Improved V3 genotyping with duplicate PCR amplification for determining HIV-1 tropism

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Objectives: To determine whether genotyping of HIV-1 by duplicate PCR amplification of the region encoding the V3 loop is more sensitive than single PCR for detecting CXCR4-using viruses.

Patients and methods: The V3 genotypes of the HIV-1 infecting 152 patients enrolled in the multicentre Geno-Tropism ANRS study were determined by all the participating laboratories using a single PCR and V3 bulk sequencing. In parallel, one laboratory determined the V3 genotype using duplicate PCR and bulk sequencing of pooled amplicons. HIV tropism was predicted with the geno2pheno10 algorithm. The phenotypes of all samples were determined with the Trolfile assay and the Toulouse tropism test (TTT) recombinant virus assay.

Results: Geno2pheno10 was 56.8% sensitive and 75.9% specific when compared with the Trofile assay for detecting CXCR4-using viruses after a single PCR. Duplicate amplification and bulk sequencing of the pooled PCR amplcons increased the sensitivity to 68.2% and specificity to 79.6%. Geno2pheno10 was 64.1% sensitive and 77.0% specific when compared with the TTT assay for detecting CXCR4-using viruses after a single PCR. Duplicate amplification and sequencing of the pooled PCR amplicons increased sensitivity to 76.9% and specificity to 80.5%.

Conclusions: The genotypic determination of HIV-1 tropism can be improved by duplicate amplifications and sequencing the pooled PCR products. This is a good compromise between improved sensitivity and reasonable cost for the genotype-based determination of tropism.

Keywords: HIV-1 tropism, phenotype, CCR5 receptor, V3 genotype, bulk sequencing

Introduction

HIV type 1 (HIV-1) enters CD4-expressing cells using one or both of the chemokine receptors CCR5 and CXCR4. CCR5-using viruses are classified as R5 variants, CXCR4-using viruses as X4 variants and viruses using both co-receptors as R5X4 dual-tropic variants.1 HIV-1 co-receptor use must be determined before using a CCR5 antagonist in clinical practice because these drugs act only against R5 viruses. HIV-1 tropism can be assessed with phenotypic assays.2,3 However, these assays are rather complex and costly for routine use. HIV-1 tropism can be determined by genotyping the V3 env region that contains the main genetic determinants of HIV-1 co-receptor usage.4,5 But studies on the algorithms for interpreting V3 sequencing have found suboptimal concordance between genotypic and phenotypic approaches.6,7

We previously evaluated the genotypic prediction of HIV-1 tropism using single amplification in the multicentre ANRS Geno-Tropism study.7 We have now compared the performances of duplicate and single PCR amplification for V3 genotyping with reference to the Trofile and Toulouse tropism test (TTT) phenotypic assays.

Materials and methods

We studied 152 patients (140 infected with HIV-1 subtype B (92%), 4 with subtype CRF02-AG, 3 with subtype G, 3 with subtype D and 2 with
Results

The 152 samples screened included 113 identified by TTT as R5 and 44 identified as R5X4 or X4. At the same time, the V3 genotype was determined in each participating laboratory by single PCR and bulk sequencing and in one laboratory by duplicate amplifications and bulk sequencing of the pooled products.

Table 1. Determination of HIV-1 tropism by two genotype-based assays using the Trofile or TTT assays as a reference

<table>
<thead>
<tr>
<th>V3 genotype</th>
<th>Trofile phenotype</th>
<th>Performance of the V3 genotype using Trofile as a reference</th>
<th>TTT phenotype</th>
<th>Performance of the V3 genotype using TTT as a reference</th>
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<tr>
<td></td>
<td></td>
<td>sensitivity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>specificity&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Single PCR and Geno2pheno10</td>
<td>R5 82</td>
<td>19</td>
<td>56.8%</td>
<td>75.9%</td>
</tr>
<tr>
<td></td>
<td>X4 26</td>
<td>25</td>
<td>68.2%</td>
<td>79.6%</td>
</tr>
<tr>
<td>Duplicate PCR and Geno2pheno10</td>
<td>R5 86</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X4 22</td>
<td>30</td>
<td></td>
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</tbody>
</table>

<sup>a</sup>Sensitivity, the capacity for detecting CXCR4-using viruses, was calculated by the number of concordant X4/R5X4 results divided by the number of viruses phenotyped as R5X4/X4.

<sup>b</sup>Specificity, the capacity for detecting exclusive CCR5-using viruses, was calculated by the number of concordant R5 results divided by the number of viruses phenotyped as R5.

Discussion

As the virological response to a treatment that includes a CCR5 antagonist is impaired by the presence of X4 viruses, these viruses must be detected by a tropism-determining assay. Population-based sequencing of the V3 env region and genotypic algorithms are useful for routine determination of HIV-1 tropism, but genotypic methods are often poorly sensitive for detecting
with each new PCR run. We also separately analysed by both single PCR run, but the probability of it being amplified increases in the quasi species. A minor variant may not be amplified in a to reduced sampling bias and better detection of minor variants improved sensitivity obtained with replicate PCRs could be due from 64.1% to 76.9% with the TTT assay as a reference. This 11.4% gain was not from the V3 genotype and two phenotypic assays. Single PCR amplifications to test 152 patients included in the GenoTropism study to assess the concordance of samples harbouring R5X4/X4 viruses. Similarly, duplicate statistically significant, probably because of the small number of samples harbouring R5X4/X4 viruses. The highest sensitivity for detecting X4 viruses was calculated for each group using both strategies of amplification and the TTT assay as a reference. The proportion of R5X4/X4 viruses accurately predicted by the V3 genotype is indicated on the bars. NA, not applicable. *McNemar’s χ² test was used for comparing the sensitivities of the genotype-based assays using single and double amplification strategies.

CXCR4-using viruses. The highest sensitivity for detecting X4 viruses was 59.3% with geno2pheno10 in the multicentre ANRS GenoTropism study, while the sensitivity was 88% in a previous study with the same genotypic algorithm after duplicate amplification of the env region. We therefore compared single and duplicate PCR amplifications to test 152 patients included in the GenoTropism study to assess the concordance between the V3 genotype and two phenotypic assays. Single bulk sequencing was performed and the V3 genotype was determined with the geno2pheno10 algorithm. Sensitivity was increased to 68.2% by duplicate amplification with the Trofile assay as a reference. This 11.4% gain was not statistically significant, probably because of the small number of samples harbouring R5X4/X4 viruses. Similarly, duplicate amplification increased the sensitivity for detecting X4 viruses from 64.1% to 76.9% with the TTT assay as a reference. This improved sensitivity obtained with replicate PCRs could be due to reduced sampling bias and better detection of minor variants in the quasi species. A minor variant may not be amplified in a single PCR run, but the probability of it being amplified increases with each new PCR run. We also separately analysed by both single and duplicate PCR amplifications 11 samples harbouring X4 variants accounting for 3%–15% of the virus population as assessed by pyrosequencing. Single PCR detected minor X4 variants in only two samples, whereas duplicate PCR detected them in the same samples and in three more samples (data not shown). However, the lower sensitivity of the single PCR approach may also be due to small differences between the different laboratories. Nevertheless, all the laboratories undergo annual quality controls, including genotypic determinations of HIV tropism.

The genotyping of samples with a low HIV RNA load can be increased by triplicate amplification. In the GenoTropism study, duplicate PCR seemed to improve the genotyping of samples with both high and low plasma HIV-1 RNA loads. Besides the efficiency of the genotypic algorithms for interpreting the V3 sequence, optimized analytical conditions could also improve the performance of the genotype-based determination of HIV-1 tropism.

The sensitivity of a triplicate PCR strategy, based on three separate PCR amplifications and three bulk sequencings, is 67.4% for predicting CXCR4-using viruses. However, this strategy is costly and time consuming. We obtained a very similar sensitivity using duplicate PCR amplification and bulk sequencing of the pooled products. The additional cost of our strategy is the price of only one RT- and nested-PCRs compared with a single PCR strategy. A recent study showed that ultra-deep sequencing detected more than twice as many maraviroc recipients as having non-R5 viruses than did Ota. In samples where ultra-deep sequencing detected the presence of non-R5 viruses accounting for 2%–20% of the virus population, 37% of them were also identified as having non-R5 virus by the triplicate strategy and 58% by Trofile. We therefore compared our duplicate approach and triplicate PCR plus three bulk sequencings in selected samples in which X4 minor variants accounted for <20% of the virus population, as assessed by pyrosequencing. The proportions of X4 viruses detected by the two strategies were the same (2/6) (data not shown).

In conclusion, our data suggest that the sensitivity of genotype-based detection of CXCR4-using viruses can be improved by duplicate amplifications of the HIV envelope gene and sequencing the pooled PCR products. This is a good compromise between improved sensitivity and reasonable cost for genotype-based determinations of tropism.

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Members of the ANRS AC11 Study Group

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Transparency declarations

None to declare.

References