High concordance of genotypic coreceptor prediction in plasma-viral RNA and proviral DNA of HIV-1 subtype C: implications for use of whole blood DNA in resource-limited settings

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Objectives: Genotypic tropism testing (GTT) of HIV is increasingly used prior to the initiation of CCR5 antagonist therapy in HIV-infected individuals. Normally performed on plasma-derived virus, the test is challenging when performed in patients with suppressed viraemia. We aimed to evaluate the performance of cell-associated proviral DNA against plasma-derived viral RNA as the genetic material for GTT in an Indian clinical setting.

Methods: From 52 HIV-1-infected individuals, the env V3 region was successfully amplified and sequenced from both proviral DNA and plasma RNA paired samples having a viral load >2500 copies/mL (n=42) and from proviral DNA only in 10 antiretroviral therapy (ART)-experienced patients with a viral load <500 copies/mL. GTT was performed using the Geno2Pheno algorithm with the interpretative false positive rate (FPR) cut-off of 10%.

Results: Among paired samples, 40 of 42 patients harboured subtype C strains. Plasma RNA tropism prediction revealed X4 tropism in 4 of 42 (9.5%). A high concordance of 97.6% in tropism prediction was noted in simultaneous RNA/DNA samples (38 R5 and 3 X4). Discordance was observed in one sample showing R5 tropism in proviral DNA and X4 tropism in plasma RNA. Comparison of Geno2Pheno FPRs in both the plasma and proviral compartments showed good correlation (overall, r=0.87; ART-naive patients, r=0.79; ART-failing patients, r=0.97). GTT was successfully performed in all 10 whole blood DNA samples having a viral load <500 copies/mL, all showing R5 tropism.

Conclusions: High concordance in tropism prediction from proviral DNA and plasma-viral RNA suggests that prediction of viral tropism using proviral DNA is accurate and feasible in resource-limited clinical settings, particularly in patients with low or suppressed viraemia.

Keywords: genotypic tropism testing, Geno2Pheno, HIV

Introduction

On the basis of their differential selection of the coreceptor used for entry, HIV strains are classified as R5-tropic (those that use CCR5), X4-tropic (those that use CXCR4) or dual-tropic (R5/X4).1 With the availability of CCR5 antagonist drugs such as maraviroc, it is recommended that viral tropism be determined prior to initiation of the drug in order to exclude the presence of X4-tropic viruses and predict better drug efficacy.1 HIV-1 tropism can be assessed by phenotypic or genotypic methods. Compared with phenotypic tropism testing, genotypic tropism testing (GTT) is a more feasible option in clinical settings due to its ease of performance, rapidity and cost-effectiveness.3 GTT is based on bioinformatic algorithms that use V3 loop sequences as the information input and have been trained on large phenotypic tropism datasets.4 The European consensus guidelines on GTT have recommended the use of a support vector machine, Geno2Pheno[coreceptor], with a false positive rate (FPR) threshold of 10% for population-based sequencing.2 The FPR is defined as the probability of falsely classifying an R5 virus as X4.

Plasma-derived viral RNA has been the most common material used for determining HIV-1 tropism by GTT; however, this is challenging in patients with a viral load <1000 copies/mL. In such situations genotypic analysis of proviral DNA is an inherently
attractive strategy.6,7 Furthermore, the use of proviral DNA in GTT can potentially solve the problem of cold-chain transport in resource-limited settings once the test is standardized on dried blood spots. Data on comparative GTT analysis using plasma virus and provirus are scarce or restricted to the analysis of HIV-1 subtype B strains,5,6,7 while worldwide the most prevalent HIV-1 strain is subtype C.9 In the present study we aimed to evaluate the performance of proviral DNA against plasma-viral RNA as the genetic material for GTT in an Indian clinical setting.

Methods

Study subjects

Fifty-two paired plasma and whole blood samples were obtained from HIV-1-infected patients (50 adults and 2 children) attending the Infectious Disease Clinic at St John’s Medical College and Hospital, Bangalore, India. Routine CD4 count was determined using a dual-platform flow cytometer (FACS Calibur, BD Biosciences, CA, USA) and plasma viral load was measured using the m2000rt RealTime PCR system (Abbott Molecular Diagnosis, Des Plaines, IL, USA).

This study was approved by the Institutional Ethical Review Board of St John’s Medical College and Hospital, Bangalore, India. Written informed consent was obtained from all participants, and the caregivers of the two children, prior to enrolment into the study.

Viral amplification and sequencing

HIV-1 RNA was extracted from plasma using a QIAamp viral RNA Mini Kit and proviral DNA was extracted from whole blood using a QIAamp DNA blood Mini Kit (Qiagen, Germany). The V3-V5 region of the env gene was amplified by nested PCR using previously described primers and PCR conditions.9,10 The purified second-round PCR amplicons obtained from both plasma-viral RNA and proviral DNA were subjected to bidirectional population sequencing in a 3730xl DNA Analyzer (Applied Biosystems, CA, USA). The sequences thus generated were manually edited in Bio-Edit version 7.0.9.0.

Genotypic prediction of coreceptor tropism

To predict the coreceptor tropism based on the env V3 sequences, we used an advanced support vector machine-based interpretation system, Geno2Pheno coreceptor with 10% FPR (Geno2Pheno10%; available at http://coreceptor.bioinf.mpi-inf.mpg.de/index.php). Sequences with an FPR <10% were deemed X4-tropic.7

Results

Patient characteristics

Among the 52 patients, both the RNA virus and provirus compartments were successfully amplified in 42 patients, among whom 26 were antiretroviral therapy (ART)-naive and 16 were failing first-line ART (Table S1, available as Supplementary data Online). Among the viral strains, 40 were typed as HIV-1 subtype C and one each as HIV-1B and A1/C (V3C3 region was A1) based on the env and pol genes.9 Both of the non-C subtype strains were found in patients failing first-line ART.

For the remaining 10 patients, who were ART-experienced and virally suppressed, plasma RNA could not be amplified. Whole blood DNA from these samples was used, and GTT was successfully performed. All the strains belonged to subtype C and showed R5 tropism.

Performance of proviral DNA against plasma RNA in predicting tropism

Using Geno2Pheno10% on plasma RNA samples, X4 tropism was predicted in four samples, at a rate of 9.5% (4/42), two each in ART-naive patients (7.7%) and ART-failing patients (12.5%), while using proviral DNA, X4 tropism was predicted in three samples at a rate of 7.1% (3/42). The concordance between GTT in the proviral and plasma-viral compartments using Geno2Pheno10% was 97.6% (41/42; 38 R5 and 3 X4). Overall, good concordance of 96.2% and 100% was observed in both ART-naive and ART-failing groups, respectively. A discordant result was seen in a single ART-naive patient, who showed R5 virus in the proviral compartment and X4 virus in the plasma-viral compartment. Comparison of Geno2Pheno FPRs in both the plasma and proviral compartments showed good correlation in both ART-naive (r=0.79) and ART-failing patients (r=0.97), with an overall correlation of 0.87 (Figure 1).

Phylogenetic analysis of the V3C3 region revealed that clusters of sequences with high bootstrap support corresponded to the paired samples from the plasma RNA and proviral DNA compartments within the same patients (Figure 2).

Discussion

We found that genotypic tropism analysis using whole blood proviral DNA as the source genetic material performed equally as well as analysis performed using plasma-viral RNA obtained from clinical samples of HIV-1C-infected patients. The use of proviral DNA to determine tropism is of particular significance in ART-experienced patients with suppressed viremia. This is evident from our observation in a subset of ART-experienced patients with undetectable plasma viral load where, despite plasma RNA being non-amplifiable, GTT was successfully performed from proviral DNA. Previous studies have determined coreceptor tropism using proviral DNA from non-viraemic patient samples,11 and some longitudinal studies have largely relied on proviral DNA as the genetic material to understand the dynamics of HIV-1 tropism under suppressive therapy.12–14 A high rate of maraviroc treatment success in patients having GTT performed on their proviral DNA sample was observed in the week 24 analysis of the Berlin maraviroc cohort.13 However, a recent study cautions that, compared with plasma virus, cell-derived viral DNA may be a less than optimal predictor of virological success on treatment with maraviroc in therapy-experienced patients.15 Proximal DNA has a clear advantage over plasma-viral RNA in resource-limited settings where cold-chain transport, storage of plasma and maintenance of RNA in deep freezers is not always possible. Whole blood can be easily stored at 4°C or can be transported as dried blood spots (DBS) from which DNA can be extracted before GTT. Hence our results may be useful in planning larger-scale studies where GTT may be performed using DBS.

Our finding of high concordance between the cellular and plasma compartments is consistent with previous studies. Most of these studies were performed in European countries, where
The subtype B population is predominant. Comparable results were obtained between the proviral and plasma-viral populations using the Geno2Pheno algorithm, with a discordance rate ranging between 4.8% and 18.2%.\textsuperscript{5–7,16,17} As in our study, two of the studies from Italy using whole blood DNA and Geno2Pheno\textsuperscript{10%} observed good concordance of 90% and 87.5%, respectively.\textsuperscript{5,7} We also observed a good correlation between FPR values of proviral DNA and plasma-viral RNA by

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scatter_plot.png}
\caption{Scatter plot for the comparison between the outcomes of plasma-viral (y-axis) and proviral (x-axis) V3-based coreceptor tropism prediction for paired RNA/DNA samples using FPR values of Geno2Pheno in (a) 26 ART-naive and (b) 16 ART-failing patients, showing a coefficient of correlation of $r=0.79$ and $r=0.97$, respectively. The overall coefficient of correlation was $r=0.87$.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{phylogenetic_tree.png}
\caption{Maximum-likelihood phylogenetic tree representing 42 provirus and plasma-virus paired sequences. The tree was constructed using the HKY-G parameter with 1000 bootstrap values in MEGA 5 software. All the samples showed bootstrap values of $>70\%$ between the pairs (i.e. samples from archived provirus and circulating plasma virus from the same patient), denoting genetic relatedness. One discordant sample pair is circled. The amino acid sequences of the discordant samples are shown in the inset box. The star-like topology observed denotes a single evolutionary origin. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.}
\end{figure}
taking the Geno2Pheno FPR values as quantitative outputs, similar to the observation of Verhofstede et al. \(^6\) of a correlation of 0.85. Although earlier studies have shown that X4-tropic strains are more frequent in proviral DNA than in plasma-viral RNA, \(^18\) we observed that our single discordant sample harboured X4-tropic strains in plasma RNA and R5-tropic strains in whole blood DNA. The probable explanation for X4 tropism in plasma and R5 tropism in provirus in this sample could be an early switch in actively replicating virus population during disease progression. Differentiating characteristics observed in the discordant sample in our study were the presence of the GPGQ motif, a higher positive charge of 6 and net charge of 4.

Since the present study focused on the use of proviral DNA for GTT we used a single algorithm, Geno2Pheno, which is the most widely used tool and is well validated against phenotypic assays. \(^8,19\) Our study was limited by the small number of paired samples included, which may have prevented true differences between the different compartments. Additionally, we have applied population-based sequencing and thus may have missed minor variants. This can be overcome by deep sequencing, which is a good predictor of the Trofile (phenotypic) assay and can be used in proviral DNA samples in patients with suppressed viral load. \(^20\) However, in resource-limited settings the technical challenges and cost associated with deep sequencing currently prohibit its easy access.

In conclusion, our results suggest that prediction of viral tropism using proviral DNA is a simple, reliable and low-cost method that may be applicable in resource-limited clinical settings. There is a need for large-scale clinical validation of GTT using proviral DNA.

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Transparency declarations
None to declare.

Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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