Prevalence and predictors of antiretroviral drug resistance in newly diagnosed HIV-1 infection

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Objectives: To determine prevalence and predictors of antiretroviral drug resistance in newly diagnosed individuals with HIV-1 infection, using a systematic approach to avoid selection bias.

Methods: Plasma samples from all persons diagnosed HIV-1 seropositive at a large London centre between April 2004 and February 2006 underwent sequencing of HIV-1 reverse transcriptase (RT) and protease genes. Subtype was assigned by phylogenetic analysis. Resistance was scored according to the IAS-USA list (2005) modified to include T215revertants and exclude isolated E44D or V118I and minor protease mutations. Recent seroconversion was identified by HIV antibody avidity testing.

Results: The cohort of 239 included 169 (70.7%) males, 126 (52.7%) homosexuals, 118 (49.5%) persons of white ethnicity and 144 (60.0%) persons born outside the UK. Subtypes included B 134 (56.1%), C 46 (19.2%), A 17 (7.1%), other non-B 42 (17.6%). The prevalence of resistance mutations was 17/239 (7.1%; 95% confidence interval 4.5–11.1%), comprising 10/239 (4.2%) nucleoside/nucleotide RT inhibitor (NRTI); 4/239 (1.7%) non-nucleoside RT inhibitor (NNRTI) and 4/239 (1.7%) protease inhibitor (PI) associated mutations. Dual-class (NRTI + PI) resistance mutations were detected in 1/239 (0.4%) person. The prevalence of resistance mutations was 7/85 (8.2%) and 10/154 (6.5%) in persons with recent and established infection, respectively. In multivariate analysis, having been born in the UK and high CD4 count, but not gender, age, risk group, ethnicity or subtype, were independent predictors of resistance.

Conclusions: In an unselected UK cohort, subtypes other than B accounted for 43.9% of new HIV-1 diagnoses. The prevalence of resistance mutations was 7.1% and highest in those born in the UK.

Keywords: mutations, avidity, subtype, HIV, drug naive

Introduction

The introduction of highly active antiretroviral therapy (HAART) has led to a dramatic improvement in rates of morbidity and mortality for individuals infected with human immunodeficiency virus type 1 (HIV-1). However, the emergence of drug-resistant strains has proved a major obstacle to the successful long-term management of the disease. The use of mono and dual therapy in the pre-HAART era, functional monotherapy resulting from the sequential introduction of single active drugs in the pre- and early-HAART era, poor potency of some triple therapy regimens,1–3 and ongoing difficulties with adherence and tolerability have led to a large pool of resistant virus becoming available to establish new infections.4 Transmission of drug-resistant viruses has been well documented and shown to occur irrespective of the route of infection.5–7 Recent data have indicated that transmitted drug-resistant strains may persist for years as dominant quasispecies and possibly for longer as minority and archived species, with the long-term potential to impact on responses to antiretroviral therapy.8–12 Several studies have demonstrated how important an impact transmitted resistance can have on responses to antiretroviral therapy13–15 an effect that can be further compounded in those patients with poor drug adherence and in those on regimens with a low genetic barrier to the evolution of further resistance.

Current guidelines recommend testing for transmitted antiretroviral drug resistance in all newly diagnosed HIV-1-infected patients,16,17 but these recommendations are not universally achievable.
followed due to uncertainty about the true prevalence of transmitted resistance in different populations and the cost–benefit of systematic testing. In Western Europe, numerous centres began resistance testing of drug-naive HIV-1-infected persons during the 1990s. However, testing has often been restricted to selected subgroups such as recent seroconverters or persons suspected to have contracted their infection from an individual with antiretroviral drug resistance, inevitably leading to an overestimation of the prevalence of transmitted resistance. A recent report suggested that the rates of transmitted antiretroviral drug resistance in the UK are among the highest worldwide, with an overall prevalence of intermediate to high-level resistance to any drug, as defined by the Stanford algorithm, of 19% in 2002–03. It is unclear to what extent selection bias influenced the prevalence estimates in that survey.

The aim of this study was to determine the prevalence and predictors of antiretroviral drug resistance in an unselected population of persons newly diagnosed with HIV-1 infection at a large clinical centre in London between April 2004 and February 2006. To our knowledge, this is the first comprehensive report from an unselected population in the UK.

Materials and methods

Patient population

All patients newly diagnosed with HIV-1 infection at the Royal Free Hospital in London between April 2004 and February 2006 underwent drug resistance testing using plasma samples collected at the time of HIV-1 diagnosis. Clinic and laboratory records were reviewed to ensure that all new diagnoses were included. Demographics and clinical data were collected as part of routine clinical care and analysed with the patients’ written consent. The timing of the infection was estimated using a previously validated Vitros-based guanidine avidity assay. Briefly, paired sera in duplicate or, where enough sample was available, triplicate wells were tested for HIV antibodies by the Anti-HIV 1 and 2 VITROS ECi assay (Ortho-Clinical Diagnostics, UK) following incubation for 10 min at a 1 : 10 dilution in either phosphate buffered saline (PBS, the reference wells) or 1 M guanidine (the test wells). The avidity index (AI) was calculated using the formula: sample to cut-off (S/CO) ratio of test (mean of replicate wells) over S/CO ratio of reference (mean of replicate wells). By testing a large panel of seroconverters, a cut-off value of ≤0.75 was found to be indicative of seroconversion within the previous 125 days (95% confidence interval, CI 85–164), with similar kinetics in B and non-B subtype infection. The reproducibility of the assay at AI values near the cut-off was calculated with 24 replicate tests of samples showing an average AI of 0.7 (±0.1). The inter-assay coefficient of variation (CV) was 3.7% (±5.8%).

HIV-1 genotyping and sequence analysis

HIV-1 pol gene sequences for codons 1–99 of the protease and 1–335 of reverse transcriptase (RT) were obtained from plasma samples using the Viroseq™ HIV-1 genotyping system (Celera Diagnostics, USA) according to the manufacturer’s instructions. Sequences were analysed using a 3100-Avant Genetic Analyzer (Applied Biosystems, UK) and scored for resistance mutations using a modified IAS-USA mutation list (2005). The following mutations were included in RT: M41L, A62V, K65R, D67NG, T69insertion, K70R, L74VI, V75I, F77L, L100I, K103N, V106A/M, V108I, Y115F, P116Y, Q151M, Y181C/I, M184V/I, Y188L, G190A/S, L210W, V215F/Y, V215revertants, K219E/QN, P225H, M230L, P236L; and in protease: D30N, V32I, L33F, M46I/L, G47A/V, I50L/V, V82A/F/S/T, I84V, N88S, L90M, E44D and V118I were excluded unless present together or with other resistance mutations; minor protease mutations were also excluded. Resistance was interpreted using the Stanford drug resistance algorithm, and resistance levels were assigned as either low (defined by the Stanford algorithm as low-level resistance) or intermediate/high (defined by the Stanford algorithm as either intermediate or high-level resistance). Subtypes were assigned by phylogenetic analysis (PAUP v4.0, Sinauer Associates) using Clustal W for sequence alignment and reference HIV-1 sequences derived from the Los Alamos database.

Statistics

The proportions with resistance in various subgroups were initially compared using Fisher’s exact test (2-tailed). Generalized linear models with log link and Poisson error [using generalized estimating equations (GEE) with an unstructured covariance] were used to assess multivariable (adjusted) relative risks for the association between various covariates and risk of a mutation being present. This was performed using SAS 8.2.

Results

Patient characteristics

Between April 2004 and February 2006, 239 patients were newly diagnosed with HIV-1 infection at the Royal Free Hospital. As shown by the baseline demographic and clinical characteristics summarized in Table 1, the study population was highly diverse. Most patients were born in a country other than the UK. Among white patients, 82/118 (69.5%) were born in the UK, followed by other European countries (23/118, 19.5%), sub-Saharan Africa (6/118, 5.1%), Oceania (4/118, 3.4%) and South America (3/118, 2.5%). Among black-African patients, 68/72 (94.4%) were born in sub-Saharan Africa and 4/72 (5.6%) in the UK. Among black-Caribbean patients, 4/10 (40.0%) were born in the UK, 3/10 (30.0%) in the Caribbean and 3/10 (30.0%) in sub-Saharan Africa. Among Asian patients, 6/8 (75.0%) were born in Asian countries (2 in China and 1 each in Singapore, Iran, Pakistan and India), 1/8 (12.5%) in the UK, and 1/8 (12.5%) in Kenya. The majority of those whose ethnicity was defined as mixed or other were born in sub-Saharan Africa (15/31, 48.4%), whereas 6/31 (19.4%) were born in European countries other than the UK, 4/31 (12.9%) in the UK, 2/31 (6.5%) in North America; 2/31 (6.5%) in Asian countries (one in Pakistan and one in South Korea), 1/31 (3.2%) in South America and 1/31 (3.2%) in the Caribbean.

HIV antibody avidity

HIV antibody avidity was determined using a guanidine-based HIV antibody assay. In previous validation studies an avidity index ≤0.75 identified seroconversion within the previous 125
days (95% CI 85–164). Based on this cut-off, in the newly diagnosed population there were 85/239 (35.6%) patients with evidence indicative of a recent infection. Of these, 19/85 (22.4%) showed seroconversion from HIV-1 antibody negative or equivocal to positive within the previous 164 days. The cohort with recent infection included 66/85 (77.6%) males, 53/85 (62.4%) homosexuals, 55/85 (64.7%) white, 48/85 (56.5%) persons born in the UK and 58/85 (68.2%) subtype B infections.

HIV-1 subtypes

A wide range of HIV-1 subtypes were found, with subtype B comprising 134/239 (56.1%) of the infections, whereas 105/239 (43.9%) showed subtypes other than B. The latter included 46 C (19.2%), 17 A (7.1%), 13 CRF02 (circulating recombinant form 02) (5.4%), 7 D (2.9%), 7 G (2.9%), 4 CRF01 (1.7%), 4 CRF06 (1.7%), 1 CRF13 (0.4%), 1 CRF16 (0.4%) and 5 (2.1%) complex mosaic sequences (cpx). The demographic and clinical characteristics of persons infected with B or non-B subtype are shown in Table 1. Overall, the cohort with B subtype included predominantly homosexual males (120/124, 96.1%), persons of white ethnicity (107/124, 86.4%) and those born in the UK (86/124, 69.2%). Conversely, patients with non-B subtypes were predominantly black-African (68/105, 64.8%) males and females, of heterosexual orientation (95/105, 90.5%) and born outside of the UK (96/105, 91.4%).

Prevalence of antiretroviral drug resistance

A total of 17/239 (7.1%; 95% confidence interval, CI 4.2–11.1%) patients had resistance mutations (Table 2). The highest prevalence of resistance mutations was observed for the nucleoside/nucleotide RT inhibitors (NRTIs) (10/239, 4.2%; 95% CI 2.0–7.6%), associated predominantly with thymidine analogue mutations (TAMs) including T215revertants. Prevalence of resistance mutations for the non-nucleoside RT inhibitors (NNRTIs) was 4/239 (1.7%; 95% CI 0.5–4.2%), represented exclusively by K103N. The prevalence of protease inhibitor (PI) resistance mutations was 4/239 (1.7%, 95% CI 0.5–4.2%), associated with the major mutations M46L, V82L and L90M. One patient (0.4%) was found to harbour dual-class resistance mutations to the NRTIs and PIs (Table 2).

Based on the Stanford interpretation algorithm, of the patients with resistance mutations, 3/17 (17.6%) had low-level NRTI resistance and 1/17 (5.9%) had low-level PI resistance. Intermediate/high-level resistance occurred in 6/17 (35.3%) persons for the NRTIs, 4/17 (23.5%) for the NNRTIs, and 2/17 (11.8%) for the PIs. Finally, 1/17 (5.9%) persons showed dual-class NRTI and PI resistance, including intermediate/high-level resistance to available PIs.

Factors associated with the presence of drug resistance mutations

The prevalence of resistance mutations was significantly higher in persons born in the UK (15/95, 15.8%) compared with those born elsewhere (2/144, 1.4%), and in persons with subtype B (15/124, 12.1%) compared with those with non-B subtypes (2/105, 1.9%) (Table 2). There was a trend for a higher prevalence of resistance in males (16/169, 9.6%) compared with females (170, 1.4%), homosexuals (13/126, 10.3%) compared with other risk groups (4/113, 3.5%), and white and other ethnic groups (15/167, 9.0%) compared with black-African persons.
<table>
<thead>
<tr>
<th>Pt</th>
<th>HIV diagnosis</th>
<th>Age (yr)</th>
<th>Risk group&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ethnicity (CoB)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>AI&lt;sup&gt;d&lt;/sup&gt;</th>
<th>CD4 (cells/mm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>VL&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Subtype</th>
<th>reverse transcriptase</th>
<th>protease</th>
<th>Level of resistance (Stanford)&lt;sup&gt;g&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>04/04</td>
<td>33</td>
<td>MSM</td>
<td>W (UK)</td>
<td>0.5</td>
<td>260</td>
<td>4.4</td>
<td>B</td>
<td>none</td>
<td>V82L</td>
<td>TPV</td>
</tr>
<tr>
<td>M2</td>
<td>07/04</td>
<td>26</td>
<td>MSM</td>
<td>W (UK)</td>
<td>0.9</td>
<td>522</td>
<td>5.4</td>
<td>B</td>
<td>K103 N</td>
<td>none</td>
<td>NVP EFV DLV</td>
</tr>
<tr>
<td>M3</td>
<td>08/04</td>
<td>30</td>
<td>MSM</td>
<td>M (UK)</td>
<td>0.6</td>
<td>669</td>
<td>4.0</td>
<td>B</td>
<td>K103 N</td>
<td>none</td>
<td>NVP EFV DLV</td>
</tr>
<tr>
<td>M4</td>
<td>09/04</td>
<td>33</td>
<td>MSM</td>
<td>W (UK)</td>
<td>0.8</td>
<td>284</td>
<td>5.1</td>
<td>B</td>
<td>none</td>
<td>L90M</td>
<td>NVP EFV DLV</td>
</tr>
<tr>
<td>M5</td>
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<td>48</td>
<td>MSM</td>
<td>W (UK)</td>
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<td>302</td>
<td>4.7</td>
<td>B</td>
<td>M41L T215S</td>
<td>none</td>
<td>ABC ddI TDF</td>
</tr>
<tr>
<td>M6</td>
<td>12/04</td>
<td>39</td>
<td>HTS</td>
<td>M (UK)</td>
<td>0.6</td>
<td>1184</td>
<td>3.0</td>
<td>B</td>
<td>M41L T215S</td>
<td>none</td>
<td>ABC ddI TDF</td>
</tr>
<tr>
<td>M7</td>
<td>03/05</td>
<td>32</td>
<td>MSM</td>
<td>W (UK)</td>
<td>0.6</td>
<td>580</td>
<td>5.4</td>
<td>B</td>
<td>M103 N</td>
<td>none</td>
<td>NVP EFV DLV</td>
</tr>
<tr>
<td>M8</td>
<td>04/05</td>
<td>39</td>
<td>MSM</td>
<td>W (UK)</td>
<td>1.0</td>
<td>829</td>
<td>4.5</td>
<td>B</td>
<td>M41LM</td>
<td>none</td>
<td>ZDV d4T</td>
</tr>
<tr>
<td>M9</td>
<td>04/05</td>
<td>39</td>
<td>MSM</td>
<td>W (UK)</td>
<td>0.9</td>
<td>519</td>
<td>5.3</td>
<td>B</td>
<td>T215D</td>
<td>none</td>
<td>ZDV d4T</td>
</tr>
<tr>
<td>M10</td>
<td>05/05</td>
<td>41</td>
<td>MSM</td>
<td>W (UK)</td>
<td>1.0</td>
<td>337</td>
<td>4.5</td>
<td>B</td>
<td>M41LM T215ST</td>
<td>none</td>
<td>ABC ddI TDF</td>
</tr>
<tr>
<td>M11</td>
<td>05/05</td>
<td>38</td>
<td>MSM</td>
<td>BC (UK)</td>
<td>1.0</td>
<td>733</td>
<td>5.0</td>
<td>B</td>
<td>D67N T69N T215V K219Q</td>
<td>L33F G48V I54T G73S V82A L90M</td>
<td>ABC ddI TDF</td>
</tr>
<tr>
<td>M12</td>
<td>06/05</td>
<td>28</td>
<td>MSM</td>
<td>W (UK)</td>
<td>0.9</td>
<td>616</td>
<td>4.6</td>
<td>B</td>
<td>M41L L210F T215D</td>
<td>none</td>
<td>IDV LPV NFV SQV TMC114 TPV</td>
</tr>
<tr>
<td>M13</td>
<td>06/05</td>
<td>20</td>
<td>MSM</td>
<td>W (UK)</td>
<td>0.8</td>
<td>401</td>
<td>2.9</td>
<td>B</td>
<td>K219QR</td>
<td>none</td>
<td>ABC, ddI, TDF</td>
</tr>
<tr>
<td>M14</td>
<td>07/05</td>
<td>66</td>
<td>HTS</td>
<td>A (Pk)</td>
<td>0.9</td>
<td>513</td>
<td>4.0</td>
<td>cpx&lt;sup&gt;f&lt;/sup&gt;</td>
<td>none</td>
<td>M46L</td>
<td>ZDV</td>
</tr>
<tr>
<td>F1</td>
<td>10/05</td>
<td>40</td>
<td>HTS</td>
<td>BA (SA)</td>
<td>0.9</td>
<td>265</td>
<td>4.3</td>
<td>C</td>
<td>K103N</td>
<td>none</td>
<td>ZDV d4T</td>
</tr>
<tr>
<td>M15</td>
<td>11/05</td>
<td>47</td>
<td>HTS</td>
<td>BA (UK)</td>
<td>0.5</td>
<td>244</td>
<td>3.6</td>
<td>B</td>
<td>D67N T69DN 219Q</td>
<td>none</td>
<td>ZDV d4T</td>
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<td>M16</td>
<td>01/06</td>
<td>47</td>
<td>MSM</td>
<td>W (UK)</td>
<td>0.2</td>
<td>587</td>
<td>7.0</td>
<td>B</td>
<td>D67N T69N K219Q</td>
<td>none</td>
<td>ZDV d4T</td>
</tr>
</tbody>
</table>

<sup>a</sup>Pt, patient; M, male; F, female.

<sup>b</sup>MSM, men who have sex with men; HTS, heterosexual.

<sup>c</sup>CoB, country of birth; W, white; M, mixed; BC, black-Caribbean; A, Asian; BA, black-African; UK, United Kingdom; Pk, Pakistan; SA, South Africa.

<sup>d</sup>AI, HIV-1 antibody avidity index, with a value ≤0.75 indicating seroconversion within the previous 125 days (95% confidence interval, CI 85-164) whereas a value of ≤0.8 indicated seroconversion within the previous 142 days (95% CI 101–183).

<sup>e</sup>VL, HIV-1 plasma RNA load (log<sub>10</sub> copies/mL).

<sup>f</sup>cpx, complex non-B mosaic of CRF02/G/CRF06.

<sup>g</sup>Antiretroviral drugs: ABC, abacavir; ATV, atazanavir; d4T, stavudine; ddI, didanosine; DLV, delavirdine; EFV, efavirenz; FPV, fosamprenavir; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; NVP, nevirapine; SQV, saquinavir; TDF, tenofovir; TMC114, darunavir; TPV, tipranavir; ZDV, zidovudine.
(272, 2.8%). There was also an association between the detection of resistance mutations and higher CD4 count at diagnosis, but there was no effect of age or HIV-1 plasma RNA load. There was also no significant difference in the prevalence of resistance in those with recent infection (7/85, 8.2%) compared with those with established infection (10/154, 6.5%), and the observation persisted when a higher cut-off value of ≤0.8 was used for the avidity assay, corresponding to seroconversion within the previous 142 days (95% CI 101–183). In multivariable models, independent predictors of the detection of resistance mutations were having been born in the UK and to a lesser extent having a higher CD4 count at diagnosis (Table 3). There was no independent effect of the other variables, including ethnicity and subtype, once country of birth was included in the model.

### Discussion

In this study we determined that the prevalence of antiretroviral drug resistance in an unselected population of HIV-1-infected individuals diagnosed between April 2004 and February 2006 was 7.1% (95% CI: 4.2–11.1%) overall, but considerably higher (15.8%) among persons born in the UK. The prevalence was close to the 10.4% rate reported in a multicentre European study of 2208 drug-naive patients tested in 1996–2002, which included data from 19 countries.28 In previous studies of selected populations of drug-naive HIV-1-infected patients in the UK, the prevalence of antiretroviral drug resistance varied from 6% in 68 patients with heterosexually acquired infection diagnosed between 1999 and 2001,29 and 7% in 60 patients with known date of seroconversion tested between 1996 and 2000,30 to 27% in a study of 26 recent (<18 months) seroconverters tested in 2000.31 A recent study reported the prevalence of resistance among 2357 drug-naive patients tested between 1996 and 2003 whose resistance data were submitted to the UK Resistance Database.19 Overall, 9.9%, 4.5% and 4.6% showed resistance mutations to the NRTIs, NNRTIs and PIs respectively, compared with 4.2%, 1.7% and 1.7% in this study, making the difference in prevalence of NNRTI and PI resistance particularly noticeable. The prevalence of intermediate to high level resistance as defined by the Stanford algorithm was 14.2% in the entire study period and substantially higher at 19.2% in 2002–03.19 These findings would indicate that the prevalence of drug resistance in drug-naive HIV-1-infected patients in the UK is among the highest worldwide and well above that reported in similar cohorts elsewhere in Europe.28,32–38 The study included only a proportion of all patients diagnosed with HIV-1 infection during the study period as clinical guidelines at the time did not recommend routine resistance testing at diagnosis or at the time of starting therapy. It is likely that in a proportion of cases, resistance testing would only have been requested for those patients in whom resistance was suspected. This was recognized by the study investigators, who suggested that selection bias may have influenced the prevalence estimate by including patients at the highest perceived risk of resistance. However, a genuine decline in the prevalence of transmitted resistance may also have occurred in recent years.39–41

In Western Europe prevalence of transmitted drug resistance continues to be highest among homosexual men of white ethnicity infected with subtype B,42 reflecting the imported origin of most heterosexual infections from countries where, until recently, access to antiretroviral therapy has been limited. In our cohort, 52.7% of newly diagnosed persons were homosexual males, 45.2% were heterosexuals and 2.1% were intravenous drug users (IVDUs) or had acquired the infection through blood or blood products. Surveillance data from the UK Health Protection Agency indicate that in 2004, 30.0% of new diagnoses occurred in homosexual males, 58.9% in heterosexuals and 11.1% in IVDUs and persons with other or unknown risk factors.41 The vast majority of heterosexual infections are believed to have been acquired abroad, especially in sub-Saharan Africa. Thus, our cohort included a greater proportion of homosexual males (mostly of white ethnicity and infected with subtype B) relative to national figures, which may have led to an overestimation of the overall prevalence of resistance.

The interpretation of HIV-1 sequence data is increasingly complex due to the significant diversity of circulating HIV-1 strains and the growing number of mutations found to be important in drug resistance. This may make it difficult in some cases to differentiate correctly between mutations selected by drug pressure and representing transmitted drug resistance, from those occurring as natural polymorphisms. Based on these considerations, it may be disputed whether protease mutations such as V82L (patient M1) and M46L (patient M14) are truly a reflection of transmitted drug resistance, although they may affect PI susceptibility. Along the same lines, it may also be difficult to

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**Table 3.** Factors associated with the detection of resistance mutations among newly diagnosed HIV-1 infected persons in univariable and multivariable analyses

<table>
<thead>
<tr>
<th>Factor</th>
<th>Relative Risk (95% CI)</th>
<th>P value</th>
<th>Relative Risk (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (women vs. men)</td>
<td>0.15 (0.02–1.12)</td>
<td>0.06</td>
<td>0.47 (0.07–3.04)</td>
<td>0.43</td>
</tr>
<tr>
<td>Country of birth (outside UK vs. UK)</td>
<td>0.09 (0.02–0.38)</td>
<td>0.001</td>
<td>0.10 (0.02–0.44)</td>
<td>0.002</td>
</tr>
<tr>
<td>Risk group (homosexual vs. heterosexual)</td>
<td>2.79 (0.94–8.29)</td>
<td>0.07</td>
<td>0.84 (0.32–2.24)</td>
<td>0.73</td>
</tr>
<tr>
<td>HIV-1 subtype (non-B vs. B)</td>
<td>0.17 (0.04–0.73)</td>
<td>0.02</td>
<td>0.67 (0.22–2.07)</td>
<td>0.49</td>
</tr>
<tr>
<td>Ethnicity (black-African vs. white)</td>
<td>0.31 (0.07–1.32)</td>
<td>0.11</td>
<td>1.75 (0.35–8.81)</td>
<td>0.50</td>
</tr>
<tr>
<td>Age (per 10 yr older)</td>
<td>0.98 (0.93–1.02)</td>
<td>0.32</td>
<td>0.97 (0.91–1.03)</td>
<td>0.28</td>
</tr>
<tr>
<td>CD4 count (per 100 cells/mm³ lower)</td>
<td>0.82 (0.73–0.92)</td>
<td>0.001</td>
<td>0.87 (0.77–0.98)</td>
<td>0.02</td>
</tr>
<tr>
<td>HIV-1 plasma RNA load (per 1 log10 copies/mL higher)</td>
<td>0.73 (0.44–1.22)</td>
<td>0.23</td>
<td>0.73 (0.41–1.27)</td>
<td>0.26</td>
</tr>
<tr>
<td>Antibody avidity index (≤0.75 vs. &gt;0.75)</td>
<td>1.24 (0.49–3.13)</td>
<td>0.66</td>
<td>1.60 (0.62–4.15)</td>
<td>0.33</td>
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differentiate between transmitted and secondary drug resistance in persons who, for a variety of reasons, may be unwilling to disclose previous antiretroviral treatment.

Infection with drug-resistant HIV-1 has important implications for the successful management of antiretroviral therapy. Only one patient in the study population had contracted a virus showing resistance to more than one class of antiretrovirals (NRTIs and PIs). While this indicates reassuringly that multiclass resistance is uncommon, this patient’s treatment options were significantly limited. Even resistance to one class of antiretrovirals can significantly restrict therapeutic options. Current British HIV Association treatment guidelines recommend initiating first-line therapy with two NRTIs and either an NNRTI or a ritonavir-boosted PI. The significance of NNRTI resistance is high, given that the presence of a single mutation such as K103N is sufficient to abrogate responses to available NNRTIs. In contrast, pharmacological enhancement with ritonavir can boost plasma concentrations of available PIs, which may overcome low to intermediate levels of resistance. The presence of TAMs, the most common resistance mutations detected in this study, affects susceptibility to multiple NRTIs, including those commonly used in first-line therapy such as zidovudine, abacavir and tenofovir. Although revertants of the T215Y/F mutation such as T215S or T215D do not show significant reductions in drug susceptibility, they have been associated with an increased risk of virological failure in persons starting therapy with thymidine analogues, as a result of the rapid re-emergence of T215Y/F under drug pressure. The presence of NRTI resistance also impacts on the use of NNRTI-based regimens, as the standard combination of two NRTIs with one NNRTI may be considered too fragile in the presence of even partial NRTI resistance.

The UK HIV-1 epidemic is increasingly diverse. In this study, 43.9% of newly diagnosed patients were infected with non-B subtypes and circulating recombinant forms. The most common non-B subtype observed was C, which reflects immigration from southern African countries such as Zambia, Zimbabwe and South Africa where subtype C predominates. However, while non-B subtypes in the UK have traditionally been associated with immigration, they are no longer restricted to non-indigenous populations and nine persons born in the UK were infected with non-B subtypes in this study. Homosexual males, persons with subtype B and those born in the UK showed the highest rates of resistance. Multivariable analysis indicated that having been born in the UK was the key predictor of resistance and neither gender, risk group, ethnicity or subtype were independently associated with the risk of resistance after accounting for place of origin. The association is consistent with the observation that most persons born outside of the UK originated from countries where antiretroviral drug use has until recently been limited, including predominantly Zimbabwe, Nigeria, Congo, Kenya, Zambia and Uganda. Prevalence surveys in the sub-Saharan African countries Burkina Faso and Cameroon have shown that resistance still remains uncommon among drug-naive persons in these regions. Despite these observations, increasing access to antiretroviral therapy with limited availability of monitoring tools indicate that ongoing surveillance is required in these populations and that baseline resistance testing in the UK should not be limited to the groups that currently show the highest prevalence rates.

Interestingly, we found no significant difference in the prevalence of resistance between those with recent and established infection. Traditionally, resistance prevalence rates have been highest among recent seroconverters. As also suggested by others, our findings point to a possible recent reduction in the prevalence of transmitted resistance. Despite this, a higher CD4 count at diagnosis was an independent predictor of having detectable resistance mutations. One possible determinant of the positive association could be the impaired fitness of resistant virus, leading to reduced pathogenicity and preservation of CD4 counts after infection. However, a probable explanation is that the higher CD4 count indicated a more recent infection and therefore a greater likelihood of detecting transmitted resistant mutants. Over time, resistant mutants tend to revert to minority species, although the reversion may occur slowly for many mutations. Currently, routine genotyping methods are only able to detect dominant resistant mutants within the quasispecies, with a lower level of sensitivity of approximately 20–30%. Use of peripheral blood mononuclear cells, allele-specific real-time PCR and single genome sequencing have been shown to improve detection of resistance in drug-naive persons. These methods remain cumbersome and labour-intensive, making them unsuitable for routine diagnostic use. Nonetheless, it should be appreciated that standard methods provide a minimal estimate of the prevalence of resistance in treatment-naive persons.

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Transparency declarations

C.L.B., A.M.G.D: none.
M.A.J. has received reimbursement for either/or: attending a symposium; a fee for speaking; a fee for organizing education; funds for research; funds for a PhD/MD student member of staff; fees for consulting from various pharmaceutical companies including Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead, Glaxo-SmithKline, Roche and Tibotec.
M.A.J. has received reimbursement for either/or: attending a symposium; a fee for speaking; a fee for organizing education; funds for research; funds for a member of staff; fees for consulting from various pharmaceutical companies including Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead, Glaxo-SmithKline, Roche and Tibotec.
A.P. has received reimbursement for either/or: attending a symposium; a fee for speaking; a fee for organizing education; funds for research; funds for a member of staff; fees for consulting from various pharmaceutical companies including Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead, Glaxo-SmithKline, Oxson Therapeutics, Roche and Tibotec.
A.M.G. has received consultancy fees or funding for speaking from Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Pfizer, Roche, Tibotec and Virco.

References


