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Estimating prevalence of accumulated HIV-1 drug resistance in a cohort of patients on antiretroviral therapy

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Objectives: Estimating the prevalence of accumulated HIV drug resistance in patients receiving antiretroviral therapy (ART) is difficult due to lack of resistance testing at all occasions of virological failure and in patients with undetectable viral load. A method to estimate this for 6498 EuroSIDA patients who were under follow-up on ART at 1 July 2008 was therefore developed by imputing data on patients with no prior resistance test results, based on the probability of detecting resistance in tested patients with similar profiles.

Methods: Using all resistance test results available, predicted intermediate/high-level resistance to specific drug classes [nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs)] was derived using the Stanford algorithm v5.1.2. Logistic regression models were then employed to estimate predicted probability of resistance to each drug class for given values of current viral load, history of virological failure and previous virological suppression. Based on these predicted probabilities and patients' covariate profiles, estimates of prevalence in 5355 patients with no prior test results were obtained. Overall prevalence of resistance was estimated by pooling these data with those observed in the remaining 1143 tested patients.

Results: Prevalence of NRTI, NNRTI and PI resistance was estimated as 43% (95% confidence interval: 39%–46%), 15% (13%–18%) and 25% (22%–28%), respectively.

Conclusions: This method provides estimates for the proportion of treated patients in a cohort who harbour resistance on a given date, which are less likely to be affected by selection bias due to missing resistance data and will allow us to estimate prevalence of resistance to different drug classes at specific timepoints in HIV-infected populations on ART.

Keywords: HIV/AIDS, antivirals, resistant, drug susceptibility, probability model, risk factors

Introduction

Antiretroviral therapy (ART) regimens containing combinations of drugs from different classes have proved highly effective at suppressing HIV replication in infected individuals.[1–4] However, mutations occurring during viral replication can lead to the emergence of virus that is less susceptible ('resistant') to specific drugs. Drug-resistant HIV strains emerge when ART fails to suppress viral replication effectively either through use of suboptimal regimens or incomplete adherence and have been a major barrier to successful treatment.[5–7] It is therefore important to estimate how widespread resistance is in treated populations and the proportion of patients carrying resistant virus at a specific time.

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The presence of resistance can be detected by HIV-RNA sequencing. However, this can generally only be done when there is a sufficiently high level of virus in the plasma (e.g. >500 copies/mL). When resistance is detected, the drugs in the regimen tend to be switched to drugs to which the virus remains susceptible. Thus after such a treatment switch, given good adherence to therapy, the viral load would tend to decrease to undetectable levels. This means that resistance mutations can be present (albeit at very low levels) even in people with undetectable viraemia, although they cannot be detected with standard population sequencing.[5,7] Guidelines recommend that genotypic resistance testing be carried out at the time of HIV diagnosis and in all cases of virological failure;[8,9] however, such tests are not always performed or the results are not always available in cohort studies.[10–12] For these reasons, it is difficult to estimate the prevalence of accumulated HIV drug resistance in patients on ART at any given time using epidemiological studies.

Published results on the prevalence of drug resistance are often difficult to compare as they differ in whether they focused on the percentage of patients with any resistant virus (even at low levels) as opposed to only resistance that could be detected in the majority population. They also differ in definitions of resistance and denominators used, which can be all patients with resistance test results available or all patients on ART. Previous studies have estimated the prevalence of HIV drug resistance in patients on ART using a variety of analytical methods resulting in a wide range of estimates from as little as 15% to a much higher value of 70%–80%.[13–19]

In this article we develop a method for estimating the prevalence of accumulated HIV drug resistance (whether as majority virus or not) to the three main drug classes, nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs), in patients receiving ART by imputing data for patients with no resistance test results available. For illustration, this method was then used to estimate the prevalence of resistance in EuroSIDA in a particular year, 2008, and to investigate the factors associated with the presence of predicted resistance.

Patients and methods

Study population

The EuroSIDA study is a prospective, observational, open cohort of 16 599 HIV-1-infected patients in 102 centres across 31 European countries, Israel and Argentina, described in detail at www.cphiv.dk.[20] Patients were enrolled into eight cohorts from May 1994 onwards with median follow-up time to August 2008. Information is collected on a standardized data collection form every 6 months, including all CD4 counts and viral loads measured since the last follow-up, starting and stopping dates of all drugs, and dates of all AIDS-defining diagnoses using the 1993 clinical definition of AIDS from the CDC.[21] Centres participating in EuroSIDA seek ethical approval according to their own local and national requirements.

EuroSIDA requests plasma samples from patients to be collected every 6 months and stored in a central repository. HIV-1 RNA is isolated from patient blood plasma using the QIAamp kit (Qiagen, Barcelona, Spain) and retrospective sequence analysis of HIV-1 reverse transcriptase (RT) and protease reading frames is performed using the Trugene HIV-1 genotyping kit and OpenGene DNA Sequencing System according to the manufacturer's recommendations (Bayer, Barcelona, Spain). Mutations are identified by comparison with a reference sequence of the subtype B isolate, HXB2. This has resulted in 6006 partial or full sequences of the RT and protease genes and the sequence database is supplemented by 3548 resistance tests performed at the clinical sites.

Inclusion criteria for derivation of models for predicting the presence of resistance

All resistance test results obtained from plasma samples dated between 1999 and 2008 from patients who had previously started ART and had a viral load measured within 3 months before the test, were included. The viral load was required to be measured before the test to avoid those measured after a treatment switch.

Inclusion criteria for estimation of prevalence of resistance in 2008

Prevalence of accumulated resistance in 2008 was estimated in patients who were under prospective follow-up in EuroSIDA and receiving ART at the mid-point (1 July) of the year, had at least 6 months previous experience of ART and a viral load measured within 3 months before 1 July. For some patients, a previous resistance test had been performed (the tested group) while for others it had not (the untested group).

Definition of resistance

The Stanford HIVdb algorithm v5.1.2 was used to define intermediate/high-level HIV resistance to the three main drug classes, NRTIs, NNRTIs and PIs, detected in the resistance tests for which results were available.[22] If a patient was predicted to have intermediate/high-level resistance to at least one drug in a drug class according to the algorithm, they were defined to have resistant HIV to that drug class. Mutations were assumed to remain present after first detection, even if they were not detected in subsequent resistance tests, and so were cumulated from previous test results. If no resistance mutations were detected in previous tests, patients were ascribed as having no resistance if the most recent test was performed in the past 6 months, but as having no test available (i.e. they were included in the untested group) if the test was done >6 months previously.

Statistical methods

Figure 1 illustrates the methodology developed for estimating resistance. Three separate logistic regression models were developed using all the resistance test results in the EuroSIDA database, each predicting resistance to one of the three main drug classes. Goodness of fit was evaluated using Hosmer–Lemeshow tests. Covariates to include in these models were identified *a priori* as likely to predict the detection of resistance:

(i) viral load, most recent within 3 months before test (<50, 50–499, 500–29 999, 30 000–99 999, ≥100 000 copies/mL);
(ii) virological failure of a drug in the class in question prior to the test date (defined by a viral load of >500 copies/mL after 4 consecutive months of being on the drug) with categories: never experienced failure on the class (including for NRTI resistance, patients who never experienced mono/dual NRTI therapy); previously experienced failure on the class but not currently receiving a drug from the class; previously experienced failure on the class and still receiving a drug from the class (for NRTIs, this last category was also split by whether or not patients had previous experience of mono/dual NRTI therapy);
(iii) whether or not patients had achieved a suppressed viral load of <500 copies/mL prior to the test date.

In order to quantify uncertainty around these predicted probabilities of resistance, a bootstrapping approach was taken. The logistic regression

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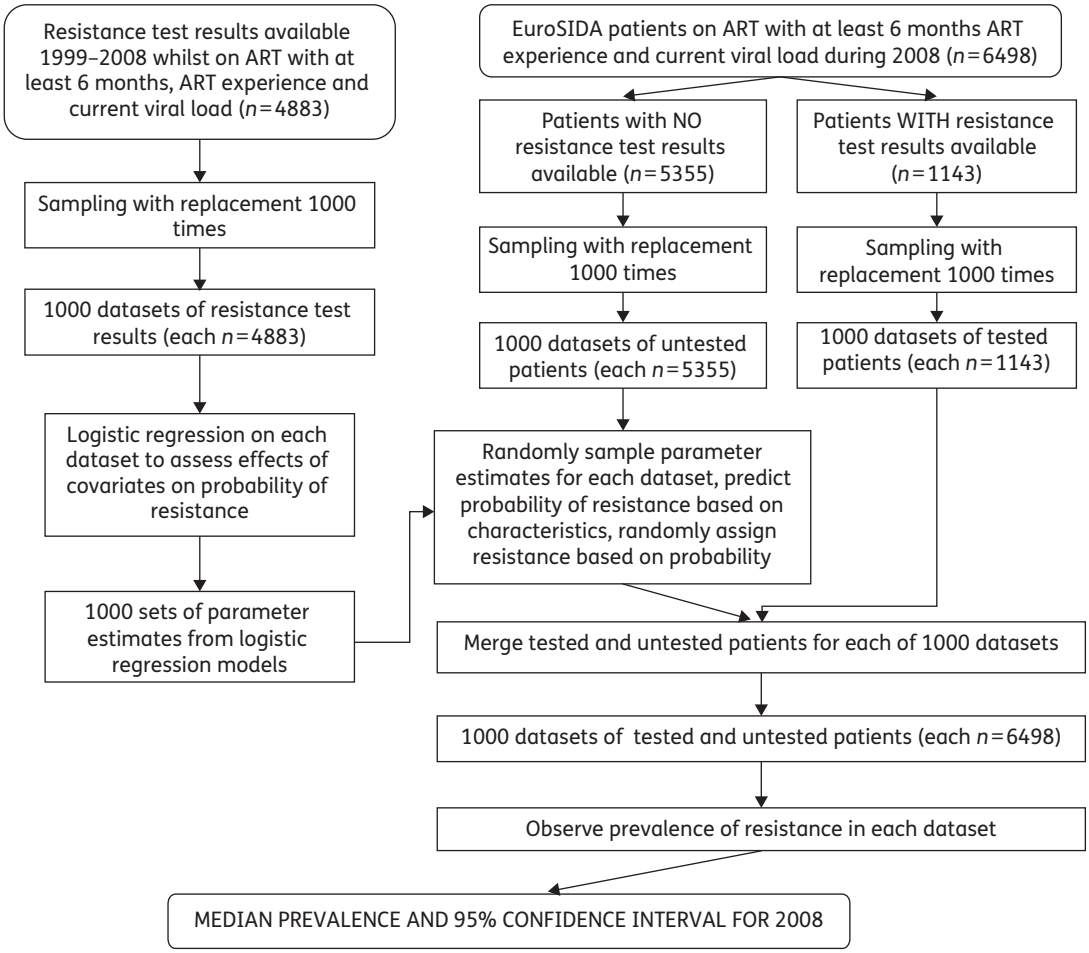

Figure 1. Methodology for obtaining estimated prevalence of accumulated HIV-1 drug resistance with 95% CIs.

models were fitted on 1000 datasets created by randomly sampling with replacement from the dataset of all resistance test results (with each dataset the same size as the original dataset) resulting in 1000 possible sets of parameter estimates for each of the three models (one for each drug class).

The second stage was to use these predicted probabilities to obtain estimates of prevalence of patients with resistance at 1 July 2008. Again, a bootstrapping approach was taken to estimate the standard error of the prevalence. One thousand datasets were created by randomly sampling with replacement from the set of ART-treated patients under follow-up at 1 July 2008. Each dataset had the same number of observations as the observed dataset and the same proportions of patients in the tested and untested groups.

In each of these 1000 datasets, for those with a resistance test available, the result of the test was used. For untested patients, resistance status for each drug class was imputed according to their covariate profiles. For each dataset, a set of parameter estimates from each of the three logistic regression models (one for each drug class) was randomly sampled from the 1000 created. Using the patient's observed data at 1 July 2008, i.e. (i) current viral load (closest within 3 months before date), (ii) previous virological failure of a drug in the class in question and (iii) whether or not a viral load <500 copies/mL had previously been achieved, the probability of resistance to each drug class was predicted. Then, each patient was randomly allocated a number between 0 and 1 from a uniform distribution and if this was less than or equal to the predicted probability from the model, they were assigned as having resistant HIV present. In this way, the presence of resistance or not was randomly assigned to each untested patient whilst taking into account their chance of actually having resistance present according to their covariate profile.

The final stage was to pool data from the tested and untested groups in each of the 1000 datasets and to obtain percentages of patients with resistance to each drug class. The denominator consisted of patients with prior positive resistance test results (the tested group) and those with no prior resistance test results (or a negative test that was older than 6 months) for whom we had a predicted assignment, as described above, of whether resistance was present or not (the untested group). The median percentage with resistance of the 1000 datasets was reported together with 2.5th and 97.5th percentiles of the bootstrap distribution to define a 95% confidence interval (CI). Analyses were also performed in NNRTI- and PI-experienced patients only.

All tests were two-sided and a P value of <0.05 was taken to be statistically significant. SAS software version 9.1 (2002–03; SAS Institute, Cary, NC, USA) was used for all analyses.

Results

Patient characteristics in 2008

A total of 6498 patients receiving ART in 2008 were included. Of these, 2277 (35%) patients had at least one set of resistance test

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Table 1. Characteristics of EuroSIDA patients receiving ART at 1 July 2008

	Total		Tested		Untested		*P*
Total patients (*n*, %)	6498	100.0	1143	17.6	5355	82.4	—
Male (*n*, %)	4842	74.5	891	78.0	3951	73.8	0.003
White ethnicity (*n*, %)	5674	87.3	932	81.5	4742	88.6	<0.001
IDU transmission risk group (*n*, %)	1251	19.3	170	14.9	1081	20.2	<0.001
VL (copies/mL)							<0.001
<50	5035	77.5	837	73.2	4198	78.4	
50–499	934	14.4	184	16.1	750	14.0	
500–29999	367	5.6	82	7.2	285	5.3	
30000–99999	83	1.3	15	1.3	68	1.3	
≥100000	79	1.2	25	2.2	54	1.0	
Previous VL < 500 copies/mL (*n*, %)	6364	97.9	1132	99.0	5232	97.7	0.004
Type of ART regimen (*n*, %)							<0.001
mono/dual NRTI	56	0.9	6	0.5	50	0.9	
single PI	349	5.4	40	3.5	309	5.8	
RTV-boosted PI	2435	37.5	460	40.2	1975	36.9	
single NNRTI	2147	33.0	166	14.5	1981	37.0	
PI + NNRTI	439	6.8	168	14.7	271	5.1	
other	1072	16.5	303	26.5	769	14.4	
>6 months on mono/dual NRTI (*n*, %)	3268	50.3	875	76.6	2393	44.7	<0.001
NNRTI experienced (*n*, %)	5036	77.5	1011	88.5	4025	75.2	<0.001
PI experienced (*n*, %)	5735	88.3	1115	97.6	4620	86.3	<0.001
Ever failed…(*n*, %)							
NRTI	4777	73.5	1117	97.7	3660	68.3	<0.001
NNRTI	1757	27.0	589	51.5	1168	21.8	<0.001
PI	2779	42.8	879	76.9	1900	35.5	<0.001
Date started first-line ART[a]	Jan 97	Sep 94–Jun 00	Apr 95	Nov 92–Oct 96	Jun 97	Apr 95–Mar 01	<0.001
Enrolment into EuroSIDA[a]	Sep 99	Feb 97–Feb 04	Mar 97	Dec 95–Mar 99	Nov 01	Apr 97–Dec 05	<0.001
Age (years)[a]	46	41–53	48	43–55	46	41–53	<0.001
CD4 count (cells/mm^3)[a]	513	355–711	472	315–671	522	365–719	<0.001

IDU, injecting drug user; VL, viral load; RTV, ritonavir.
P values obtained from χ^2 tests for categorical data and Kruskal–Wallis tests for continuous data.
[a]Median and interquartile range are reported.

results before 1 July 2008; however, 1134 patients had no resistance detected in any previous tests and their last resistance test was >6 months before this date. They were therefore included in the untested group instead, leaving 1143 (18%) of the 6498 patients in the tested group. Table 1 displays some of the patient characteristics and treatment experience at 1 July 2008. The majority of patients included were male and of white ethnicity. Similar viral load distributions were observed in both tested and untested groups with three-quarters having an undetectable HIV-RNA of <50 copies/mL. In the untested group, ART was generally started more recently (median, June 1997 versus April 1995) and CD4 count was higher (median, 522 versus 472 cells/mm^3).

Most patients (71%) were receiving single NNRTI-based or ritonavir-boosted PI-based regimens. The untested group had less previous mono/dual NRTI experience (45% versus 77%; $P<0.001$), less NNRTI experience (75% versus 89%; $P<0.001$) and less PI experience (86% versus 98%; $P<0.001$). Corresponding to this, the untested group had fewer patients who had experienced virological failure on NRTIs, NNRTIs or PIs, whereas in the tested group, almost all patients had experienced NRTI failure, half had experienced NNRTI failure and three-quarters, PI failure.

Probability of accumulated HIV-1 drug resistance

In order to estimate the prevalence of accumulated resistance in the 5355 untested patients at 1 July 2008, we used the results of 4883 resistance tests from 2410 patients who had at least 6 months experience of ART and a viral load measurement (most recent) in the 3 months prior to the time of the test, over the years 1999–2008. A small proportion of the tests (9%) were carried out on samples where the viral load was <500 copies/mL [of which 156 (34%) had <50 copies/mL]; however, HIV-RNA amplification was still successful. Virological suppression (<500 copies/mL) was achieved prior to 3868 (79%) of the

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Table 2. Median and range of predicted probability of accumulated HIV-1 drug resistance according to selected characteristics in EuroSIDA patients with resistance tests whilst receiving ART, 1999–2008

		Viral load (copies/mL) at time of resistance test														
		<50			50–499			500–29999			30000–99999			≥100000		
		median	range	*n*	median	range	*n*	median	range	*n*	median	range	*n*	median	range	*n*
NRTI resistance																
Previously had VL<500																
Ever failed an NRTI?	no	0.05	0.01–0.10	10	0.07	0.02–0.15	21	0.11	0.03–0.19	55	0.09	0.03–0.17	38	0.11	0.03–0.21	33
	yes, not on NRTI[a]	0.30	0.18–0.43	8	0.38	0.28–0.49	24	0.50	0.43–0.58	223	0.45	0.35–0.53	121	0.50	0.44–0.58	169
	yes, still on NRTI, ART naive[b]	0.31	0.21–0.43	25	0.40	0.29–0.51	38	0.52	0.46–0.59	354	0.47	0.39–0.58	60	0.52	0.46–0.59	56
	yes, still on NRTI, prior mono/dual ART[c]	0.47	0.31–0.60	112	0.56	0.47–0.67	218	0.67	0.64–0.70	1772	0.63	0.57–0.68	318	0.68	0.62–0.74	213
No previous VL<500																
Ever failed an NRTI?	no	0.07	0.02–0.14	0	0.10	0.02–0.20	0	0.16	0.05–0.27	10	0.14	0.04–0.24	4	0.16	0.05–0.29	4
	yes, not on NRTI[a]	0.40	0.23–0.53	0	0.50	0.37–0.64	1	0.61	0.53–0.70	25	0.57	0.47–0.65	32	0.62	0.53–0.70	63
	yes, still on NRTI, ART naive[b]	0.42	0.28–0.56	0	0.51	0.38–0.68	1	0.63	0.55–0.70	40	0.59	0.49–0.67	33	0.64	0.55–0.71	39
	yes, still on NRTI, prior mono/dual ART[c]	0.58	0.39–0.71	1	0.67	0.57–0.79	0	0.77	0.71–0.82	371	0.73	0.66–0.80	206	0.77	0.71–0.84	185
NNRTI resistance																
Previously had VL<500																
Ever failed an NNRTI?	no	0.03	0.01–0.07	121	0.08	0.05–0.12	182	0.10	0.08–0.12	1112	0.11	0.08–0.15	277	0.13	0.11–0.17	229
	yes, not on NNRTI	0.25	0.10–0.42	15	0.45	0.33–0.58	62	0.52	0.46–0.57	558	0.54	0.46–0.60	131	0.59	0.51–0.66	160
	yes, still on NNRTI	0.24	0.10–0.41	19	0.45	0.35–0.55	57	0.51	0.46–0.55	734	0.53	0.47–0.62	129	0.59	0.51–0.66	82
No previous VL<500																
Ever failed an NNRTI?	no	0.06	0.02–0.13	1	0.14	0.09–0.20	2	0.18	0.14–0.22	262	0.19	0.14–0.25	122	0.23	0.17–0.28	113
	yes, not on NNRTI	0.38	0.16–0.59	0	0.61	0.48–0.73	0	0.67	0.60–0.73	62	0.69	0.62–0.75	66	0.73	0.64–0.79	98
	yes, still on NNRTI	0.38	0.16–0.58	0	0.61	0.47–0.71	0	0.67	0.60–0.72	122	0.69	0.63–0.75	87	0.73	0.67–0.79	80
PI resistance																
Previously had VL<500																
Ever failed a PI?	no	0.06	0.03–0.10	77	0.05	0.03–0.08	95	0.07	0.05–0.11	597	0.06	0.04–0.09	125	0.07	0.04–0.09	130
	yes, not on PI	0.33	0.18–0.47	25	0.31	0.22–0.40	67	0.39	0.34–0.44	472	0.35	0.27–0.44	137	0.36	0.29–0.43	141
	yes, still on PI	0.47	0.29–0.62	53	0.45	0.33–0.55	139	0.54	0.51–0.57	1335	0.49	0.42–0.55	275	0.50	0.43–0.56	200
No previous VL<500																
Ever failed a PI?	no	0.12	0.06–0.20	0	0.11	0.07–0.17	0	0.15	0.11–0.21	114	0.13	0.09–0.17	52	0.13	0.09–0.18	46
	yes, not on PI	0.52	0.35–0.67	1	0.50	0.38–0.61	2	0.59	0.52–0.65	63	0.55	0.44–0.64	44	0.56	0.47–0.64	66
	yes, still on PI	0.66	0.49–0.78	0	0.64	0.52–0.74	0	0.72	0.68–0.77	269	0.68	0.61–0.74	179	0.69	0.62–0.75	179

n, number of tests; VL<500, viral load <500 copies/mL.
[a]Not on an NRTI at time of resistance test.
[b]Still on an NRTI at time of resistance test and ART naive when started cART.
[c]Still on an NRTI at time of resistance test and has experienced prior mono or dual ART.

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Table 3. Number of EuroSIDA patients receiving ART and estimated prevalence of accumulated HIV-1 drug resistance according to selected characteristics in 2008; total patients in subgroup (prevalence of resistance, % within subgroup)

			Viral load (copies/mL) at 1 July 2008				
		Total	<50	50–499	500–29999	30000–99999	≥100000
NRTI resistance							
Previous VL < 500							
Ever failed an NRTI?	no	1675 (6)	1445 (6)	196 (8)	24 (17)	3 (0)	7 (0)
	yes, not on NRTI[a]	266 (33)	184 (34)	56 (30)	15 (20)	7 (57)	4 (50)
	yes, still on NRTI, ART naive[b]	1019 (43)	680 (40)	207 (45)	101 (55)	22 (64)	9 (44)
	yes, still on NRTI, prior mono/dual ART[c]	3404 (60)	2711 (57)	468 (67)	169 (74)	33 (61)	23 (83)
No previous VL < 500							
Ever failed an NRTI?	no	46 (17)	8 (0)	4 (0)	18 (17)	4 (50)	12 (25)
	yes, not on NRTI[a]	2 (100)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)
	yes, still on NRTI, ART naive[b]	60 (60)	2 (0)	2 (50)	33 (73)	9 (67)	15 (40)
	yes, still on NRTI, prior mono/dual ART[c]	26 (69)	5 (40)	2 (50)	7 (71)	4 (75)	8 (88)
Previous NRTI experience[d]							
	yes	6488 (42)	5027 (39)	933 (47)	366 (60)	83 (60)	79 (53)
	no	10 (20)	8 (13)	1 (100)	1 (0)	0 (0)	0 (0)
Time from starting NRTI, years[e]							
	<3	346 (19)	192 (8)	73 (23)	48 (50)	13 (39)	20 (20)
	3–5	711 (20)	506 (13)	136 (27)	40 (48)	13 (77)	16 (44)
	6–8	851 (22)	679 (18)	117 (29)	40 (48)	5 (80)	10 (60)
	9–11	1852 (38)	1477 (35)	235 (45)	102 (58)	22 (55)	16 (69)
	12–14	1586 (59)	1275 (56)	198 (71)	84 (71)	19 (53)	10 (80)
	≥15	1142 (60)	898 (59)	174 (62)	52 (75)	11 (82)	7 (86)
NNRTI resistance							
Previous VL < 500							
Ever failed an NNRTI?	no	4655 (5)	3844 (4)	600 (9)	153 (10)	31 (10)	27 (19)
	yes, not on NNRTI	1074 (43)	735 (37)	209 (53)	95 (63)	26 (65)	9 (67)
	yes, still on NNRTI	635 (33)	441 (28)	118 (46)	61 (36)	8 (63)	7 (71)
No previous VL < 500							
Ever failed an NNRTI?	no	86 (16)	11 (18)	6 (33)	39 (15)	7 (29)	23 (9)
	yes, not on NNRTI	20 (80)	1 (100)	1 (100)	8 (50)	2 (100)	8 (100)
	yes, still on NNRTI	28 (75)	3 (33)	0 (0)	11 (91)	9 (67)	5 (80)
Previous NNRTI experience[d]							
	yes	5036 (18)	3933 (13)	691 (29)	288 (39)	65 (48)	59 (49)
	no	1462 (5)	1102 (4)	243 (9)	79 (8)	18 (22)	20 (5)
Time from starting NNRTI, years[e]							
	<3	575 (12)	385 (4)	101 (21)	53 (30)	16 (63)	20 (40)
	3–5	1088 (11)	860 (7)	151 (23)	57 (35)	10 (50)	10 (50)
	6–8	2007 (16)	1624 (13)	241 (27)	103 (35)	21 (43)	18 (56)
	≥9	1366 (28)	1064 (23)	198 (42)	75 (52)	18 (39)	11 (55)
PI resistance							
Previous VL < 500							
Ever failed a PI?	no	3627 (7)	3069 (7)	446 (6)	85 (13)	14 (21)	13 (0)
	yes, not on PI	624 (38)	486 (38)	97 (39)	26 (31)	9 (0)	6 (67)
	yes, still on PI	2113 (53)	1465 (53)	384 (51)	198 (56)	42 (52)	24 (42)
No previous VL < 500							
Ever failed a PI?	no	92 (14)	12 (17)	5 (0)	39 (15)	14 (14)	22 (14)
	yes, not on PI	7 (57)	0 (0)	0 (0)	5 (80)	1 (0)	1 (0)
	yes, still on PI	35 (69)	3 (33)	2 (50)	14 (71)	3 (100)	13 (69)

Continued

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Table 3. *Continued*

	Total	Viral load (copies/mL) at 1 July 2008				
		<50	50–499	500–29999	30000–99999	≥100000
Previous PI experience[d]						
yes	5735 (28)	4449 (26)	830 (31)	319 (43)	71 (38)	66 (38)
no	763 (7)	586 (5)	104 (7)	48 (23)	12 (25)	13 (8)
Time from starting PI, years[e]						
<3	447 (14)	283 (10)	99 (15)	44 (34)	9 (22)	12 (25)
3–5	695 (16)	506 (13)	127 (18)	38 (40)	8 (0)	16 (31)
6–8	694 (19)	542 (16)	92 (25)	42 (41)	8 (50)	10 (20)
9–11	3317 (31)	2641 (29)	438 (36)	175 (46)	39 (44)	24 (50)
≥12	582 (41)	477 (39)	74 (46)	20 (55)	7 (57)	4 (75)

VL<500, viral load <500 copies/mL.
[a]Not on an NRTI at time of resistance test.
[b]Still on an NRTI at time of resistance test and ART naive when started cART.
[c]Still on an NRTI at time of resistance test and has experienced prior mono or dual ART.
[d]Started drug at least 6 months before 1 July 2008.
[e]In patients with experience of the drug class.

tests. A total of 96% of the tests occurred after virological failure of an NRTI, 50% after NNRTI failure and 75% after PI failure.

To test the goodness of fit of the model predicting resistance, which included the covariates named in the Patients and methods section, each of which was significantly associated with the outcome ($P<0.001$), we calibrated predicted probabilities of resistance against observed percentages of resistance. The resistance test data were divided into four groups based on the value of their predicted probability of resistance (repeated for NRTI, NNRTI and PI resistance): <0.25; 0.25–0.49; 0.5–0.74; and ≥0.75. The percentages of observed resistance were then examined in each group to check that they were within the boundaries of the predicted probability for the group, e.g. for NRTI resistance, the percentage of observed resistance in each group (<0.25, 0.25–0.49, 0.5–0.74 and ≥0.75, respectively) was 11%, 46%, 65% and 77%. This showed that low predicted probabilities of resistance were correctly being assigned to a group with low prevalence of observed resistance and similarly, higher predicted probabilities were assigned to groups with higher prevalence. Hosmer–Lemeshow tests were performed and no significant differences were found in the observed and expected probabilities of NRTI resistance ($P=0.626$), NNRTI resistance ($P=0.129$) or PI resistance ($P=0.315$).

Table 2 displays the median predicted probabilities (and ranges) of resistance from the 1000 datasets (all of size $n=4883$) created by randomly sampling from the original observed dataset. As expected, the risk of accumulated drug resistance was high when there was a previous history of treatment failure, especially when the patient was still receiving a drug from the same drug class at the time of the test. For NRTI resistance, prior mono/dual therapy also increased the chance of resistance, compared with when patients started a combination ART (cART) regimen from ART naivety. These associations remained significant after controlling for viral load at the time of the test and for previous viral suppression, which were also both independently associated with probability of resistance detection. The risk of detected resistance increased according to a higher viral load and was greater in those with no previous viral suppression.

Estimated prevalence of accumulated HIV-1 drug resistance in 2008

Using the methods described, the prevalence of accumulated resistance to NRTIs, NNRTIs and PIs at 1 July 2008 was estimated in the 6498 patients receiving ART. The percentages of tested patients harbouring NRTI, NNRTI and PI resistance (to at least one drug in the class) were observed to be 94%, 35% and 50%, respectively. Note that patients with no previous resistance mutations detected and whose last resistance test was done >6 months previously were excluded from the tested group, therefore these observed percentages represent a worst case scenario. The percentages of untested patients (including those excluded from the tested group) predicted to be harbouring NRTI, NNRTI and PI resistance were much lower at 31%, 11% and 20%, respectively. Combined, these gave estimates of 43%, 15% and 25%, respectively, in 2008. Percentages of NRTI, NNRTI and PI resistance were also predicted in the tested group (40%, 17% and 32%) that were similar to the actual observed results before exclusion of those with no previous resistance mutations (47%, 18% and 25%).

In Table 3, these estimates were stratified by current viral load (most recent in 3 months before 1 July 2008) and other selected characteristics. Estimated prevalence of resistance was lowest when the current viral load was <50 copies/mL compared with other viral loads and unsurprisingly, higher in patients with previous experience of the drug class. They also increased according to time since starting a drug in that class. This was most pronounced for NRTI resistance where 19% of those starting <3 years before had NRTI resistance compared with 60% of those starting at least 15 years before.

In those with a current viral load of <50 copies/mL ($n=5035$, 77%), the prevalence of resistance to a drug class was greatly increased in those with previous treatment failure of a drug in that class. Of those with no previous NRTI failure, only 6%

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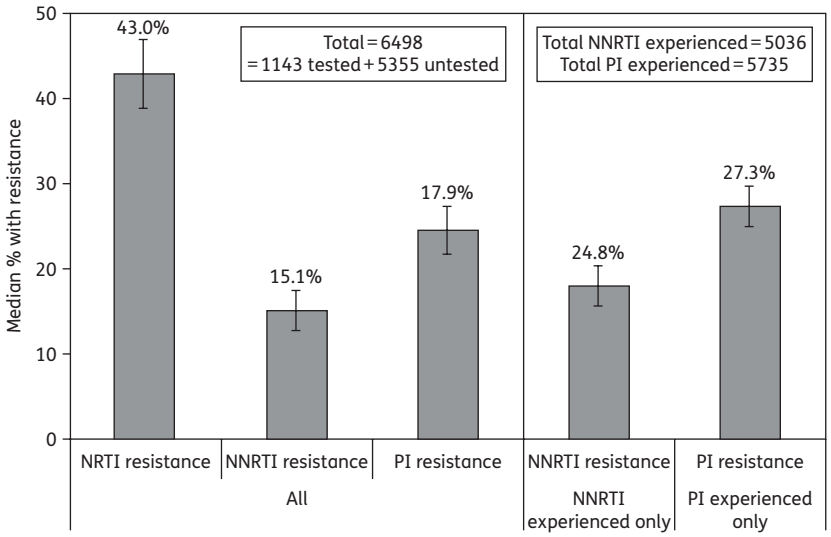

Figure 2. Median estimated prevalence of accumulated HIV-1 drug resistance in 1000 randomly sampled datasets of EuroSIDA patients receiving ART in 2008.

were estimated to have NRTI resistance, 4% of those with no previous NNRTI failure were estimated to have NNRTI resistance and 7% of those with no previous PI failure were estimated to have PI resistance.

Figure 2 shows the median estimates of drug resistance prevalence, together with 95% CIs (2.5th and 97.5th percentiles) obtained using the bootstrapping method. According to our method, the point estimates are fairly precise, as indicated by the narrow 95% CIs.

Discussion

A method of estimating the extent of accumulated HIV-1 drug resistance in ART-treated EuroSIDA patients in a particular year was developed by imputing data on untested patients, based on the probability of detecting resistance in tested patients with a similar covariate profile. The results of using this method revealed that in 2008, there was a 43% (95% CI: 39%–46%) prevalence of NRTI resistance, 15% (13%–18%) prevalence of NNRTI resistance and a 25% (22%–28%) prevalence of PI resistance. Much of this resistance may be archived and to drugs that were no longer being taken. As the majority of patients were NNRTI and PI experienced, estimates did not change greatly in these subsets: 18% (15%–21%) of NNRTI-experienced patients with NNRTI resistance and 27% (25%–30%) of PI-experienced patients with PI resistance. These results illustrate how estimates based on tested patients under follow-up in an epidemiological study at a given point in time might be misleading as they tend to greatly overestimate the proportion of patients with resistance.

Comparing estimates of prevalence of HIV drug resistance from the analysis of observational studies is complicated due to the variability in analytical methods and denominators used. A number of studies have investigated prevalence of resistance in ART-experienced patients with resistance test results available.[13,15–19,23–27] The key limitation of these studies is that the interpretation of the results is difficult because, as our analysis suggests, the prevalence is likely to be an overestimate of the true prevalence of resistance in everyone treated and the trend over time is affected by variations in the composition of the group that gets tested. For example, patients may be selected for resistance testing due to suspected resistance and criteria for selection may vary over time (at different levels of viral load, etc.) so may not be representative of the treated population at any point in time.

The UK Collaborative Group on HIV Drug Resistance used a single-timepoint analysis to find the proportion of tests with resistance (~80% over 1998–2002) with an alternative cumulative model in the context of all treated patients showing an increase in the prevalence over time reaching 17% in 2002.[14] However, this assumes that all patients harbouring resistance were tested, which is not always possible if a patient's viral load is suppressed.[28] Therefore the difference between our estimates and theirs could reflect a reduction in the size of the population that could be tested.

The Swiss Cohort Study, using a similar approach to ours, extended estimates of drug resistance in ART-experienced patients with genotypic information available to those who did not by estimating their risk of harbouring resistant virus on the basis of treatment history.[29] The method produced lower and upper estimates of drug resistance, 37% and 45% in 2007. Given that most of the patients with any resistance have an NRTI-resistant virus, our estimate of 43% in 2008 is very consistent with these. At the Conference on Retroviruses and Opportunistic Infections, 2010, Abraham *et al.*[30] presented a novel method for quantifying resistance, which, again, was similar to ours and involved imputing data for individuals without resistance test results according to characteristics such as viral load, CD4 count and prior use of NRTIs before cART. The two methods differ in the set of predictors considered, our approach accounting for only three factors that were considered *a priori* to be most important for predicting drug resistance.

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As expected, our results showed that in 2008, estimates of the prevalence of accumulated resistance were lowest when there was no previous history of virological failure, in particular for patients who had a currently suppressed viral load. Estimates were obtained that are consistent with those observed in epidemiological surveillance of transmitted resistance in ART-naive patients, consistent with the interpretation that the resistance observed in people with this profile is due to transmitted drug resistance, and supporting the validity of the model.[31] These estimates were based on low numbers of resistance tests due to the difficulty in HIV-RNA sequencing in patients with low viral load and so unexplained variation in the sample cannot be ruled out. A further approach was explored where all patients with a currently suppressed viral load and no prior virological failure were imputed to have no resistance and similar overall findings were obtained.

The risk of accumulated resistance to a particular drug class was greatly increased in patients with at least 6 months experience of a drug in that class. Furthermore, in those with experience, risk of resistance increased according to length of time since the patient had first started a drug of that class. This can be attributed to accumulation of mutations as the viral load increased on treatment.[5,7] In particular, the striking increase in the prevalence of NRTI resistance linked to a longer time since starting an NRTI can be linked to the change from mono/dual NRTI therapy to cART in the mid-1990s.

EuroSIDA is one of the largest international HIV cohort studies and has a vast genotypic sequence database, which allowed the development of our new method. There are limitations to our approach. Our analysis only included patients in whom viral load had been measured over the 3 months prior to a genotypic test (or to 1 July 2008) and therefore a population of patients who are monitored more frequently than the average patient in the cohort may have been selected. Nevertheless, our method has its merits, as it is likely to reduce selection bias introduced by the exclusion of patients with no test available. Our logistic regression models included only three main predictors of resistance. It cannot be ruled out that the accuracy of the model in predicting detection of resistance could be improved by including additional covariates resulting in better estimates. Our approach is based on the assumption that test results were missing at random (i.e. that the reason for not having the test was not related to the amount of resistance present). An alternative approach that was not explored in this analysis is sample selection modelling, e.g. Heckman's two-stage regression, which aims to account for non-random sample selection bias.[32] A limitation of this is that it would be necessary to have information on at least one variable associated with having resistance test results available only, or associated with detection of resistance only, to avoid problems of co-linearity between the variables included. We aim to investigate and compare results of this and other approaches in future analyses.

In conclusion, estimates of prevalence in HIV drug resistance vary greatly between studies due to differences in the definitions of resistance, analysis methods, selection of samples and denominators. We have developed a method that should allow us to more reliably estimate the prevalence of HIV-infected individuals harbouring resistance to different drug classes at a specific timepoint, including resistance mutations that were archived. Our results, which are less likely to be affected by selection bias due to missing resistance test data, show lower overall estimates of prevalence of resistance compared with studies that assume resistance testing at all occurrences of virological failure.

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