Promoting early detection of HIV and anal dysplasia in Thai men who have sex with men
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CHAPTER 4

Incidence and characterization of acute HIV-1 infection in a high-risk Thai population


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Objective: The objective of this study was to investigate the incidence, demographics, HIV subtype, and genotypic resistance of acute HIV infections in a high-risk Thai population.

Methods: Between March 2006 and September 2007, 6426 stored samples at the Thai Red Cross Anonymous Clinic were screened for acute HIV infection by 2 methods: pooled nucleic acid testing (NAT) of fourth-generation enzyme immunoassay (EIA)–negative samples (n = 5402) and subsequent first-generation EIA testing of fourth generation EIA-positive samples (n = 1024).

Results: Eleven acute HIV–infected subjects were identified by pooled NAT (n = 7) and serial EIA (n = 4). Mean age was 28 years; 9 were male; and 60% were men who have sex with men. Median HIV RNA was 99,601 copies per milliliter (log10 Viral load (VL) = 5.00). Eight samples could be genotyped: 6, CRF01_AE; 1, subtype B; and 1, CRF01_AE/B recombinant. No resistance to antiretroviral therapy was found. The HIV incidence per 100 person-years, calculated from the pooled, antibody-negative samples, was 2.7% (95% confidence interval, 2.2%–4.3%).

Conclusions: This is the first report of antibody-negative, NAT-positive, acute HIV infection in Thailand. The majority were men who have sex with men, which reflects the current epidemic in Thailand and justifies prevention programs aimed at this group. This high-risk population may be suitable for future studies on acute HIV infection, HIV treatment, vaccine, and prevention of onward transmission strategies.
**Introduction**

Increasing attention is focused on the diagnosis of antibody-negative, “acute” HIV infection as recent evidence shows significant CD4+ T-cell depletion occurring within the first 3 weeks of onset.1,2 Understanding the pathogenesis and characterizing early clinical events are critical to the evaluation of breakthrough infection, postvaccination, and the impact of early antiretroviral therapy on HIV disease progression. As persons with acute HIV infection have an 8–20 times greater risk for transmitting HIV compared with those with chronic infection, medical and behavioral intervention in these persons and their social networks could be crucial in limiting the spread of HIV.3,4

It is now possible to diagnose HIV infection within the first 3 weeks of onset using nucleic acid testing (NAT).5 Acute HIV infection was identified at a rate of 4.9 per 10,000 low-risk blood donors in North Carolina using this strategy.6 In addition, use of an HIV testing algorithm employing an initial HIV antigen/antibody combination direct enzyme immunoassay (EIA) using recombinant HIV antigens (fourth generation), followed by a less sensitive antibody-only EIA with viral lysate (first generation) on fourth-generation EIA-reactive samples would also provide an indication of acute HIV infection if the first-generation test was nonreactive.7 In Asia, almost 1 million persons were newly diagnosed with HIV in 2005 alone,8 but little is known about the incidence of acute HIV infection in Asia. A recent study from China reported a 1.2% acute HIV infection prevalence among almost 12,000 clients of sexually transmitted diseases (STDs) clinics.9 In this study, we investigate the incidence, demographics, HIV subtype, and genotypic resistance in acute HIV infection within a high-risk Thai population at the Thai Red Cross Anonymous Clinic (TRCAC), which has an HIV prevalence of about 17%.10

**Materials and methods**

The TRCAC is a voluntary counseling and testing center (VCT) in Bangkok, Thailand. Clients seeking VCT are not required to provide name and contact information. Data and samples are stored without any personal identifying information. Clients are asked to complete a self-administered demographic and risk behavior questionnaire in which they may choose to answer some or none of the questions. HIV diagnosis is routinely established using a fourth-generation (HIV antigen/antibody combination detection assay) EIA (AxSYM; Abbott Laboratories, Wiesbaden, Germany). Positive samples are confirmed with a recombinant antigen sandwich EIA, Genscreen HIV 1/2 (Marne-la-Coquette, France), and a particle agglutination assay, Serodia HIV 1/2 (Fujirebio, Tokyo, Japan).

Between March 2006 and September 2007, 6426 VCT-stored samples were screened for acute HIV infection at the Armed Forces Research Institute of Medical Sciences in Bangkok by 2 methods. First, AxSYM-negative samples (n = 5402) were pooled (40 subjects per pool), and NAT was performed using Roche Amplicor v1.5 ultrasensitive assay, with a quantitation limit of 50 copies per milliliter (Roche Diagnostics, Branchburg, NJ), according to methods previously described,5 with a modification in which pools were tested in a qualitative assay format. Samples from reactive pools were then tested individually according to the manufacturer’s instructions. Acute
HIV infection samples were AxSYM negative and NAT positive. Second, AxSYM positive samples (n = 1024) were tested with first-generation US Food and Drug Administration–approved HIV-1 EIA (HIV-1 Microelisa System; Organon Teknika, Durham, NC). Acute HIV infection samples were AxSYM positive, first generation sensitive EIA negative, and NAT positive. A standard HIV-1 p24 antigen assay (ABL Inc, Kensington, MD) without immune complex dissociation was performed on samples from acute HIV–infected subjects if sufficient sample was available. Laboratory staging for acute HIV infection was performed according to Fiebig et al. Multiregion hybridization assays and TRUGENE HIV-1 genotyping (Bayer, Leverkusen, Germany) were performed to determine HIV subtype and resistance, respectively. Informed consent was not obtained from the subjects in this study as the specimens were discarded as anonymous samples and could not be linked to the tested subjects. The study was approved by the Chulalongkorn University, the University of Hawaii, and the Walter Reed Army Institute for Research institutional review boards.

Data on the demographics and risks were collected for the acute HIV–infected subjects if available. The information from the questionnaire and the samples was linked by a unique 7-digit identification number that the clients reused. This number was not linked to any personal or contact information (name, phone number, address), and the clients were asked for their birth date and place for verification. At every visit, this information was obtained from the client and then compared with the TRCAC database to determine if the client was a newcomer. The prevalence of acute HIV infection was calculated using the number of first generation–negative samples in the denominator. Similarly, an estimate of the annual HIV incidence and 95% confidence intervals (CIs) were calculated using the method described by Brookmeyer et al.

### Results

Plasma from the 6426 VCT clients was tested, and 11 subjects had acute HIV-1 infection. Seven of the 5402 AxSYM-negative samples were NAT positive. Three had sufficient samples for p24 antigen assay, and all were positive (Fiebig stage II). Four subjects without sufficient specimens were either Fiebig stage I or II, as p24 antigen level could not be determined. Four of 1024 AxSYM-positive samples had negative first-generation EIA, and all had detected p24 antigen (Fiebig stage III). The acute HIV
infection prevalence was 20.3 per 10,000 persons at risk (95% CI, 10.1 to 36.4), and the estimated HIV incidence was 2.7 per 100 person-years (95% CI, 2.2 to 4.3).

Table 1 shows the demographics and laboratory data. The subjects’ mean age was 28 years (range 17–45 years), and 9 were male. Sixty percent were men who have sex with men (MSM). Seven subjects gave additional information: 4 were single, 1 married, and 2 cohabitating. Two subjects reported condom use at all times, 6 sometimes, and 2 not at all. One subject did not answer. No one reported having sex with either male or female commercial sex worker. For 4 subjects, this was their first HIV testing. Three subjects had had a prior negative HIV testing, and 2 did not answer. Six said that they never had any STD, 1 had condyloma acuminata, and three did not answer. Seven subjects provided information about alcohol and illicit drug use. Of these, 5 consumed alcohol less than 1–4 times per month, 1 consumed 2–6 times per week, and 1 does not use alcohol, whereas 6 never used illicit drugs, and 1 had inhaled illicit drug in the past. Five subjects had bachelor degrees, 1 technical diploma, 3 high school, 1 secondary school, and 1 did not answer. Five clients were company employees, 2 business owners, 2 unemployed, 1 student, and 1 did not answer. The average monthly income based on 6 subjects who provided the information was 833 USD (range 303–1515 USD).

Median HIV RNA was 99,601 copies per milliliter (log10 VL = 5.00, range 130 to >100,000), but there was insufficient sample to allow testing of the samples by the Amplicor 1.5 standard assay, which would have increased the upper limit of the HIV RNA data up to 750,000 copies per milliliter. HIV-1 subtype was assigned in 8 of the 11 samples analyzed by the multiregion hybridization assays, as shown in Figure 1. The results revealed that 75% (n = 6) of the infections were caused by B/CRF01_AE strains, whereas 25% (n = 2) of the infections were caused by non-CRF01_AE viruses, 1 by B subtype, and 1 by B/CRF01_AE recombinant. Of note, 2 of the nontypeable samples showed reactivity with a subtype B–specific probe, and 1 putative dual infection was also detected. All the samples analyzed (n = 10) using the TRUGENE HIV-1 genotyping assay showed mutations in the protease gene that are weakly associated with resistance or are polymorphisms common among non-B subtype viruses.
Discussion
In this first acute HIV infection study in Thailand, 11 of the 6426 subjects were identified using pooled NAT and sequential sensitive/less sensitive EIA algorithm in a high-risk Thai population over an 18-month period. Our pooled NAT strategy demonstrated a limit of detection of 3 HIV RNA copies per milliliter. Fiebig staging in these subjects corresponded with the reported average (95% CI) cumulative window period for HIV diagnosis of 5.0 days (3.1 to 8.1), 10.3 days (7.1 to 13.5), and 13.5 days (10.0 to 17.0) for Fiebig stages I, II, and III, respectively. The prevalence of acute HIV infection in our study is significantly higher than that reported in low-risk blood donors in the United States and in STD clinic attendees in China.6,9

The acute HIV–infected subjects in our study were mostly young MSM. This supports the current epidemiological trends in Thailand where the observed prevalence of HIV among MSM in Bangkok rose from 17% in 2003 to 28% in 2005. Lower education, recruitment from a park, self-identification as homosexual, receptive and insertive anal intercourse, more years since first anal intercourse, and more male sex partners were significantly and independently associated with HIV prevalence.14 The majority of our subjects were educated, employed, and had income 3 times higher than the average Thai household monthly income. Similar to previous reports14,15 few consistently used condoms. This is worrisome as the majority had very high HIV RNA plasma levels. Unlike MSM in Western countries, a significant number of Thai MSM also have sex with paid and unpaid female partners, which further complicates the design of an effective prevention program.16

Knowledge of the HIV virology in acutely infected subjects is important for understanding HIV epidemics and for prevention studies and vaccine development.17,18 Using a population-based phylogenetic approach, Brenner et al19 demonstrated that almost half of primary HIV infections in the mainly MSM cohort in Quebec were likely transmitted from others with primary HIV infection, thereby supporting the notion that intervention during this early infection period is important for infection containment.19,20 Previous studies have indicated that HIV recombinant forms are frequently found in high-risk populations.21 CRF01_AE viruses continue to represent the majority of circulating viruses in Thailand.22 The results of this study, however, are in agreement with 2 recent studies, which indicated that the occurrence of non-CRF01_AE strains in Thailand might be increasing among high-risk groups.23 The mutations in the protease gene in our subjects are commonly found natural polymorphisms among non–subtype B viruses.24 It is reassuring that none of the 10 subjects tested had antiretroviral resistance, despite antiretroviral therapy being widely available in Thailand since 2003. About 10% of newly infected persons in developed countries harbor resistant viruses.25,26

Current treatment guidelines defer to the judgment of treating physicians to start antiretroviral therapy for acute HIV infection.27,28 Several small nonrandomized studies suggested that if HIV infection is treated early, therapy can later be stopped while maintaining a high CD4 and low plasma HIV RNA levels.29–31 In addition, treatment of early HIV infection may shorten the half-life of the latently infected,
memory CD4 reservoir.\textsuperscript{32} Many of the subjects in the aforementioned studies, however, are treated after a few months of HIV infection rather than during acute (seronegative) infection. Given the profound loss of CD4 T cells within the first days of infection, these 2 groups may be pathogenetically distinct. It is possible that greater immune preservation would be seen in subjects who are treated with antiretroviral and/or immune-based therapy within the seronegative window of the first 3 weeks before significant immune destruction occurs.\textsuperscript{1,33} Antiretroviral treatment during the highly viremic phase of acute HIV infection deserves further investigation as an added strategy to behavioral approaches in reducing HIV transmission.\textsuperscript{19,20}

One limitation of this study is that the estimated HIV incidence applies to a group that seeks anonymous VCT at the TRCAC and at higher risk of HIV infection than the general Thai population. Additionally, due to the selection of this population, the low number of subjects and missing data, demographic descriptions, and virological data about resistance and subtypes may not be representative as well.

In conclusion, our study confirms that acute HIV infection can be identified using pooled NAT and sequential EIA in a high-risk Thai population. Men who have sex with men are at highest risk, warranting prevention programs aimed at this group. This high-risk population may be suitable for future studies of acute HIV infection, HIV treatment, and preventive vaccine trials.

References


Appendix 1. The South East Asia Research Collaboration with Hawaii 004 Protocol Team