected in PBMC of NR (10/10) than in those of SVR (5/9), P = 0.032. Mixed genotype infection was detected in 6/15 (40%) PBMC-positive cases and was not associated with P–R treatment response. By multivariate analysis, MG in plasma samples was the most important virological factor affecting the treatment response (P = 0.0237).

Detection of MG in plasma of HIV/HCV coinfected patients seems to represent the major determinant of response to P–R treatment. This finding may have important clinical implication in light of the new therapeutic approach in HIV/HCV coinfected individuals suggesting that combination treatment with direct acting antivirals could be less effective in MG.

INTRODUCTION

Hepatitis c virus is an enveloped, positive strand RNA virus belonging to the Hepacivirus genus in the Flaviviridae family. Seven confirmed genotypes are generally distinguished by phylogenetic methods and pairwise distance calculations. Hepatitis C virus genotypes diverged from each other by a pairwise distance of >30%. Individual genotypes can be further divided into more closely related subtypes that diverged by a pairwise distance of 15% to 30%. All viral genotypes retain their repertoire of collinear structural and nonstructural genes, thereby facilitating preliminary genotype classification on the basis of partial genome sequences in the core, 5’noncoding region (5’NC) and the nonstructural-5B domain. It is well known that Peginterferon and Ribavirin (P–R) antiviral treatment yields success rates <50% depending on the infecting genotype. Its correct assessment, in combination with viral load, serves to optimize the therapeutic regimen.

It is difficult to determine the prevalence of mixed genotype infections (MG) by the commercial assays, or DNA sequencing because these assays are designed to identify the dominant genotype.

A previous study involving chronically infected intravenous drug users, hemodialysis patients and hemophiliacs from Sweden and Russia used 2 genotyping methods based on the use of type-specific primers to detect MG and suggested that the frequency of MG is very low, even in these high-risk groups. Tuveri et al identified multiple HCV genotypes in 13% of French patients with hemophilia or von Willebrand’s disease using the same methodological approach of Viazov. Two other studies revealed the presence of MG by using restriction fragment length polymorphism (RFLP) in 45% and 50.8% parenterally transmitted infection risk groups.

Altogether, these studies demonstrated the difficulties of assessing the real prevalence of the minor genotypes within the MG, due to the absence of highly sensitive sequencing strategies to detect the low-frequency genomes.

With next-generation sequencing (NGS) platforms, it is now possible to investigate viral heterogeneity at much greater
detail. Their high throughput allows for generation of millions of reads in a single sequencing run. Next-generation sequencing can detect variants at low frequencies, which would go undetected by standard sequencing methods. Recently, 2 studies investigated reliability and reproducibility of NGS technologies to detected HCV minor variants within quasispecies, and their possible implication in the response to treatment with direct acting antivirals (DAAs).

One other study evaluated the infection with multiple HCV genotypes by NGS analysis showing high accuracy of this technique in revealing mixed genotype infection. As an HCV viral reservoir, peripheral blood mononuclear cells (PBMC) might harbor viral variants distinct from the genotype detected in plasma. One study showed that, although mixed infections at HCV genotype level became evident in only 5.6% of PBMC the culture methodology increased HCV infections with multiple genotypes to 62.5%.

Although PBMC are not the primary site of HCV replication, previous reports emphasize their role as viral reservoirs whereas others speculated that HCV diversity in PBMC might be an important determinant of treatment response.

A number of studies suggested that extra-hepatic HCV replication at the end of treatment may be an important factor predicting viral relapse. A recent report suggests that HCV–RNA clearance in PBMC after 1 month of treatment may be a reliable predictor of SVR, mainly in patients lacking rapid virological response.

To date, most of these analyses of HCV replication in PBMC have involved a small number of patients. Therefore, it remains unclear if HCV replication in PBMC affects the treatment efficacy, whereas the effect of anti-HCV treatment on the viral population heterogeneity in this compartment is still unknown.

Recently, the development of DAAs has become the standard of care for HCV genotype 1 infection. The association treatment with different antivirals in interferon-free and interferon-based therapy represent the future treatment strategy to eradicate the virus. A number of DAAs exhibit variable activity and potency on different genotypes and subtypes. So, most treatment protocols require the correct identification of the infecting HCV genotype to provide the association and duration of antiviral therapy. In addition to natural occurring resistant strains, also MG may play a role in the success rate of DAAs-based treatment. In this context, the role of these innovative treatments toward patients with MG is unknown so far.

The aim of this study is to determine the presence of MG and the temporal dynamic of HCV infecting genotype in different compartments in HIV/HCV-infected patients under P–R treatment, and speculate on the role of these factors in the treatment response with new direct antivirals differently active on different genotypes and subtypes.

METHODS

Patients

Nineteen HIV/HCV coinfected patients belonging to the pilot, randomized, prospective, multicenter, open label, controlled clinical trial KaMON were studied. Briefly, the trial enrolled individuals with age >18 years, confirmed diagnosis of HIV/HCV coinfection, naive for HCV treatment, without liver cirrhosis or with liver cirrhosis with a CD4+ absolute number >350 cells/mm³, on stable HAART and with HIV-RNA <50 copies/mL at least 6 months before enrollment, no previous anti-HIV protease inhibitor (PI) mutations, no virologic failure to prior PI treatment. The patients were randomized to ritonavir-boosted lopinavir monotherapy (LPV/r; Arm A: 400/100 mg BID) plus anti-HCV therapy (pegylated-IFN-alpha 2a plus ribavirin 0.8–1.2 g/day depending on body weight) or HAART (Arm B: LPV/r and tenofovir/emtricitabine) plus anti-HCV therapy (pegylated-IFN-alpha 2a plus ribavirin 0.8–1.2 g/day depending on body weight). It endured 72 weeks: 48 weeks (W) with HIV/HCV therapy and 24 weeks of HIV treatment only. Hepatitis C virus virological response (HCV–RNA <12 U/mL) was evaluated at W4, W12, W24, W48 (end of treatment), and W24 post-treatment (W24-PT). Four/nine SVR patients showed a rapid virological response (RVR; HCV–RNA <12 IU/mL at W4 of treatment).

Coupled plasma samples and PBMC for virological analysis were available at baseline in 19/30 cases (8 nonresponder patients, NR, 2 relapser patients, RE, and 9 SVR) included in the study KaMON. In SRV virus population was investigated at baseline (BL) because HCV–RNA was undetectable at W12 of treatment and thereafter.

In 5 nonresponder (NR), dynamic of viral population was evaluated in paired plasma samples and PBMC obtained at BL, W12, and W24 of treatment. In 2 of these 5 NR, viral population was analyzed also at W24-PT. In 3 NR W24 plasma samples were not available. Additionally, in 2 RE with HCV–RNA undetectable (<12 IU/mL) during P–R treatment, viral population was evaluated at W24-PT. Overall, 36 plasma samples and 32 PBMC specimens were studied by NGS. The patients were considered infected by a difficult to treat genotype (DTG) in the case of infection with HCV-genotype 1 or 4 or infected by an easy to treat genotype (ETG) in the case of infection with genotype 2 or 3.

The study was conducted in accordance with the Good Clinical Practice guidelines and the ethical principles stated in the Declaration of Helsinki. The enrolled patients gave their written informed consent, and the study was approved by the Ethics Committees of all of the participating institutes (ClinicalTrials.gov registration No. NCT00437684).

HCV–RNA detection in Plasma and PBMC compartments

Total RNA was extracted from plasma using the QIAamp Viral RNA kit (QIAGEN) and from 1 × 10⁶ PBMC, using a commercially available kit (TRIzol LS; Gibco, BRL, NY) according to the manufacturer’s instructions, and amplified by means of reverse transcriptase polymerase chain reaction (PCR) with primers spanning the highly conserved 5’ NC. Briefly, reverse transcription was performed using an appropriate primer in a volume of 25 µL containing 10 µL isolated RNA and 20 pmol downstream outer primer. After incubation at 37 °C for 60 minutes (min), the complementary DNA was amplified in 50 µL of the PCR mixture containing 10 pmol of an outer primer set: SF1: 5’-GCCATGGCGTTAGTTA GTATG-3’; 82–101 nt and SR1: 5’-TGACAGGCTACAGGAC CTGCTC-3’; 339–320 nucleotide (nt, amplicon length 240 base pairs, bp). Amplification was carried out for 25 cycles of 1 min at 94 °C, 30 (s) at 55 °C, and 1 min at 72 °C, with a final 3 min extension at 72 °C. For the second PCR, 2 µL of the first amplification products were amplified with 10 pmol of the inner primer set: SF2: 5’-GGCTACAGGCTATGTTAGT-3’; 104–123 nt and SR2: 5’-GGGACATCTCAGCAGCACCC TAT-3’; 316–296 nt (amplicon length 210 bp) under the same cycling conditions. All measures to prevent PCR contamination
were strictly applied. Furthermore, at least 2 different PCR products were produced from the same sample in order to exclude the cross contamination of different specimens.

Next-Generation Sequencing

The NGS analysis of 5’NC region was used to determine the presence of MG in different compartments.

First, the 5’NC region was amplified by reverse transcriptase (RT)-nested-PCR using the same set of primers (SF1, SR1) for HCV–RNA detection. All primers used for the second round of amplification included a 10-base molecular barcode (MID, multiplex identifier) found, Figure, Supplemental Content 1, http://links.lww.com/MD/A485). To generate amplicons the first round of amplification was performed as described above for HCV–RNA detection, whereas, for the second round, 35.5 μL H2O, 5 μL buffer 10×, 1 μL 10 mM dNTP mix, 3 μL 25 mM MgCl2, 0.5 μL Taq DNA polymerase, and 2 μL 10 μM SR2, and 10 base molecular barcode (1 for each sample analyzed) oligonucleotides was mixed with 1 μL of the first round amplicon. The list of MID used for second round amplification for each sample was summarized in Table, Supplemental Content 2, http://links.lww.com/MD/A485. To verify the quality of amplicons, PCR products were analyzed by using chip Agilent Bioanalyzer DNA 1000. The purified amplicons were quantitated with a fluorescence assay (Quantifluor–ST, Promega) following the manufacturer’s instruction. Briefly, 1 μL of each purified amplicon was mixed with 2 μL of Hoechst-TNE Working Solution. For clonal amplification purified amplicons were pooled in equimolar amounts.

Clonal amplification on beads (emulsion PCR), beads isolation, and sequencing was performed according to the manufacturer’s protocol for the GS FLX platform (454 Life Sciences, Roche company, Branford, CT), obtaining a total of ~4000 sequences with a mean length of 141 bp. Data analysis was performed by using GS sequencer (version 2.6) for the images and AVA software for the sequences. About 2200 of 4000 sequences obtained from UDPs run were included in our analysis on the basis of amplicon length (140 bp at least). A mean of 30 (range 10–61) sequences were analyzed for each sample.

To determine HCV infecting genotype and to identify MG, a phylogenetic analysis of nucleotide sequences obtained from PCR-positive specimens (plasma samples and PBMC) of each patient was performed. Clustal X, version 1.64 b was used to infer the multiple alignments of the nucleotide sequences. Prototype sequences for each genotype and subtype (GenBank accession numbers: Genotype 1a M62321, Genotype 1b D90208, Genotype 1c AY651061, Genotype 2a D00944, Genotype 2c D31972, Genotype 3a D28917, Genotype 4a Y11604, Genotype 4c FJ462436, and Genotype 4d DQ516083) were added and phylogenetic tree was constructed using Phylip package 3.67. Of note, by using the 5’NC region for genotyping it was not possible to distinguish between 4c and 4d subtypes because sequences of these 2 genotypes are identical within the evaluated region.

Statistical Analysis

Clinical data were considered for statistical analysis at BL of all 19 patients included in the study. Viral population in plasma and PBMC were considered in SVR at BL, in NR at BL, and during treatment and in RE at BL and W24-PT. The MG information changed for patient (PT) 4 from single to MG during the period of observation. Therefore, this patient was considered as infected by MG since BL. Continuous variables were described by median and interquartile range (IQR), whereas categorical variables by absolute counts and percentages (%).

Univariate Analysis

Data were analyzed using Chi-Square, Fisher exact, or Mann-Whitney U test, when appropriate. A P value of <0.05 was considered statistically significant.

Multivariate Analysis

The aim of the multivariate analysis was to determine the role of covariates in affecting the treatment response variable, as the presence of MG, the HCV viral load (expressed as the logarithm of the result of the HCV–RNA quantitative test), age, HCV infecting genotype, sex, and HCV–RNA detectability in PBMC.

We performed the analysis using a logistic regression model to predict the binary response to treatment with the covariates mentioned above, measured at BL. A P value of <0.05 was considered statistically significant.

RESULTS

Frequency of MG in the KaMON Study

By NGS analysis a high frequency (11/19 cases, 58%) of MG was detected in plasma of this group of HIV/HCV coinfected persons. No differences in regard of demographic data, immune status assessed by CD4+ T cells count, transaminase levels, and HCV load were found between individuals with mixed or single infection (Table 1).

Frequency of MG According to Antiviral Treatment Response

A different distribution of MG was detected in NR with respect to SVR: MG was present in 9/10 patients with no response to P–R treatment, including 8 NR and 1 RE, and in 2/9 SVR patients (P = 0.005).

Four patients with SVR had RVR and none of these 4 patients had MG. A different frequency of RVR was found between individuals with or without MG, P = 0.02.

HCV–RNA was positive in PBMC of 15/19 (73%) patients, at BL. This positivity was more frequent in NR (10/10) than in SVR (5/9); P = 0.03.

As in plasma, also in PBMC a DTG was more frequently detected (9/10 cases) in NR patients, whereas ETG (4/5 cases) was prevalent in SVR patients; P = 0.01.

By univariate analysis, in addition to MG, older age, higher HCV–RNA levels, the absence of RVR, infection with DTG as dominant strain and HCV–RNA positivity in PBMC, were significantly associated with treatment failure (P = 0.02, P = 0.007, P = 0.03, P = 0.0007, and P = 0.03, respectively, Table 2).

Multivariate analysis (Table, Supplemental Content 3, http://links.lww.com/MD/A485) showed that HCV MG reported a significant negative impact on the probability of treatment response (regression coefficient associated to HCV MG = −3.815, Table, Supplemental Content 3, http://links.lww.com/MD/A485), which can be interpreted as a significant reduction of the probability of response of 0.022 (OR = 0.022, 95% CI = [0.001–0.602]; P = 0.029), whereas HCV–RNA levels (evaluated as unitary increase) showed a
trend toward significance (OR 0.17, 95% CI, OR 0.025–1.231; 
\( P = 0.07 \)). Hepatitis C virus viral load was retained by the model, 
meaning that it is a no-redundant variable to allow for the whole 
model to be significant. The genotype, which is considered to be 
an important factor in the treatment response process, did not 
result significant in the multivariate analysis. The infecting 
genotype, classified as DTG or ETG, was the first variable 
excluded by the selection procedure (\( P = 0.999 \)).

Dynamic of MG in Plasma Compartment

The dynamic of viral population in NR/RE during P–R 
treatment is summarized in Table 3 and Figure 1. In particular, 
PT1, in whom ETG strain was already present at BL as minor 
variant, showed ETG as unique dominant strain during treat- 
ment. In this patient at W24-PT, in the absence of P–R 
pressure, the genomic pattern was the same of that detected 
at BL. In PT4, ETG was not detected at BL but emerged as 
unique dominant strain at W24 of therapy and remained as 
minor variant at W24-PT. In PT6, G4a was present as dominant 
virus at BL and W12 of treatment, whereas at W24 was 
replaced, as dominant strain by G4c/d. Unfortunately, in this 
case it was not possible to evaluate W24-PT viral population 
because the plasma sample was not available at this time point. 
During the observation period, 3 NR patients (PT3, PT5, PT7) 
maintained the same DTG detected as dominant at BL. Two 
other patients (PT2, PT8) showed a conserved virological 

### TABLE 2. Clinical Characteristics of HIV/HCV Coinfected Patients on the Basis of Response to P–R Treatment

|                  | Mixed Infection N = 11 | Single Infection N = 8 | P     |
|------------------|------------------------|------------------------|-------|
| Age, years (median, IQR) | 46 (44–49)             | 43 (41–45)             | 0.08  |
| Gender (male/female)    | 8/3                    | 5/3                    | >0.99 |
| Risk factor for HIV infection Parenteral/sexual | 8/3                    | 5/3                    | >0.99 |
| Duration of HIV infection, years (median, IQR) | 17 (15–22)             | 16 (13–19)             | 0.43  |
| CD4\(^+\), cells count/μL (median, IQR) | 758 (458–1171)         | 475 (436–602)          | 0.23  |
| CD8\(^+\), cells count/μL (median, IQR) | 878 (654–919)          | 1108 (847–1368)        | 0.14  |
| ALT, U/L (median, IQR) | 72 (52–98)             | 99 (71–194)            | 0.41  |
| AST, U/L (median, IQR) | 42 (34–55)             | 49 (37–98)             | 0.56  |
| HCV–RNA, log IU/mL (median, IQR) | 6.22 (5.75–6.37)       | 5.68 (4.61–6.10)       | 0.16  |
| DTG/ETG               | 10/1                   | 2/6                    | 0.006 |
| SVR/NR or RE          | 2/9                    | 7/1                    | 0.005 |
| RVR                   | 0                      | 4                      | 0.020 |

ALT = alanine amino transferase, AST = aspartate amino transferase, ETG = easy to treat genotype, DTG = difficult to treat genotype, HCV = hepatitis C virus, HIV = human immunodeficiency virus, IQR = inter quartile range, NR = nonresponder, RE = relaper, RNA = ribonucleic acid, RVR = rapid virological response (HCV–RNA < 12 IU/mL at W4 of treatment), SVR = sustained virological response.

### TABLE 3. Clinical Characteristics of HIV/HCV Coinfected Patients on the Basis of Mixed or Single HCV Infection Evaluated in Plasma Compartment

|                  | Mixed Infection N = 11 | Single Infection N = 8 | P     |
|------------------|------------------------|------------------------|-------|
| Age, years (median, IQR) | 46 (44–49)             | 43 (41–45)             | 0.08  |
| Gender (male/female)    | 8/3                    | 5/3                    | >0.99 |
| Risk factor for HIV infection Parenteral/sexual | 8/3                    | 5/3                    | >0.99 |
| Duration of HIV infection, years (median, IQR) | 17 (15–22)             | 16 (13–19)             | 0.43  |
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| DTG/ETG               | 10/1                   | 2/6                    | 0.006 |
| SVR/NR or RE          | 2/9                    | 7/1                    | 0.005 |
| RVR                   | 0                      | 4                      | 0.020 |

ALT = alanine amino transferase, AST = aspartate amino transferase, ETG = easy to treat genotype, DTG = difficult to treat genotype, HCV = hepatitis C virus, HIV = human immunodeficiency virus, IQR = inter quartile range, NR = nonresponder, RE = relaper, RNA = ribonucleic acid, RVR = rapid virological response (HCV–RNA < 12 IU/mL at W4 of treatment), SVR = sustained virological response.
pattern during treatment. Finally, PT 10 had evidence of HCV single infection during all the observation period. Concerning 2 SVR patients with MG, an ETG was dominant in one subject and a DTG in the other one.

Of the other 7 SVR, a single infection with ETG was detected in 6, whereas DTG was found in the remaining one. The distribution of HCV genotype identified at BL and the pattern of viral population in SVR are summarized in Figure 2.

Dynamic of MG in PBMC Compartment

Dynamic of HCV infection in PBMC of NR/RE patients is summarized in Table 3 and Figure 1. A similar distribution of MG in PBMC compartment was detected between NR/RE and SVR; 6/15 (40%) HCV–RNA positive cases had an MG infection: 4 of whom were NR/RE (PT2, PT3, PT6, PT9). Among these, 3 patients harbored a DTG as dominant strain, and the remaining 1 (PT2) an ETG. Among SVR, 2/5 HCV–RNA PBMC-positive cases had an MG with dominance of ETG and DTG, respectively (Fig. 2). Of note, in 2 NR patients (PT1, PT10) with single infection at BL, an MG was revealed during treatment. In PT1 HCV–RNA became undetectable at W24-PT.

Interestingly, HCV–RNA was found negative in PBMC at W12 and W24 of treatment in the 2 patients that relapsed the infection after treatment.

Comparison of Viral Population Between Two Different Compartments (Plasma and PBMC)

At BL evaluation, among individuals found positive in both compartments (plasma and PBMC) a concordant genotype as dominant was observed in 5/8 NR, 1/2 RE, and 4/5 SVR, \( P = 0.60 \).

During treatment, in 3 NR (PT1, PT4, PT6) a discordant genotype was detected as dominant in plasma and PBMC. Two (PT4, PT6) of these 3 patients also had a discordant genotype in the 2 compartments at BL.

DISCUSSION

Hepatitis C genotype and viral kinetics on treatment have been suggested to be the most important viral factors affecting the treatment response. The dynamic of HCV MG in different compartments and its correlation with response to P–R treatment has never been set up before in HIV/HCV coinfected persons. These patients, depending on the risk factor, may be

TABLE 3. Viral Population Analysis by Next-Generation Sequencing During Treatment With P–R and Posttreatment Follow-Up (HCVG–W24PT) in HIV/HCV Coinfected Individuals

| HCVG–BL | HCVG–W12 | HCVG–W24 | HCVG–W24PT |
|---------|----------|----------|------------|
| Plasma  | 1b-1a-1c-3a | 3a | 3a | 1b-1a-1c-3a |
| PBMC    | 1b | 1b | 1b-1a | neg |
| Plasma  | 1a-1c | 1a-1c | – | – |
| PBMC    | 3a-1b | 1a | – | – |
| Plasma  | 4c/d-1b-1a-3a-2a | 4c/d-2a-1a | 4c/d-3a | – |
| PBMC    | 4c/d-1b-3a-2a | neg | neg | – |
| Plasma  | 1a | 1a | 3a | – |
| PT1     | 1a | 1a | 3a | 1a-4c/d-1b-3a |
| Pt2     | 1b | 1b | 1b-2a | – |
| Pt3     | 1b | – | – | – |
| Pt4     | 1c | 1a | 1c | 1a |
| Pt5     | 1b-1a-3a-2a | 1b-2a | – | – |
| Pt6     | 1b | – | – | – |
| Pt7     | 1a | – | – | – |
| Pt8\^\* | 1b | neg | neg | 1b |
| Pt9\^\* | 1b-1a-1c | neg | neg | 1c-1a-1b |
| Pt10    | 4c/d-4a-3a | neg | neg | – |
| Pt11    | 4c/d | 4c/d | 4c/d | – |

Dominant genotype is indicated in bold. G = genotype, PT = patients. – = specimen not available.

\(^\*\) RE patients.
more frequently exposed to MG.28 A possible explanation for the lack of information concerning the extent and the response rate in MG infection might be the low sensitivity of biological approach to detect MG. Second, in clinical trials the inclusion of patients with MG is not allowed, rendering this data not available in large groups of patients. In the present study, we had the opportunity to precisely estimate the rate of MG by using a highly sensitive method "NGS", showing a high frequency (58%) of MG in a group of HIV/HCV coinfected patients under P–R treatment.

Our result is in line with that of other studies8,9 performed in parenterally transmitted infection risk groups and using highly sensitive strategies to detect MG.

By univariate analysis, MG was found to be associated with virological response to P–R treatment, in addition to some factors already known to be involved in the response to P–R treatment: age, HCV-viral load, genotype, and RVR.

In this context, RVR is known to be a strong predictor of SVR,29–30 whereas no data are available on the correlation between RVR and MG. In the present study we found that RVR was more frequent in individuals with single infection respect to those with MG. One other viral factor, which could influence the response rate, was the presence of HCV genomic sequences in PBMC. Inglot et al31 suggested that the replication of HCV in this compartment could be a potential marker for treatment response. A number of studies20–22 showed that HCV–RNA positivity in PBMC of patients that attained end of treatment virologic response is associated with relapse of infection in the post-treatment phase.

In the present study, HCV–RNA was more frequently detected in PBMC of NR than in SVR. Only 2 RE patients were included; therefore, it was not possible to evaluate the correlation between HCV–RNA persistence in PBMC and reactivation of the infection in the post-treatment phase.

Our data, although obtained in a small group of patients, suggested that replication of HCV in PBMC rather than viral population complexity in this compartment assessed by detection of MG, may play a role in the treatment success rate.

By multivariate analysis we showed that the probability of response to P–R treatment was negatively and significantly affected mainly by an MG, whereas HCV load showed a trend toward significance and HCV infecting genotype per se together with HCV–RNA positivity in PBMC did not reach statistical significance. To our knowledge, only 1 study32 evaluated the
effect of mixed genotypes 1 to 2 infection on the response during P–R treatment, showing that individuals infected with multiple genotypes had a response rate similar to those infected by a single genotype. The study was retrospective, conducted in Japan where genotype 1 is prevalent, and performed in HIV-negative patients. Additionally, clinical data obtained in MG were compared with those of historical controls. Therefore, the different geographic origin of their and our patients, the different population analyzed in terms of risk factor for HCV infection, the concurrent infection with HIV in our study group, and the fact that Huang et al...
exploring by next-generation sequencing the extent of MG and plasma and PBMC. To our knowledge, this is the first study to examine the alternance of DTG and ETG could be related to a different strain after treatment. It is unlikely a super-infection with a new virus because these 2 patients denied unprotected sexual contact and intra venous drug injection. Additionally, the genotype detected during P–R treatment was present, albeit as minor variant, at baseline evaluation. We formed the hypothesis that the presence/absence of MG in plasma may be responsible for the discrepant results.

Interestingly, 2 patients showed during P–R treatment, the emergence of an ETG as unique dominant strain in the plasma of interest to evaluate the presence/absence of MG infection in SVR patients infected by DTG (G1–4). However, our data generated a precise information on mixed infection dynamic during the course of P–R treatment in different compartments. We showed that HIV/HCV coinfected patients may harbor multiple and discordant HCV genotypes in the different compartments explored and that the presence of MG in plasma samples may be a potential marker of poor treatment response. This finding could have important clinical implication especially in the case of association treatment with new antivirals targeting a specific genotype, because these drugs could be less effective in MG.

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