

Transmitted Antiretroviral Resistance among Patients with Acute and Recent HIV Infection Identified in North Carolina between 1998 and 2004

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673 - Transmitted antiretroviral resistance among patients with acute and recent HIV infection identified in North Carolina between 1998 and 2004. Hicks C, Eron J, Fiscus S, Petch L, Nguyen T, Menezes P, Giner J, Leone P, Cachafeiro A, Stalzer B, Williams D, McPherson T, Sebastian J, Pilcher C. Duke Univ Med Ctr, Durham NC; Univ NC, Chapel Hill, NC; NC, Stae DHHS, Raleigh, NC, LabCorp, Burlington NC.

Background: Most existing data examine transmitted resistance among patients who are identified as acutely-infected based on clinical suspicion. North Carolina (NC) recently introduced a nucleic acid program (STAT) for all state-funded HIV testing in which all HIV EIA (-) specimens are tested for acute HIV using RNA, and all HIV EIA (+) specimens are tested for "recent" infection using a detained HIV EIA. We compared this novel VCT-based surveillance with more traditional, clinically driven acute HIV surveillance in the same geographic area.

Methods: Two groups of patients had genotypic resistance testing: 1) 26 symptomatic patients referred from across NC to the Duke-UNC-Emory Acute HIV Consortium (n=26) diagnosed between 1998-2004; 2) 76 patients requesting publicly funded HIV VCT from November 2002 to April 2004, with no previous HIV-test result. Both "acute" (HIV RNA=Ab – at testing; n=19) or "recently acute" (HIV Ab= at testing but negative on a detained HIV EIA; n=57) specimens were sequenced. Bulk sequencing of RT and protease was done using Genouse or Trugene. Only major resistance mutations were included in analyses, as defined by 2000 IAS-USA guidelines.

Results: Overall, major resistance mutations were detected in 11 (10.8%; 95% CI: 5.5-18.5%) of 102 patients in NC, coding resistance to NRTI (3, 2.9%), NNRTI (6, 5.9%), PI (5, 4.9%) or multiple classes (3, 2.9%). Overall frequencies of mutations were similar among "acute" (0.45, or 8.9%) and "recent" (0.57, or 12.3%) infections. Prevalence estimates for the VCT-based population (9/76 or 11.8%, 95%CI 5.6-21.3%) were much more precise than for the referal population (2/26 or 7.7%, 95%CI 0.9-25.1%). Major PI mutations and multi-class resistance were only detected in recent infections from the STAT VCT population.

Conclusions: Observed rates of transmitted resistance in North Carolina (around 10%) and the detection of MDR transmission argue for an important role of HIV genotypic screening in the evaluation of newly/recently HIV infected patients in the state and underscore the public health importance of monitoring systems. VCT programs incorporating active laboratory-based surveillance for early HIV infection can more effectively illuminate patterns of transmitted resistance in defined geographic areas than collection of data on patients with clinically suspected acute HIV. These data provide NC with a baseline for prospective monitoring of trends in transmission of ARV resistance over time.

Background

- Transmission of HIV resistant to antiretroviral agents has been reported to be occurring with increasing frequency among patients with acute HIV infection.
- Between 1996-97 and 2000-01 in San Francisco, rates of transmitted resistance increased from 0% to 13.2% for non-nucleoside RT inhibitors (NNRTI) and from 2.5% to 7.7% for protease inhibitors (PI) [Grant]
- Between 1995-98 and 1999-2000, a 10-city study of 377 newly infected patients showed an increasing frequency of high-level resistance over time (from 3.4% to 12.4%) [Little].
- Recommendations for resistance testing include testing patients with acute HIV infection [Hirsch]. Rationale for such testing includes:
 - Transmitted resistance is likely most detectable soon after infection
 - Transmitted resistance likely persists in reservoirs
 - Persons who acquire viruses with transmitted resistance may respond less well to subsequent therapy.
- North Carolina has implemented statewide surveillance for acute HIV infection – the STAT program - increasing case recognition [Pilcher]
- Samples from persons undergoing voluntary counseling and HIV testing (VCT) at 110 NC public HIV testing sites (n = 120,000 tests annually) are assessed as part of the STAT program.
- Specimens are tested by Vironostika HIV-1 EIA (bio/Merieux, Durham, NC) and western blot (BioRad, Hercules, CA) for HIV antibodies; pooled HIV Ab- samples assayed for HIV-1 RNA by NucliSENS HIV-1 QL NAAT (bio/Merieux, Durham, NC)
- HIV antibody + samples are also tested by a less-sensitive, or "detuned" EIA based on the Vironostika kit to identify recently HIV-infected persons

Study Design

Acute HIV infection in North Carolina residents was identified from among two cohorts:

- Duke-UNC-Emory Acute HIV Infection Research Consortium (Jan 1998-Dec 2004) – persons included in this population were North Carolina residents diagnosed based on clinical presentation of acute HIV infection

- NC DHHS/UNC Screening & Tracing Active Transmission (STAT) program for public HIV VCT (Nov 2002-Oct 2003) – persons included in the population were identified based on NAAT testing of pooled HIV Ab-negative samples from the NC state HIV test centers

Recent HIV infections were identified from among a population of HIV EIA/WB positive persons who underwent HIV testing at the publicly funded NC HIV VCT centers between Nov 02 - Oct 03. Persons were categorized as having been recently infected if the HIV EIA/WB was positive on standard testing, but a less sensitive "detuned" antibody test was negative.

The study was approved by the Institutional Review Boards at all participating institutions. Informed consent was obtained from all study participants prior to enrollment.

Specimens for resistance testing were obtained prior to the initiation of antiretroviral therapy.

Genotypic HIV resistance testing was done by bulk sequencing of reverse transcriptase and protease using the Genouse or Trugene technology. Genotypic resistance was defined by the presence of ≥1 mutation based on the IAS-USA 2003 definitions [Hirsch].

Nucleosides (nRTI):	41L, 44D, 65R, 67N, 69A/S, 70R, 74V, 118I, 151M, 184I/V, 210W, 215Y/E/D/N/S/C/E, 219Q/E
Non-nucleosides (NNRTI):	100I, 103N, 106A/M, 108I, 181C/L, Y188C/L/H, 190A/S, 225H, 236L
Protease Inhibitors (PI):	10F, 30N, 32I, 33I/F, 46I/L, 48V, 50L/V, 54V/M/L, 82A/E/T/S, 84V/A/C, 90M

Results

127 persons were identified and tested

- 40 from the clinical Acute HIV Consortium cohort
- 89 from the STAT program for acute surveillance
- 20 with acute HIV infection as defined by HIV Ab-/NAAT+
- 69 with recent HIV infection as defined by "detuned" EIA
- [2 persons were part of both groups (both without resistance)]

Patient Characteristics*

	Duke-UNC(40)	STAT(88)
Age	Med (Range)	27 (17-59)
Sex	Med (Range)	30 (15-59)
	Male	36 (90%)
	Female	4 (10%)
Race/	Black	17 (43%)
	White	52 (61%)
Ethnicity	White	19 (48%)
	Latino/Latina	20 (24%)
	Native American	12 (14%)
	Other/UNK	0
Mode of	MSM	26 (65%)
	Heterosexual	33 (39%)
Transmission	Heterosexual	10 (25%)
	Other/UNK	4 (10%)
		14 (16%)

* No data available on 4 STAT patients
Characteristics of patients within groups were similar whether resistance was present or not

**25 cases of resistance detected overall (19.7%)
Rates of resistance to specific antiretroviral classes were:**

nRTI	7.1%
NNRTI	6.3%
PI	3.1%
MDR	3.1%

Prevalence of Transmitted Resistance

	Duke/UNC		STAT Program	
	All	Acute	Recent	
Sequences	40	89	20	69
Any ARV	8	17	3	14
nRTI	4	5	1	4
NNRTI	3	5	2	3
PI	1	3	0	3
MDR	0	4	0	4
NRTI + PI	0.0%	2	0.0%	2
3-class	0.0%	2	0.0%	2

Patterns of Mutations for Individual Patients

	Duke/UNC		STAT Program	
	Acute	Recent	Acute	Recent
nRTI	118 IV	118I	41L	44D
			67N	219E
	41L,215D		103N	103N
NNRTI	103N	108I	103N	103N
	103N	103N, 225H	103N	103N
PI	33F		46I	46L
			46L, 84V, 90M	118I, 46L
MDR			184V,215F/S,190E, 54L,84V,90M	215C,103N,90M
			70R, 190A, 33F, 84V	

- Estimates of the prevalence of transmitted resistance were similar for patients identified by the Duke/UNC clinical cohort (8/40 – 20%) compared to those detected by either NAAT testing or detained EIA in the VCT group (overall 17/89 – 19.1%).
- However, 3 of the 4 cases of protease inhibitor resistance and all 4 of the cases of multi-drug resistance (MDR) were found in patients classified as "recent" HIV infections based on the detained EIA in the VCT group.
- The current list of genotypic resistance mutations considered significant by the IAS-USA guidelines includes the reverse transcriptase mutation V118I. Although sometimes associated with decreased susceptibility to lamivudine, this mutation also exists as a relatively common polymorphism (present in ~3% of isolates from ARV treatment-naïve patients) and should probably not be included as evidence of transmitted resistance

Results if 118I mutation not included

21 (vs. 25) cases of resistance detected overall (16.5% vs. 19.7%)
Rates of resistance to specific antiretroviral classes were:

nRTI	3.9% (vs. 7.1%)
NNRTI	6.3% (no change)
PI	3.9% (vs. 3.1%)
MDR	2.4% (vs. 3.1%)

Conclusions/Discussion

- Rates of transmitted genotypic resistance in North Carolina were high (approaching 20%) and did not differ significantly among the three main classes of antiretroviral agents.
- In this study, clinical referral- and VCT-based systems for identification of acute HIV infection yielded similar estimates of the prevalence of transmitted resistance.
- The detection of these significant rates of resistance underscore an important role for HIV genotypic resistance testing in the evaluation of persons with acute and recent HIV infection.
- In North Carolina, the STAT program offers a unique system for acute HIV infection surveillance, facilitating the prospective monitoring of transmitted resistance.
- Generalizing our results to the full population of persons with new HIV infection in North Carolina is limited because of the characteristics of the tested populations
 - Samples tested from the STAT program are from persons who volunteer for testing at publicly funded testing sites (e.g. persons evaluated in STD clinics, persons self-identifying as at risk for HIV infection, prenatal/gynecologic patients, persons seeking family planning services, etc).
 - Clinical diagnoses of symptomatic acute HIV infection are infrequent (Weintrob), thus the clinical cohort is also likely biased towards persons with "traditional" risk factors.
- Patients with long term infection whose HIV antibody titers are relatively low can be misclassified as a "recent" infection by the detained EIA approach. Patients tested in the STAT program with a prior positive HIV test are excluded from further analysis.
- Nonetheless, it is possible some persons with chronic HIV infection in our study may have been incorrectly categorized as having recent HIV infection.
- In an attempt to avoid misinterpreting genotypic resistance related to ongoing antiretroviral therapy from transmitted resistance, we plan additional assessments of all cases of resistance detected in the recently HIV infected persons. Residual blood remaining from the original tested sample will be screened for the presence of antiretroviral agents in a future planned analysis.
- It is important to develop a consensus regarding the classification of mutations as significant for purposes of monitoring transmitted resistance. Ideally such consensus should be based on responses to treatment of persons with identified mutations

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