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Does HIV-1 co-receptor tropism correlate with fibrosis progression in HIV/HCV co-infected patients?

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ABSTRACT

Background: In HIV/HCV co-infected patients, HIV-1 gp120 activates human hepatic stellate cells (HSCs) which play a key role in fibrosis pathogenesis. It is still unclear whether pro-fibrogenic effects are more attributable to X4 or R5 strains *in vivo*.
Objective: To assess if HIV-1 X4 or R5 variants are associated with a different progression of fibrosis.
Study design: A total of 105 HIV/HCV co-infected patients were submitted to gp120 sequencing on proviral DNA and classified as X4 or R5 based on g2p (20% false positive rate). The fibrosis evolution was retrospectively determined by means of APRI and FIB-4 scores at 3-month intervals from the first anti-HCV-positive test. The association of co-receptor tropism with increased fibrosis scores was evaluated by linear mixed models.
Results: X4 variants were found in 41 patients (39%). The median observation period was similar in X4 and R5 patients (17 years). No difference was observed between the two groups of patients, except for nadir CD4 which was lower in X4 compared to R5 (percentage, $p = 0.005$, and absolute number, $p = 0.005$). X4 and R5 patients did not significantly differ for FIB-4 and APRI score over time ($p = 0.5$, and $p = 0.1$, respectively). No association between HCV-RNA levels over time and co-receptor tropism was noted ($p = 0.9$). Conversely, a significant correlation of fibrosis scores with gamma-glutamyl transferase levels, lower current CD4 count, HIV viremia and use of antiretrovirals was observed.
Conclusions: This retrospective analysis of fibrosis evolution did not support the evidence of a differing pro-fibrogenic activity for X4 and R5 HIV-1 variants in HIV/HCV co-infected patients.

1. Background

HIV infection modifies the natural course of chronic hepatitis C by increasing the likelihood of cirrhosis, liver failure and HCV-related mortality [1,2]. The biological basis for this interaction, however, is still poorly understood and many factors, both direct and indirect, are probably involved [3]. Among the indirect mechanisms, the most important is based on an impaired T-cell response to HCV [4], which can be only partially reverted by antiretroviral treatment [2]; in fact, end-stage liver disease actually represents the leading cause of mortality among HIV-positive patients, regardless of the use of ART [5]. Moreover, HIV itself and certain antiretrovirals may contribute to liver disease by inducing the metabolic syndrome [6]. In addition, HIV infection of the gastrointestinal tract amplifies microbial translocation which can stimulate hepatocytes, Kupffer cells (KCs), and hepatic stellate cells (HSC) to produce pro-inflammatory cytokines and chemokines. These factors attract activated lymphocytes and monocytes to the liver, thus promoting the fibrosis process [3].

Among the direct mechanisms, HIV cannot infect hepatocytes, KCs and HSC, as these cells do not express CD4 receptors, even if alternative primary receptors have been suggested [7]. However, HSCs, which play a key role in the pathogenesis of fibrosis, express both HIV CCR5 and CXCR4 co-receptors, which pertain to the family of 7-transmembrane-helix beta-chemokine receptors. CCR5 and CXCR4 are mainly adopted by HIV, together with the CD4, for entry into human host cells and, based on their differential use, HIV-1 isolates are classified as R5 and X4, respectively. It has been shown that, even in the absence of productive infection, the HIV-1 gp120 binding to CXCR4 [8,9] and CCR5 [10] is able to activate HSCs. However, whether X4 and R5 viruses exert a different profibrogenic effect on HSCs is debated. Based on *in vitro* studies, Bruno et al. [10] reported that R5 gp120, more than X4,

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promotes the secretion of IL-6 and MCP-1 (monocyte chemoattractant protein-1), thus inducing HSC chemotaxis, and to a lesser extent, determining some pro-fibrogenic effects. On the contrary, the study of Hong et al. [9] focused on the effect of X4-tropic gp120 and found that the ERK1/2 (extracellular signal-regulated kinase) pathway was important for collagen-I induction. The expression of CXCR4 was found to be enhanced in HCV infected livers and increased with culture-induced HSC activation [8]. Moreover, SDF-1a (stromal cell-derived factor-1 alpha), the endogenous ligand for CXCR4, was shown to promote HSC collagen-I expression, activation, and proliferation [8]. Therefore, it could be hypothesized that R5 gp120 exert more pro-inflammatory effects while X4 gp120 is more pro-fibrogenic, but further investigation is required. In addition, CXCR4 binding with HIV-1 gp120 was also demonstrated to induce *in vitro* hepatocyte apoptosis (which can conversely trigger pro-fibrotic activity of HSC) [11,12], and to enhance HCV replication [13], also by interacting with HCV E2 protein [14].

Notwithstanding these *in vitro* studies, it is still unclear whether the pro-fibrogenic effects *in vivo* are more attributable to X4 or R5 HIV-1 strains. Therefore, we decided to consider the working hypothesis that CXCR4 viruses, which are associated to a faster HIV disease progression, are also responsible for a more rapid evolution of liver fibrosis in co-infected patients.

2. Objective

The aim of the study was to assess if, in a group of HIV/HCV positive patients, HIV-1 co-receptor tropism (CRT, X4 or R5) was associated with a different fibrosis progression over time, retrospectively assessed by means of surrogate serological markers.

3. Study design

A total of 105 HIV/HCV co-infected patients were enrolled and consecutively subjected to blood sampling for genotypic co-receptor testing (CRT). As most patients were treated and therefore expected to have low or undetectable plasma viral load, CRT was performed for all subjects on proviral DNA which furnishes highly comparable results with those from viral RNA [15]. Concurrently, patients were also submitted to routine blood exams for the assessment of immune-virological (CD4+ cell count and HIV viral load) and liver function parameters. Retrospective clinical data were extracted from the database of the institute and included: date of first HIV- and HCV-positive test, demographic and behavioral (alcohol and smoking habits) data, risk factors for HIV acquisition, onset of opportunistic infections (AIDS events), CDC staging (revised 1993), blood biochemical parameters, CD4+ cell count, plasma HIV-1 and HCV viral load, liver function parameters, clinical and therapeutic history and reported side effects.

3.1. Gp120 sequencing and CRT assignment

HIV-DNA was extracted and amplified from PBMCs and gp120 sequencing on proviral DNA was performed as previously described [16]. Only one PCR product per sample was subjected to standard population sequencing. Sequences were analyzed with Seqscape software v2.5 (Applied Biosystems, Foster City, CA). Nucleotide mixtures were considered if the second highest peak in the electropherogram was >25%. CRT was inferred with the geno2pheno[co-receptor] algorithm (http://co-receptor.bioinf.mpi-inf.mpg.de/), setting the false positive rate (FPR) at 20%, according to the 2011 European Guidelines [17] since interpretation was based on a single DNA amplification and sequencing; isolates were classified as R5 (FPR >20%) or X4 (FPR ≤20%). Only clonal prediction was employed for classifying sequences.

3.2. HIV-1 subtyping

The HIV subtype was assigned by phylogenetic analysis (neighbor-joining method using Kimura two-parameter distances and Simplot analysis) of *pol* (complete protease and reverse transcriptase) (Viroseq HIV Genotyping Kit; Applied Biosystems, Foster City, CA) and *env* sequences.

3.3. Fibrosis assessment

The fibrosis evolution was retrospectively determined by means of APRI (aspartate aminotransferase/platelet ratio index) and FIB-4 scores at 3-month-intervals starting from the first anti-HCV positive testing to the date of CRT testing (or HIV positive testing if successive to HCV positivity) for untreated HCV patients and up to the last available determination before initiating anti-HCV therapy for treated patients.

APRI was calculated using Wai's formula: (AST/upper limit of normal considered as 40 IU/L)/platelet count (expressed as platelets $\times 10^9$ L^{-1}) $\times$ 100. FIB-4 was calculated using Sterling's formula, as follows: age [years] $\times$ AST [IU/L]/platelet count [expressed as platelets $\times 10^9$/L] $\times$ (ALT1/2[IU/L]).

In a subgroup of 76 patients, liver stiffness at time of CRT testing was also evaluated by a single certified operator (trained by the manufacturer) using transient elastography (FibroScan®; EchoSens, Paris, France).

3.4. Statistical methods

Descriptive statistics were produced for all variables. The Mann–Whitney test was used to compare X4 and R5 groups in terms of quantitative variables at time of CRT testing, while the Fisher exact test was adopted for categorical variables. The association of CRT with an increase in fibrosis scores over time, adjusting for baseline values, was evaluated by linear mixed models, including random intercept (patient) and random slope (time in years from HIV infection). Results were then back-exponentiated for better clarity.

Stata computer software version 12.0 (Stata Corporation, 4905 Lakeway Drive, College Station, TX 77845, USA) was used for statistical analysis.

3.5. Ethics

The research did not require approval from the ethics committee according to the Italian law since it was performed as an observational retrospective study in the context of normal clinical routines. However, all patients provided informed consent for the use of their data for research purposes.

4. Results

The characteristics of the 105 enrolled HIV-1-infected patients according to CRT are summarized in Table 1. A total of 41 (39%) patients showed a X4 virus in their HIV-1 proviral DNA while the remaining 64 patients was classified as R5. The median period of observation was similar in the two groups (median 17 years, IQR 13–21 years). Ten patients were also HBV infected (equally distributed between the two groups, see Table 1); all of them were treated with tenofovir/emtricitabine and had a negative HBV-DNA.

A total of 90/105 patients (86%) were on antiretroviral therapy, 61 of whom (68%) had plasma viral load (pVL) <25 copies/ml at time of CRT. The duration of antiretroviral therapy (overall, and for each