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Research paper

Risk Factors for HIV sero-conversion in a high incidence cohort of men who have sex with men and transgender women in Bangkok, Thailand

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ABSTRACT

Background: We measured Human Immunodeficiency (HIV) incidence, retention, and assessed risk factors for seroconversion among two previously unreported cohorts of men who have sex with men (MSM) and Transgender Women (TGW) in Bangkok, Thailand between 2017 and 2019.

Methods: We conducted an 18-month prospective cohort study of HIV-uninfected Thai cisgender men and TGW aged between 18 and 35 years who reported sex with men in the past six months and at least one additional risk factor for HIV infection. HIV and syphilis testing and computer-based behavioral questionnaires were administered at each visit. We utilized Poisson regression to calculate HIV incidence rates. A survival random forest model identified the most predictive risk factors for HIV sero-conversion and then used in a survival regression tree model to elucidate hazard ratios for individuals with groups of selected risk factors. Cox proportional hazards (pH) regression evaluated the strength of association between individual covariates and risk of sero-conversion.

Findings: From April 2017-October 2019, 1,184 participants were screened, 167 were found ineligible, and 1,017 enrolled. Over the 18-month study, visit retention was 93·4% (95% CI 91·6%–94·8%) and HIV incidence was 3·73 per 100 person-years (95% CI 2·79–5·87). Utilizing survival regression tree modeling, those who were 18–20 years of age, reported sexual attraction to mostly or only men, and had five or more lifetime sexual partners were 4·9 times more likely to seroconvert compared to other cohort participants. Factors associated with HIV incidence utilizing Cox pH regression included sexual attraction to mostly or only men (adjusted hazard ratio (aHR) 14·9 (95% CI 20·1–107·9), younger age (18–19 years, aHR 10·88 (95% CI 4·12–28·7), five or greater lifetime sexual partners (aHR 2·0, 95%CI 1·1–3·6), inconsistent condom use with casual partners (aHR 2·43, 95% CI 1·3–4·5), and prior HIV testing (adjusted HR 2·0, 95% CI 1·1–3·5).

Interpretation: Interpretation HIV incidence remains high among Bangkok-based MSM and TGW. These key populations expressed high interest in participating in efficacy evaluation of future prevention strategies and had high retention in this 18 month study.

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Research in context

Evidence before this study

MSM and TGW bear a disproportionately high burden of new HIV infections as compared to other reproductive age adults. HIV prevention interventions need to be developed and tested for efficacy in these key populations. Two of the six completed efficacy trials of candidate preventive HIV vaccines were completed in Thailand, which has large MSM and TGW populations that have been impacted by HIV, particularly in urban centers such as Bangkok.

We searched PubMed with the terms "HIV", "MSM", "TGW", and "Asia" for articles published in English up to December 31, 2020 and tracked studies enrolling MSM and/or TGW in Thailand in clinicaltrials.gov and the Thai Clinical Trials Registry. In 2014, the median HIV prevalence among MSM in Thailand was 9·2%, but in Bangkok, this was 28·6%; this prevalence was the highest in the Asia-Pacific Region. While fewer data are available for TGW, national surveys have estimated HIV prevalence ranging between 11 and 15·7% during 2014–2018.

The Bangkok Men's Cohort Study (BMCS) followed MSM between 2005 and 2015 and found an overall HIV incidence of 4·8 per 100 PY, incidence peaked around 2011 and then declined to 3·8 per 100 PY in 2015. Other cohort studies among MSM in Thailand have identified the following risk factors for HIV seroconversion: inconsistent condom use with steady male partners, receptive anal intercourse with male partners, use of erectile dysfunction and club drugs, prior Hepatitis A and Hepatitis B infection, ulcerative sexually transmitted infections, and rectal chlamydia infection.

Added value of this study

This prospective study of >1000 volunteers with high retention over 18 months provides current disaggregated HIV incidence estimates from a cohort of Bangkok-based MSM and TGW who reported anal intercourse in the six months prior to enrollment. In addition, by utilizing survival tree regression models, this study allowed for the identification of subgroups at highest risk for seroconversion. Being mostly or only sexually attracted to men, being of younger age, reporting a higher number of lifetime sexual partners, having a history of prior HIV testing and inconsistent condom use were significantly associated with HIV seroconversion in this cohort.

Implications of all available evidence

Young MSM and TGW continue to experience high HIV incidence and low PrEP uptake in Bangkok despite ongoing prevention efforts. These associated risk factors can be utilized to specifically reach populations at highest risk for future preventive research or public health interventions.

1. Introduction

In Thailand, men who have sex with men (MSM) have made up the majority of new HIV diagnoses since 2010 [1]. Additionally, the Asian Epidemic Model forecasts that MSM will represent over half of all new HIV infections by 2025 [1]. While fewer data are available on TGW, they also experience a disproportionate burden of HIV, with prevalence of 11·0% in 2018 as compared to 1·1% for all Thai adults [2]. Despite their high burden of disease, TGW are historically underrepresented in HIV research and often conflated with MSM, so updated HIV incidence estimates among TGW are particularly necessary to inform future interventions in this key population [3, 4].

While the first Thai National Guidelines on oral PrEP were released in 2016, the cost of medication and follow-up were only covered under the national health insurance program starting in 2019, and uptake has been low [5]. Other novel prevention interventions, such as an HIV preventive vaccine, could represent an important option to decrease HIV transmission for those unwilling or unable to take PrEP.

The continued development of novel strategies to prevent HIV infection such as preventive vaccines, monoclonal antibodies, long-acting pre-exposure prophylaxis (PrEP), and microbicides relies on the ability to conduct efficacy trials in populations with high HIV incidence. Successful conduct of such trials requires 1) access to populations with high HIV incidence 2) willingness to participate in interventional trials and 3) high retention over a prolonged follow up period. Two of the six completed efficacy trials of candidate preventive HIV vaccines have been conducted in Thailand: VAX003 from 1999 to 2000 and RV144 from 2003 to 2009 [6,7]. However, as these trials were both conducted prior to the approval of oral PrEP, updated HIV incidence estimates are needed to quantify current HIV transmission dynamics and elucidate factors associated with sero-conversion. We therefore conducted a prospective cohort study among MSM and TGW in Bangkok, Thailand from 2017 to 2019 in order to assess site feasibility for future HIV preventive vaccine trials. We measured HIV incidence, retention, and willingness to participate in vaccine trials and identified factors associated with HIV acquisition in these key populations in order to inform participant selection and study design for future trials of HIV prevention interventions.

2. Methods

2.1. Study design and participants

Participants for this prospective observational cohort were enrolled at the Vaccine Trial center at Mahidol University (VTC) and Royal Thai Army Clinical Research center (RTA) in Bangkok, Thailand. The VTC is an established center with decades of vaccine clinical trial experience. Participants for this trial were seen in a dedicated location apart from other clinical trial participants. Participants were recruited between April and October 2017. Each participant was followed for up to 18 months, with follow up visits conducted at three, six, twelve, and eighteen months after enrollment. Each site sought to enroll 500 participants and independently have at least 80% power to detect at least 3·1% incidence (point estimate 4·82, 95% CI 3·1–6·55) with assumed loss to follow up of 10% of the cohort every six months.

2.2. Procedures

Eligible participants were Thai cisgender men or TGW between 18 and 35 years of age, residing in the Bangkok metropolitan area, willing to be followed for 18 months, willing to undergo study procedures, and considered high-risk for HIV infection. High risk was defined as engaging in both anal intercourse and reporting at least one of the following in the past six months: engaging in condomless anal intercourse with a male or TGW partner of unknown or HIV positive status; having three or more sexual partners; exchanging sex for money, goods, or drugs; or being diagnosed with a new sexually transmitted infection (STI), which included gonorrhea, chlamydia, herpes, or syphilis. TGW were self-identified through a two-step question process asking sex at birth, followed by current gender identity. Exclusion criteria included receiving active product in a previous candidate HIV vaccine trial. All participants received education about HIV transmission, condoms, including PrEP indications and benefits, and given guidance on procuring PrEP through the local

healthcare system or as part of a demonstration project at every cohort visit. Participation in other trials or use of PrEP were not exclusion criteria, and study team asked participants to disclose if they were taking any medications or participating in other HIV prevention studies.

Each site recruited participants independently. Both sites asked participants to refer their peers or contacts and recruited participants at local universities and vocational institutes. These sites were within the greater Bangkok metropolitan area and served as a site of peer referral to the clinic. However, each site also employed its own unique recruitment strategies. RTA advertised free HIV testing for MSM and TGW on social media and conducted direct outreach at entertainment venues frequented by the target populations, including bars, clubs, saunas, and fitness centers. VTC, on the other hand, recruited during community health campaigns and festivals, and also asked for referrals from community-based organizations serving the target populations. Replacements were allowed during the accrual period, defined as the period of time when active enrollment was occurring, for participant withdrawal or loss to follow-up. Upon completion of enrollment, replacements were not permitted.

HIV testing was conducted using sequential rapid tests according to the Thai National Guidelines [5]. Alere Determine HIV-1/2 Ag/Ab Combo (Orgenics Ltd., Yavne, Israel) was the initial test conducted. Positive tests were confirmed with First Response HIV-1−2−0 (Premier Medical Ltd, Daman, India) and SD Bioline HIV 1/2 3·0 (Standard Diagnostics, Inc., Gyeonggi-do, Korea). At the study screening visit, participants also were tested for hepatitis B surface antigen with Genetic Systems HbsAg EIA 3·0 (Bio-Rad Laboratories, Redmond, WA), Hepatitis C Virus (HCV) antibody using the ORTHO HCV 3·0 ELISA Test System (Ortho Clinical Diagnostics, Raritan, NJ), and syphilis using the Rapid Plasmia Reagin (RPR) Test Kit (Lab21 Healthcare, Kentford, Suffolk, UK). Syphilis and HIV testing were repeated at all follow-up visits.

For participants who tested positive for HIV, referral was made to the hospital clinic which the participant had access to under their national health care benefit, and staff provided counselling to assist in starting antiretroviral treatment. Those who were HIV positive ended participation in future cohort visits. For those who tested positive for syphilis, referral was made to local clinics for treatment.

All participants received voluntary counseling and testing from trained research nurses to review the interpretation of positive or negative HIV test results. In addition, extensive computer assisted self-interviewing questionnaires were administered at each visit. Questionnaires were administered via a tablet or computer in the clinic in the absence of clinical site staff to minimize bias or coercion when answering questions about potential risk factors for HIV acquisition, including sexual behaviors, condom use, and drug and alcohol use. A short questionnaire assessing willingness to participate in preventive vaccine trials was administered at study entry and at month eighteen. Questionnaires are detailed in Appendix C. The study was reviewed and approved by the institutional review boards at Walter Reed Army Institute of Research (WRAIR), Faculty of Tropical Medicine at Mahidol University, and the Royal Thai Army. All participants provided written informed consent prior to enrollment.

2.3. Outcomes

The primary objectives were to measure HIV incidence and participant retention over an eighteen-month period. Secondary objectives included determining prevalence of hepatitis B, hepatitis C, syphilis, and HIV; assessing risk factors associated with incident HIV; measuring willingness to participate in a future HIV preventive vaccine trial; evaluating knowledge, attitudes, and practices regarding PrEP; and studying HIV-1 molecular patterns in participants living with HIV.

2.4. Statistical analyzes

HIV incidence rates and 95% confidence intervals (CIs) were estimated by fitting a Poisson regression model with a log person-time offset term and using HIV seroconversion as the response variable. Participants at risk for HIV contributed person-time from enrollment until either an HIV infection or a censoring event. The censoring events were end of study observation, study withdrawal, loss to follow-up, and death. The censoring time for individuals who were lost to follow-up was set equal to the visit time of the last completed visit. Retention confidence intervals were evaluated assuming a normal approximation interval [8]. Individuals were considered retained at 18 months if they completed the final study visit. No censoring occurred in analyses of retention. Retention and visit completion were estimated with a 95% CI for each study visit. The majority of missingness was addressed by coding 'parent-child' dependencies appropriately. For example, if the answer to a parent question was no, the dependent child answer was coded as no. In cases where this was not possible, we developed 'if-then' statements for each with team review of code logic.

Descriptive statistics summarized data for each cohort by site. Willingness to participate in HIV vaccine trials and their respective 95% CIs were estimated using a normal approximation interval.

To evaluate variables associated with HIV sero-conversion, a survival analysis was conducted that included all participants from both study sites. A total of 137 socio-demographic and behavioral variables as listed in Appendix B were considered as possible predictors of incident HIV with the most impactful variables identified using a two-step process. First, a survival random forest model was used as a down selection tool, with two measures employed to rank the impact of each variable: minimal depth and variable importance (VIMP) as measured by Brieman-Cutler permutation [9]. The minimal depth indicates the average depth of the variable among all survival trees in the forest; smaller values of depth indicate greater importance. VIMP measures the change in the predictiveness of the random forest model when the variable is randomly permuted; large scores indicate importance. Since both minimal depth and VIMP measured the impact of a variable in different ways, we selected the union of the top K variables as scored by depth together with the top K variables in terms of VIMP. To select a proper value of K, we utilized the average out-of-bag (OOB) error rate as a measure of overall model predictiveness. To assess the error rate of models under consideration we utilized Harrell's predictive error rate to measure how well each model correctly ranked two random individuals in terms of their survival times [10]. For each bootstrapped sample we estimated the prediction on the OOB samples for models that were trained on in-the-bag data and averaged the OOB error over 1000 bootstrap simulations. The selected tree model and associated value of K was determined in accordance with the minimum plus one standard error rule, which selects a value K that is slightly larger than the value that minimizes the OOB error rate [11].

Variables from the concatenated list were used as candidate variables in several survival regression tree models. The final survival regression tree was selected by fitting a survival tree model to the union of the top K variables as identified by the VIMP or Depth metric. Predictor variables that were selected by the final survival regression tree model were used in unadjusted and adjusted Cox proportional hazards models to estimate hazard ratios and associated 95% CIs. Kaplan-Meier failure curves were generated for statistically significant predictors of incident HIV. The software used for the statistical analysis was R, and the packages used included rpart for the survival tree models and Random Forest SRC for the random forest models.

We chose the tree model for the following reasons: 1) Because of the many predictor variables from the risk questionnaire, the tree model was well-suited to handle high dimensionality regression tasks as a non-linear regression model and does not suffer from

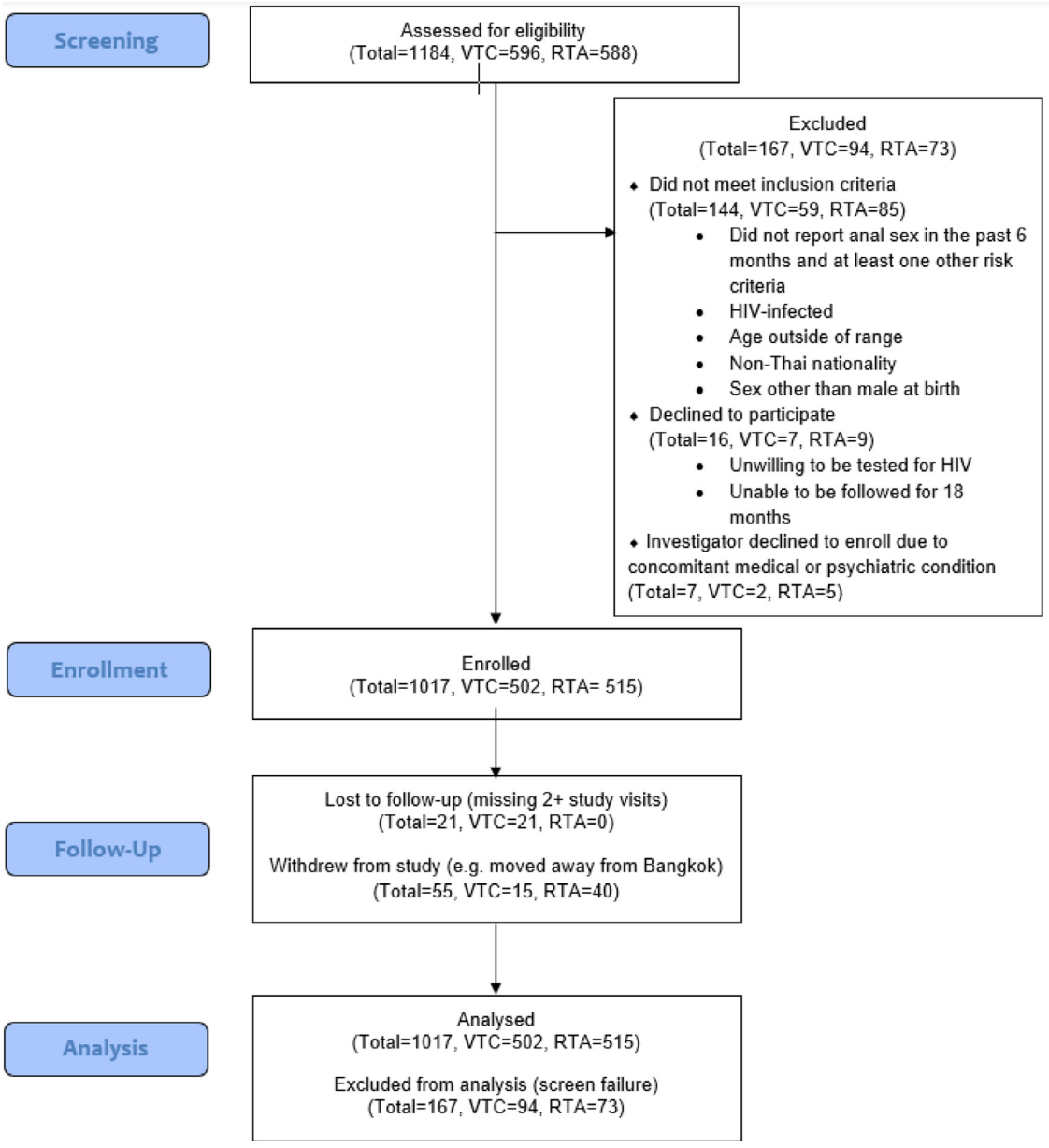

Fig. 1. Participant Flow Diagram.
This participant flow diagram displays the progression of participants through screening, enrollment, follow-up and analysis. A total of 1184 potential participants were screened and 1017 were eligible and enrolled. Twenty-one participants were lost to follow-up and 55 participants withdrew from the study. In total, 1017 participants were included in the study analysis.

multi-collinearity issues. 2) The prediction from a random forest model is the aggregate prediction from numerous tree models, so the prediction error is often much smaller than individual tree models, and 3) The method produces a decision tree that partitions risk in a way that is easy to interpret.

2.5. Role of the funding source

The funder was involved in approving study design, analysis, interpretation, and writing of the report. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

3. Results

A total of 1184 potential participants were screened and 1017 were eligible and enrolled (Fig. 1). Of the 1184 screened, 87 (7·1%) were excluded due to prevalent HIV (95% CI 5·9%–9·0%); HIV prevalence was 8% (95% CI 5·2–8·8%) among TGW compared to 6·8% for MSM (95% CI 5·7–11.0%; p = 0·46). The median age of enrolled participants was 22 years (interquartile range [IQR] 20–25) and 349 (34·3%) identified as TGW (Table 1). Over 75% of participants had completed at least some university, with most having income less than 15,000 baht (approximately USD 480) a month. Median age at sexual debut was 17 years (IQR 15–18). At enrollment, syphilis was observed in 39 (3·8%), hepatitis B surface antigen in 15 (1·5%), and hepatitis C antibody in 2 (0·2%). Willingness to participate in an HIV vaccine trial at study entry was 79·1% (95% CI 76·4%–81·5%). A total of 436 participants (43·9%) reported that they were previously aware of PrEP as an HIV prevention tool, and 42 (3·5%) participants reported using PrEP in the past, but no participants reported current PrEP use at enrollment. During the study, 41 (4·0%) participants reported PrEP use. The largest increase in PrEP use was noted between the Month 6 and Month 12 visits, with 30 participants indicating that they used PrEP during that time.

Table 1
Demographics of Study Population.

Characteristics	VTC (*n* = 502)	RTA (*n* = 515)	*p*-value
Age (years)			
18–20	188 (37·5%)	154 (29·9%)	0·0284
21–23	168 (33·4%)	182 (35·3%)	
23+	146 (29·1%)	179 (34·8%)	
Gender identity			
Cisgender man	339 (67·3%)	321 (62·3%)	0·1535
Transgender woman	161 (32·1%)	186 (36·1%)	
Other gender identity	3 (0·6%)	8 (1·6%)	
Highest level of education completed			
High school	45 (9·0%)	73 (14·2%)	0·0036
Vocational training	38 (7·6%)	27 (5·2%)	
Some university	288 (57·4%)	254 (49·3%)	
University	91 (18·1%)	117 (22·7%)	
Other education	40 (7·9%)	44 (8·6%)	
Income			
<15,000 Baht per month	352 (70·1%)	287 (55·7%)	0·0002
≥15,000 Baht per month Missing	146 (29·1%)	198 (38·4%)	
	4 (0.8%)	30 (5.9%)	
Length of Current Residence			
Less than 1 year	127 (25·3%)	111 (21·6%)	0·3632
1–2 years	88 (17·5%)	96 (18·6%)	
2+ years	279 (55·6%)	304 (59·0%)	
Missing	8 (1.6%)	4 (0.8%)	
Number of Sex partners in past six months			
1 to 3	214 (42·6%)	85 (16·5%)	<0·0001
4 to 7	178 (35·5%)	222 (43·1%)	
8 or more	102 (20·3%)	198 (38·5%)	
Missing	8 (1·6%)	10 (1·9%)	
Types of Sexual partners (lifetime)			
Cisgender men only	332 (66·1%)	418 (81·2%)	<0·0001
Cisgender women only	71.4)%	1	
		(0.2%)	
Transgender women only	21 (4·2%)	12 (2·3%)	
Cisgender women and cisgender men but not transgender women	40 (8·0%)	34 (6·6%)	
Cisgender men, cisgender women, and transgender women	42 (8·4%)	22 (4·3%)	
Cisgender men and transgender women but not cisgender women	13 (2.6%)	6 (1.1%)	
Cisgender women and transgender women but not cisgender men	47 (9.3)	22 (4.3%)	
History of sex work in past six months			
No	406 (80·9%)	389 (75·5%)	0·0392
Yes	96 (19·1%)	126 (24·5%)	
Condom use during anal sex			
Always or almost always	114 (22·7%)	113 (21·9%)	0·0289
Sometimes	77 (15·3%)	105 (20·4%)	
Rarely or Never	58 (11·6%)	92 (17·9%)	
Missing	253 (50.4%)	205 (39.8%)	
Receptive anal sex in past year			
No	233 (46·4%)	161 (31·3%)	<0·0001
Yes	269 (53·6%)	354 (68·7%)	
Lubricant use during anal sex			
Always or almost always	295 (58·8%)	351 (68·2%)	0·0005
Sometimes	120 (23·9%)	117 (22·7%)	
Rarely or Never	76 (15·1%)	41 (8·0%)	
Missing	11 (2.2%)	6 (1.1%)	
Sexually attracted to mostly men or only men			
No	15 (3·0%)	10 (1·9%)	0·3062
Yes	483 (96·2%)	504 (97·9%)	
Don't know/Refuse to answer	4 (0.8%)	1 (0.2%)	
Ever injected drugs			
No	464 (92·4%)	46,590·3%)	0·0574
Yes	33 (6·6%)	45 (8·7%)	
Missing	5 (1·0%)	5 (1·0%)	
Ever used methamphetamine			
No	440 (87·6%)	442 (85·8%)	0·0952
Yes	51 (10·2%)	68 (13·2%)	
Missing	14 (2·2%)	5 (1·0%)	
Used drugs prior to sex in past six months			
No	302 (60·2%)	301 (58·4%)	0·9183
Yes	189 (37·6%)	203 (39·4%)	
Missing	11 (2·2%)	11 (2·1%)	
Ever tested for HIV			
No	323 (64·3%)	270 (52·4%)	<0·0001
Yes	177 (35·3%)	244 (47·4%)	
Missing	2 (0·4%)	1 (0·2%)	
Ever heard of PrEP			
No	288 (57·4%)	220 (42·7%)	<0·0001
Yes	179 (35·7%)	267 (51·8%)	
Missing	35 (6·9%)	28 (5·5%)	

Enrollment characteristics of Thai men who have sex with men and transgender women by study site, Vaccine Trial center (VTC, Mahidol University), and RTA (Royal Thai Army). Responses were labeled 'missing' for those who responded 'don't know' as well as those that refused to answer. Other abbreviation: PrEP = pre-exposure prophylaxis, used for HIV prevention.

3.1. HIV incidence and retention

Over the eighteen-month study period, 53 incident HIV infections were identified, for an overall incidence rate of 3·73 per 100 person-years (PY) (95% CI 2·79–5·87). Participants aged 18–21 years experienced higher incidence (9·4 per 100 PY, 95% CI 6·3–13·4) compared to those 22 or older (4·6 per 100 PY, 95% CI 2·6–7·6; p = 0·003). HIV incidence was significantly higher at the RTA site compared to the VTC site (5·02 (95% CI 3·52–6·95) vs. 2·41 per 100 PY (95% CI 1·40–3·86, p = 0·03). However, HIV incidence among TGW and MSM was nearly identical; TGW had an incidence of 3·8 per 100 PY (95% CI 2·3–6·0) while MSM had an incidence of 3·7 per 100 PY (95% CI 2·5–5·1; p = 0·94). Median time to HIV seroconversion was 12 months (IQR 6–13). Overall retention was 93·4% (95% CI 91·6%–94·8%) and did not differ significantly by site (Fig. 2).

3.2. Risk factors for HIV seroconversion

To calculate the optimal number of variables to use in the statistical model for predictors of HIV incidence, we first calculated the OOB error rate by number of candidate variables. As shown in Fig. 3, the minimum OOB error rate was observed when K = 6. Adding one standard error to this revealed K = 7 as the optimal number of variables to include in the survival regression tree model. Since there were two separate measures for variable importance, the top 7 variables in terms of VIMP (Fig. 4A) and depth (Fig. 4B) were identified and de-duplicated to yield 12 unique variables for further evaluation. These variables were then used as candidate variables in a survival regression tree model that ultimately identified six key predictors of HIV acquisition (Fig. 5).

Sexual attraction mostly or only to men was the first variable that the tree selected. Among 227 participants who were not sexually attracted to mostly or only men (i.e., those attracted to mostly or only women, or to both men and women), only 1 acquired HIV over the course of the study, yielding a hazard ratio of 0·15. Among the 790 participants who were sexually attracted to mostly or only men, 52 acquired HIV, with a hazard ratio of 1·3.

The terminal nodes at the bottom of the tree revealed the highest risk group to be participants who were sexually attracted to mostly or only men, were younger than 20 years of age, and had sex with five or more partners in their lifetime. Out of 9 participants in this subgroup, 5 acquired HIV with a hazard ratio of 4·9. The next highest risk subgroup included participants who were sexually attracted to mostly or only men, were aged between 20 and 22 years, and reported prior HIV testing. Finally, the last subgroup with increased seroconversion risk included those who were sexually attracted to

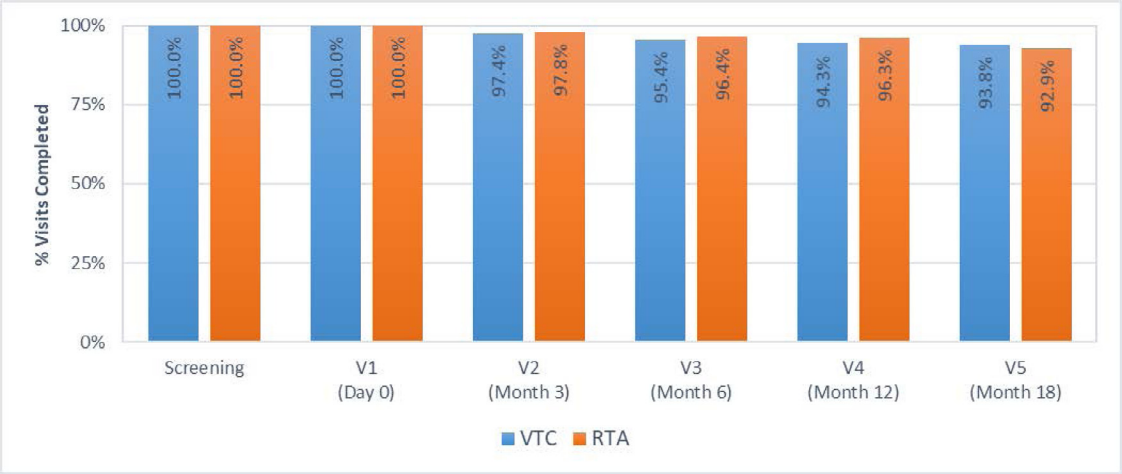

Fig. 2. Retention rate by visit and site.

Retention was defined as attending and completing the study visit. The x-axis shows the visit number and corresponding month of the visit. The Y axis shows the percentage of visits completed for all eligible cohort participants at that visit. Overall retention was 93·4% (95% CI 91·6%–94·8%) and did not differ significantly between Mahidol University (VTC) and Royal Thai Army Clinical Research center (RTA).

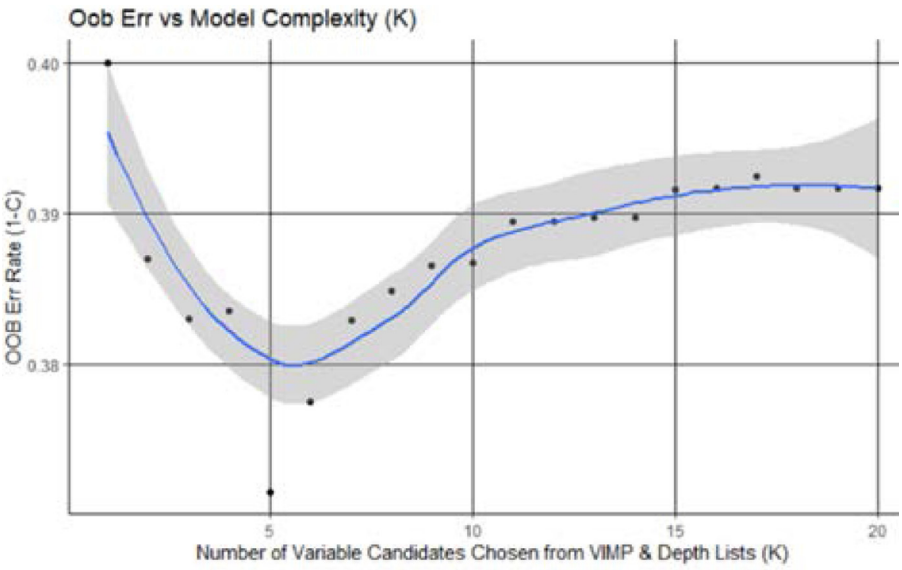

Fig. 3. Determination of the optimal number of variables to include in survival tree model.

In the plot of the out of bag (OOB) error rate versus model complexity (K), the dots reflect the OOB error for tree models built with K candidate variables and the blue line (along with grey confidence interval) is a LOWESS smoother which attempts to smooth out the randomness and estimate the mean OOB error rate curve.

mostly or only men, were aged 20 to 22 years, did not report prior HIV testing, and reported receptive anal sex in the past year.

In the multivariable Cox proportional hazards model, factors independently associated with incident HIV included younger age, prior HIV testing, being mostly or exclusively sexual attracted to men, reporting receptive anal intercourse in the past year, and having greater than five lifetime sexual partners (Table 2). Kaplan-Meier curves for risk of HIV seroconversion were constructed with the variables that were predictive of HIV in Cox proportional hazard regression models (Fig. 5, panels A-F). Specifically, age group, sexual attraction, number of lifetime sexual partners, frequency of condom use during anal sex with casual male partners, prior HIV testing, and history of receptive anal intercourse in the past 12 months were predictive of risk. While most variables were dichotomized as yes/no, age group and number of lifetime partners were automatically partitioned categorization by the survival regression tree model, indicating that those at highest HIV risk were aged 18–19, followed by those aged 20–22, and then ages 23 and above Fig. 6.

4. Discussion

Our study demonstrated both high HIV incidence and excellent retention over eighteen months among Bangkok-based MSM and TGW. Notably, MSM and TGW in our cohort had equivalent HIV incidence. This is important because prior studies of HIV incidence in Bangkok-based populations were unable to report disaggregated data and estimates of HIV risk among TGW have relied on cross-sectional data alone [12]. These incidences are similar to the HIV incidence of MSM reported by the Bangkok Men's Cohort Study (BMCS) study in 2015 (3·8 per 100 PY), suggesting that HIV incidence in Bangkok had remained sustained at this level through 2019, and slightly higher than HIV incidence (3·5 per 100 PY), measured in four provinces outside of Bangkok among a similar MSM/TGW population during 2015–2018 [11,12]. While there was some PrEP availability through demonstration projects, clinical trials offering access to PrEP such as HPTN 078, and self-pay programs, MSM and TGW in our cohort continued to seroconvert, underscoring limited PrEP knowledge and uptake in these populations [13, 14].

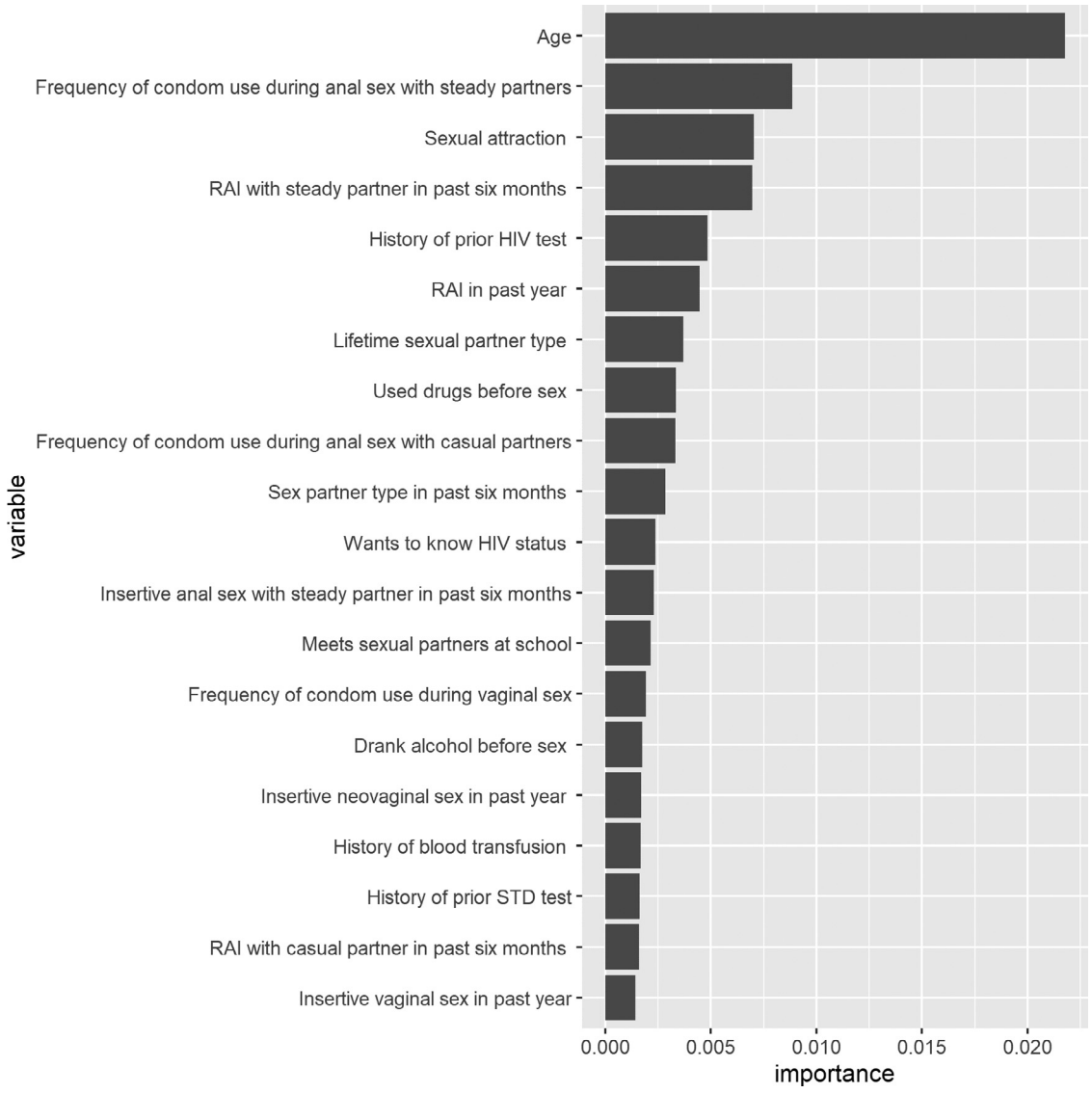

Fig. 4. Risk Factors for HIV Sero-conversion.

Figure 4a ranks the top 20 random forest variables in terms of Variable Importance (VIMP). VIMP measures the change in the predictiveness of the random forest model when the variable is randomly permuted; large scores indicate importance.

Figure 4b ranks the top 20 variables in terms of minimal depth. The minimal depth indicates the average depth of the variable among all survival trees in the forest; smaller values of depth indicate greater importance.

Both sites demonstrated their ability to retain MSM and TGW with an overall retention rate of 93·4%. Study teams noted the following strategies in helping to maintain retention: utilizing multiple platforms to keep in touch with participants (e.g. phone, text message, LINE© chat, Facebook™ messenger), updating contact information at each visit, providing a safe space for participants with snacks available, and emphasizing confidentiality. The BMCS, which recruited between 2006 and 2010, reported a loss-to-follow-up rate of 9·6% at three years in 2014 [15]. Factors associated with being lost to follow up in the BMCS included young age (18–21 years), low educational attainment, no previous HIV testing, living outside Bangkok, and identification as heterosexual or bisexual. More recently, MSM clients who attended an urban testing clinic in Bangkok were evaluated to measure testing adherence to Thai Ministry of Public Health regulations, which recommend twice annual testing for MSM. Of 1927 clients who attended the clinic for an initial HIV test, only 19% repeated at least one HIV test within six months. Notably, those who were aged 18–24 and had an HIV test were more likely to return for repeat testing [16].

By utilizing survival tree models that captured conditional associations, we were able to identify subgroups at highest risk for HIV sero-conversion that would not have been identified using traditional Cox proportional hazards models alone. Tree models are conditional models that do not suffer from multi-collinearity as do linear regression models [17,18]. In addition, the tree determines whether the node should be split by a variable, wherein all candidate variables have the opportunity to make splits. In the tree model, those with the highest risk of HIV sero-conversion were those who were attracted to men exclusively, between the ages of 18–20, and had five or more lifetime sexual partners. The characteristics of the specific subgroup of participants identified to have highest HIV risk could be considered as inclusion criteria for future HIV prevention studies.

Those who were 18–21 years had the highest risk of seroconversion, with HIV incidence of 9·4 per 100 PY (95% CI 6·3–13·4). This finding is consistent with prior studies of HIV incidence in Bangkok-based MSM, with the BMCS demonstrating highest HIV incidence in MSM less than 21 years of age between 2006 and 13 [19]. Prior HIV testing was conditionally important only for those who were aged

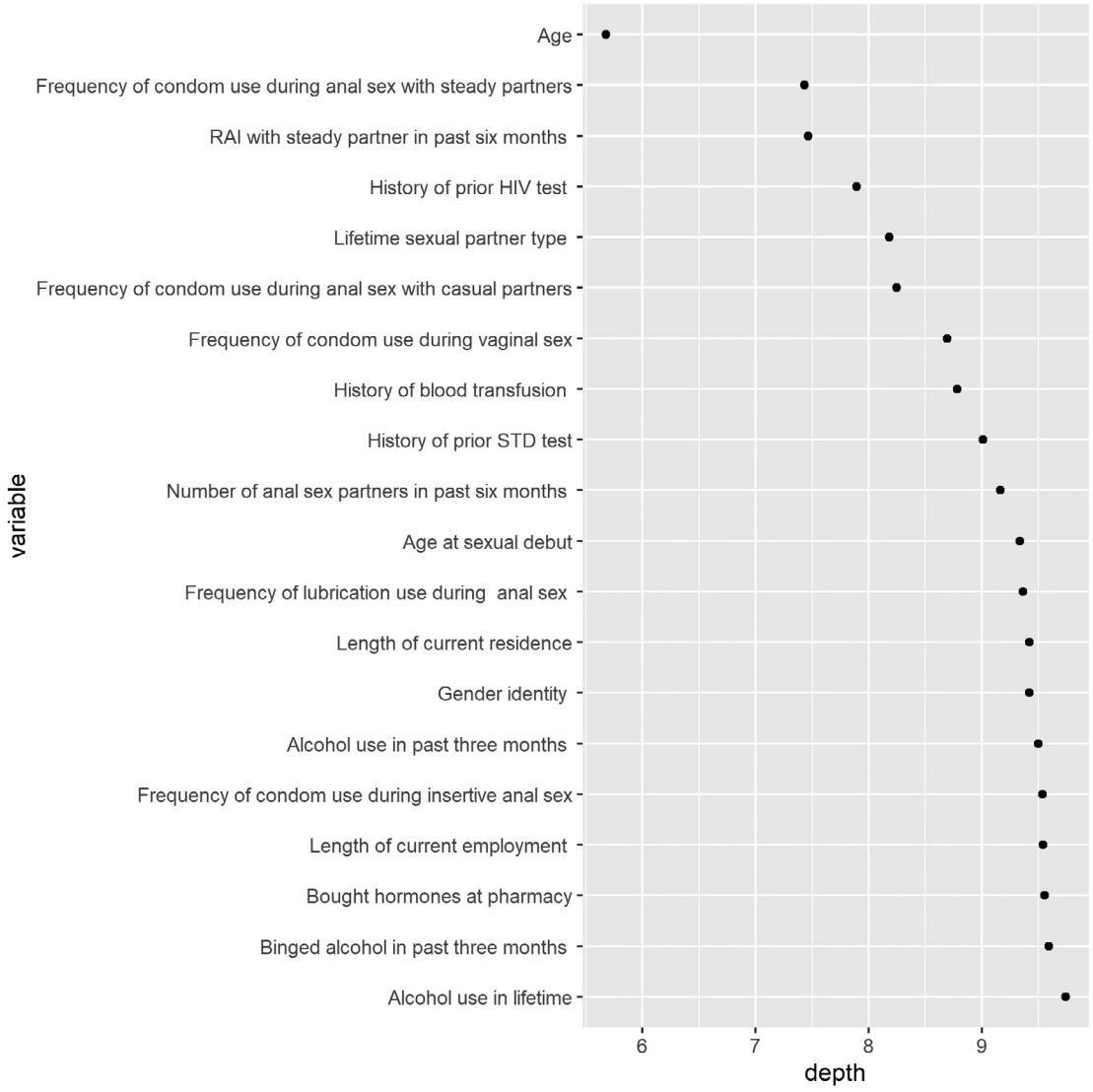

Fig. 4. Continued.

between 20 and 22 and mostly or only sexually attracted to men in the tree model for predictors of HIV seroconversion; participants who met these criteria were more than twice as likely to seroconvert as those who did not meet these criteria. The marginal effect derived from the Cox proportional hazards model was also about two-fold across the larger cohort population. In our cohort, 41·4% reported a prior HIV test. This was higher than a nationally reported average of 29% of MSM reporting prior HIV testing in 2018, and may be indicative of greater access to HIV testing in Bangkok relative to other locations through not only hospitals and clinics, but also key population-led health services targeting MSM and TGW [20].

Being mostly or only sexually attracted to men was the most strongly predictive risk factor for HIV sero-conversion in both tree and Cox proportional hazards models. While sexual attraction may not perfectly correlate with sexual behavior, near or exclusive attraction to men could be a surrogate marker for those who have sex with men only compared to those who have sexual partners of other genders, including transgender and cisgender women.

An Asia-based meta-analysis comparing men who have sex with men only (MSMO) and men who have sex with men and women (MSMW) revealed that MSMW had lower odds of reporting a prior HIV test than MSMO [21]. Although no difference in HIV prevalence was noted between MSMO and MSMW in the overall analysis, authors noted that in Southeast Asia, MSMO had higher HIV prevalence than MSMW. In addition, a US-based 2014 meta-analysis revealed that MSMW were less likely to engage in condomless anal intercourse with men. HIV prevalence among MSMW was lower than MSMO and men who have sex with women only [22]. In a recent survey of men who have sex with Asian men in Sydney and Melbourne, those who were tested for HIV regularly were more likely to have multiple partners, engage in condomless anal intercourse with both casual and regular partners, and were more likely to discuss HIV with both types of partners [23].

Initial PrEP awareness was low during our study. While all cohort participants received counseling regarding PrEP, few initiated PrEP during our study. We did not collect specific reasons for not accepting PrEP, but previous research in Thailand found that no or low HIV risk perception was the most reported reason to decline PrEP among MSM and TGW who were eligible [22].

One limitation of our study was that it intentionally sampled MSM and TGW who reported high risk of HIV acquisition in Bangkok, so may not be generalizable to those populations who do not engage in these behaviors and live in other geographic areas. Additionally, because the behavioral questionnaire and PrEP use relied on self-

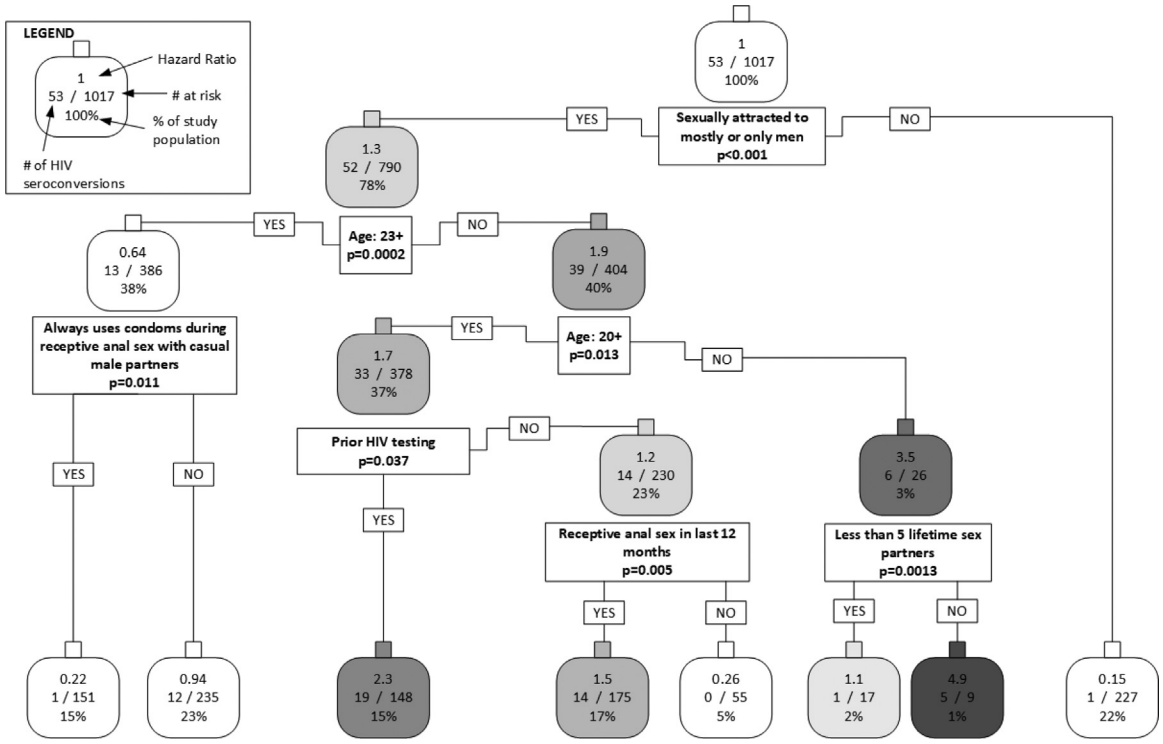

Fig. 5. Survival tree model with hazard ratios for risk of HIV sero-conversion.

Survival tree model with hazard ratios for risk of HIV sero-conversion utilizing top seven variables from the union of depth and variable importance lists. The "splitting" decisions made in survival regression trees are based on the log-rank statistic and each split in the tree represents a log rank test with a statistically significant difference. Thus, the first (dichotomous) variable that a tree model splits by is arguably the most important as it makes the largest difference in the hazard ratio. Below the root node at the top of the survival tree model, if the criterion below the node equals yes, the tree splits to the left; if it equals no, it splits to the right. Within each node, the first number is the hazard ratio relative to the overall study population. The second row in each node describes the number of people who seroconverted by the end of the study out of the number of people at risk in that node subgroup. The last row depicts the percentage of the total population represented by the node. The color of the node depicts the strength of the hazard ratio, with higher hazard ratios having darker shades.

Table 2
Unadjusted and adjusted hazard ratios of risk factors for HIV seroconversion.

Covariate	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Proportion by Covariate (%)*	Incidence rate and 95% CI per Response Category
Sexual Attraction				
Women/Both Men and Women	REF	REF	21.8%	0.3 (0.01–1.8)
Mostly or Only Men	8·7 (1·15–65·63)	14·91 (2·06–107·89)	77.7%	4.7 (3.5–6.2)
Age (Years)				
23+	REF	REF	41.5%	2.0 (1.0–3.5)
20–22	3·03 (1·59–5·8)	2·9 (1·53–5·52)	43.2%	5.3 (3.6–7.5)
18–19	13·97 (5·2–37·49)	10·88 (4·12–28·71)	15.3%	4.1 (1.9–7.7)
Number of Lifetime Sexual Partners				
Less than 5	REF	REF	43.5%	2.4 (1.3–4.0)
5 or more	2·02 (1·1–3·72)	2·01 (1·11–3·62)	54.8%	4.9 (3.5–6.7)
Condom Use Frequency During Receptive Anal Intercourse with casual male partners				
Always	REF	REF	11.7%	4.2 (1.7–8.7))
Not Always	1·53 (0·8–2·92)	2·43 (1·31–4·48)	52.8%	5.1 (3.6–7.0))
HIV Testing History				
Never tested for HIV	REF	REF	58.3%	2.6 (1.6–3.9)
Prior HIV testing	1·99 (1·14–3·48)	1·99 (1·14–3·46)	41.4%	5.4 (3.7–7.6)
Receptive Anal Intercourse (RAI) in Past Year				
No RAI	REF	REF	38.7%	1.7 (0.8–3.1)
RAI	1·4 (0·65–2·99)	2·89 (1·41–5·94)	61.3%	5.0 (3.7–6.8)

This table presents unadjusted and adjusted hazard ratios (HR) for HIV seroconversion with the reference category for each risk factor represented as REF. In the multivariate Cox proportional hazards model, significant risk factors for HIV seroconversion included sexual attraction to mostly or only men, younger age, higher number of lifetime sexual partners, inconsistent condom use during receptive anal intercourse with casual male partners, a history of prior HIV testing, and reporting receptive anal intercourse in the past year.

* Totals do not equal 100% due to non-applicability and/or missingness.

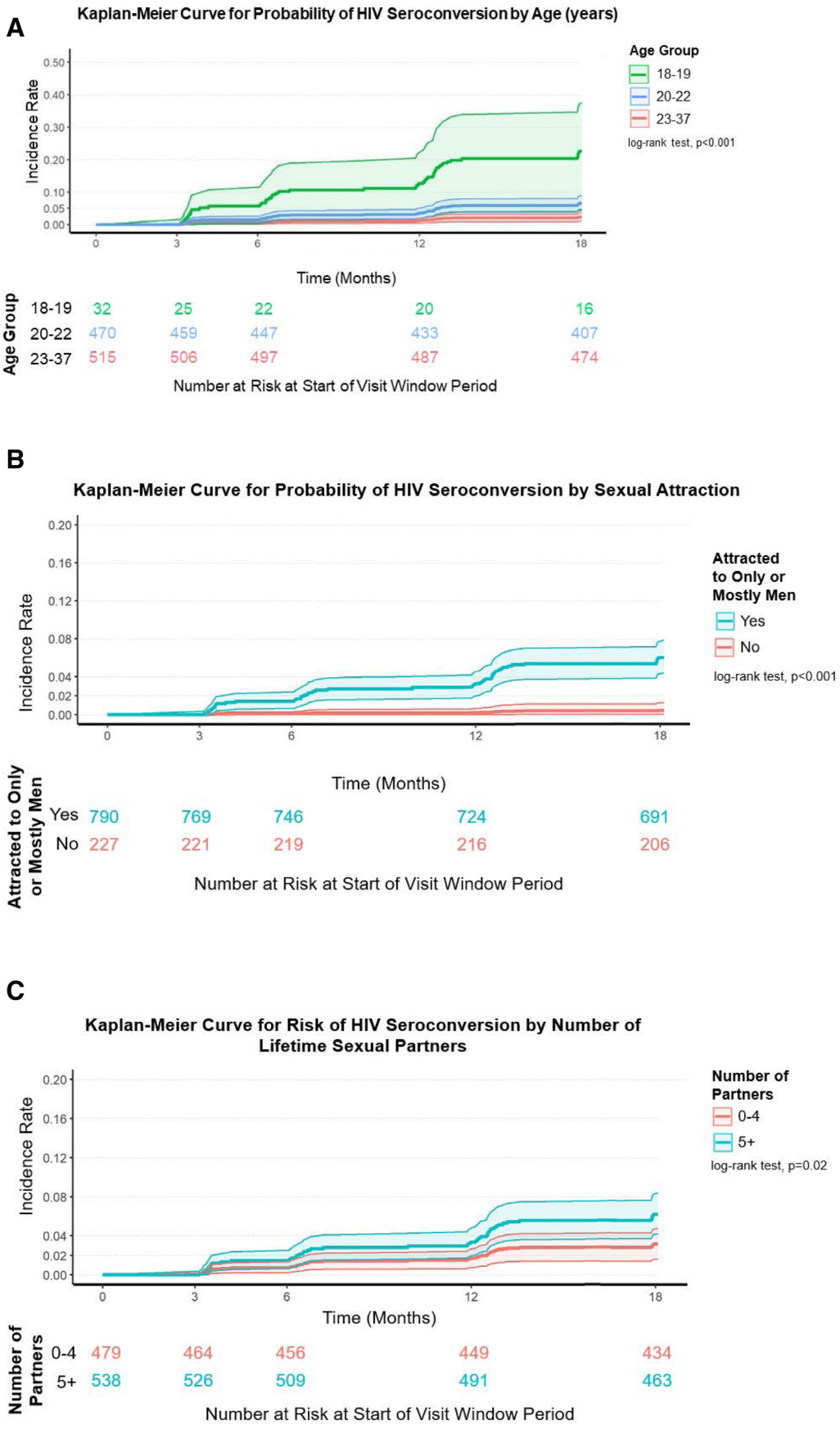

Fig. 6. Kaplan-Meier Curves for Risk of HIV Sero-conversion by Covariate.

This figure shows the Kaplan-Meier Curves for Risk of HIV Sero-conversion by Covariate. Panel A, Age. Panel B, Sexual Attraction Men. Panel C, Number of Lifetime Sexual Partners. Panel D, Frequency of Condom Use during Receptive Anal Sex with Casual Male Partners. Panel E. History of Prior HIV Testing; Panel F. History of Receptive Anal Intercourse in the Past Twelve Months. Note: to facilitate interpretation of distinctions between subgroups, we chose an upper Y-axis limit of 0.02, with the exception of Age, which has an upper limit of 0.05.

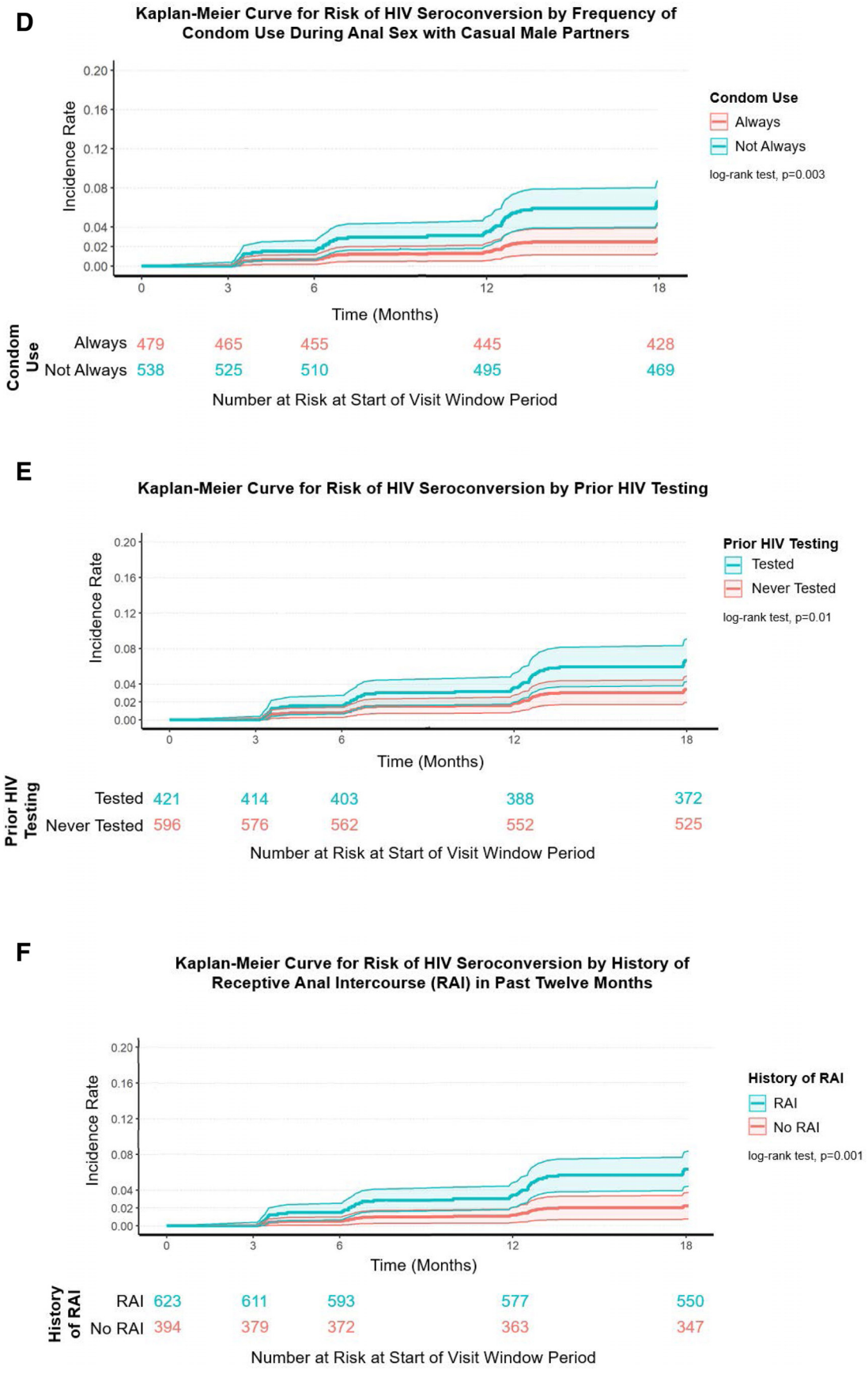

Fig. 6. Continued.

report, it may be subject to bias with underreporting of risk and/or PrEP use or recall bias. To minimize social desirability bias, the questionnaire was administered through a tablet or computer without clinic staff present, and only a few questions related to inclusion criteria were designated as mandatory, allowing the participant to choose to not answer questions that they did not want to answer.

In conclusion, our study documented high HIV incidence among MSM and TGW engaged in high risk behaviors in Bangkok, Thailand. More targeted HIV testing and prevention education geared at young MSM is necessary, and inclusion of youth in prevention trials should be further encouraged to capture this critical key population. Importantly, through this approach to risk analysis, a significant difference in HIV risk was identified between participants aged 18–19 years versus those aged 20–23 years. This highlights the critical need to targeting education, behavioral interventions, PrEP, and clinical trials toward a young age group. Since 2012, youth no longer need parental permission to test for HIV in Thailand, and waivers of consent (from parents or guardians) have been successfully utilized in adolescent sexual health trials. The populations reached by our two clinics in Bangkok had exceptional retention over 18-months and expressed high willingness to participate in future vaccine studies, indicating that they could serve as potential sites for efficacy testing of candidate HIV vaccines and other preventive interventions. The risk factors for HIV seroconversion observed in this study can inform the targeting of HIV prevention interventions and the development of inclusion criteria for studies to test these interventions, which should focus on young MSM and TGW who are sexually attracted to men and engage in condomless receptive anal sex.

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Contributors

T.W., M.L.R, K.S., E.A.H., P.P., R.J.O., and S.V. designed the study. T. W., S.M., S.N., S.K., P.G., D.R., J.D., K.S., S.A., P.P., R.J.O., and S.V. conducted the clinical study and data collection. T.W., S.K., T.C., L.F., Q.L., D.K., M.L.R, E.A.H., P.P., R.J.O., and S.V. conducted data interpretation. T.W., T.C., L.F., Q.L., D.K., and S.V. conducted data analysis. T.W., T.C., L. F., Q.L., D.K., and S.V. wrote the manuscript.

Declaration of Competing Interests

T.C. reports grants from U.S. Army, grants from NIH, M.R. and S.V. reports grants from US Army Medical Research and Materiel Command with Henry M. Jackson Foundation for the Advancement of Military Medicine, during the conduct of the study. All other authors report no potential conflicts.

Data sharing

Study protocol and informed consent documents are available online. Deidentified participant-level data and accompanying research resources are available upon request. Distribution of data will require compliance with all applicable regulatory and ethical processes.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2021.101033.

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