Net benefits of resistance testing directed therapy compared with standard of care in HIV-infected patients with virological failure: A meta-analysis

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BACKGROUND. We incorporated the latest available information to evaluate the net benefit of using resistance testing in HIV-infected patients with virological failure. Methods. Meta-analysis of randomized controlled trials comparing the clinical impact of selecting antiretroviral therapy according to results of resistance testing (phenotype or genotype) or according to the standard of care. The population studied included HIV-infected patients with virological failure. The outcome measures were the proportion of patients with HIV-1 RNA below the detection limit, and the decline in HIV-1 RNA and increase in CD4 lymphocyte count at the end of follow-up (≤ 24 weeks). Clinical trials were identified through searches in MEDLINE, EMBASE and proceedings from major infectious diseases meetings.

RESULTS. Eight trials including a total of 1810 patients were eligible. Therapy guided by resistance testing resulted in a higher percentage of patients with HIV-1 RNA below the detection limit, and the decline in HIV-1 RNA and increase in CD4 lymphocyte count at the end of follow-up (≤ 24 weeks). Clinical trials were identified through searches in MEDLINE, EMBASE and proceedings from major infectious diseases meetings.

The number needed to treat (NNT) was 13 (95% CI: 9-25). Subgroup analysis showed greater benefits in therapy guided by genotype testing with expert interpretation, when compared with standard of care (NNT: 5; 95% CI: 3-9; p = 0.06). The heterogeneity among trials for evaluating HIV-1 RNA decline and CD4 lymphocyte cell count increase made unfeasible pooling the results across studies.

CONCLUSION. Genotype testing with expert interpretation showed the greatest benefit for guiding therapy in patients with HIV infection and virological failure.

Key words: Drug resistance. Randomized controlled trials. Viral genotype. Viral phenotype. Viral load. Meta-analysis.
Introduction

Testing for HIV resistance to antiretroviral drugs has been recommended to help guide the use of new regimens after failure of treatment1-7. HIV resistance testing involves genotypic assays, phenotypic assays or both8. Genotypic assays detect drug resistance mutations in the relevant viral genes (e.g. reverse transcriptase and protease) that are known to confer drug resistance. Interpretation of test results requires knowledge of the mutations or consultation with an expert in HIV drug resistance in order to select active drugs. Phenotyping assays measure virus' ability to grow in different concentrations of antiretroviral drugs. Drug concentrations that inhibit 50% (IC50) and 90% (IC90) of viral replication (i.e. the median inhibitory concentration [IC]) are calculated, and the ratio of the IC50 of test and reference viruses is reported as the fold increase in the IC50 (i.e. fold increase). Interpretation of pheno- typic testing is complicated by the paucity of data regarding the specific resistance level that is associated with drug failure. Again, consultation with a specialist can be useful for interpreting test results.

Resistance testing, both genotyping and phenotyping, has some drawbacks that include the lack of uniform quality assurance for all available assays, relatively high cost and insensitivity for minor viral species9. Moreover, randomized clinical trials have shown conflicting results when the efficacy of antiretroviral regimens guided by resistance testing was compared with standard of care.

A previous meta-analysis has compared the efficacy of resistance testing with the standard of care to achieve a HIV-1 RNA below the detection limit10. In the present meta-analysis, we updated the information available, and in addition we assessed the magnitude of HIV-1 RNA decrease and the CD4 lymphocyte recovery in the intervention and control groups.

Methods

Eligibility criteria

Studies of HIV infected patients with viral load > 400 copies/mL after at least 12 weeks of HAART therapy were randomized to changes in therapy according to results of resistance testing (geno- type, phenotype) or therapy changes according to the standard of care. Outcomes evaluated were the proportion of patients with HIV-1 RNA below the detection limit, changes in HIV-1 RNA and, changes in CD4+ cells at the end of follow-up.

Search strategy

We searched (1996-October 2004) MEDLINE and EMBASE using the search strategy: drug resistance OR genotypic resistance OR phenotypic resistance OR type of resistance testing used, genotype interpretation by experts, time to outcome evaluation, proportion of patients with HIV-1 RNA below the limit of detection, at the end of follow-up, and difference in CD4+ lymphocytes and HIV-1 RNA between baseline and end of follow-up. Disagreement was resolved by consensus. Duplicate or updated publications were identified. We included only the most complete data set in our review. The methodological quality of the included trials was scored according to the validated Jadad 5 point scale11. The scale consisted of three items describing randomization (0-2 points), masking (0-2 points), and description of dropouts and withdrawals (0-1 points) in the report of randomized-controlled trials. Higher scores indicate better reporting.

Data extracted

Two of us (J.E. and R.F.R.A.) independently selected studies and collected data regarding study quality, baseline HIV-1 RNA, lower limit of detection of HIV-1 RNA, drug resistance testing group and the standard of care treatment was chosen by clinicians or investigators. The description of standard of care was based on the use of standard HAART as per the standard of care at the time of the study12-14. The study quality assessment was based on the validated Jadad scale11. The Jadad scale consists of three items describing randomization (0-2 points), masking (0-2 points), and description of dropouts and withdrawals (0-1 points). The results were combined using a random-effect meta-analysis. Forest plots of the summary statistic were generated for each drug regimen and drug resistance testing. A funnel plot was generated for each drug regimen and drug resistance testing to assess publication bias.

Results

Trial flow

Figure 1 describes the flowchart diagram. We identified 6 full-length reports in peer-reviewed journals, and two abstracts from conferences proceeding meetings with usable information for the meta-analysis10,11.

Study characteristics

Study characteristics are summarized in table 1. Among the studies, 5 used genotype testing, 2 phenotype testing and 1 both types of resistance testing. The description of standard of care varied among the trials: two studies did not define this term10,11, three described that standard of care treatment was chosen by clinicians or investiga-
The length of follow up was equal or less than 24 weeks in all studies. The quality of methods of 3 trials was rated high (H11350 3 points), in the remaining 5 studies allocation was not conceal or was unknown. The funnel plot showed no significant publication bias.

Outcomes
The primary outcome of all studies was the proportion of patients with HIV-1 RNA below the detection limit at the end of follow up. Five trials described the reduction observed in HIV-1 RNA (mean and standard deviation, log10 copies/mL) and three the changes in CD4 lymphocyte count (mean and standard deviation, cells ×109/L) observed at the end of follow-up (table 2).

Undetectable virus load
A total of 413 (40.21%) patients in whom therapy was guided by resistance testing achieved a not detectable viral load at end of follow up compared with 258 (32.95%) patients treated according the standard of care (Relative Risk 1.23; 95% CI: 1.09-1.40, p = 0.0009) (fig. 2). Although there were differences among trials in type of resistance test used, rate of virological failure at entry and lower

**Table 1. Characteristics of Clinical Trials selected**

<table>
<thead>
<tr>
<th>Study (Ref)</th>
<th>Baseline median number of patients</th>
<th>Baseline median CD4 cells ×109/L</th>
<th>Baseline HIV-1 RNA log10 copies/mL</th>
<th>Previous exposure to antiretroviral therapy</th>
<th>Only one ART failure (%)</th>
<th>Intervention</th>
<th>Follow-up (%)</th>
<th>Primary endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIRADAPT12</td>
<td>4 108</td>
<td>214</td>
<td>4.7</td>
<td>39 mos. with NRTI, 12 mos. with PI, NNRTI 10%</td>
<td>40</td>
<td>Genotype test vs. standard of care</td>
<td>92</td>
<td>HIV-1 RNA &lt; 200 copies/ml at 24 weeks</td>
</tr>
<tr>
<td>GART13</td>
<td>3 153</td>
<td>230</td>
<td>4.42</td>
<td>13 mos. with antiretroviral therapy NNRTI 10%</td>
<td>48</td>
<td>Genotype test + expert advice vs. standard of care</td>
<td>97</td>
<td>HIV-1 RNA &lt; 500 copies/ml at 12 weeks</td>
</tr>
<tr>
<td>NARVAL14</td>
<td>3 541</td>
<td>280</td>
<td>&gt; 3.0</td>
<td>56 mos. with antiretroviral therapy NNRTI 10%</td>
<td>30</td>
<td>Genotype test vs. phenotype test vs. standard of care</td>
<td>78</td>
<td>HIV-1 RNA &lt; 500 copies/ml at 12 weeks</td>
</tr>
<tr>
<td>HAVANA15</td>
<td>1 328</td>
<td>387</td>
<td>4.6</td>
<td>≥ 2 PI 67%, ≥ 1 NNRTI 26%</td>
<td>35</td>
<td>Genotype test + expert advice vs. no expert advice</td>
<td>97</td>
<td>HIV-1 RNA &lt; 400 copies/ml at 24 weeks</td>
</tr>
<tr>
<td>ARGENTA16</td>
<td>3 174</td>
<td>265</td>
<td>&gt; 3.30</td>
<td>24 mos. therapy with NRTI NNRTI 25% Failure to 2 PI 15%</td>
<td>47</td>
<td>Genotype test + expert advice vs. standard of care + expert advice</td>
<td>91</td>
<td>HIV-1 RNA &lt; 500 copies/ml at 24 weeks</td>
</tr>
<tr>
<td>CCTCG151</td>
<td>1 218</td>
<td>NA</td>
<td>4.10</td>
<td>36 mos. with NRTI NNRTI 25% Failure to 2 PI 15%</td>
<td>70</td>
<td>Phenotype test vs. standard of care</td>
<td>83</td>
<td>HIV-1 RNA &lt; 400 copies/ml at 24 weeks</td>
</tr>
<tr>
<td>VIRA300118</td>
<td>1 226</td>
<td>327</td>
<td>5.92-4.18</td>
<td>Exposure to NNRTI 4%</td>
<td>100</td>
<td>Phenotype test vs. standard of care</td>
<td>93</td>
<td>HIV-1 RNA &lt; 400 copies/ml at 16 weeks</td>
</tr>
<tr>
<td>Rubini19</td>
<td>1 44</td>
<td>NA</td>
<td>NA</td>
<td>Exposed to 2-6 antiretroviral regimens, 48% failure to PI</td>
<td>NA</td>
<td>Genotype test vs. standard of care</td>
<td>100</td>
<td>HIV-1 below limit of detection at 24 weeks</td>
</tr>
</tbody>
</table>

NA: not available; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.
limit of HIV-1 RNA detection, we did not detect significant heterogeneity among trials (\(p = 0.46; I^2 = 0\%\)). The Absolute Risk Reduction was 8.1% (95% CI: 3%-12%), and accordingly the Number Needed to Treat was 13 (95% CI: 9-25).

Among our "a priori" hypotheses tested in the subgroup analysis, we found that patients having genotype testing interpreted by experts received the greatest benefit (table 3). The proportion of patients achieving a not detectable viral load at the end of follow-up in patients with therapy guided by genotype resistance testing interpreted by experts compared with the standard of care was 51.6% vs. 29.6, respectively (\(p = 0.06\)). Accordingly, the number of patients needed to treat was 5 (95%CI: 3-9).

Studies with intrinsically smallest bias (rated as "high quality") showed the strongest association between resistance testing guided antiretroviral therapy and achieving a not detectable viral load at the end of follow up (table 3). Subgroup analysis did not show differences between genotype and phenotype resistance testing, neither in virological response according length of follow-up, nor in virological response regarding the number of previous antiretroviral failing regimens.

Decrease in HIV-1 RNA

Five of the eight identified trials, with a total of 1,272 patients, contributed data to this outcome 10,12-14,17. Pooling the results there was a significant (\(p < 0.000001\), although clinically modest difference in favor of resistance testing directed therapy compared with the standard of care (weighted mean difference in HIV-1 RNA reduction: 0.36 log10 copies/mL, 95% CI: from 0.25 to 0.46). Nevertheless, there was a significant heterogeneity among trials (1\% = 79.7%, \(p = 0.0006\)). The observed decrease in HIV-1 RNA (weighted mean difference) varied from 0.57 to 1.5 log10 copies/mL in the resistance testing group, and

<table>
<thead>
<tr>
<th>Study</th>
<th>Resistance Testing n/N</th>
<th>Standard of Care n/N</th>
<th>RR (fixed) 95% CI</th>
<th>Weight %</th>
<th>RR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIRADAPT12</td>
<td>21/65</td>
<td>6/43</td>
<td>2.56</td>
<td>2.32 [1.00, 5.26]</td>
<td></td>
</tr>
<tr>
<td>GART13</td>
<td>26/78</td>
<td>10/75</td>
<td>5.79</td>
<td>1.58 [0.91, 2.67]</td>
<td></td>
</tr>
<tr>
<td>NARVAL14</td>
<td>147/382</td>
<td>55/159</td>
<td>27.55</td>
<td>1.11 [0.87, 1.43]</td>
<td></td>
</tr>
<tr>
<td>HAVANA15</td>
<td>78/161</td>
<td>60/165</td>
<td>21.02</td>
<td>1.30 [1.03, 1.62]</td>
<td></td>
</tr>
<tr>
<td>ARGENTA16</td>
<td>16/85</td>
<td>15/93</td>
<td>5.20</td>
<td>1.26 [0.68, 2.33]</td>
<td></td>
</tr>
<tr>
<td>CCGT57517</td>
<td>57/119</td>
<td>57/119</td>
<td>20.22</td>
<td>1.09 [0.77, 1.50]</td>
<td></td>
</tr>
<tr>
<td>VIR3001</td>
<td>52/114</td>
<td>36/112</td>
<td>13.60</td>
<td>1.34 [0.97, 1.86]</td>
<td></td>
</tr>
<tr>
<td>Rubini19</td>
<td>14/23</td>
<td>11/21</td>
<td>4.08</td>
<td>1.18 [0.69, 1.96]</td>
<td></td>
</tr>
<tr>
<td>OVERALL (95% CI)</td>
<td></td>
<td></td>
<td>100.00</td>
<td>1.23 [1.00, 1.40]</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Relative risk of achieving a not detectable viral load for all eight trials.
from 0.39 to 2.0 log_{10} copies/mL in the standard of care group. Patients treated according to genotype tests interpreted by experts had a reduction in HIV-1 RNA (weighted mean difference 0.37 log_{10} copies/mL, 95% CI: from 0.12 to 0.62) greater than those in treated by the standard of care (weighted mean difference 0.24 log_{10} copies/mL, 95% CI: from –0.01 to 0.48).

CD4 lymphocyte recovery

Three trials, with a total of 823 patients, contributed to this outcome [11,14,17]. Pooling the results there was a significant (p < 0.0001) but clinically modest smaller CD4 recovery in the group of patients with therapy directed by resistance testing compared with those who received therapy according the standard of care weighted mean difference –7.63 × 10^9 cells/L, 95% CI: from –11.03 to –4.22). The observed differences in lymphocyte CD4 counts between the resistance testing and the standard of care groups varied from –12.00 × 10^9 cells/L (95% CI: from –11.03 to –4.22) to 0.00 × 10^9 cells/L (95% CI: from –16.77 to 16.77).

Discussion

Our intention was to examine clinical outcomes in terms of proportion of patients achieving a not detectable HIV-1 RNA, quantitative reduction in HIV-1 viral load, and increase in CD4 lymphocytes, after comparing treatment guided by resistance testing or by standard of care. Patients failing antiretroviral therapy. Resistance testing confers a statistically significant benefit for achieving a not detectable viral load compared with the standard of care in patients failing antiretroviral therapy. Resistance testing has been recommended by several agencies for patients switching therapy due to treatment failures in order to maximize the number of active drugs in the new regimen. Several retrospective studies showed the predictive value of genotypic and phenotypic testing. However, in occasional clinical history alone has been sufficient to predict the failure of subsequent therapy, for example, with abacavir in heavily nucleoside-experienced patients [18] or with nelfinavir in patients with prolonged protease inhibitor experience [19]. Zolopa and colleagues [20] found that clinical history alone predicted 40 percent of the treatment response in univariate analysis, genotype alone predicted 67 percent of the treatment response, and adding the clinical history into the model in a multivariate analysis the value was raised to 71%. It has been shown that efficacy of a new antiretroviral regimen in patients with previous virological failure is related with the number of available drugs that still maintain activity [21]. Our study did not show the inverse association between exposure to antiretroviral drugs (expressed by percent of patients failing a first antiretroviral regimen) and efficacy of salvage regimens (table 3). Nevertheless, we could not properly test this observation in our meta-analysis due to lack of enough information available from individual studies.
major drug classes-nucleoside-reverse transcriptase inhibitors (NRTI), protease inhibitors (PI), and non-nucleoside reverse transcriptase inhibitors (NNRTI) - maintain stable or rising CD4-cell counts and remain clinically well. An observational study showed that a complete suppression of HIV viremia is not absolutely necessary for achieving immunologic and clinical stability in patients with multiple antiretroviral regimens’ failures20.

Limitations

Reasons for virological failure are multi-factorial, and emergence of viral resistance is just one of them. Poor penetration of drugs into certain body compartments, insufficient adherence, and variable pharmacokinetics within and between individuals can contribute to virological failures. Moreover, the most common shortcomings of genotypic and phenotypic resistance test include unreliable standardization, variable reproducibility of testing methodology, disorganized or confusing reporting formats for test results, and lack of physician expertise in interpretation.

The major limitation of this meta-analysis was the paucity of randomized clinical trial data, and the short follow-up period. The sparseness of the data, and the heterogeneity among trials prevented the assignment of definitive conclusions in terms of potency of antiretroviral therapy guided by resistance testing to reduce HIV-1 viral load and to increase CD4 lymphocyte counts. To avoid selection bias, a systematic and comprehensive search was conducted and two reviewers independently evaluated trials for inclusion. There is a possibility of publication and selection bias in any meta-analysis, however, significant publication bias was excluded according the funnel plot.

Conclusion

Resistance testing is a useful tool to gather knowledge about “in vitro” resistance mechanisms to antiretroviral drugs, and to delineate algorithms to manage patients with virological failure. Our study showed that patients with virological failure received greatest benefit from genotype resistance testing with expert advice when compared with the standard of care. However, it should be taken into account that HIV-1 virological failure is a complex problem in which viral suppression and immunologic benefit are related not only to viral resistance, but also to adherence, drug concentration and the number of active available antiretroviral drugs required to build up a salvage regimen.

References