Characterization of Acute HIV-1 Infection in High-Risk Nigerian Populations

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Background. Acute phase of human immunodeficiency virus (HIV) infection (AHI) may account for a significant proportion of HIV-1 transmission. We identified and characterized individuals in Nigeria with AHI.

Methods. Individuals were tested using a combination of rapid HIV testing in mobile units and laboratory-based specimen pooling for nucleic acid amplification testing. Genome sequences were characterized. A linear segmented regression model was fit to serial viral load (VL) measurements to characterize early VL profiles.

Results. Sixteen AHIs were identified from 28,655 persons screened. Specimens were genotyped: 7 (43.8%) were CRF02_AG, 6 (37.5%) were subtype G, 1 (6.3%) was CRF06_cpx, and 2 (12.5%) were unique recombinant forms. No antiretroviral resistance mutations were detected. The mean duration of high VL burden from peak to nadir was 76 days (95% confidence interval [CI], 58–93 days), and the mean rate of viremic control was $-0.66 \log_{10} \text{VL per month}$. The mean VL at set-point was $4.5 \log_{10} \text{copies/mL}$ (95% CI, 3.9–5.1 $\log_{10} \text{copies/mL}$).

Conclusions. This study is the first to characterize AHI among Nigerians identified as HIV infected before seroconversion who would be otherwise missed by conventional HIV testing. Infections by HIV subtypes in Nigeria exhibit long periods of high viral burden, which can contribute to increased transmissibility.
**METHODS**

**Nigerian Acute HIV Infection Cohort**

The Recruiting Acute Cases of HIV (REACH) study was conducted between May 2003 and March 2010 in Abuja and Jos, Nigeria. The study population included adults aged ≥18 years who volunteered for HIV counseling and testing and provided informed consent for study participation. Institutional review boards at the University of Maryland, Baltimore, the US Centers for Disease Control and Prevention, and the Federal Ministry of Health’s National Institute for Pharmaceutical Research and Development approved the study protocol.

The study was conducted in 2 phases. In the first phase, screening for AHI was integrated at healthcare facilities and on mobile testing units targeting individuals at high risk for HIV infection, including antenatal clinic attendees, negative partners in serodiscordant couples whose HIV-infected partners were receiving care at the hospital, brothel-based sex workers, nonbrothel-based sex workers, and motorcycle taxi drivers. Individuals who tested HIV antibody positive were referred for staging for ART. Individuals with evidence of HIV-1 RNA with no antibody to the HIV virus at first screening and individuals who tested HIV antibody and RNA negative at first screening but had a positive HIV antibody test during subsequent testing were enrolled in the second phase of the study for longitudinal follow-up. This phase included clinical examination and collection of 10 mL of plasma, 40 mL of serum, and oral fluid at 7–10 days, 3 weeks, 5 weeks, 7 weeks, 9 weeks, and then 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 months after the screening with documented HIV antibody–negative and HIV RNA–positive status.

**Testing Algorithms for Screening and Detection of Acute HIV Infection**

Serum samples that were reactive by the initial rapid HIV test (Determine HIV, Abbott) were tested using a second rapid HIV test (Unigold HIV, Trinity) possessing a different antigen source from that of the first rapid test. Samples that were repeatedly reactive by both rapid assays were considered confirmed positive for HIV antibodies. Samples that exhibited discordant results by the 2 rapid tests underwent a tie-breaker third rapid HIV test (Stat-Pak HIV, Chembio) for the purpose of same-day, posttest counseling. After collection and rapid testing in the field, samples were processed within 6 hours of collection time to maintain the ranges required for RNA testing.

Plasma samples that were HIV antibody negative or discordant based on 2 rapid antibody tests underwent the multistage, pooled RNA polymerase chain reaction (PCR) to screen for the presence of HIV RNA. A 3-stage 25:1, 5:1, and 1:1 pooling strategy, adapted from techniques used by Quinn et al [10] and Pilcher et al [11] was used, and samples were tested by Roche Amplicor version 1.5 (Roche Diagnostics); an absolute lower limit of detection of 400 copies/mL permits detection of individual specimens with ≥10 000 copies/mL in a master pool using a 1:25 dilution. Single PCR was performed on all HIV RNA–positive pool specimens and on positive plasma collected at follow-up visits. To confirm seroconversion, HIV rapid tests and Western blots (Genetic System HIV-1 Western blot, Bio-Rad Laboratories) were performed on longitudinal serum collected from individuals enrolled in the second phase of the study.

Multiple measures were taken to ensure and maintain quality of the pooled NAAT. Positive and negative controls were incorporated in each run using known standards in both pooled and single samples. Additionally, 2 known external control plasma samples were run biweekly to verify the continued effectiveness of the procedure. These 2 quality-control samples consisted of a negative master pool and a positive pool. The positive pool contained 24 negative samples and 1 sample containing 20 000–100 000 HIV RNA copies/mL.

**HIV-1 Sequencing**

Proviral HIV sequencing was used to characterize the virus in the individuals with AHI. Peripheral blood mononuclear cells (PBMCs) were purified from the rest of blood components by the Ficoll Hypaque procedure, and the PBMCs were used for DNA extraction. High-molecular-weight DNA was extracted from at least $2 \times 10^8$ PBMCs following the procedures of the QIAamp Nucleic Acid Extraction Assay (Qiagen). Extracted DNA was stored in elution buffer at $-20^\circ C$ until use. The proviral DNA was subjected to nearly full-length genome sequencing using HIV-specific primers. PCR amplification of one 9-kilobase segment of the genome (including the whole provirus except for the 5′ region of the long term repeat) can be done routinely from PBMC DNA using a nested strategy. Limiting template dilution into the first round was performed to allow for direct sequencing of the second-round PCR product. The virtually full-length genome was amplified using MSF12b (5′- AAATCTCTTAGCAGTGCCGCCCAGACAG) and OFMR1 (5′–TGAGGGA TCTCTAGTTACACAGTC) followed by F2nst (5′- GCGGAGCCTAGAAAAGGAGAGATGG) and ofm19 (5′- GCAC TCAAGGCCAGTTATTTGAGGCTTA). PCR was performed as described [12, 13], using the Expand Long Template kit (Boehringer-Mannheim) and a hot-start method with a melting wax barrier (Dynaxaw). Multiple second-round PCR amplifications were combined to provide a sufficient template for sequencing.

PCR amplification products were fully sequenced on both strands using fluorescent dye terminators and an Applied BioSystems Model 3100 DNA sequencer. DNA sequences were assembled using Sequencher software (GeneCodes).
Phylogenetic Analysis
A multiple alignment of the Nigerian AHI sequences, 6 samples (01NG.PLs) collected in 2001 from seroprevalent individuals in Jos, Nigeria [14], and selected HIV-1 reference sequences was constructed using MacGDE 1.9.5 [15]. Reference isolates from the different subtypes and circulating unique recombinant forms (URFs) were used to classify the sequences. Phylogenetic trees were constructed using the neighbor-joining method, and the consistency of branching order was evaluated using the parsimony bootstrap by MEGA3 software [16]. Hypermutated sequences were identified using Hypermut 2.0 software from the National HIV Sequence Database (http://www.hiv.lanl.gov/) and were deleted from appropriate analyses.

Recombinant analysis was done with boot scanning [17] and distance scanning [12] using SimPlot software version 3.5 [18]. The nucleotide positions of recombinant breakpoints were designated relative to HXB-2 (GenBank accession no. K03455). The significance of the breakpoint assignment was assessed by the bootstrap value of the relevant node in the phylogenetic tree, which was 95% for significance.

To investigate the antiretroviral (ARV) drug-resistance patterns of the strains, the pol sequences were analyzed for resistance mutations using the Stanford HIV Drug Resistance Database.

Data Analyses
Among AHI individuals with documented HIV seroconversion, the estimated dates of seroconversion were calculated by assuming that the date of seroconversion was the midpoint between the last negative and first positive HIV antibody test results. For the majority of the AHI individuals, time to seroconversion was determined from the first visit in which HIV RNA was positive but HIV antibody was negative.

In the analysis of longitudinal plasma VL, a linear segmented regression of time since infection, originally used by Gange et al [19] to model longitudinal CD4+ T-lymphocyte counts and adapted by Blattner et al [2] to model HIV-1 RNA during AHI, was fitted for each individual to obtain estimates of time of nadir approaching VL set-point and rates of change pre- and postnadir time point. In this equation, $Y_{ij} = \beta_0 + \beta_1 (t_{ij} - T_{nadir_i})^{\text{Pre}} + \beta_2 (t_{ij} - T_{nadir_i})^{\text{Post}} + \epsilon_{ij},$ $Y_{ij}$ represented log10 VL of the $i$th individual collected at time $t_{ij}$, $T_{nadir_i}$ denoted the eligible time of nadir between 2 consecutive VLs for the $i$th individual, $(t_{ij} - T_{nadir_i})^{\text{Pre}}$ equals 0 if $t_{ij} < T_{nadir_i}$, $(t_{ij} - T_{nadir_i})^{\text{Post}}$ equals $t_{ij} - T_{nadir_i}$ if $t_{ij} > T_{nadir_i}$, and $\epsilon_{ij}$ represented a normal error term with mean 0 and $\sigma_i$. The $T_{nadir_i}$ that resulted in the smallest residual variability between the data and the fitted line was chosen as the VL nadir for the $i$th individual.

Distributions of variables were tested for normality using the Kolmogorov-Smirnov test. Fisher exact test and 2-sample $t$ test were used to test for differences in the proportion and mean values, respectively.

RESULTS
Study Population
A total of 28 655 individuals were tested (Figure 1), and 3837 (13.4%) were confirmed seropositive. Of 24 184 individuals who were negative for HIV antibodies at the time of first HIV

Figure 1. The Recruiting Acute Cases of HIV (REACH) study. The first phase of the study (white boxes) screened for individuals with evidence of human immunodeficiency virus type 1 (HIV-1) RNA with no HIV antibody. The second phase of the study (shaded boxes) followed individuals for seroconversion. Abbreviation: AHI, acute HIV infection.
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<th>Western Blot Results</th>
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Abbreviations: AHI, acute HIV infection; ANC, antenatal clinic attendees; CSW, commercial sex workers; DC, serodiscordant couples; HIV, human immunodeficiency virus; N, negative; NA, not applicable; OCT, outreach community testing; P, positive; PCR, polymerase chain reaction; SC, seroconversion; STI, sexually transmitted infection; VCT, voluntary counseling and testing.

For interval seroconverter, the last seronegative and RNA PCR negative was used.

Time to seroconversion defined as [(Date of first HIV positive antibody – Date of last HIV negative antibody) / 2] – Date at HIV RNA positive but HIV antibody negative.
testing, 9 were HIV RNA positive and 3 individuals were HIV uninfected at first screening but became HIV antibody positive during subsequent testing of 1299 individuals. Of 634 individuals who were serodiscordant by 2 rapid tests, 4 were HIV RNA positive.

Sixteen individuals with documented HIV seroconversion were followed. Of these 16, 12 individuals were negative on the first rapid HIV test; 4 individuals had positive results on the first rapid HIV test but subsequently had negative results on the second and third rapid HIV tests (Table 1). The subjects’ mean age was 29 years (range, 18–40 years), and 7 were males. Six (37.5%) were married, 2 reported condom use at all times, and 4 women were pregnant. For all subjects, this was their first HIV testing. Nine subjects reported having symptoms of a sexually transmitted infection (STI).

**Subtype Distribution Based on Full-length Genome Analysis**

HIV-1 subtype based on nearly full-length genomes was determined for 14 of the 16 AHI individuals; the remaining 2 were subtyped based on partial genomes. Shown on the phylogenetic tree are the 14 full-length genomes of AHI individuals identified in this study (Figure 2). In addition, there were 2 proviral samples for which only partial genome sequences (pol and env) were available: 1 (SC19) was CRF06_cpx in both genomic regions and the other (SC16) was a recombinant between CRF02_AG (in pol) and subtype G (in env). When the partial results are added to the full genome results, the distribution of the subtypes is CRF02_AG, 43.8% (n = 7); subtype G, 37.5% (n = 6); CRF06_cpx, 6.3% (n = 1); and unique recombinant forms, 12.5% (n = 2). Both the AHI individuals and 6 samples (01NG.PLs) collected from seroprevalent individuals in 2001 showed domination by CRF02_AG and subtype G.

The isolate from subject SC27 contained a very interesting URF. The parental strains of this recombinant were subtype A1 (clustering with the East African A’s), CRF02_AG, and CRF25_cpx. CRF25_cpx is itself a recombinant between subtype A, subtype G, and some part of the genome that we were unable to classify. The isolate from subject SC16 could be sequenced only partially. In pol it was CRF02_AG, whereas in env it was subtype G. From those results it could be either a dual infection or a recombinant between the 2 dominant
genetic forms in the epidemic. To determine which was the case, the quasispecies of the env were sequenced, and all viruses were subtype G. This suggested that SC16 was a recombinant rather than a dual infection.

None of the 16 AHI individuals who were genotyped exhibited any of the reported resistance mutations (data not shown).

**Plasma HIV-1 RNA During the First 18 Months**

Heterogeneity in plasma VL patterns was evident among AHI individuals. Median HIV RNA at the time of first testing was 534,430 copies/mL (mean log_{10} VL, 5.7; range, 4.2–6.4). A mean peak HIV RNA at 5.5 log_{10} copies/mL (95% confidence interval [CI], 4.7–6.3) was observed with a well-defined decrease to 3.5 log_{10} copies/mL over an average of 76 days from the time of first testing. The rate of viremic control to nadir was 0.66 log_{10} per month (95% CI, .27–1.05), whereas the rate of increase postnadir was 0.12 log_{10} per month (95% CI, .004–.23). Figure 3 illustrates longitudinal plasma HIV RNA for all 13 individuals identified as HIV infected before seroconversion.

The rate of initial clearance to nadir did not differ between subtype G and CRF02_AG (0.84 log_{10} per month vs 0.57 log_{10} per month, respectively; P = .63); however, the time to nadir was longer for infection with CRF02_AG (53 days vs 92 days; P = .04). No differences in the rate of change postnadir (0.07 log_{10} per month vs 0.21 log_{10} per month; P = .23) were observed between the subtype G and CRF02_AG, respectively. For 2 subjects, SC16 and SC27 (Figure 3D, 3H), who were infected with URFs, the viral RNA set-point was achieved at 77 and 68 days, respectively, and maintained at 4.7 log_{10} copies/mL and 5.6 log_{10} copies/mL, respectively, until the end of follow-up.

We did not observe any differences between women and men in peak VL (5.9 vs 5.5 log_{10} copies/mL; P = .50), time to nadir (86 vs 72 days; P = .42), rate of viremic control (0.53 vs 0.41 log_{10} per month; P = .16), or VL level at set-point (4.2 vs 4.6 log_{10} copies/mL; P = .32). There were also no differences in these VL parameters between risk groups (data not shown).

**DISCUSSION**

To our knowledge, this is the first study describing acute HIV-1 infection in Nigeria where individuals were identified in the preseroconversion window period using a combination of HIV rapid tests and pooled NAAT. The AHI subjects were mostly women, 4 of whom acquired the infection during pregnancy. Most of the AHI subjects were infected with either CRF02_AG or subtype G viruses, and the majority exhibited very high HIV RNA plasma levels during the window period.

CRF02_AG and subtype G viruses continue to represent the majority of circulating viruses in Nigeria. Our findings suggest that the relative frequencies of CRF02_AG and subtype G in current circulation are similar to the relative frequencies in prevalent cases, both in high- and low-risk groups [20, 21]. Coupled with our previous data on HIV subtypes transmitted during mother-to-child transmission,
both subtypes appear to have similar transmission efficiencies, and there are no data so far that point to 1 genetic form overtaking the other. With 1 of the URF isolates, it is possible that this individual was infected with multiple strains of HIV-1, spinning off multiple recombinants, of which only 1 was targeted for sequencing. With the other URF, quasispecies analysis suggested that only 1 form was present. The high VL set-point associated with these recombinants warrants further investigations with a larger number of cases.

Nigeria is the only country in which subtype G is commonly observed, and the phylogenetic analysis reveals that there are 2 major clusters of subtype G among the Nigerian isolates—a smaller cluster that contains all 3 of the reference subtype G strains and 1 of the strains from an AHI individual and a second cluster that contains 9 Nigerian samples (including 5 AHI individuals) and no reference strains and is a significantly distinct cluster with a bootstrap value of 100%. In our sampling frame, the second most common subtype among the AHIs was this distinctive subtype G. Further analyses on hundreds of partial genomes are being undertaken to characterize the frequency of this strain in Nigeria.

In using the same segmented regression approach we had previously published to characterize 22 individuals [22] with HIV-1 subtype B infection [23] in the Trinidad Acute HIV Infection Study, we also compared the VL profiles of the Nigerian AHIs to HIV-1 subtype B AHIs and found that AHI subjects in our study population appeared to have a longer period of high VL characterized by a longer time to VL nadir (mean of 76 days vs 54 days) and less robust control of VL from viremia (mean rate of initial clearance of 0.66 vs 1.19 log10 per month). Elevated VL levels have been reported for

![Figure 3. Continued.](https://academic.oup.com/jid/article-abstract/205/8/1239/876909)
subtype C and CRF01_AE compared with subtype B [24–27]. A longer period of high VL during AHI may increase an individual’s infectiousness. This has implications for the transmission dynamics of HIV and, if true for other subtypes, may make identification of and intervention during AHI even more critical. Although more in-depth investigations are needed, especially by accessing larger cohorts of AHIs, our preliminary finding has implications for the transmission dynamics of HIV and may make identification of and intervention during AHI even more critical. Early HIV case finding and prevention is an important aspect of disease control efforts that could reduce both morbidity and further disease transmission [1, 28].

The screening of high-risk individuals provides an important opportunity for promotion of primary prevention of disease, even among those found not to be infected. Our approach of using mobile medical services in Nigeria represents an innovative approach to case finding and disease control by bringing testing services and medical care services directly to those populations at increased risk for HIV and other STIs and their sexual partners. Currently, little is known about the effectiveness of mobile medical services in the control of HIV and other STIs in resource-limited settings; therefore, it is important to further evaluate the contribution of mobile medical services to disease control efforts by linkage to care and treatment of HIV-infected, high-risk individuals.

In summary, we confirm that AHI can be identified using standard of care rapid HIV tests with pooled NAAT in a limited-resource setting. Furthermore, we were able to demonstrate the utility of this approach within the context of mobile HIV counseling and testing for high-risk populations. Because our study was conducted under a research protocol, future studies should examine the costs and yield of implementing AHI screening more widely for high-risk populations. We also found that the distribution of subtypes affecting incident cases of HIV-1 in Nigeria was similar to the distribution of prevalent cases, providing evidence that, currently, neither of the 2 major genetic forms is overtaking the other. Identifying individuals with acute HIV infections who are otherwise missed by conventional HIV testing algorithms is critical for prevention programs and may be even more important when infections are caused by HIV subtypes with longer periods of high transmissibility.

**Genbank Accession Numbers**

The following are the accession numbers for the seroprevalent infection sequences: 01NG.674 (DQ-013278), 01NG.PL669 (DQ013280), 01NG.PL760 (DQ013283), 01NG.PL567 (DQ013274), 01NG.PL754 (DQ013282), 01NG.PL710 (DQ013281). The following are the accession numbers for the AHI sequences: SC_Full.sequin 06NG.SC11 JN248580, SC_Full.sequin 07NG.SC12 JN248581, SC_Full.sequin 08NG.SC13 JN248582, SC_Full.sequin 09NG.SC20 JN248583, SC_Full.sequin 09NG.SC21 JN248584, SC_Full.sequin 09NG.SC24 JN248585, SC_Full.sequin 09NG.SC26 JN248586, SC_Full.sequin 09NG.SC27 JN248587, SC_Full.sequin 09NG.SC28 JN248588, SC_Full.sequin 09NG.SC29 JN248589, SC_Full.sequin 09NG.SC30 JN248590, SC_Full.sequin 09NG.SC31 JN248591, SC_Full.sequin 09NG.SC61 JN248592, SC_Full.sequin 09NG.SC62 JN248593.

**Notes**

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