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Frequency of Long-Term Nonprogressors in HIV-1 Seroconverters From Rakai Uganda

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Abstract

Objective—Studies on long-term nonprogressors (LTNP) have been conducted in the USA and Europe. This study examined the frequency of LTNPs and HIV controllers among 637 HIV-1 seroconverters in rural Uganda.

Design and Methods—LTNPs were defined as being infected for more than 7 years with a CD4⁺ T-cell count above 600 cells per microliter, and HIV controllers as having undetectable viral loads on 3 separate occasions without antiretroviral treatment. HIV-1 viral load and subtype distribution between LTNP and non-LTNP populations were determined.

Results—Of the HIV seroconverters, 9.1% (58/637) were LTNPs and 1.4% (9/637) were HIV controllers. LTNPs had a significantly lower viral load at set point than non-LTNP participants ($P < 0.001$). The Kaplan–Meier joint probability of surviving to 7 years with a CD4 count >600 was 19.2%. Individuals who survived 7 years had a significantly higher frequency of HIV-1 subtype A ($P < 0.05$), but seroconverters infected with HIV-1A did not have a significantly higher probability of becoming an LTNP.

Conclusions—The frequency of LTNPs appears to be relatively high in Uganda and it may be important to take this into account when designing studies of viral pathogenesis and performing HIV vaccine trials in sub-Saharan Africa.

Keywords

Africa; HIV; HIV disease progression; long-term nonprogressors

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INTRODUCTION

In 2008, 33 million people worldwide were estimated to be infected with HIV type 1 (HIV-1) with approximately 66% of these individuals living in sub-Saharan Africa.¹ It is estimated that up to 70% of eligible infected adults in Africa do not have access to antiretroviral drug treatment (ART) and are at high risk of death from AIDS. However, a portion of HIV-infected individuals progress to AIDS slowly or not at all although they have actively replicating virus, and these individuals are classified as long-term nonprogressors (LTNP). Additionally, HIV-infected individuals who continually present with undetectable viral loads over a multiyear period are classified as HIV controllers.² The proportion of newly infected individuals who ultimately become LTNPs is estimated from cohorts in the USA and Europe to be approximately 5–8%.^{3–5} If this proportion is applicable to infected populations elsewhere, then over 1 million LTNPs may be living in sub-Saharan Africa alone. LTNPs and HIV controllers may provide insights into the biological mechanisms of effective HIV control, which is essential for designing appropriate therapies and vaccines.² However, little is known about adult LTNPs in Africa, and whether they differ from their counterparts in the developed world.

Theories regarding the biology of LTNP include infection with attenuated viruses, mutations in the host genes that code for the viral coreceptors, host allelic differences in promoter regions of the ligand specific for the viral coreceptors, and the presence of specific human leukocyte antigen class 1 alleles.^{2,6–11} An effective HIV-specific CD4 and CD8 cytotoxic T-cell responses have also been associated with control of virus replication early in infection.^{2,12,13} Even with these multiple factors associated with LTNPs, there are many patients who lack these known protective factors, and it has been suggested that LTNP and HIV-positive controllers should be separated into groups according to their mechanism of viral control.¹⁴

Studies from Africa have shown that HIV-1 subtypes can differ significantly in their rates of progression to AIDS and death, and it is possible that rates of LTNPs and HIV controllers may also be associated with HIV-1 subtype.^{15–17} Data from participants in the Rakai Community Cohort in Uganda from 1994 to 2000 were used to determine the frequency of LTNPs and HIV controllers among HIV-1 seroconverters.

METHODS

Study Population

The Rakai cohort is an open cohort of 15-to 49-year-old residents in 50 communities in the rural Rakai district of southwestern Uganda. This cohort has been described in detail previously.¹⁸ Briefly, 10,000–14,000 people are annually surveyed with a sociodemographic and behavioral interviews, and each participant is asked to provide a venous blood sample for HIV testing. All participants have a unique identifier that allows them to be followed longitudinally. Free treatment for sexually transmitted infections and general medical conditions, and free condoms and health education were provided. Voluntary HIV counseling and testing for individuals and for couples were promoted and provided at no cost. Participants were encouraged to share their HIV results with their partners. The cohort was approved by institutional review boards in Uganda (the Uganda Virus Research Institute's Science and Ethics Committee and the Uganda National Council for Science and Technology) and from the institution review boards of collaborating US institutions (Walter Reed Army Institute of Research, Columbia University, and Johns Hopkins University).

Seroconverters were participants with a negative HIV test followed by a positive result in a subsequent year, as described in Lutalo et al.¹⁸ The time of infection was calculated as the midpoint between these 2 visits. A total of 837 seroconverters were identified between 1994 and 2004, of whom 637 (76.1%) seroconverted before 2000 allowing for 7+ years of follow-

up for identification of LTNP in this study.¹⁸ Data were available on deaths, clinical diagnosis of AIDS, migration, loss to follow-up, and CD4⁺ T-cell (CD4) counts from 2004 to 2007. LTNP were classified as antiretroviral naive HIV-infected individuals who had CD4 counts >600 at 7 years or more after their first HIV-positive test, and no CD4 count <600 before that time point.⁴ The CD4 counts used for this classification were not available for this population before 2004 and were performed semiannually after that point. Seroconverters who were found to have an undetectable viral load at any time point tested were further analyzed by testing all available time points for viral load. Those participants who had at least 3 undetectable viral loads (<400 copies/mL) over a multiyear period with at least one of these being an undetectable by an ultrasensitive viral load reading (<50 copies/mL) and no detectable levels in any other time points were classified as HIV controllers.² Viral set point was determined to be the viral load level at the earliest annual sample postseroconversion that was available. Subjects were also classified as those who died, progressed to AIDS, those who had not developed AIDS, and those who had migrated out of the area or were lost to follow-up.

Laboratory Testing

HIV serostatus was determined by two enzyme-linked immunosorbent assays (Organon Teknika, Charlotte, NC; Cambridge Biotech, Worcester, MA). Discordant enzyme-linked immunosorbent assay results and all HIV seroconversions were confirmed by Western blot (BioMerieux Vitek, St. Louis, MO). The HIV-1 serum viral load was tested using the standard or ultrasensitive Roche Amplicor 1.5 assay (Roche Diagnostics Corporation, Indianapolis, IN). CD4 counts were assessed by fluorescent-activated cell sorter-based assay (Becton Dickinson, Inc., San Diego, CA). HIV-1 subtype was determined by multiple region hybridization assay or direct sequence analysis of p24 and gp41 gene fragments.¹⁹

Statistical Analysis

Comparisons were done using the Mann–Whitney rank sum test for continuous variables, and χ^2 test for discrete ones. Kaplan–Meier (KM) survival analysis was used to estimate the probability of surviving to 7 years after seroconversion, and the probability of having a CD4 count of >600 per microliter after surviving up to 7 years. These joint probabilities of survival >7 years and CD4 >600 were then used to estimate the cumulative probability of becoming an LTNP in this population.

RESULTS

Of the 637 individuals who seroconverted between 1994 and 2000, 58 (9.1%) were found to be LTNP. LTNP had a significantly lower viral load at set point than non-LTNP seroconverters ($P < 0.001$) (Table 1). However, there were no significant differences in the distribution of subtypes ($P = 0.66$), age, or gender between the LTNP and non-LTNP seroconverters (Table 1). Of the 637 seroconverters, 9 subjects were identified as HIV controllers (1.4%), of whom 5 were also LTNP. The other 4 HIV controllers were lost to follow-up, so their survival beyond 7 years could not be determined. The remaining seroconverters were classified as HIV-positive progressors with CD4 counts <600 [8.6% (55/637)], individuals who progressed to AIDS or death [44.1% (281/637)], or were lost to follow-up [37.5% (239/637)] so their status could not be defined.

There were no significant differences in the distribution of subtypes between seroconverters followed for 7 years and those lost to follow-up ($P = 0.80$) (Table 2). However, those lost to follow-up were more likely to be women (63.2% vs 52.3%, $P < 0.05$), younger (24.9 vs 28.1, $P < 0.05$), and had a lower median viral load set point (4.35 vs 4.53 log cps/mL, $P < 0.05$, respectively) compared with those who were followed up for 7 years (Table 2).

To account for individuals lost to follow-up, a KM analysis was performed to estimate the cumulative probability of becoming an LTNP in this population (Fig. 1).²⁰ Using this analysis, it was determined that the probability of seroconverters surviving to 7 years after HIV infection was 62%, and of these subjects 31% (58/186) had CD4 counts >600. Therefore, the joint probability of surviving 7 years and having a CD4 count >600 is 19.2% (ie, 0.62×0.31).

Individuals who survived for >7 years had a significantly higher frequency of subtype A infection [20.0% (24/120)] than those who died in <7 years [7.7% (7/90)] ($P < 0.05$). However, seroconverters infected with HIV-1A had only a slightly higher frequency of becoming LTNP [11.8% (6/51)] vs non-A-infected seroconverters [9.8% (29/297)] ($P = 0.85$). Additionally, of the those individuals who survived up to 7 years, persons infected with HIV-1A had a similar frequency of being an LTNP [31.6% (6/19)] compared with non-A-infected survivors [31.5% (23/73)] ($P = 1.0$).

DISCUSSION

This study is the first to provide estimates of the prevalence and cumulative probability of becoming an LTNP in an African setting. The LTNP selection parameters used in this study were first described in the USA and Europe and were utilized to provide comparability between studies.⁴ However, it has been shown that healthy HIV-negative Africans have lower CD4 levels compared with US patients, suggesting that these criteria may not be generalizable to the African context.²¹ The LTNP did have significantly lower viral load set points, which almost certainly plays a role in disease progression.

The seemingly high proportion of individuals lost to follow-up in this analysis is due to the relatively long timeframe (7+ years) needed to reach the study endpoint of LTNP, and the annual retention rate was 95.3%. The differences in age and gender between the individuals lost to follow-up and those with defined outcomes in part reflects the fact that younger people in this population are more likely to move out of the area, and that women who become ill have a higher likelihood of migrating out of the region to stay with a relative in a different area of Uganda. It is possible that these differences are biasing the rates of LTNPs in this population; however, this bias should be minimal.

The estimates that 9.1% of all seroconverters were LTNPs and 1.4% were HIV controllers is similar to the rates reported in western cohorts.²² However, this is likely to be an underestimate due to censoring by loss to follow-up over 7 years of observation. This is supported by the lower median viral load set point in the lost to follow-up group, who are more likely to be selectively healthier and more mobile. Also, individuals with higher viral loads are more likely to progress to disease quickly and therefore have a higher likelihood of having a defined outcome. Thus, the joint probabilities of survival up to 7 years and having a CD4 count of >600 might provide a better estimate of LTNP, which was found to be 19.2%. This is substantially higher than the estimated proportions of LTNP reported elsewhere,^{4,5,23} despite the fact that disease progression rates were found to be high in this Ugandan population.¹⁵ It should be noted that the KM methods were used previously to determine survival probabilities in LTNP populations but not to estimate the probability of becoming an LTNP.²⁰ Therefore, it is difficult to directly compare this estimate of probability to previous studies. Also, many previous studies examined seroprevalent populations, which cannot account for lead time from infection to enrollment.

The relatively high probability of becoming an LTNP in this population could be influenced by the prevalence of HIV-1A-infected individuals, who have been shown to have slower disease progression than patients with HIV-1D.¹⁵ However, the rates of LTNP in the HIV-1A-infected seroconverters were not significantly higher than the non-A-infected individuals. It

should be noted that only 60.3% of the LTNP identified in this population were subtyped because LTNPs are more likely to have low viral loads that are less likely to amplify for sequencing. Of those individuals who survived 7 years, those infected with HIV-1A had an almost identical frequency of being an LTNP compared with non-A-infected survivors. These data support the theory that host-genetic and immunological factors may play an important role in LTNP development, and the differences seen in disease progression between HIV-1A and HIV-1D may be independent of these factors,^{8-10,15,24} although it is possible that these host and viral subtype factors have a synergistic effect in the establishment of long-term nonprogression.

In conclusion, when analyzed by survival methods, which account for censoring, LTNP appears to be relatively frequent in this African population, and this finding may influence the design of future studies examining LTNPs, HIV controllers, and vaccine trials in sub-Saharan Africa.

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FIGURE 1.

KM analysis of survival of HIV-1 seroconverters. The probability of survival of HIV-1 seroconverters is shown with the number of surviving individuals indicated below according to year postseroconversion. The probability of survival was calculated as survivors divided by the total number of individuals with known outcomes at that year postseroconversion (survivors and deaths).

TABLE 1

Characteristics of HIV Seroconverters Who Became LTNP Compared With Non-LTNP Seroconverters

	LTNP (n = 58)	Non-LTNP (n = 579)
Median age (IQR)	25.0 (22.1–30.6)	27.2 (22.6–34.3)
Sex (% female)	53.4	56.2
Viral load log ₁₀ (IQR)	3.56 (2.89–4.07) *	4.58 (3.99–5.16) *
HIV-1 subtype		
A	17.1% (6/35)	14.4% (45/313)
D	65.7% (23/35)	62.0% (194/313)
Recombinants	17.1% (6/35)	23.6% (74/313)

IQR, interquartile ratio.

* Significant difference in viral load between the 2 groups ($P < 0.05$).

TABLE 2

Characteristics of HIV Seroconverters Lost to Follow-Up and With Known Outcomes

	Lost (n = 239)	Known Outcome (n = 398)
Median age (IQR)	24.9 (21.4–33.3) *	28.1 (23.7–34.3) *
Sex (% female)	63.2 *	52.3 *
Viral load log ₁₀ (IQR)	4.35 (3.45–4.91) *	4.53 (3.89–5.17) *
HIV-1 subtype		
A	16.1% (14/87)	14.2% (37/261)
D	63.2% (55/87)	62.1% (162/261)
Recombinants	20.7% (18/87)	23.8% (62/261)

IQR, interquartile ratio.

* Significant difference between the 2 groups is shown ($P < 0.05$).