**Methods**

*Study Design*

This cross-sectional study was an identity-unlinked HIV seroprevalence study nested within a prospective randomized trial of ED-based HIV testing comparing targeted and non-targeted testing strategies. The HIV Testing using Enhanced Screening Techniques in EDs (HIV TESTED) trial was a multi-center, pragmatic randomized trial of different opt-out HIV screening strategies (ClinicalTrials.gov identifier: NCT01781949). Inclusion criteria for this trial were adults persons (at least 18 years of age) presenting to the ED without known HIV who were capable of consenting for medical care.1 We determined HIV status using an automated electronic medical record program that reviewed each chart and excluded patients with positive HIV antibody or viral load testing. ED patients were excluded from HIV TESTED by the following criteria: critical illness; altered level of consciousness; presenting to the ED as a victim of sexual assault, or occupational exposure. Eligible patients presenting to the ED were randomized to one of three arms: (1) non-targeted HIV testing, wherein all subjects were offered an HIV test by a triage nurse; (2) targeted HIV screening based on conventional risk characteristics as defined by the CDC in a Behavioral Risk Screening Tool (BRST);2 and (3) enhanced targeted HIV screening based on the Denver HIV Risk Score, a validated quantitative HIV risk prediction instrument.3 HIV TESTED operated 24 hours per day and seven days per week during the trial period.

*Risk Criteria for Targeted Testing*

Targeted testing using conventional risk characteristics in the BRST are shown in Table 1.2 Testing was offered if any of the following were present: injection drug use; sexual intercourse with someone with HIV; sexual intercourse with more than one partner without barrier protection; history of sexually transmitted infection, viral hepatitis, or tuberculosis; or history of an opportunistic infection. Subjects who denied any of these risk factors or who did not respond to the questions were not offered testing. Enhanced targeted screening used the Denver HIV Risk Score, which included age, sex, race/ethnicity, sex with a male, injection drug use, and HIV testing history (Table 1).3 Subjects whose Denver HIV Risk Score were ≥ 30 were considered at increased risk and offered HIV testing.

*Setting and Participants*

The Johns Hopkins Hospital (JHH) ED served as a study site for the HIV TESTED trial and was the setting for this identity-unlinked seroprevalence study. The JHH ED had 66,000 annual visits and a historically high seroprevalence of undiagnosed HIV.4 The JHH ED HIV testing and linkage-to-care program has been in operation since 2005. It offers and performs routine HIV screening to all eligible patients who were ages 18 and older but not critically ill, known HIV positive, or with altered mentation. Tailored post-test counseling is performed with all tested non-reactive patients and comprehensive counseling and the linkage-to-care services are provided for new HIV diagnoses, or for ED patients living with HIV who are not currently in care.

During the course of the trial, we conducted an identity-unlinked HIV seroprevalence study using methods previously described.5,6 Briefly, we collected remnant blood samples from all ED patients at this site for a six-week period from December 10, 2015, through January 21, 2016, during which time the HIV TESTED trial was ongoing. We stripped blood samples of identifiers and tested for HIV antibody using the Genetic Systems 3rd generation ELISA assay (BioRad, Redmond, WA). Positive results were confirmed by Western Blot (BioRad, Redmond WA). We performed ART testing of plasma specimen on all HIV-positive specimens with sufficient volume using HPLC-high resolution accurate mass spectrometry by the Clinical Pharmacology Laboratory and Pathology Reference Laboratory at our institution.7 Antiretroviral drugs in this analysis include abacavir, amprenavir, atazanavir, darunavir, efavirenz, emtricitabine, indinavir, lamuvidine, lopinavir, maraviroc, nelfinavir, nevirapine, raltegravir, rilpivirine, ritonavir, saquinavir, stavudine, tenofovir, tipranavir, and zidovudine.

We linked de-identified HIV testing results to a blinded identification code, which allowed for comparison of a limited set of demographic information (i.e., age, sex, race, and ethnicity) and information regarding patient’s ED disposition (admission versus discharge). We also linked de-identified HIV testing results to information regarding the subject's participation in the HIV TESTED study (i.e., ineligible or eligible). For those who participated in the trial, we determined their trial arm and whether they were offered, accepted, and completed HIV testing. Because of the de-identified nature of the data collected, no follow-up data were obtained on subjects for this seroprevalence study. Our primary outcome was the proportion of subjects with previously undiagnosed HIV who completed each component of the screening cascade.

*Statistical Analysis*

Previously undiagnosed HIV infection was operationally defined as no documented HIV diagnosis in the electronic medical record system within our institution by manual chart review (performed by AVP) and no ART detected in the remnant specimen if the subject had a new diagnosis of HIV in the identity-unlinked seroprevalence study. Descriptive data analyses were performed, followed by bivariate analyses using chi-square or Fisher’s exact tests for categorical data or t-tests for continuous data.

Ethics Approval

This study was approved by the Johns Hopkins Hospital Institutional Review Board.

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