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Predicting, Diagnosing, and Treating Acute and Early HIV Infection in a Public Sector Facility in Eswatini

Bernhard Kerschberger, MD, MPH,[a] Aung Aung, MD, MSc,[a] Qhubekani Mpala, BScN, MSc,[a] Nombuso Ntshalintshali, BSc,[a] Charlie Mamba, BSc,[a] Michael Schomaker, PhD,[b,c] Marie Luce Tombo, MD,[a] Gugu Maphalala, BSc, MSc,[d] Dumile Sibandze, MSc,[d] Lenhle Dube, BSc, MPH,[e] Rufaro Kashangura, MD, MClinEpi,[f] Simangele Mthethwa-Hleza, MBChB, MSc,[e] Alex Telnov, MD,[g] Roberto de la Tour, MSc, PhD,[g] Alan Gonzalez, MD, MSc,[g] Alexandra Calmy, MD, PhD,[h,i] and Iza Ciglenecki, MD[g,i]

Background: The lack of acute and early HIV infection (AEHI) diagnosis and care contributes to high HIV incidence in resource-limited settings. We aimed to assess the yield of AEHI, predict and diagnose AEHI, and describe AEHI care outcomes in a public sector setting in Eswatini.

Setting: This study was conducted in Nhlangano outpatient department from March 2019 to March 2020.

Methods: Adults at risk of AEHI underwent diagnostic testing for AEHI with the quantitative Xpert HIV-1 viral load (VL) assay. AEHI was defined as the detection of HIV-1 VL on Xpert and either an HIV-seronegative or HIV-serodiscordant third-generation antibody-based rapid diagnostic test (RDT) result. First, the cross-sectional analysis obtained the yield of AEHI and established a predictor risk score for the prediction of AEHI using Lasso logistic regression. Second, diagnostic accuracy statistics described the ability of the fourth-generation antibody/p24 antigen–based Alere HIV-Combo RDT to diagnose AEHI (vs Xpert VL testing). Third, we described acute HIV infection care outcomes of AEHI-positive patients using survival analysis.

Results: Of 795 HIV-seronegative/HIV-serodiscordant outpatients recruited, 30 (3.8%, 95% confidence interval: 2.6% to 5.3%) had AEHI. The predictor risk score contained several factors (HIV-serodiscordant RDT, women, feeling at risk of HIV, swollen glands, and fatigue) and had sensitivity and specificity of 83.3% and 65.8%, respectively, to predict AEHI. The HIV-Combo RDT had sensitivity and specificity of 86.2% and 99.9%, respectively, to diagnose AEHI. Of 30 AEHI-positive patients, the 1-month cumulative treatment initiation was 74% (95% confidence interval: 57% to 88%), and the 3-month viral suppression (<1000 copies/mL) was 87% (67% to 98%).

Conclusion: AEHI diagnosis and care seem possible in resource-limited settings.

Key Words: acute HIV infection, AHI, AEHI, ART, HIV, viral load

(*J Acquir Immune Defic Syndr* 2021;88:506–517)

Received for publication April 23, 2021; accepted August 12, 2021.

From the [a]Médecins Sans Frontières (OCG), Mbabane, Eswatini; [b]Centre for Infectious Disease Epidemiology and Research, School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa; [c]Institute of Public Health, Medical Decision Making and Health Technology Assessment, UMIT—University for Health Sciences, Medical Informatics and Technology, Hall in Tirol, Austria; [d]Ministry of Health (NRL), Mbabane, Eswatini; [e]Ministry of Health (SNAP), Mbabane, Eswatini; [f]Ministry of Health (Nhlangano Health Centre), Nhlangano, Eswatini; [g]Médecins Sans Frontières (OCG), Geneva, Switzerland; [h]HIV/AIDS Unit, Division of Infectious Diseases, Geneva University Hospitals Geneva, Switzerland; and [i]Institute of Global Health, University of Geneva, Geneva, Switzerland.

The authors have no conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

Correspondence to: Bernhard Kerschberger, Médecins Sans Frontières (MSF), P.O. Box 18, Eveni, Lot No. 331, Sheffield Road, Industrial Area, Mbabane, Eswatini (e-mail: bernhard.kerschberger@gmail.com).

INTRODUCTION

The World Health Organization (WHO) recommends antiretroviral therapy (ART) initiation at the time of HIV diagnosis to improve patient-level outcomes and reduce onward HIV transmission.[1] This policy (treat-all) has been implemented by 93% of countries in resource-limited settings (RLS) in 2019.[2]

The impact of treat-all on reduction of HIV incidence remains inconclusive,[3] possibly due to the inability to diagnose acute and early HIV infection (AEHI) in RLS.[4] AEHI—the time between HIV acquisition and detection of HIV antibodies or early seroconversion—presents with high HIV viral load (VL), is highly infectious, and contributes 3%–20% of all HIV infections in sub-Saharan Africa.[4] Diagnosing AEHI, prompt ART initiation, and identification of further contacts may reduce HIV transmission, particularly in settings with risky sexual networks and high HIV incidence.[5,6] At the patient level, ART initiation during AEHI reduces viral reservoirs and chronic inflammation and improves the immune function.[7–10]

Barriers to AEHI care in RLS include lack of awareness of AEHI among health workers, with it being rarely suspected in symptomatic patients.[4,11] Diagnostic barriers are the inability to detect AEHI with routinely used third-generation antibody-based rapid diagnostic tests (RDTs), the suboptimal performance of fourth-generation antibody/antigen RDT, and the high costs of HIV VL assays.[4] Finally, WHO has not provided specific policy guidance for AEHI care in RLS.[1]

Eswatini has expanded HIV care and, despite high ART coverage among people living with HIV (74.1%), HIV incidence (1.36% per 100 person-years) in individuals aged 15 years or older remained high in 2016/2017.[12] To further reduce HIV transmission, Médecins Sans Frontières and the Ministry of Health operationalized AEHI diagnosis and care in a public sector facility. We aimed (1) to assess the burden of AEHI, (2) to establish a screening algorithm to predict AEHI, (3) to evaluate the performance of a novel RDT to diagnose AEHI, and (4) to describe AEHI care outcomes.

METHODS

Setting

This study was conducted in the outpatient ward of Nhlangano facility with approximately 7000 general outpatient consultations each month. It is located in the small Nhlangano town, the capital of the southern predominantly rural Shiselweni region (population approximately 204,000) of Eswatini that had a high HIV prevalence (27%) and incidence (1.36%) in individuals aged 15 years or older in 2016/2017.[12,13]

Study Eligibility and Definitions

Patients (aged 18–49 years) at risk of AEHI were recruited after routine HIV testing at Nhlangano outpatient department from March 2019 to March 2020. The HIV serostatus was determined in a serial testing algorithm with the third-generation antibody-based AlereDetermine RDT, followed by Uni-Gold RDT. Being at risk of AEHI was defined as presenting with (1) an HIV-serodiscordant RDT result (AlereDetermine positive and Uni-Gold negative); or (2) an HIV-seronegative RDT result (AlereDetermine negative) and symptoms suggestive of AEHI within the past 3 days [sore throat, complaints suggestive of sexually transmitted infection (STI), self-reported fever, or current axillary temperature $>37.5°C$][14–18]; or (3) transfer in as an HIV-seronegative/HIV-serodiscordant patient from the pre-exposure prophylaxis (PrEP)/postexposure prophylaxis (PEP) program due to suspicion of AEHI.

The AEHI status of study participants was determined through diagnostic VL testing with the Xpert HIV-1 VL assay (lower limit of detection: 40 viral copies/mL). The pragmatic definition of AEHI required both a detectable HIV-1 VL and either an HIV-seronegative or HIV-serodiscordant RDT result as according to the standard third-generation antibody-based serial testing algorithm. To rule out possible false-positive initial VL results,[19,20] AEHI was confirmed only if the VL measurement was 10,000 copies/mL or the second PCR-tested sample was also detectable for participants with the first VL measurement between 40 and 9999 copies/mL.VL results indicating that the target was detected but not quantifiable were considered undetermined, and a VL follow-up test was recommended.

Procedures

Figure 1 summarizes the study flow. At outpatient care registration, a nurse identified patients at risk of AEHI by asking about study eligibility criteria and HIV status. Irrespective of study eligibility, provider-initiated RDT testing was performed for patients with unknown or undisclosed HIV status using whole blood finger stick by HIV testing counselors. Only study eligible patients were referred to the study room to give informed written consent.

The nurse performed a physical examination and administered a questionnaire assessing sociodemographic, behavioral, and clinical factors. Paired whole venous blood samples were also obtained through venepuncture.

A laboratory technician prepared the blood samples and performed diagnostic VL testing (Xpert). Paired leftover whole blood and plasma specimens were tested with the HIV-Combo RDT.

Patients diagnosed with acute HIV infection (AHI) received counseling and were offered immediate ART initiation with tenofovir, lamivudine, and dolutegravir.[21] Baseline laboratory tests (CD4, biochemistry, and hemoglobin) were performed routinely. Dried blood spot collection cards were prepared and shipped for HIV genotypic drug-resistance testing at the Geneva University Hospital, Switzerland.

ART care visits were scheduled at 2 and 4 weeks, followed by monthly visits until 6 months. At each visit, CD4 and VL tests were performed. Patients were followed up until the first occurrence of an unfavorable treatment outcome (no-show to facility, death), transfer out of the facility, or database closure (March 31, 2020). Patients were encouraged to bring sexual partners with unknown HIV status to the facility for RDT and Xpert testing.

Analyses and Statistics

Analyses were performed in Stata 16. Multiple imputation by chained equations was used to account for missing baseline covariate values with 10 imputed data sets created. Occupation status had 27.2% missing values, 4 covariates had between 8% and 10% missing data, and 8 covariates had less than 3% missing data (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/B719). Predictors of study enrollment among eligible presumptive AEHI cases were assessed with logistic regression.

Yield of AEHI

From the cross-sectional sample of presumptive AEHI cases, we obtained the prevalence of AEHI by dividing the total number of AEHI cases with the total number of study participants. To also obtain the yield of VL-positive but

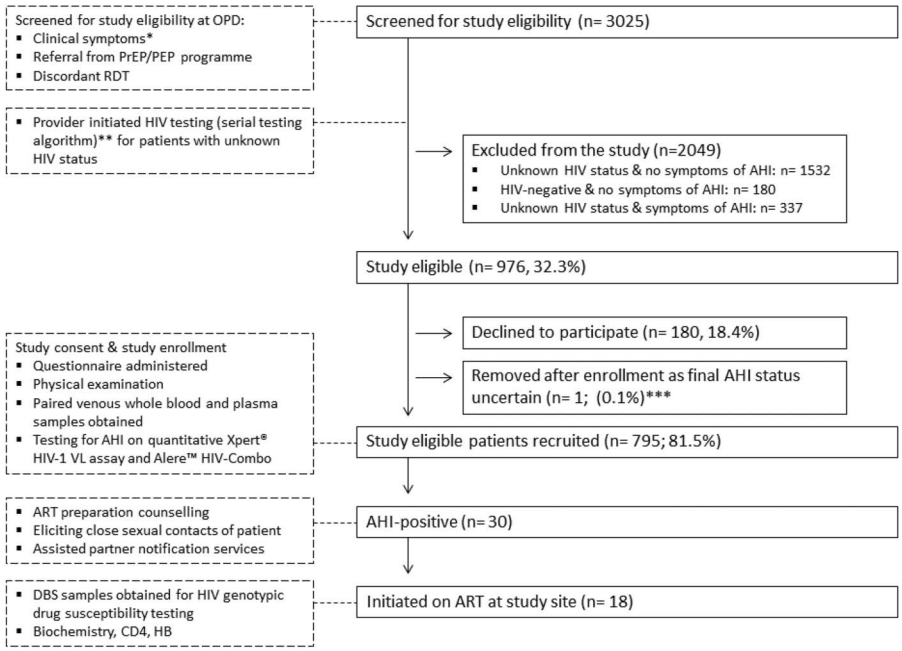


FIGURE 1. Study flow and main procedures.

antibody-negative AHI cases, we excluded cases from the study sample who were HIV-serodiscordant as per the third-generation RDT testing algorithm. Then, we divided the number of AHI cases by the total number of HIV-seronegative patients enrolled.

Predictors Risk Score

From the cross-sectional sample, we developed a symptom-based and behavior-based predictors risk score (PRS) algorithm for the prediction of AEHI. The least absolute shrinkage and selection operator (Lasso)[22] for logistic regression was used on the imputed data sets to determine these predictors (see Text 1, Supplemental Digital Content, http://links.lww.com/QAI/B719). Model fitting and variable selection with Lasso have the advantage over traditional logistic regression that the model's tuning parameters are based on cross-validation; thus, the model is built to optimize predictive performance. Standardized beta coefficients of factors identified by Lasso were averaged across imputed data sets (ie, combined according to Rubin rules[23]), multiplied by 10, and then rounded to the first decimal place[24] to obtain predictor scores that were summed up for each patient for the overall PRS. The optimal diagnostic cut-off levels of the PRS were obtained by receiver operating characteristic (ROC) curve and the area under ROC (AUC) statistics. Other test characteristics [sensitivity, specificity, positive predictive value (PPV)/negative predictive value (NPV)] were evaluated at the 2 main identified cut-off levels of the PRS. Second, performance of external PRS reported from 3 studies in sub-Saharan Africa (Kenya, Malawi, and South Africa)[14–16] that use a similar definition of AEHI was assessed in our patient sample through ROC/AUC statistics.

Evaluation of HIV-Combo RDT

We assessed the performance of the lateral flow point-of-care fourth-generation RDT AlereHIV-Combo[25] for the diagnosis of AEHI when compared with diagnostic VL testing. The HIV-Combo RDT detects free HIV-1 p24 antigen and HIV-1/HIV-2 antibodies.[25] VL results from Xpert were categorized as AEHI-positive and AEHI-negative as per the study definition of AEHI. Results of the HIV-Combo were classified as AEHI-positive for reactivity on the p24 antigen and/or antibodies bars. Test characteristics were evaluated separately for plasma (HIV-Combo-plasma) and whole blood (HIV-Combo-wb) testing compared with VL testing. Supplementary analysis restricted the analysis to patients presenting with AHI.

AEHI Care Outcomes

We describe baseline factors including pretreatment antiretroviral resistance among patients diagnosed with AEHI. Kaplan–Meier estimates and plots were computed to describe crude programmatic outcomes for time from AHI diagnosis to ART initiation and viral outcomes (viral suppression defined as <1000 copies/mL and undetectable VL defined as <40 copies/mL or VL target detected but not quantifiable) and for time from ART initiation to care retention. Cox regression models were fitted to identify associations for time from AEHI diagnosis to undetectable VL. Finally, partner notification outcomes were described.

Ethics

The study was approved by Médecins Sans Frontières Ethics Review Board and the Eswatini National Health Research Review Board.

RESULTS

Enrollment

We screened 3025 outpatients for being at risk of AEHI, of whom 1612 were study ineligible and 337 (11.1%) showed symptoms suggestive of AEHI but remained with an undocumented HIV status (Fig. 1). Of the remaining 976 (32.3%) HIV-seronegative/HIV-serodiscordant presumptive AEHI patients, 180 (18.4%) declined to participate and 1 (0.1%) was excluded from analysis because of undetermined AEHI classification after VL testing. A total of 795 (81.5%) patients were retained for analysis. The only predictor clearly decreasing the odds of study enrollment among presumptive AEHI cases (n = 976) was later study enrollment period (see Table S2, Supplemental Digital Content, http://links.lww.com/QAI/B719).

Presumptive AEHI Cases

Of presumptive AEHI cases (n = 795) enrolled, the median age was 26 [interquartile range (IQR) 23–30] years and 56.1% was women. Screening symptoms at care registration comprised complaints suggestive of STI (50.2%), self-reported fever during the previous 3 days (41.5%), sore throat (38.6%), and current temperature ≥37.5°C (8.6%). Fourteen (1.8%) patients had an HIV-serodiscordant RDT result. Eight (1.0%) asymptomatic patients were referred from the PrEP/PEP program. Other baseline characteristics of patients enrolled are sumarized in Table 1.

Burden of AEHI

Of 795 patients undergoing VL testing, 30 [3.8%, 95% confidence interval (CI): 2.6% to 5.3%] presented with AEHI with a median VL of 132,400 (IQR 18,500–903,000) copies/mL. Seven (23%) patients had 2 detectable VLs <10,000 copies/mL and the remaining 23 had a high VL more than 10,000 copies/mL, and 16 had more than 100,000 copies/mL. None of the patients had an undetermined VL result (target detected but not quantifiable). Excluding patients with HIV-serodiscordant RDT results (n = 14) at enrollment, 21 of the remaining 781 patients (2.7%, 95% CI: 1.8% to 4.1%) presented with AHI (as according to our post hoc definition).

Predicting AEHI

The univariate odds of presenting with AEHI was higher for HIV-serodiscordant RDT result, some sociodemographic (women, being pregnant/lactating) and behavioral (sexual partner ≥10 years older, desire for children, feeling at risk of HIV) factors, self-reported symptoms (swollen glands), and physical examination signs (pharyngitis, oral ulcer, oral thrush, and herpes simplex infection) (Table 1).

Study-specific PRS

The PRS comprised the following variables with standardized beta coefficients/risk scores (Table 2): serodiscordant RDT result (4.9), women (1.3), feeling at risk of HIV (0.1), self-reported swollen glands (1.0), and fatigue (0.5). The ROC curve and test characteristics of the PRS are presented in Figure 2, with 2 main cut-off levels identified. At the cut-off of ≥1.4 points, sensitivity and specificity were 83.3% and 65.8%, respectively. At the cut-off of ≥1.6 points, sensitivity decreased to 53.3% and specificity increased to 88.1%. Although NPV was high (≥97.7%) for both cut-off levels, the PPV was low (8.7% at cut-off ≥1.4; 15.0% at cut-off ≥1.6).

Comparison With External PRS

No single predictor factor was found in all PRS (Table 2). The study-specific predictors of serodiscordant RDT result and fatigue were reported in 2 and one external PRS, whereas other factors were identified only in the study-specific PRS (women, feeling at risk of HIV, self-reported swollen glands). The study-specific PRS (AUC 0.83, 95% CI: 0.76 to 0.90) had the highest ability to predict AEHI, followed by the external PRS-1 (AUC 0.74, 95% CI: 0.63 to 0.84) (Fig. 2).

Performance of HIV-Combo RDT

AEHI-Positive Cases

Of paired test results available for HIV-Combo-plasma (n = 745) and HIV-Combo-wb (n = 429), 3.9% (n = 29) and 4.4% (n = 19) were AEHI-positive according to Xpert testing. Among AEHI-positive cases, 25 of the 29 (86.2%) and 15 of the 19 (78.9%) were also detected by HIV-Combo-plasma and HIV-Combo-wb, respectively (see Table S3, Supplemental Digital Content, http://links.lww.com/QAI/B719). Most of the cases detected by HIV-Combo were reactive to antibodies only, followed by reactivity to p24 antigen only and reactivity to both p24 and antibodies (Fig. 3). There was one false-positive reactive case for both HIV-Combo-plasma and HIV-Combo-wb when compared with Xpert testing. The AUC was 0.93 and 0.89 for HIV-Combo-plasma and HIV-Combo-wb, respectively. The sensitivity tended to be slightly higher for HIV-Combo-plasma (86.2% vs 78.9%), and specificity was high for both the tests (≥99.8%) (Table 3). The NPV was above 99.0% for both the tests, and the PPV was 93.8% for HIV-Combo-wb and 96.2% for HIV-Combo-plasma.

AHI-Positive Cases

Restricting analysis to AHI-positive cases only, the test characteristics of the HIV-Combo for the detection of AHI were similar to AEHI although the point estimates of AUC, sensitivity, and PPV were slightly lower (Fig. 3 and Table 3, Table S3, http://links.lww.com/QAI/B719).

AEHI Care Outcomes

Baseline Characteristics

The median age of the 30 AEHI patients was 25.5 (IQR 23–29) years, and 80% was women (see Table S4, Supplemental Digital Content, http://links.lww.com/QAI/B719). Overall, 80% of patients felt at risk of HIV infection. Almost all (n = 29, 97%) reported current

TABLE 1. Baseline Characteristic of AEHI-Negative and AEHI-Positive Patients and Univariate Predictors of AEHI Infection (n = 795)

	AEHI-Negative		AEHI-Positive			Univariate Analysis (n = 795)	
	n	%	n	%	*P*	cOR	95% CI
Enrollment factors							
Calendar time							
2019-q1	31	4.1	1	3.3	0.239	2.12	0.32 to 13.98
2019-q2	200	26.1	4	13.3		1	
2019-q3	193	25.2	12	40.0		2.88	0.96 to 8.61
2019-q4	157	20.5	8	26.7		2.40	0.75 to 7.68
2020-q1	184	24.1	5	16.7		1.33	0.38 to 4.69
Referral from PrEP/PEP program	18	2.4	0	0.0	0.395	0.66	0.04 to 11.25
Discordant RDT	**5**	**0.7**	**9**	**30.0**	**<0.001**	**61.10**	**19.65 to 189.94**
Sore throat	295	38.6	12	40.0	0.874	1.08	0.52 to 2.24
Reported symptomatic STI	382	49.9	17	56.7	0.469	1.30	0.63 to 2.68
Reported fever	321	42.0	9	30.0	0.192	0.61	0.28 to 1.33
Temperature ≥37.5°C (9.2%)	59	8.5	3	10.7	0.682	1.36	0.43 to 4.33
Sociodemographic factors							
Age, yrs							
18–25	270	35.3	11	36.7	0.713	1	
25–34	407	53.2	17	56.7		1.01	0.47 to 2.16
35–49	88	11.5	2	6.7		0.66	0.17 to 2.66
Women	**422**	**55.2**	**24**	**80.0**	**0.007**	**3.06**	**1.28 to 7.36**
Pregnant/breastfeeding	14	1.8	2	6.7	0.064	**4.55**	**1.13 to 18.31**
Education (2.8%)							
None/primary	79	10.6	2	6.9	0.645	1	
Secondary	588	79.0	25	86.2		1.44	0.39 to 5.38
Tertiary	77	10.3	2	6.9		1.16	0.19 to 6.88
Occupation (27.2%)							
Employed	327	58.9	15	62.5	0.552	1	
Partial income	43	7.7	3	12.5		1.75	0.54 to 5.73
Unemployed	185	33.3	6	25.0		0.88	0.36 to 2.15
Migrant	11	1.4	0	0.0	0.508	1.08	0.06 to 18.68
MSM	7	0.9	0	0.0	0.599	1.66	0.09 to 29.70
Sex worker	4	0.5	0	0.0	0.691	2.77	0.15 to 52.69
Drug user	24	3.1	0	0.0	0.325	0.50	0.03 to 8.35
Behavioral factors							
Sexual partners							
0	36	4.7	0	0.0	0.425	1	
1	455	59.5	20	66.7		3.29	0.19 to 55.43
≥2	274	35.8	10	33.3		2.79	0.16 to 48.66
≥1 HIV-positive partner(s) (8.2%)	56	8.0	3	10.0	0.694	1.35	0.43 to 4.26
≥1 partner(s) with unknown HIV status (8.2%)	411	58.7	19	63.3	0.615	1.24	0.59 to 2.62
Main partner's age difference (0.9%)							
≥10 yrs younger	**26**	**3.8**	**1**	**3.4**	**0.040**	1.26	0.23 to 7.02
5–9 yrs younger	**119**	**17.2**	**1**	**3.4**		0.29	0.05 to 1.57
Same age (±4 yrs)	**416**	**60.1**	**16**	**55.2**		1	
5–9 yrs older	**100**	**14.5**	**7**	**24.1**		1.83	0.75 to 4.48
≥10 yrs older	**31**	**4.5**	**4**	**13.8**		**3.35**	**1.12 to 10.05**
Sex without condom (2.3%)	261	34.9	10	33.3	0.856	0.95	0.44 to 2.03
Paid sex (1.3%)	70	9.3	4	13.3	0.455	1.64	0.59 to 4.60
Sex under alcohol, (1.1%)	112	14.8	4	13.3	0.822	0.96	0.35 to 2.67
Pervious PrEP/PEP use (2.0%)	42	5.6	1	3.3	0.593	0.83	0.16 to 4.39
Intended PrEP use (2.4%)	410	54.8	18	64.3	0.322	1.44	0.67 to 3.09
Desire for children (1.1%)	**267**	**35.3**	**16**	**53.3**	**0.044**	**2.07**	**1.01 to 4.25**
At risk of HIV infection (1.4%)	**387**	**51.3**	**23**	**76.7**	**0.006**	**2.94**	**1.28 to 6.78**
Reported symptoms							

TABLE 1. (*Continued*) Baseline Characteristic of AEHI-Negative and AEHI-Positive Patients and Univariate Predictors of AEHI Infection (n = 795)

	AEHI-Negative		AEHI-Positive			Univariate Analysis (n = 795)	
	n	%	n	%	*P*	cOR	95% CI
Headache	272	35.6	10	33.3	0.803	0.93	0.43 to 1.98
Red eyes	70	9.2	5	16.7	0.167	2.13	0.82 to 5.52
Ulcer in mouth	22	2.9	1	3.3	0.883	1.68	0.31 to 9.14
Diarrhea	48	6.3	0	0.0	0.157	0.24	0.01 to 4.03
Weight loss	46	6.0	4	13.3	0.105	2.63	0.93 to 7.45
Body pain	87	11.4	5	16.7	0.374	1.67	0.65 to 4.32
Fatigue	119	15.6	8	26.7	0.103	2.04	0.91 to 4.61
Swollen glands	**27**	**3.5**	**4**	**13.3**	**0.007**	**4.56**	**1.57 to 13.29**
Cough	117	15.3	3	10.0	0.427	0.70	0.23 to 2.17
Night sweat	41	5.4	3	10.0	0.276	2.22	0.70 to 7.06
Abdominal pain	180	23.5	10	33.3	0.217	1.66	0.78 to 3.56
Anogenital discomfort	236	30.8	9	30.0	0.921	0.99	0.45 to 2.16
Physical examination signs							
Conjunctivitis	19	2.5	2	6.7	0.161	3.36	0.85 to 13.20
Pharyngitis	28	3.7	3	10.0	0.078	**3.29**	**1.02 to 10.65**
Oral ulcer	**6**	**0.8**	**3**	**10.0**	**<0.001**	**14.87**	**3.84 to 57.59**
Oral thrush	**6**	**0.8**	**2**	**6.7**	**0.002**	**10.25**	**2.27 to 46.20**
Herpes simplex	**11**	**1.4**	**2**	**6.7**	**0.027**	**5.76**	**1.39 to 23.76**
Body rash	18	2.4	0	0.0	0.395	0.66	0.04 to 11.25
Lymphadenopathy	17	2.2	1	3.3	0.688	2.17	0.39 to 12.00
Hepatomegaly	2	0.3	0	0.0	0.779	5.01	0.24 to 106.55
Abdominal tenderness	10	1.3	0	0.0	0.529	1.18	0.07 to 20.60
Genital warts	11	1.4	0	0.0	0.508	1.08	0.06 to 18.68
Genital ulcer	86	11.2	5	16.7	0.360	1.69	0.66 to 4.37
Genital discharge	231	30.2	12	40.0	0.253	1.56	0.75 to 3.25

cOR, crude odds ratio; n, number; q, quarter.
Significant values are indicated in bold.

symptoms, with 16 (53%) showing clinical signs of STI coinfection. Of patients with available laboratory results, the median baseline CD4 cell count was 371 (IQR 269–553) cells/mm^3, one patient had elevated liver enzymes, and none had hemoglobin levels <9.5 g/dL or elevated plasma creatinine (>110 μmol/L).

Of 19 patients with baseline drug resistance testing available (Table 4), none had treatment-relevant resistance against nucleoside reverse transcriptase inhibitors (NRTIs) and integrase strand transfer inhibitor (INSTI) antiretroviral drugs. Three patients (16%) showed high-level resistance against non-NRTI (NNRTI) coinciding with high VL. All patients had resistance against the protease inhibitor tipranavir/ritonavir (36I, 69K, 89I/M).

Programmatic Outcomes

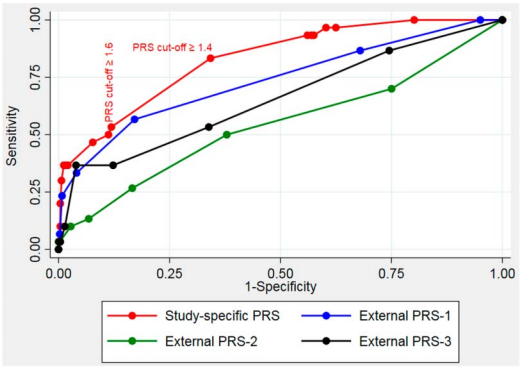
Figure 4 displays programmatic outcomes. Of 30 patients with AEHI, 23 patients initiated ART at a median of 3.5 (IQR 0–15) days. The 1-month hazard of treatment initiation was 74% (95% CI: 57% to 88%), and 37% (95% CI: 22% to 56%) initiated on the same day as AEHI diagnosis. Of patients initiated on ART in the study clinic and with a minimum follow-up of ≥1.5 months (n = 18), the 6-month hazard of retention was 82% (95% CI: 54% to 94%).

From the time of diagnosis (n = 30), the 3-month hazard of viral suppression <1000 copies/mL and an undetectable VL were 87% (95% CI: 67% to 98%) and 73% (95% CI: 50% to 91%), respectively. The median time to viral suppression (below 1000 copies/mL) and an undetectable VL (below 40 copies/mL) were 14 (IQR 14–27) and 56 (IQR 27–62) days, respectively. We did not identify associations for time from diagnosis to an undetectable VL (see Table S3, Supplemental Digital Content, http://links.lww.com/QAI/B719).

Forty-four sexual partners were reported by 30 AEHI patients. Only 13 (30%) sexual partners presented to the facility: 2 declined HIV testing, 4 tested RDT-negative with an undetectable VL, and 7 tested newly RDT-positive (Fig. 5).

DISCUSSION

We provided AEHI diagnosis and care in a public sector setting. AEHI was prevalent, and most AEHI patients achieved favorable treatment outcomes. The



A

Study-specific PRS: AUC 0.83 (95% CI 0.76–0.90); PRS-1: AUC 0.74 (95% CI 0.63–0.84); PRS-2: AUC 0.54 (96% CI 0.42–0.66); PRS-3: AUC 0.66 (95% CI 0.55–0.77).

	Study-specific PRS (n=795)			
	Cut-off ≥1.4		Cut-off ≥1.6	
	Point estimate	95% CI	Point estimate	95% CI
Sensitivity	83.3%	65.3–96.4	53.3%	34.3–71.7
Specificity	65.8%	62.3–69.1	88.1%	85.6–90.3
AUC	0.75	0.68–0.82	0.71	0.62–0.80
PPV	8.7%	5.7–12.6	15.0%	8.8–23.1
NPV	99.0%	97.7–99.7	98.0%	96.6–98.9

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; PRS, predictor risk score; AUC, area under receiver operating characteristic curve.

B

FIGURE 2. Receiver operating characteristic (ROC) curves of study-specific and external PRSs (panel A) and test characteristics of study internal PRS at the cut-off levels ≥1.4 and ≥1.6 (panel B). full color online

HIV-Combo RDT had the ability to diagnose most cases of AEHI, and the PRS showed potential to predict patients at risk of AEHI.

Explanation of Findings

Burden of AEHI

AEHI prevalence was 3.8% and higher than that in clinical settings in East Africa (<2%)[16,26,27] and comparable with antibody-negative AHI yield in febrile outpatients in Mozambique (3.3%)[18] and high-risk populations (eg, men who have sex with men).[28] Higher AEHI yield can be attributed to differences in recruitment strategies, targeted screening and testing algorithms, higher HIV transmission in population tested (eg, key populations), other prevalent diseases with symptoms similar to AHI (eg, malaria), and different definitions applied (eg, combining AEHI).[4,28] Our pragmatic definition of AEHI included patients with AEHI and possibly some cases of chronic HIV infection missed by routine third-generation–based RDT algorithm (false-negative cases).[29] Of importance, our study eligibility criteria allowed targeting and enrollment of at-risk populations, likely resulting in a study population enriched for AEHI and, therefore, higher testing yield of AEHI. This targeted approach, however, was also likely to miss some cases of AEHI because they were not eligible for VL testing.

TABLE 2. Study-specific and 3 External PRS-1 to PRS-4 of AEHI

Study Characteristics	Study-specific PRS	External PRS		
		PRS-1[14]	PRS-2[15]	PRS-3[16]
Country	Eswatini	Kenya	South Africa	Malawi
Target population	General	MSM	Women at risk of HIV, SW	STI clients
Year(s) of data collection/ analysis		2005–2012	2004–2005	2003–2004
Study design	Cross-sectional	Prospective cohort	Prospective cohort	Cross-sectional
Enrollment criteria*				
Serodiscordant RDT	4.9	4		4
Sore throat			1	
Reported fever		1		1
Reported symptomatic STI		1		
Sociodemographic factors				
Women	1.3			
Young age		1 (≤29 yrs)	1 (≤24 yrs)	
Behavioral factors				
≥2 sexual partners				1
At risk of HIV infection	0.1			
Reported symptoms				
Swollen glands (reported)	1.0			
Fatigue	0.5	1		
Body pain				1
Diarrhea		1		2
Weight loss			1	
Loss of appetite†			2	
Physical examination signs				
Oral ulcer				
Body rash			2	
Vaginal discharge			2	
Genital ulcer			2	2

SW, sex worker; yrs, years.
*These were specific eligibility criteria for this study, but no enrollment criteria for other studies.
†Loss of appetite was not reported in our study.

Symptoms of AEHI and PRS

Our PRS included similar (discordant RDT, fatigue) and new (women, at risk of HIV, self-reported swollen glands) predictive factors for AEHI compared with PRS from other studies in Africa. Predictors of AEHI are context-specific depending on aforementioned factors, the risk profile of the

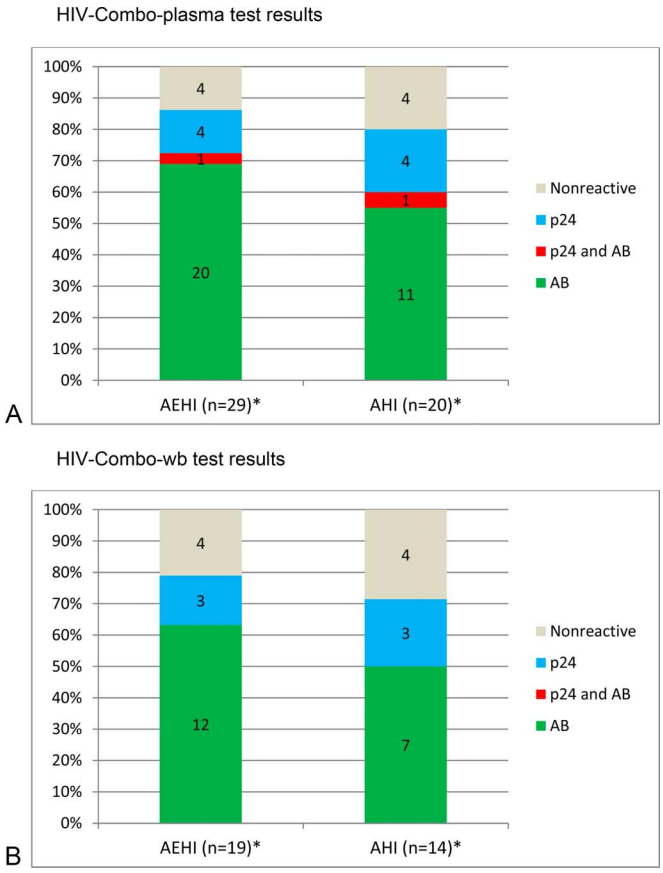


FIGURE 3. Test results of the Alere HIV-Combo RDT for plasma and whole-blood specimens with confirmed AEHI and AHI (as per Xpert testing). AB, reactive to antibodies; p24, reactive to p24 antigen. *AEHI and AHI positive test results as according to Xpert testing. full color online

target population, and viral subtype.[5,27,30–33] For instance, HIV subtype C—dominant in Southern Africa—causes less acute retroviral symptoms than subtype A (eg, dominant in Eastern Africa).[33,34] Patient-level determinants may include differences in behavioral and socioeconomic factors, and we assessed factors not evaluated in all other studies (eg, feeling at risk of HIV) and vice versa (eg, loss of appetite). Our context is characterized by concurrent sexual partnerships in the general population,[35] high incidence of HIV and STI,[12,36] and low malaria incidence.[37]Finally, because our study population was enriched for AEHI, our PRS may not be fully comparable with studies applying broader eligibility criteria and should only be considered for settings using similar targeted screening criteria.

Our PRS performed better than PRS from other settings. Overall, external PRS have lower predictive ability in validation studies than score development studies for various reasons (eg, different study designs and definitions of AEHI).[28] Another limitation of PRS may be their suboptimal use if they are too complicated[28] or health workers are faced with multiple screening algorithms for different diseases (eg, tuberculosis). However, PRS used in digital health interventions could be an alternative in mobilizing populations at risk of AEHI (eg, web-based self-assessment of AEHI risk[28]).

TABLE 3. Test Characteristics of the HIV-Combo Rapid Diagnostic Test Using Plasma and Venous Whole Blood for the Detection of AEHI and AHI When Compared With Paired Xpert HIV-1 Viral Load Testing

	HIV-Combo-Plasma (Point Estimate and 95% CI)		HIV-Combo-wb (Point Estimate and 95% CI)	
AEHI*	n = 745		n = 429	
Prevalence of AEHI	3.9%	2.6 to 5.5%	4.4%	2.7 to 6.8%
Sensitivity	86.2%	68.3 to 96.1%	78.9%	54.4 to 93.9%
Specificity	99.9%	99.2 to 100%	99.8%	98.6 to 100%
AUC	0.93	0.87 to 0.99	0.89	0.80 to 0.99
PPV†	96.2%	80.4 to 99.9%	93.8%	69.8 to 99.8%
NPV†	99.4%	98.6 to 99.8%	99.0%	97.5 to 99.7%
AHI‡	n = 731		n = 423	
Prevalence of AHI	2.7%	1.7 to 4.2%	3.3%	1.8 to 5.5%
Sensitivity	80.0%	56.3 to 94.3%	71.4%	41.9 to 91.6%
Specificity	99.9%	99.2 to 100%	99.8%	98.6 to 100%
AUC	0.90	0.81 to 0.99	0.86	0.73 to 0.98
PPV†	94.1%	71.3 to 99.9%	90.9%	58.7 to 99.8%
NPV†	99.4%	98.6 to 99.8%	99.0%	97.5 to 99.7%

AB, antibody; n, number; wb, whole blood.

*For AEHI: All patients who provided paired whole blood and plasma specimens for testing on HIV-Combo in the laboratory by the laboratory technician had tested HIV-seronegative (Alere Determine RDT negative) or HIV-serodiscordant (Alere Determine RDT positive and Uni-Gold RDT negative) using whole blood finger stick by HIV testing counselors at the outpatient department. Finger-stick testing with Alere Determine/Uni-Gold was performed on the same day as blood collection for HIV-Combo testing.

†Assuming the sample-specific prevalence of AEHI and AHI.

‡For AHI: The sample from AEHI was further restricted to patients with HIV-seronegative test results only as according to the third-generation RDT algorithm (and, therefore, patients with HIV-serodiscordant test results were removed from analysis).

Performance of HIV-Combo RDT

The HIV-Combo RDT detected AEHI cases missed by the routinely used third-generation RDT. Some cases were clearly cases of AEHI because there was reactivity to the p24 bar with or without reactivity to the antibody bar. However, most AEHI-positive cases that were missed by the third-generation serial testing algorithm were reactive to the antibody bar only and not the p24 antigen bar of the HIV-Combo RDT. Several explanations exist. First, reactivity to antibody bar only during AEHI has been reported before for immunoblot-negative and p24-positive samples (Fiebig stage II/III).[38] It does not rule out AEHI because p24 antigen may become undetectable on HIV-Combo RDT during early seroconversion due to formation of undetectable antigen/antibody immune complexes and undetectable low levels of free p24 antigen.[25,39] Second, other explanations for antibody detection by HIV-Combo but missed by the third-generation AlereDetermine RDT may be different detection ability of low antibody titer (during early seroconversion, undisclosed long-term ART), variation in titers by specimen type (finger stick vs plasma), lower performance of third-generation vs fourth-generation immunoassays, and user errors by different qualified staff (lay cadres vs laboratory technicians).[26,40,41] False HIV-negative results in RDT testing algorithms have also been reported from other routine care settings.[29] In our study, the HIV-Combo RDT was performed by a laboratory

TABLE 4. Patients With AEHI, Baseline Characteristics and Baseline HIV Drug Resistance, HIV-Combo Test and Viral Load Test Results, and Viral Outcomes

	Baseline Data					HIV-Combo Testing		Mutations with Treatment Relevant Resistance			
ID	Sex	Age	RDT*	VL	CD4	Plasma	Whole Blood	NRTI	NNRTI	PI	INSTI
2090	M	21	Disc	5.6	251	AB	—	S	S	R(4)	S
2373	M	21	Disc	5.3	377	AB	AB	S	R(1)	R(4)	S
1177	M	28	NR	6.8	—	NR	NR	S	S	R(4)	NA
1122	M	32	NR	3.6	768	AB	—	S	S	R(5)	S
1222	M	35	NR	7.0	—	AB	NR	—	—	—	—
1097	M	39	Disc	6.4	269	AB	—	S	S	R(4)	NA
1335	F	19	NR	5.0	270	AG	AG	S	S	R(4)	S
2392	F	20	Disc	5.8	261	AB	AB	S	R(2)	R(4)	NA
1187	F	21	NR	4.8	—	AG	AG	—	—	—	—
1125	F	22	NR	7.0	—	AG	—	S	S	R(4)	S
2285	F	22	Disc	5.2	495	AB	AB	S	S	R(4)	NA
1152	F	23	NR	3.1	616	AG, AB	—	—	—	—	—
1238	F	23	NR	4.9	844	AB	AB	S	S	R(4)	S
2188	F	24	Disc	1.9		AB	—	—	—	—	—
2329	F	24	NR	5.2	—	NR	NR	—	—	—	—
1421	F	25	NR	8.3	—	NR	NR	—	—	—	—
1257	F	25	NR	4.6	—	AB	AB	—	—	—	—
1338	F	25	Disc	4.3	95	AB	AB	S	S	R(4)	S
2218	F	25	NR	5.3	—	AB	AB	—	—	—	—
2282	F	26	Disc	6.3	305	AB	AB	S	S	R(4)	S
1239	F	27	NR	3.9	436	AB	AB	S	R(3)	R(4)	S
2042	F	27	NR	6.0	338	AB	—	S	S	R(4)	S
1212	F	27	NR	3.7	476	AB	AB	S	S	R(4)	S
2265	F	28	NR	4.5	837	AB	AB	S	S	R(4)	S
2205	F	29	NR	5.4	365	—	—	S	S	R(4)	S
2002	F	29	NR	4.3	—	AB	—	—	—	—	—
1217	F	30	NR	3.3	—	AB	AB	—	—	—	—
2236	F	30	NR	7.0	553	AG	AG	S	S	R(4)	NA
1086	F	31	Disc	3.7	240	AB	—	S	S	R(4)	S
1171	F	31	NR	4.3	—	NR	—	—	—	—	—

AB, antibody; AG, p24 antigen; disc, discordant; F, female; M, male; NA, not available; NR, nonreactive; PIs, protease inhibitors; R, HIV drug resistant; S, HIV drug susceptible; STI, sexually transmitted infection (clinically diagnosed); WB, whole blood.

*HIV testing was performed with Alere Determine and Uni-Gold RDT on plasma specimens by the laboratory technician in a laboratory setting.

The ANRS (French National Agency for AIDS Research) dHIV-1 drug resistance interpretation algorithm (version 2019.30/sg/b) was used to interpret genotypic drug susceptibility testing results for nucleoside reverse transcriptase inhibitors (NRTIs), NNRTI, protease inhibitors (PIs), and INSTIs.

Resistance test results: R(1) EFV/R3/103N; NVP/R3/103N. R(2) EFV/R3/103S; ETR/R2/106I,138G; NVP/R3/103S; RPV/R3/103S,138G. R(3) DOR/R3/103N,225H; EFV/R3/103N,225H; NVP/R3/98S,103N. R(4) TPV/R3/36I, 69K, 89M. R(5) TPV/R3/36I, 69K, 89I.

technician based in a laboratory setting using whole blood and plasma samples vs third-generation Alere Determine testing being performed by less qualified lay HIV testing counselors in a busy clinic using finger-stick sampling. Finally, our study eligibility criteria allowed for the inclusion of patients with HIV-serodiscordant test results according to the third-generation serial testing algorithm; thus, p24 antigen may already have become undetectable.

AEHI Cohort

Some patients had high-level resistance against NNRTI (16%) coinciding with a high VL, suggesting patients on ineffective (undisclosed) ART, previous exposure to antiretroviral drugs, or the transmission of drug-resistant strains. Comparable levels of pretreatment resistance against NNRTI (16.1%) were reported in Eswatini in 2016[42] and from an AEHI cohort in Malawi (20%).[43] Of importance, first-line dolutegravir-based treatment is considered effective in the context of high-level pretreatment NNRTI mutations.[44] All patients had high-level resistance against tipranavir (mutations 36I, 69K, 89I), which are likely naturally occurring polymorphisms in HIV-1 subtype C isolates with sustained phenotypic susceptibility to tipranavir.[45,46]

Although most of the patients initiated ART (74%) within 1 month, same-day ART initiation was low with reported reasons being that patient did not feel ready for treatment and delay in performing VL testing and releasing test results. It is encouraging that most patients were retained on therapy (82% at 6 months) and that viral suppression was quickly achieved, reducing the likelihood of onward HIV

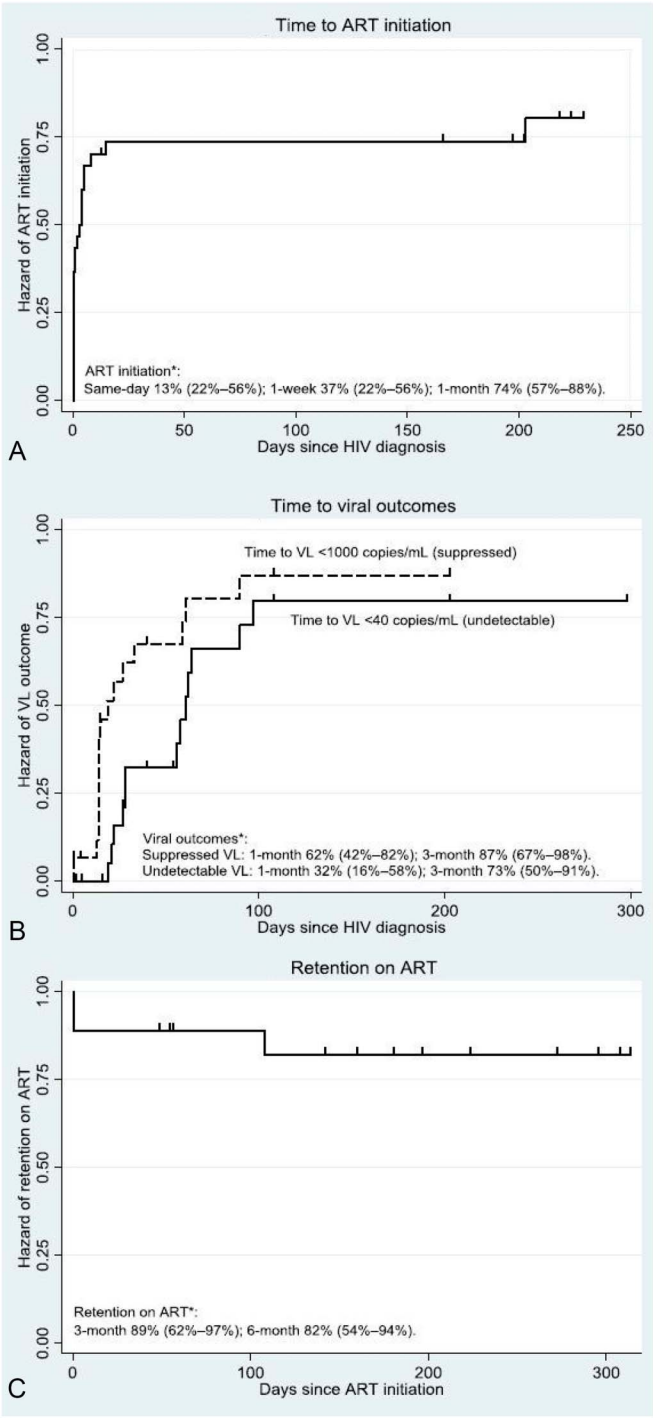


FIGURE 4. Crude programmatic outcomes for time to ART initiation (A) and to viral outcomes (B) since diagnosis of HIV and retention on ART (C). *Kaplan–Meier point estimates and 95% CIs. VL results of patients with AEHI were classified as follows: VL suppressed (VL < 1000 copies/mL) and VL undetectable (VL < 40 copies/mL or VL target detected but not quantifiable). Patients were removed from retention on ART analysis if ART initiation occurred in another facility (n = 3) and minimum follow-up time <1.5 months before database closure (n = 2). full color online

transmission. AEHI care outcome data are scarce from routine contexts, but VL outcomes of our study population appear comparable with outcomes from high-resourced and low-resourced settings.[47,48] Patients with AEHI seemed to be healthy (higher CD4, normal laboratory test results) overall, possibly explaining the lack of associations detected between baseline factors and viral outcomes.

Although the yield of newly diagnosed HIV was high among sexual contacts presenting to the facility, many partners did not present to the clinic for unknown reasons. Partner notification services seem to be acceptable in RLS, but setting-specific barriers may limit larger-scale implementation.[4] Further research should assess how to effectively operationalize contact tracing and linkage interventions in the public sector in the context of AEHI programming.

Findings in Context

AEHI care may contribute to HIV epidemic control in high-incidence settings. The contribution of AEHI to HIV transmission is likely higher when most people living with HIV access ART. For instance, although Eswatini surpasses the UNAIDS 95-95-95 targets,[49] HIV transmission remains high.[12] VL reduction combined with postdiagnosis behavioral changes during AEHI reduced onward HIV transmission by 89% within 1 year in men who have sex with men in Thailand.[48]

The HIV-Combo RDT has received WHO prequalification for the diagnosis of HIV.[50] The sensitivity is high for detection of HIV antibodies, and the newer version has increased ability to detect p24 antigen.[51–54] This test could be considered in RLS for the diagnosis of AEHI in combination with VL testing. Routine AEHI testing can also reduce the risk of false-negative HIV test results in PEP/PrEP programs, thus avoiding the emergence of HIV drug resistance.[4,55] Because evaluation studies of the HIV-Combo RDT were mostly laboratory based,[38,52,54] further field testing (eg, lay providers using finger-prick blood sampling) is required to assess its performance under routine conditions.[4]

Side findings were observed. First, in our study sample enriched for AEHI, almost half of all patients presented with STI coinfection. Because STI is associated with HIV infections specifically among young women in Eswatini,[56] greater focus on quality STI care is warranted in RLS to reduce the risk of HIV acquisition. Second, approximately two-thirds of patients with AEHI felt at risk of HIV infection and intended to use PrEP, whereas previous PrEP/PEP use was rare, indicating suboptimal access to this biomedical prevention service. Interestingly, AEHI was not diagnosed in any presumptive AEHI case referred from the PrEP/PEP program, suggesting that AEHI is rare and/or remains undetected in routine PrEP/PEP programs.

Limitations and Strengths

First, because we used a programmatic definition of AEHI and additional analyses (eg, Western blotting) was not performed, some patients diagnosed with AEHI were likely not true cases of AEHI as defined according to the original Fiebig classification of HIV infection.[4,57] This limitation may not affect considerations for the use of HIV-Combo RDT and

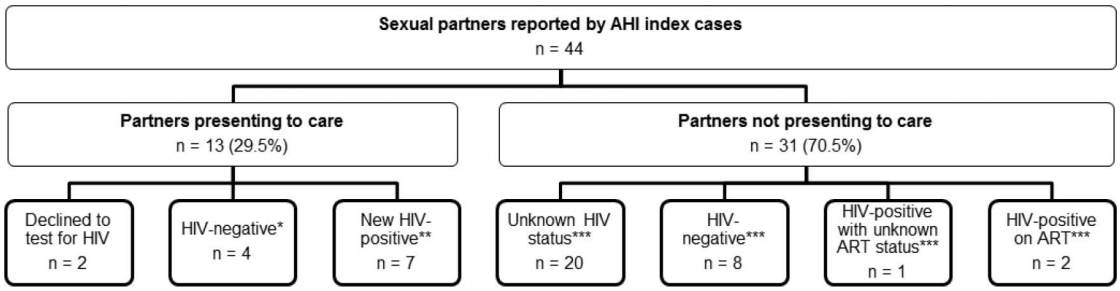


FIGURE 5. Outcomes of assisted partner notification services for sexual partners reported by the AEHI index case. n, number. *Partners of AHI patients who tested negative on RDT also routinely received a VL test. For these patients, the VL test showed an undetectable result. **The RDT were positive, so VL testing was not required. ***According to the information as provided by the AEHI patient. Footnote: Of 31 (70.5%) sexual partners who did not attend to care, the index cases reported 8 of them to be HIV-negative, one HIV-positive with unknown ART status, 2 on ART, and 20 with unknown HIV status (Fig. 5).

PRS in routine settings such as ours where third-generation RDT may miss some early HIV-seroconverters and some chronic HIV-positive patients. Second, although the integration of PRS and HIV-Combo RDT into routine care may show public health benefit, further studies should evaluate the cost-effectiveness of this intervention in the public sector.

A strength of the study was that the patient sample was obtained from the general population in a routine outpatient context, thus being representative for settings in Southern Africa with high HIV prevalence and high treatment coverage. Our findings may inform health policy in RLS that lack experience in AEHI care.[4]

CONCLUSIONS

The diagnosis and treatment of AEHI is a key public health intervention to decrease HIV transmission early during infection. Contextualized screening and testing algorithm with improved RDT are warranted for high HIV incidence settings. Our study suggests the potential of the novel HIV-Combo RDT for the diagnosis of AEHI and the feasibility of treating AEHI in routine practice in RLS. However, more effective public health strategies are required to effectively target partners of AEHI patients for onward HIV transmission control.

ACKNOWLEDGMENTS

The authors thank all the patients and health care workers who were involved in piloting the diagnosis and treatment of AHI, specifically patients in the outpatient department of Nhlangano health zone. The authors also thank all the MSF teams involved in data collection and data cleaning and the laboratory staff.

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