

ORIGINAL ARTICLE

Prospective Study of Acute HIV-1 Infection in Adults in East Africa and Thailand

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ABSTRACT

BACKGROUND

Acute human immunodeficiency virus type 1 (HIV-1) infection is a major contributor to transmission of HIV-1. An understanding of acute HIV-1 infection may be important in the development of treatment strategies to eradicate HIV-1 or achieve a functional cure.

METHODS

We performed twice-weekly qualitative plasma HIV-1 RNA nucleic acid testing in 2276 volunteers who were at high risk for HIV-1 infection. For participants in whom acute HIV-1 infection was detected, clinical observations, quantitative measurements of plasma HIV-1 RNA levels (to assess viremia) and HIV antibodies, and results of immunophenotyping of lymphocytes were obtained twice weekly.

RESULTS

Fifty of 112 volunteers with acute HIV-1 infection had two or more blood samples collected before HIV-1 antibodies were detected. The median peak viremia (6.7 log₁₀ copies per milliliter) occurred 13 days after the first sample showed reactivity on nucleic acid testing. Reactivity on an enzyme immunoassay occurred at a median of 14 days. The nadir of viremia (4.3 log₁₀ copies per milliliter) occurred at a median of 31 days and was nearly equivalent to the viral-load set point, the steady-state viremia that persists durably after resolution of acute viremia (median plasma HIV-1 RNA level, 4.4 log₁₀ copies per milliliter). The peak viremia and downslope were correlated with the viral-load set point. Clinical manifestations of acute HIV-1 infection were most common just before and at the time of peak viremia. A median of one symptom of acute HIV-1 infection was recorded at a median of two study visits, and a median of one sign of acute HIV-1 infection was recorded at a median of three visits.

CONCLUSIONS

The viral-load set point occurred at a median of 31 days after the first detection of plasma viremia and correlated with peak viremia. Few symptoms and signs were observed during acute HIV-1 infection, and they were most common before peak viremia. (Funded by the Department of Defense and the National Institute of Allergy and Infectious Diseases.)

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EVENTS DURING ACUTE HUMAN IMMUNODEFICIENCY TYPE 1 (HIV-1) infection may modulate the long-term course of HIV-1 disease.¹⁻⁴ Acute and early HIV-1 infection is a major contributor to the epidemic spread of HIV-1,⁵⁻⁷ and limiting this spread through “test and treat” strategies may require treatment of persons during the acute phase of infection.⁸⁻¹⁰ The HIV-1 reservoir, which confounds efforts to cure infection,¹¹ may be more responsive to antiviral therapy during acute HIV-1 infection than during chronic infection.¹²⁻¹⁴ Intervention during this stage of infection could dramatically reduce epidemic spread,¹⁵ reduce the size of the HIV-1 reservoir, and potentially achieve long-term control of plasma viremia without the use of long-term antiviral treatment.¹⁶

Studies of the clinical presentation and kinetics of viremia in persons with acute HIV-1 infection and of the role of these factors in predicting long-term outcomes show conflicting results. Initial descriptions of acute HIV-1 infection were based on cohorts of persons who were identified on the basis of symptoms that were often characterized as those of seronegative mononucleosis.^{1,17-21} The use of pooled nucleic acid testing has permitted broader identification of acute HIV-1 infection, and classification systems for the staging of acute HIV-1 infection have been developed on the basis of the sequential reactivity of nucleic acid testing, the presence of the p24 antigen in plasma, and results of antibody testing.^{22,23}

We performed a study involving volunteers who were at high risk for HIV-1 infection. Plasma nucleic acid testing was performed twice weekly, and a systematic analysis of the clinical, virologic, and immunologic characteristics of the earliest stage of HIV-1 infection was conducted.

METHODS

STUDY DESIGN AND POPULATION

RV 217 is a prospective natural-history study conducted at the Makerere University Walter Reed Project, Kampala, Uganda; the Walter Reed Project, Kericho, Kenya; the Mbeya Medical Research Centre, Mbeya, Tanzania; and the Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. The protocol (available with the full text of this article at NEJM.org) was approved by the local ethics review boards and the Walter Reed Army Institute of Research. Written informed consent was obtained from all participants.

Participants were recruited from bars, clubs, and other locations associated with transactional sex. Men and women, 18 to 50 years of age, who were at high risk for HIV-1 infection were identified with the use of an audio computer-assisted self-interview. To be eligible for study entry, participants had to meet at least one of the following four criteria within the previous 3 months: had exchanged goods for sex, had unprotected sex with a known HIV-positive partner, had unprotected sex with three or more partners, and had symptoms of a sexually transmitted infection. In the first part of the study, which involved surveillance of participants who were not infected, volunteers who had at least one of these high-risk criteria underwent small-volume blood collections by fingerstick measurement twice weekly and large-volume blood collections of 26 to 67 ml every 6 months. Small-volume blood samples were tested for HIV-1 RNA within 24 to 48 hours after collection.

Volunteers in whom tests for HIV-1 RNA were reactive entered the second part of the surveillance phase, during which large-volume blood samples were obtained and a structured medical evaluation was performed twice weekly for 4 weeks. Volunteers with confirmed HIV-1 infection were enrolled in the long-term follow-up phase. Full details of the study design and statistical analysis plan are provided in the protocol.

MEDICAL MANAGEMENT

Volunteers with HIV-1 infection were referred to a local care provider for treatment, including antiretroviral therapy. Counseling regarding HIV risk reduction was provided every 3 months and informally during small-volume blood collections. Condoms and lubricants were provided to participants at the study sites. The study team encouraged care providers to initiate treatment promptly if the volunteers had clinically significant symptoms of acute retroviral syndrome, were pregnant, or met national guidelines for the initiation of antiretroviral therapy.

NUCLEIC ACID TESTING

Approximately 600 μ l of whole blood measured with a fingerstick device was collected into a BD Microtainer (Becton Dickinson) containing EDTA. Whole blood was centrifuged at 9000 \times g for 3 minutes and plasma was separated into aliquots for same-day or next-day testing. Plasma was diluted (in a 1:5 ratio) in phosphate-buffered

saline (pH, 7.0 to 7.5) and tested for HIV-1 RNA with the use of the Aptima HIV-1 RNA Qualitative Assay (Hologic).

HIV SEROLOGIC TESTING, MEASUREMENT OF VIRAL RNA, AND FLOW CYTOMETRY

HIV serologic testing with the use of standard diagnostic methods was performed at screening, every 6 months, and during the second part of the surveillance phase (see the Supplementary Appendix, available at NEJM.org). Plasma HIV-1 RNA levels were measured in batches with the use of the RealTime HIV-1 Assay (m2000 RealTime System, Abbott Molecular). EDTA-anticoagulated samples of whole blood were analyzed with the use of the BD Multitest on an FACSCalibur flow cytometer (Becton Dickinson). HIV-1 subtyping was performed as described previously (see the Supplementary Methods section in the Supplementary Appendix).

DATA ANALYSIS

Day 0 was defined as the day on which the first blood sample was reactive for HIV-1 RNA. Viral RNA levels below the lower limit of quantitation were imputed by dividing the limit of quantitation by two. The viral upslope was calculated from the date of the last negative sample to the peak viral load, excluding data from participants for whom the period between the last negative sample and the first sample that was positive for HIV-1 RNA was more than 10 days. The early nadir in the HIV-1 RNA viral load was defined as the lowest viral load after the peak viral load through day 42. Viral downslope was calculated from the peak viral load to the early nadir viral load. The viral-load set point was defined as the average viral load of all samples collected before antiretroviral therapy was administered between days 42 and 365 among participants in whom at least two viral-load values were measured during this period.

Results of physical examinations and reported clinical symptoms are described according to the study visit and per patient. Data on participants were censored at the initiation of antiretroviral therapy.

STATISTICAL ANALYSIS

We performed an exploratory analysis of viral-load dynamics in acute HIV-1 infection without prespecified hypotheses. Correlations between

Figure 1 (facing page). Enrollment and Outcomes.

Single false reactive results on qualitative nucleic acid testing were common, but acute HIV infection was confirmed in all participants who had two consecutive plasma samples that were reactive for HIV-1 RNA on qualitative nucleic acid testing. Among 112 participants with acute infection, peak viremia could be accurately defined in 54 participants who had at least two samples in which testing for HIV-1 RNA was reactive and enzyme immunoassay was nonreactive. Four of these participants were excluded from virologic and immunologic analyses because they received early antiretroviral therapy (ART). Most infections were of the subtype HIV-1 CRF01_AE in Thailand and of the HIV-1 subtype A and recombinant form of the A, C, and D strains in East Africa. Fiebig stages range from I through VI, with higher stages indicating a more mature stage of antibody response to HIV. CI denotes confidence interval, EIA enzyme immunoassay, and NAT nucleic acid test.

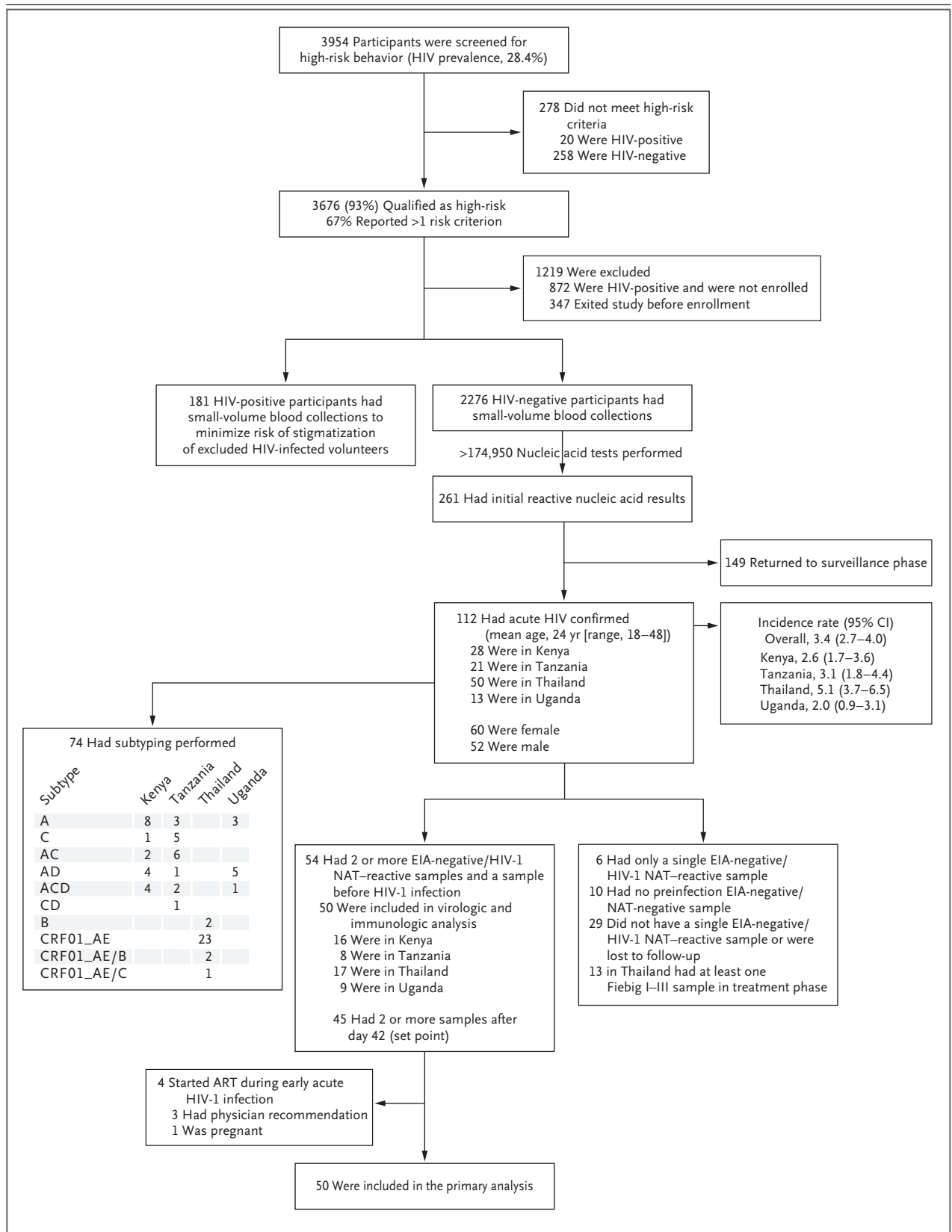
viral load and immune factors were assessed with the use of Spearman's rank-correlation coefficients (ρ). Regional differences in viral loads in East Africa and Thailand were evaluated with the use of Wilcoxon rank-sum tests. We used Wilcoxon signed-rank test to assess changes from baseline. Log-transformed viral RNA dynamics during the first year were assessed with the use of regression splines with participant-specific intercepts and slopes. Lymphocyte data were assessed with the use of repeated-measures models with adjustment for region and study visit. (Details of the statistical analysis are provided in the Supplementary Methods section in the Supplementary Appendix.)

Clinical signs (abnormal physical findings on examination) and symptoms were described primarily with comparisons between geographic regions for individual findings with the use of Fisher's exact test. All analyses were performed with the use of SAS software, version 9.3 (SAS Institute) and GraphPad Prism, version 6.0a (GraphPad Software).

RESULTS

STUDY PARTICIPANTS

From June 2009 through June 2015, a total of 3954 volunteers were screened (Fig. 1) and 2276 of 3676 high-risk participants (61.9%) with negative results on an enzyme immunoassay for HIV antibodies entered the surveillance phase.



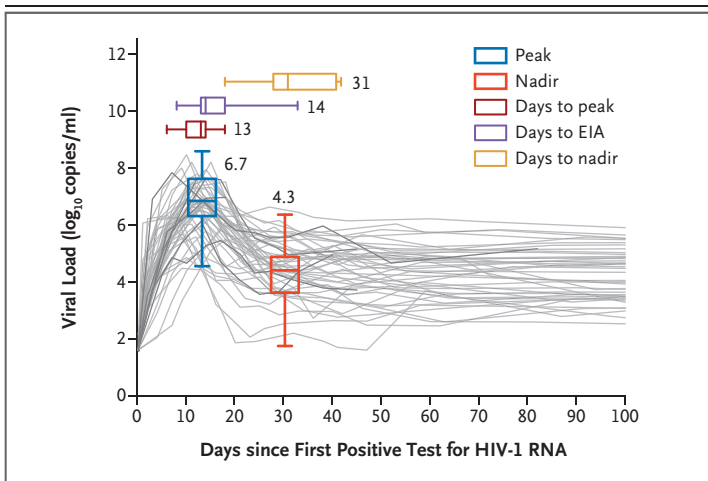


Figure 2. Viral Loads over the First 100 Days of HIV-1 Infection in 50 Participants.

Longitudinal viral-load values are plotted against the number of days since the first blood sample was reactive for HIV-1 RNA in 33 participants from East Africa and 17 from Thailand who had two or more blood samples that were nonreactive on enzyme immunoassay (EIA) and were reactive on nucleic acid testing. Day 0 is the day of the first positive nucleic acid test. The box-and-whisker plots show the median, interquartile range, and range for each variable. The vertical box plots show peak and nadir viral loads, and the horizontal box plots show the number of days from the first reactive result on nucleic acid testing to the peak viral load, to reactivity on the EIA, and to the nadir viral load. Median values are shown for each variable.

The majority of participants reported receiving goods for sex (64%), having symptoms of a sexually transmitted infection (61%), or both (Table S1 in the Supplementary Appendix). Most participants with acute HIV-1 infection in the three African sites were heterosexual women, whereas most participants with acute infection from Thailand were homosexual men or transgender women.

To accurately define peak viremia, we restricted the analysis to the 50 participants in whom at least two large-volume blood samples showed detectable HIV-1 RNA and a nonreactive enzyme immunoassay, who had had at least one study visit before detection of viral RNA, and who had quantitative HIV-1 RNA data. Analysis of the viral-load set point in 45 participants who had not received antiretroviral treatment required two blood samples obtained after day 42. In these participants, a median of 4 days (range, 2 to 162 days) occurred between the last negative sample and the first sample that was reactive for HIV-1 RNA.

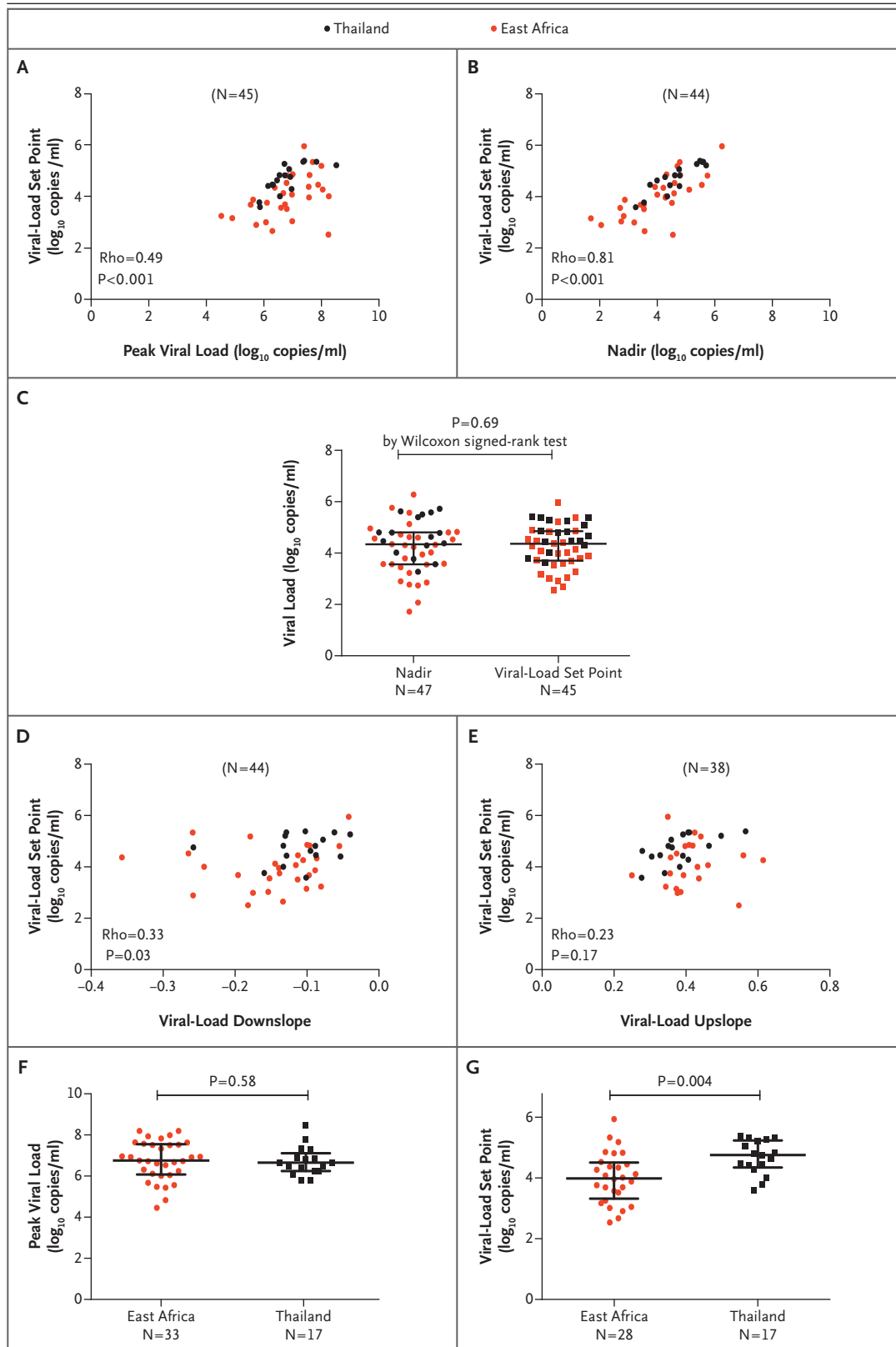
Figure 3 (facing page). Viral-Load Associations.

Spearman's correlations of peak (Panel A) and nadir (Panel B) viremia with the viral-load set point in participants from Thailand (black dots) and East Africa (red dots) are shown. Five participants were excluded because of missing viral-load data or the initiation of antiretroviral therapy. The early viremic nadir and viral-load set point were highly correlated (Panel B) and did not differ significantly (Panel C). A significant positive Spearman's correlation of viral-load downslope with the viral-load set point was observed in 44 participants with acute HIV-1 infection (Panel D). Viral-load upslope did not show significant Spearman's correlation with the viral-load set point in the overall sample (Panel E). Seven of 45 participants were excluded from the upslope analysis because the interval between the last test that was negative for HIV-1 RNA and the first test that was reactive for HIV-1 RNA was longer than 10 days. Comparisons with the use of a Wilcoxon rank-sum test did not show regional differences between East Africa and Thailand with respect to peak viral load (Panel F); however, the viral-load set point was higher in participants in Thailand, with a narrower range of values (Panel G). Sex, race or ethnic group, and HIV-1 subtypes also differed between the two groups.

HIV-1 VIRAL DYNAMICS

Figure 2 shows HIV-1 viremia during the first 100 days of HIV-1 infection, including the median days to the peak viral load, enzyme immunoassay reactivity, and early nadir viral load. The median initial viral RNA level was 4.0 \log_{10} copies per milliliter (range, 1.3 to 7.3), and a median peak of 6.7 \log_{10} copies per milliliter (range, 4.5 to 8.5) was reached 13 days (range, 6 to 18) after the first sample showed reactivity for RNA on nucleic acid testing. A third-generation enzyme immunoassay was reactive at a median of 14 days (range, 8 to 48). The median early nadir viral RNA level, 4.3 \log_{10} copies per milliliter (range, 1.7 to 6.3), occurred at a median of 31 days (range, 18 to 42). The median viral-load RNA set point was 4.4 \log_{10} copies per milliliter (range, 2.5 to 6.0) (Tables S3 and S4 in the Supplementary Appendix).

The spline models showed a significant interaction between viremia and geographic region and indicated differences in viral RNA dynamics among regions. The models also showed that the viral-load set point was established at the conclusion of acute viremia and remained stable subsequently (Fig. S1 in the Supplementary Appendix). Peak viremia was positively correlated



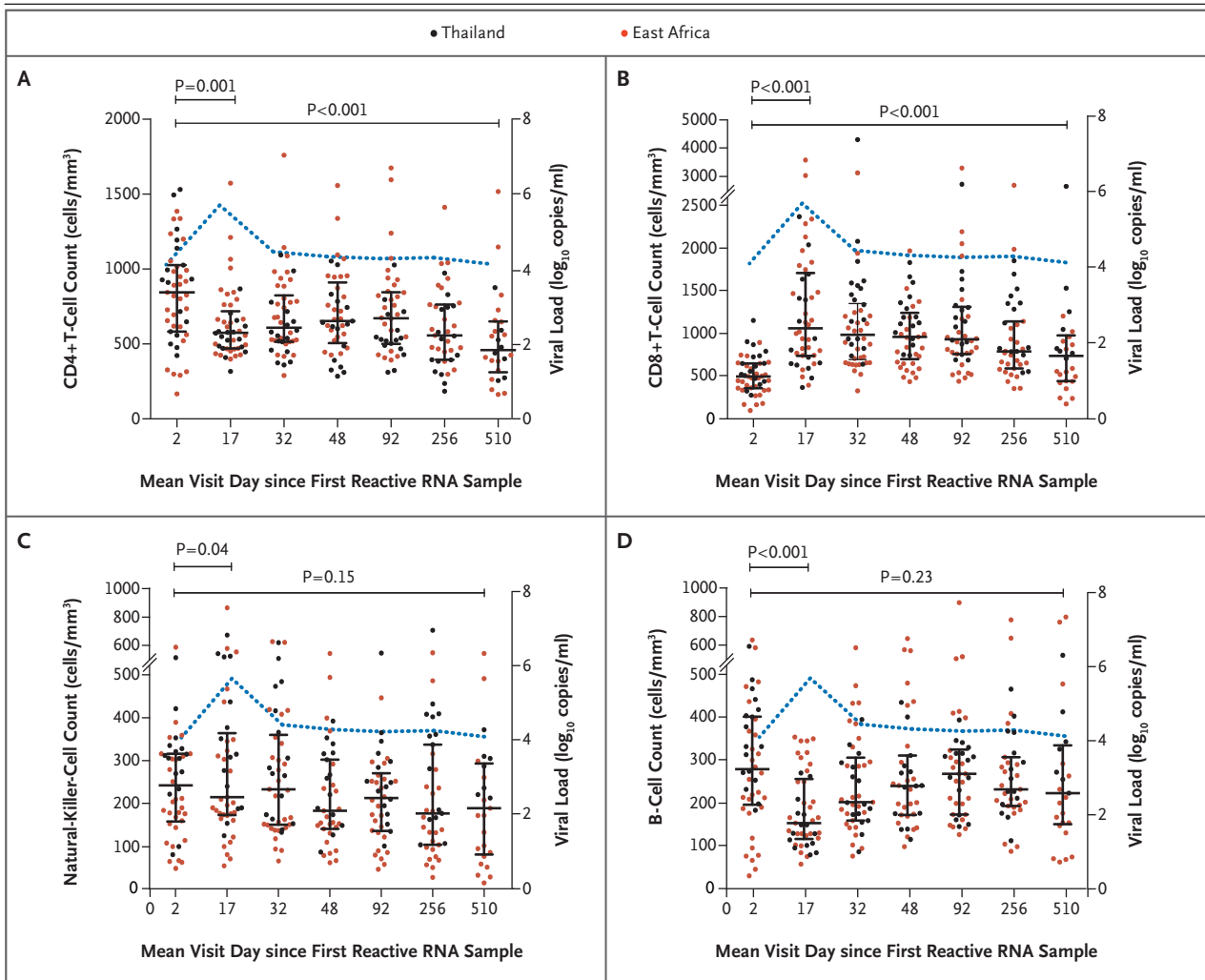


Figure 4. Immune-Cell Counts over 510 Days of Follow-up in 50 Participants.

The absolute counts of CD4+ T cells (Panel A), CD8+ T cells (Panel B), natural killer cells (Panel C), and B cells (Panel D) are plotted against the mean visit day. The red dots indicate 33 participants from East Africa, and the black dots 17 participants from Thailand. The blue dotted line indicates the mean viral load. Wilcoxon signed-rank tests were performed on available paired data before the initiation of antiretroviral treatment. A significant decrease in absolute CD4+ counts and a significant increase in absolute CD8+ counts at day 17 roughly coincided with the timing of the peak viral load in 44 participants with data that could be evaluated. By day 510, absolute CD4+ and CD8+ counts had not returned to initial levels in 26 participants. Absolute natural-killer-cell counts decreased at day 17 in 42 participants; however, these counts returned to original levels by day 510 in 24 participants. There was a significant decrease in absolute B-cell counts ($P<0.001$) at day 17 in 42 participants; however, these counts recovered by day 510 in 24 participants.

with the viral-load RNA set point in the total cohort ($\rho=0.49$, $P<0.001$) (Fig. 3A) and independently in each geographic region (Fig. S2A in the Supplementary Appendix). There was a strong correlation between an early viral RNA nadir at the end of acute HIV-1 infection and the viral-load set point ($\rho=0.81$, $P<0.001$) (Fig. 3B); this correlation remained significant within each region (Fig. S2B in the Supplementary Appendix). The values of the early nadir and set point did

not differ significantly (Fig. 3C); this shows that the viral-load RNA set point was established within the first 42 days after viremia was detectable.

The downslope of viral RNA was correlated with the viral-load RNA set point ($\rho=0.33$, $P=0.03$) (Fig. 3D). The upslope of viral RNA was not correlated with the viral-load set point in the overall cohort (Fig. 3E); it was strongly correlated in Thailand only ($\rho=0.66$, $P=0.004$) (Fig.

S2D in the Supplementary Appendix). Although the peak viral RNA level was nearly the same in Thailand and East Africa (Fig. 3F), the viral-load set point differed significantly between the two regions (Fig. 3G).

IMMUNOPHENOTYPE

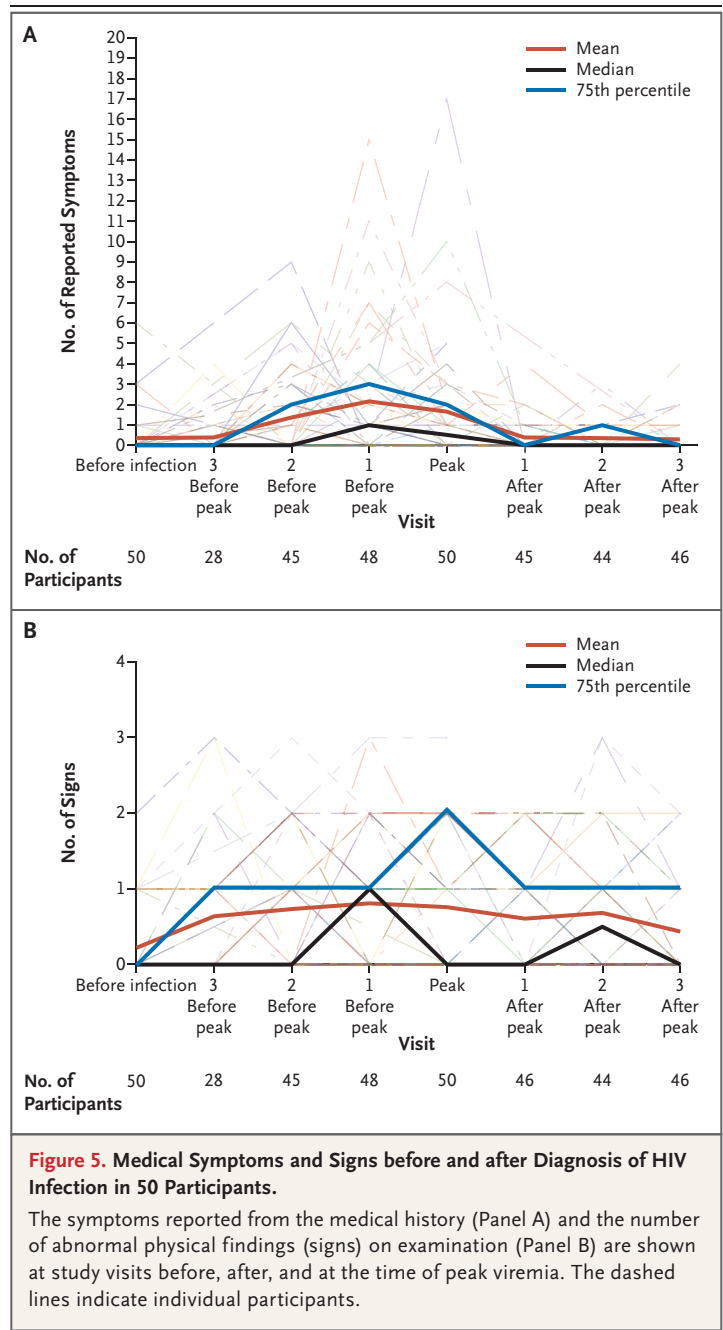
Immunophenotyping of lymphocytes showed no change or a minimal variation from normal values at the first study visit after the onset of plasma viremia (Fig. 4A through 4D, and Fig. S3 in the Supplementary Appendix). Subsequently, levels of both B cells and CD4+ T cells decreased sharply at the time of peak viremia, while levels of CD8+ T cells increased significantly. Changes in levels of natural killer cells during acute infection were variable. After peak viremia, levels of CD4+ T cells increased and levels of CD8+ T cells decreased, but these levels never returned to a normal range.

Levels of CD4+ T cells and B cells were inversely correlated with contemporaneous viral RNA levels, whereas the increase in the number of CD8+ T cells was correlated directly with contemporaneous viral RNA levels in a model adjusted for geographic region and study visit. After adjustment for other cell counts, region, and visit, a 100-cell increase in the CD4+ T-cell count was associated with an average decrease of 0.1 in \log_{10} viral RNA across visits. The nadir CD4+ T-cell count was correlated with the CD4+ T-cell count at 12 months ($\rho=0.59$, $P<0.001$).

CLINICAL PRESENTATION

A structured history was obtained and a physical examination was performed at study entry, every 6 months, and every 3 or 4 days throughout the period of acute infection. Symptoms and signs were identified at least once during observation in 94% of the participants with acute HIV-1 infection (88% reported at least one symptom and 78% reported at least one sign). However, during the period of acute infection, in 367 of 518 visits in which participants underwent a physical examination (71%), participants reported no symptoms, and 50% of these participants had neither symptoms nor signs.

Fever, headache, and malaise were the most common symptoms, and tachycardia, lymphadenopathy, and other head and neck findings were the most common signs (Table S5 in the Supplementary Appendix). The greatest number of



symptoms was reported at the study visit before the peak viral RNA level (median, 1; range, 0 to 15) and was reported at a median of two visits (Fig. 5A). Observed signs on physical examination peaked at the visit before the peak viremia (median, 1; range, 0 to 3) and were recorded for a median duration of three visits (Fig. 5B). Heat maps enumerating each volunteer's symptoms or signs at each visit show that most findings

occurred before and at the time of peak viremia but waned quickly thereafter (Fig. S4A and S4B in the Supplementary Appendix). Although fever was the most common reported symptom, only six volunteers were found to be febrile on physical examination.

Lymphadenopathy was more common among participants in Thailand than among those in East Africa ($P < 0.001$) (Table S5 in the Supplementary Appendix). The magnitude of the lymph-node enlargement was minimal; the maximal lymph-node diameter was greater than 2 cm in only five volunteers.

DISEASE PROGRESSION

The CD4+ T-cell count at 12 months after the diagnosis of HIV-1 infection and the last available CD4+ T-cell count were used to evaluate the disease course. The start of antiretroviral therapy was not used as an end point because it was most frequently initiated because of pregnancy.

After exclusion of five participants without a CD4+ T-cell count within 120 days before or after the 12-month time point, the CD4+ T-cell count was inversely correlated with the viral-load RNA set point ($\rho = -0.65$, $P < 0.001$). Similarly, the last available CD4+ T-cell count was highly and inversely correlated with the viral-load RNA set point ($\rho = -0.50$, $P = 0.004$).

A surrogate clinical end point was the number of days to two consecutive visits during which a CD4+ T-cell count of less than 350 cells per cubic millimeter was recorded. After exclusion of volunteers who were pregnant or who had started to receive antiretroviral therapy for reasons other than a CD4 T-cell count below 350 cells per cubic millimeter, 15 of 50 participants reached this surrogate clinical end point in a median of 306 days (range, 7 to 1083).

There was no difference in follow-up time between participants who reached the end point and those who did not ($P = 0.68$). The peak viral RNA level did not differ significantly between participants who reached the end point and those who did not; however, the viral-load RNA set point was significantly higher among those who reached the end point than among those who did not (median, $4.83 \log_{10}$ copies per milliliter vs. $4.02 \log_{10}$ copies per milliliter, $P = 0.002$), absolute CD4+ T-cell counts at 1 year were lower (median, 356 cells per cubic millimeter vs. 639.5 cells per cubic millimeter, $P = 0.002$), and CD8+ T-cell peaks were higher (median, 1661 cells per

cubic millimeter vs. 1202 cells per cubic millimeter, $P = 0.06$).

DISCUSSION

In contrast to previous studies of acute HIV-1 infection in which volunteers were evaluated less often, we evaluated high-risk volunteers twice weekly in order to systematically describe both the clinical disease and host-virus interactions, with precise determination of the onset and dynamics of acute plasma viremia. The upslope, peak, and downslope of viremia in acute infection were defined with precision and at a high frequency among observed cases of HIV-1 infection. These variables were significantly associated with the viral-load set point; this association underscored the crucial role of the very earliest interactions between the host and virus in determining the long-term course of the disease. Our study showed that the viral-load RNA set point was established within 42 days after detectable viremia, was steady over the period of observation, and was associated with the early clinical outcome as measured by the CD4+ T-cell count 12 months after infection and a CD4+ T-cell count below 350 cells per cubic millimeter.

The peak viremia reported here was of a higher magnitude (median, \log_{10} 6.7; range, \log_{10} 4.5 to 8.5) than that which is commonly reported,²⁴⁻²⁸ probably because of the frequency of assessment. Although the peak viremia was nearly equivalent in East Africa and Thailand, there was a significant difference of $0.8 \log_{10}$ copies per milliliter in the viral-load RNA set point. Differences in viral-load RNA set points according to sex have been reported; set points in men are generally approximately three times as high as set points in women who have infections of the same viral subtype.²⁹ Because of multiple confounding variables, including host genetic factors, viral subtype, endemic disease, and risk characteristics, a mechanistic explanation for this regional variation in the viral set point remains undefined.

This study showed the alterations in cell phenotype before and during peak viremia. At the onset of plasma viremia, immunophenotypes were largely normal, but subsequently, CD4+ T-cell counts decreased and CD8+ T-cell counts increased around the time of the peak viral RNA level, were highly correlated with the viral RNA level during acute HIV-1 infection, and did not

fully recover as the viral RNA level decreased to the set point. Ndhlovu et al. recently described an association between the timing and magnitude of CD8+ T-cell activation and the viral-load set point in 11 cases of acute infection.³⁰ The observed, dramatic loss of B cells has been reported in simian immunodeficiency virus,³¹⁻³³ but data on the role of this loss in acute HIV-1 infection in humans are lacking.³⁴

In many studies of HIV-1, patients had symptoms before an evaluation for the diagnosis of acute infection was initiated.^{35,36} As in other studies of acute HIV-1 infection, most persons in our study (94%) had clinical manifestations sometime during acute infection. However, non-specific symptoms and signs were most common, severe manifestations were not observed, volunteers reported symptoms in only 29% of visits, and on any given visit day the likelihood of observing a symptom or sign was only 50%. The frequency of clinical manifestations of disease clustered before the peak viremia and at the time of peak viremia and resolved quickly, but the median number of symptoms and signs was only 1.

Since the study scheduled visits throughout the period of acute HIV-1 infection, the proportion of participants who would have sought medical care is unknown. Sullivan et al. reported symptoms and signs of acute infection among discordant couples who were prospectively followed every 3 months and found that a majority of patients with incident HIV-1 infection could not recall an illness and did not pursue medical care.²¹ Thus, systematic identification of acute HIV-1 infection may be challenging and will probably require nucleic acid testing with a rapid turnaround and frequent evaluation of high-risk groups rather than clinical presentation in a health care setting.

The contribution of acute HIV-1 infection to HIV-1 transmission may be substantial.^{5-7,37,38} Presumably, a high viral load plays a part, but the biologic characteristics of transmitted founder

viruses, the homogeneity of viral sequence during acute HIV-1 infection, and the incomplete or immature host immune response may create a transmission diathesis. HIV-1 may evolve to maintain efficient replication in the host and lose characteristics that are favorable for transmission. Recent data show that transmitting viruses are qualitatively distinct from those emerging under host immune pressure.³⁹ Viral RNA is a dominant factor in the risk of transmission in various patient groups (e.g., among infants who may become infected through perinatal transmission and among heterosexuals), and if a high level of viral RNA in acute HIV-1 infection is the primary variable contributing to increased infectiousness in early and acute HIV-1 infection, the duration of this risk appears to be brief because the viral-load RNA set point is achieved within a few weeks after peak viremia. This observation may have important consequences and may limit the effect of test-and-treat strategies on overall rates of transmission.

The data reported here emphasize the importance of acute infection to our understanding of the pathogenesis of HIV-1. They also provide evidence that during the acute phase, identification of cases of HIV-1 on the basis of clinical criteria may prove to be difficult.

The views expressed are those of the authors and should not be construed to represent the positions of the Departments of the Army or Defense or the National Institutes of Health (NIH).

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No potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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