

1 **Performance of two-stage cervical cancer screening with primary high-risk HPV testing in women**  
2 **living with HIV**

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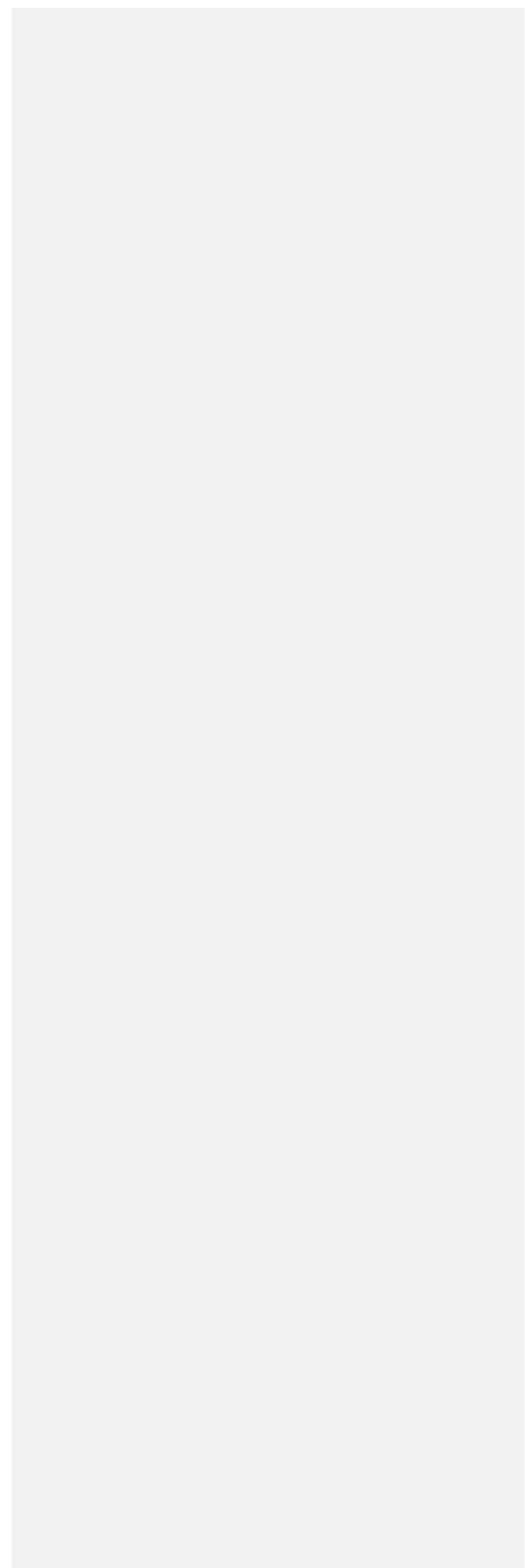
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35 **Precis**

36 Colposcopy following positive high-risk human papillomavirus testing maintained sensitivity and  
37 improved positive predictive value of high-grade cervical dysplasia among women living with human  
38 immunodeficiency virus.

39 **Abstract**

40 *Objective:* To evaluate the performance of cervical cancer screening algorithms for women living with  
41 human immunodeficiency virus (HIV), utilizing primary high-risk human papillomavirus testing (hrHPV)  
42 testing followed by cytology, visual inspection with acetic acid (VIA), or colposcopy.

43 *Methods:* Prospective cohort study of women living with HIV in Botswana. All participants underwent  
44 hrHPV testing. Participants with positive hrHPV results underwent cytology, VIA, colposcopy, and  
45 biopsy. Participants with negative hrHPV results also underwent cytology. Histopathology was the  
46 reference standard for determination of pre-invasive cervical disease and cervical cancer. Sensitivity,  
47 specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratios (LR) of  
48 hrHPV-based two-stage screening algorithms were calculated.

49 *Results:* Among 300 women screened, 88 (29%) had a positive hrHPV test, and 29 of the 88 (35%)  
50 hrHPV-positive women had CIN2+ on histopathology. hrHPV followed by colposcopy resulted in a  
51 sensitivity of 83%, specificity of 49%, PPV of 47%, LR+ of +1.6 and LR- of -0.4. hrHPV followed by  
52 VIA resulted in a reduced sensitivity of 59%, specificity of 49%, PPV of 39%, LR+ of +1.2 and LR- of -  
53 0.8. hrHPV testing followed by cytology also resulted in a reduced sensitivity of 62%, specificity of 77%,  
54 PPV of 60%, LR+ of +2.7 and LR- of -0.5. Stratification by HPV 16/18/45 did not improve performance  
55 of the algorithms.

56 *Conclusion:* In a high-risk HIV population, hrHPV testing followed by colposcopy demonstrated the  
57 highest sensitivity and PPV in detecting high-grade cervical dysplasia. Allocating resources to colposcopy  
58 in resource-limited settings may be more effective than other screening strategies.

59 *Clinical Trial Registration:* 2-stage Cervical Cancer Screening in Botswana,

60 <https://clinicaltrials.gov/ct2/show/NCT03324009>, NCT03324009

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61 **Introduction**

62 Cervical cancer is the fourth leading cause of cancer death in women worldwide and the leading cause of  
63 cancer death in women in Botswana.<sup>1,2,3</sup> The disease burden in Botswana is impacted by the high prevalence  
64 of human immunodeficiency virus (HIV), which is 22% among people aged 15-49 years and is a well-  
65 established risk factor for cervical cancer.<sup>4,5,6</sup> Most cervical cancers are associated with infection with high-  
66 risk human papillomavirus (hrHPV) types.<sup>7,8,9</sup> Globally, HPV prevalence is variable, ranging from 15-45%,  
67 with higher prevalence in women living with HIV.<sup>10,11,12</sup> HPV 16, 18, and 45 are the high-risk types most  
68 commonly associated with cervical cancer in Africa.<sup>13,14,15</sup> Among women living with HIV, persistent  
69 hrHPV positivity and infection with multiple types are strong risk factors for cervical cancer.<sup>16</sup>

70 Cervical cancer is largely preventable and treatable where screening and treatment programs are  
71 available.<sup>17,18,19,20</sup> Cervical cancer screening strategies are most effective when based on local evidence and  
72 tailored to the population and resource infrastructure.<sup>21</sup> Current programming in Botswana utilizes a  
73 combination of cytology (Pap smear) and visual inspection with acetic acid (VIA). However, there is  
74 mounting evidence that primary hrHPV testing is the most effective screening strategy because of its high  
75 sensitivity (95%).<sup>22</sup> hrHPV testing is increasingly included in some national guidelines.<sup>23,24,25</sup> hrHPV  
76 testing is planned for future national programming in Botswana, but the guidelines for managing positive  
77 hrHPV results remain unclear, particularly among women living with HIV.<sup>26,27,28</sup> Appropriate triage of a  
78 positive hrHPV result is necessary to prevent overtreatment of hrHPV when it is associated with no or low-  
79 grade cervical dysplasia. The best two-stage screening strategy is unknown for women living with HIV in  
80 resource-limited settings<sup>29,30,34</sup>

81 In this study, we investigated the performance of primary hrHPV testing followed by cytology, VIA and  
82 colposcopy impression to predict pre-invasive cervical disease in women living with HIV in Botswana. We  
83 hypothesized that VIA, cytology and colposcopy would perform similarly as a triage test in women living  
84 with HIV who test positive for hrHPV. Evaluating cervical cancer screening algorithms with primary

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85 hrHPV testing in women living with HIV is essential for establishing an evidence-based screening strategy  
86 in this high-risk population.

87

## 88 **Methods**

89 We conducted a prospective cohort study of women seeking care at the infectious disease care clinic at  
90 Princess Marina Hospital in Gaborone, Botswana. The infectious disease care clinic provides care to  
91 people living with HIV at Princess Marina Hospital, the regional tertiary referral hospital. Women  
92 included in the study were HIV-positive, greater than 24 years of age, and competent to understand study  
93 procedures and give informed consent. Women were excluded if they were currently pregnant, currently  
94 menstruating heavily or with persistent vaginal discharge, had a previous hysterectomy, or had a previous  
95 diagnosis of cervical cancer.

96 Eligible women were provided study information by a research assistant or study nurse while waiting for  
97 their scheduled clinical visit at infectious disease care clinic and offered voluntary participation. After  
98 obtaining informed consent, we administered a questionnaire including demographic data, HIV treatment  
99 history, history of cervical cancer screening, and knowledge about cervical cancer. In addition to patient  
100 report, the electronic medical record was searched for results of prior cervical cancer screening. The  
101 institutional review boards of the Botswana Ministry of Health and Wellness, the University of Botswana,  
102 and the Beth Israel Deaconess Medical Center approved this study. The ethics committee of Princess  
103 Marina Hospital also approved this study.

104 All participants underwent a speculum examination of the cervix by a trained study nurse, at which time  
105 samples were collected from the cervix for hrHPV testing and for cervical cytology using a Cervex-brush<sup>®</sup>.  
106 HPV specimens were placed in a PreservCyt<sup>®</sup> transport medium and testing was performed using the Xpert<sup>®</sup>  
107 HPV Assay (Cepheid, Sunnyvale, CA) at the Botswana Harvard AIDS Initiative Partnership Laboratory.

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108 The Xpert® HPV assay tests for 14 hrHPV types, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,  
109 66, and 68. Cytology was prepared by spreading collected cervical cells from a Cervex-brush® onto a glass  
110 slide and fixing with a spray fixative at the collection site. Cytology was sent to the National Health  
111 Laboratory for processing and pathologist evaluation and reported using the revised Bethesda  
112 classification.<sup>31</sup> Abnormal lower genital tract cytology was evaluated at two thresholds: abnormal  
113 squamous cells of undetermined significance (ASC-US) or worse, and high-grade squamous intraepithelial  
114 lesion (HSIL) or worse.

115 Because there are no clinical guidelines for management of positive hrHPV results in Botswana, we also  
116 collected cytology at the time of hrHPV sample collection to ensure that all participants were screened  
117 according to current cervical cancer screening guidelines in Botswana. We referred participants who tested  
118 negative for hrHPV to colposcopy if they had a study cytology of HSIL or had a prior abnormal cytology  
119 result and study cytology result of ASC-US or worse ( $\geq$ ASC-US) in accordance with current Botswana  
120 National Cervical Cancer Prevention Programme algorithms. We referred all participants who tested  
121 positive for any hrHPV type to VIA and colposcopy, regardless of their cytology result. At the time of the  
122 colposcopy visit, participants underwent a speculum examination of the cervix with both VIA and  
123 colposcopy performed by providers who were blinded to the HPV test results and cytology results. VIA  
124 was performed by a trained nurse midwife who had participated in the Botswana Ministry of Health and  
125 Wellness national VIA training program and was experienced in performing VIA in the clinical setting.  
126 Visual assessment was performed after applying 5% acetic acid to the cervix using a cotton swab and  
127 findings were categorized as normal, abnormal with recommendation for cryotherapy, or abnormal with  
128 recommendation for loop electrosurgical excision procedure (LEEP). In the analysis, we considered lesions  
129 recommended for cryotherapy as “low-grade” and lesions recommended for LEEP as “high-grade”.  
130 Subsequently, a gynecologist blinded to the VIA assessment performed colposcopy and normal, low-grade  
131 or high-grade impression was recorded. All participants had a biopsy collected at the time of colposcopy.  
132 If there was a visible lesion, a punch biopsy or LEEP was performed according to current best practice in

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133 Botswana. If no lesion was visible, a small endocervical excision or an endocervical curettage was  
134 performed. All women with cervical intraepithelial neoplasia  $\geq$  CIN2 (CIN2+) on biopsy or endocervical  
135 curettage were referred for an excisional procedure. Women with histopathology showing CIN3 with  
136 microinvasion or invasive cervical cancer were referred to gynecologic providers for further assessment  
137 and treatment.

138 The primary outcome was performance of two-stage cervical cancer screening algorithms in detecting high  
139 grade cervical dysplasia. We defined high-grade cervical dysplasia as a colposcopy result of cervical  
140 intraepithelial neoplasia grade 2 or higher (CIN2+). Using histopathology collected at time of colposcopy  
141 as the gold standard, we calculated the sensitivity, specificity, positive predictive value (PPV) negative  
142 predictive value (NPV), and likelihood ratios (LR) to detect high-grade cervical dysplasia for 1) cytology  
143 following a positive hrHPV test, 2) VIA impression following a positive hrHPV test and 3) colposcopy  
144 impression following a positive hrHPV test. For each two-stage screening strategy, we evaluated test  
145 performance at two cutoffs. For cytology, we evaluated cut-offs of ASC-US and HSIL. For VIA and  
146 colposcopy, we evaluated cut-offs of low-grade and high-grade impressions. In addition, we repeated this  
147 analysis stratified by hrHPV type (16/18/45 and other hrHPV).

148 Data were entered into a REDCap electronic database by a designated research assistant and accuracy of  
149 data entry were verified by the study nurse and principal investigator. Descriptive statistics are presented  
150 as median with interquartile range or proportion. We compared categorical variables with the chi-square  
151 or Fisher's exact test and continuous variables with the Wilcoxon rank sum test. We considered two-  
152 sided p values  $<0.05$  statistically significant and used SAS 9.4 (SAS Institute, Cary, North Carolina) for  
153 analyses.

154 The goal of a two-stage algorithm to detect high-grade cervical dysplasia is to increase PPV while  
155 maintaining sensitivity and specificity. In a prior cervical cancer screening study among a population of  
156 women with a relatively high HIV prevalence, the PPV of a hrHPV positive test for high-grade cervical

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157 dysplasia was 24% (Denny, 2000). Our sample size calculation was targeted to detect an improvement in  
158 PPV from 24% for hrHPV testing alone to 49% for the two-stage algorithms. Assuming a two-sided alpha  
159 of 0.05, a sample size of 81 participants with hrHPV was needed to yield 80% power to detect the  
160 specified difference. Based on preliminary data from a recent study of women living with HIV in  
161 Botswana, we assumed hrHPV-positivity would be 30% (unpublished data). Thus, we needed to enroll  
162 270 participants with HIV to yield 81 who would be hrHPV positive. To allow for 10% loss to follow-up  
163 between the primary hrHPV testing and colposcopy we aimed to enroll at least 300 participants.  
164

#### Authors' Data Sharing Statement

Will individual participant data be available (including data dictionaries)? Yes

What data in particular will be shared? All of the individual participant data collected during the trial, after deidentification.

What other documents will be available? Study protocol

When will data be available (start and end dates)? Beginning 3 months and ending 5 years following article publication.

With whom? Investigators whose proposed use of the data has been approved by an independent review committee identified for this purpose.

For what types of analyses? To achieve aims in the approved proposal

By what mechanism will data be made available? Proposals should be directed to [rluckett@bidmc.harvard.edu](mailto:rluckett@bidmc.harvard.edu). To gain access, data requestors will need to sign a data access agreement.

165

166 **Results**

167 We recruited participants from April to July 2018, and all follow-up colposcopy visits were completed by  
168 August 2018. Of the 312 women living with HIV enrolled, 12 were lost to follow-up, deemed ineligible or  
169 withdrawn before cervical samples were collected at the first study visit, leaving 300 (96%) who underwent  
170 hrHPV testing and cytology collection. Of those participants, 88 (29%) had a positive hrHPV result. Among  
171 those 88 who were hrHPV positive, we did not have colposcopy results for 6 (3 were lost to follow-up, 1  
172 withdrew, 1 became ineligible due to pregnancy, and 1 biopsy specimen was lost in the laboratory) and had  
173 histopathology results from colposcopy for 82 women for this analysis. Additionally, two participants who  
174 were hrHPV-negative underwent colposcopy for cytology of HSIL (Figure 1).

175 Baseline characteristics were similar among women who tested positive and negative for hrHPV (Table 1).  
176 The majority of women reported having undergone prior cervical cancer screening (95%). There were no  
177 differences between groups in prior abnormal screening results or cervical excisional procedures. Only 5  
178 women had a recent CD4 count of  $< 200/\mu\text{L}$ , and all of the participants were taking antiretroviral therapy.  
179 Only two women reported a history of smoking, and both tested negative for all hrHPV types.

180 Of the 88 (29%) women who were positive for any hrHPV type, 15 of the 300 screened had HPV 16  
181 (prevalence 5%); 21 of the 300 screened had HPV 18/45 (prevalence 7%); and 66 of the 300 screened had  
182 other hrHPV types (prevalence 22%). Among the 82 women with a positive hrHPV test who had  
183 histopathology results, 29 (35%) had CIN2+ (Table 2). The prevalence of CIN2+ by hrHPV type was  
184 31%, 21%, and 43% for HPV 16, HPV 18/45, and other hrHPV types, respectively. Among the 11  
185 participants co-infected with multiple hrHPV types, the prevalence of CIN2+ was 45%.

186 We compared the performance of the two-stage cervical cancer screening algorithms. hrHPV followed by  
187 colposcopy impression had a sensitivity of 83%, specificity of 49%, PPV of 47%, LR+ of +1.6 and LR-  
188 of -0.4. hrHPV testing followed by VIA resulted in a reduced sensitivity of 59%, specificity of 49%, PPV

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189 of 39%, LR+ of +1.2 and LR- of -0.8 at the low cut-off point of “low-grade impression”. hrHPV testing  
190 followed by cytology also resulted in a reduced sensitivity of 62%, specificity of 77%, PPV of 60%, LR+  
191 of +2.7 and LR- of -0.5 at the ASC-US threshold (Table 3). Triaging hrHPV positive women with  
192 colposcopy impression, VIA and cytology missed CIN2+ diagnoses in 5, 12, and 11 women in our cohort,  
193 respectively. Evaluation of the two-stage algorithms stratified by HPV 16/18/45 versus other hrHPV  
194 types did not improve the performance of any algorithm (Table 4).

195 Four women had histopathology results of cancer or microinvasive CIN 3. One of these women had  
196 HPV18/45 and the other three had other hrHPV types. All four had a cytology result of HSIL. Three had  
197 low-grade impressions on both VIA and colposcopy, while one had a high-grade impression on both VIA  
198 and colposcopy.

199 **Discussion:**

200 Primary hrHPV testing followed by colposcopy was the most sensitive two-stage algorithm for cervical  
201 cancer screening among women living with HIV in Botswana. Both VIA and cytology as second-stage  
202 screening methods had unacceptably low sensitivity, missing approximately one-third of women with  
203 high-grade cervical lesions. Triaging hrHPV positive results with VIA or cytology eliminated the benefit  
204 of the high sensitivity that primary hrHPV testing provides. Further, triaging of hrHPV positive results  
205 based on type did not improve the performance of any two-stage algorithm.

206 One third of women in our study with positive hrHPV primary screening had high-grade cervical disease,  
207 which is a higher proportion than found in other populations living with HIV.<sup>32</sup> Our population also had a  
208 higher prevalence of high-grade dysplasia among women with other hrHPV compared to women with  
209 HPV 16 or 18/45. This is consistent with prior studies in Botswana that showed heterogeneous HPV types  
210 associated with high-grade precancerous cervical lesions among women living with HIV (16, 18, 35, 58,  
211 and 61) and a lower prevalence of HPV 16 and 18 positivity in cervical cancer specimens.<sup>33,34,35</sup> This

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212 cross-sectional data does not support triaging strategies based on hrHPV type, as may be considered in  
213 other African settings.<sup>36</sup>

214 Primary hrHPV testing followed by colposcopy results in a high number of referrals for colposcopy,  
215 presenting challenges in resource-limited settings.<sup>37</sup> Guidelines for low- and middle-income countries have  
216 presumed that scaling up colposcopy is not feasible.<sup>38,39</sup> Recent trends in cervical cancer screening in the  
217 region have focused on visual inspection strategies as opposed to colposcopy training.<sup>40</sup> However,  
218 consideration of available data to plan effective screening programs is vital. Our findings support concerns  
219 raised in prior studies that VIA and cytology triaging of women with hrHPV may have variable or low  
220 sensitivity, particularly in women living with HIV, and that referral to colposcopy may be a better  
221 alternative.<sup>41,42,43,44</sup> Building on the infrastructure that visual inspection has developed may facilitate roll-  
222 out of colposcopy, if coupled with the training of nurses and general practice providers in the region. In  
223 Botswana, for instance, the VIA programming has equipped a number of facilities with capability to  
224 perform LEEP, and many LEEP sites have colposcopes not currently in use. If rapid hrHPV testing were  
225 available in the future, same-day triage with colposcopy and treatment at these sites would be feasible.

226 This study highlights the acute need to improve screening for cervical cancer and raises concern about the  
227 frequency of screening in women living with HIV in low- and middle-income countries. Current national  
228 strategy in Botswana recommends screening with cytology or VIA in women living with HIV every three  
229 years. While many of the participants had been screened before (over 90%), only 11% of women reported  
230 a prior abnormal result and 2-3% reported a prior excisional procedure. Our high prevalence of high-  
231 grade pre-invasive cervical disease supports the need for frequent screening to ensure diagnosis of disease  
232 prior to progression to malignancy. In addition to high rates of pre-invasive cervical disease, the rate of  
233 detection of cervical cancer in our screening cohort was relatively high at 2%. This included 3 women  
234 enrolled but immediately referred for suspicion for clinical stage IB cervical cancer on examination and 4  
235 women with histopathology concerning for Stage IA cervical cancer (cervical cancer or microinvasive

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236 CIN3). This rate was similar to another screening cohort in Zambia where 6 of 200 (3%) women living  
237 with HIV had invasive cervical cancers detected at the time of screening, but higher than other settings.<sup>45</sup>  
238 In a large cervical cancer screening cohort of 79,506 women in India, 238 (0.3%) invasive cervical  
239 cancers were detected (Sankaranarayanan, 2009). In a cervical cancer screening cohort of 1128 women  
240 living with HIV in India, 5 (0.4%) invasive cervical cancers were detected.<sup>46</sup>

241 We found lower rates of hrHPV prevalence among women living with HIV than reported in the literature,  
242 which may highlight the improvement in HIV management over time with higher antiretroviral therapy  
243 utilization and viral suppression.<sup>47,48</sup> Botswana has had continuous access to antiretroviral therapy in the  
244 public sector since 2002, with initiation of antiretroviral therapy at graduated CD4 counts over time  
245 (initially 200 then 350) until a test-and-treat policy was initiated in 2016. Demographic differences in  
246 study populations may also contribute to this difference. Our study had a higher median age than in  
247 studies conducted in the United States, Kenya and Brazil. Additionally, the population in New York had  
248 higher risk behaviors, as indicated by high rates of smoking and on-going intravenous drug use.<sup>49</sup> The  
249 study population in Brazil was pregnant which may have resulted in increased immunosuppression and  
250 higher hrHPV detection rates.<sup>50</sup> Rates of hrHPV prevalence among women living with HIV in the region  
251 generally range from 47-57%, however, the prevalence is lower in women aged 40-49.<sup>51,52</sup> In a similarly-  
252 aged cohort of women in Zambia, where 90% of participants were on antiretrovirals and only 77% virally  
253 suppressed, hrHPV positivity was 47% (Chibwasha, 2016). On-going evaluation of hrHPV rates in  
254 women living with HIV in the modern antiretroviral therapy are necessary to understand if our findings  
255 are generalizable.

256 Our study has limitations. Our confidence intervals are wide around sensitivity, specificity, PPV and NPV  
257 as a result of our relatively small sample size. Further, research in larger populations will help to clarify if  
258 the difference in performance detected in this study is significant. The cohort was recruited from an HIV  
259 treatment center, which may represent a unique population of health-seeking individuals and may not be

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260 representative of a broader population. Ease of communication and follow-up of abnormal results may  
261 not therefore be replicated in a larger population. However, we found many women were not only  
262 reachable, but proactively followed-up their results, indicating that improved education about cervical  
263 cancer may reduce loss to follow-up and maximize dissemination of results. While the goal of this study  
264 was to evaluate screening algorithms that would be possible with pathology services currently available,  
265 external validation of cytology and histopathology specimens was not performed and thus accuracy  
266 compared to an expert gynecologic cytopathologist and pathologist was not evaluated. History of cervical  
267 cancer screening is primarily self-reported with limited ability to confirm results in the electronic medical  
268 record. In regards to study design, the effect of co-infection with multiple hrHPV types could not be  
269 assessed because the study sample was not sufficiently powered for this subgroup. Finally, one VIA nurse  
270 and colposcopist conducted the evaluations; therefore, performance of these tests may not be  
271 generalizable.

272 Follow-up of this cohort is currently underway to evaluate the best interval and modality for longitudinal  
273 screening. Further research on the performance of technology-based cervical cancer screening methods  
274 compared to current available methods in low- and middle-income countries is also being planned in a  
275 larger population. Balancing the cost of these strategies with clinical effectiveness is essential and a cost-  
276 effectiveness evaluation of these strategies in Botswana is being explored. Finally, regional adoption of a  
277 test-and-treat policy for HIV may continue to impact cervical cancer rates in the long-term as long-  
278 standing antiretroviral therapy use and initiation of treatment at higher CD4 levels may reduce incidence  
279 of cervical dysplasia, progression of dysplasia, and increase the likelihood of CIN regression.<sup>53</sup> On-going  
280 research in our population living with HIV is essential to understand this impact.

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**Table 1: Baseline characteristics of the study population**

Characteristic	All n = 300*	hrHPV positive n = 88	hrHPV negative n = 212	p
Age, years [interquartile range]	46 [42-52]	44 [40-51]	47 [42-52]	0.05
<b>Education</b>				0.40
≤Primary	94 (31)	24 (27)	70 (33)	

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≥Secondary	206 (69)	64 (73)	142 (67)	
<b>Employed</b>	197 (66)	63 (72)	134 (63)	0.21
<b>Marital status</b>				0.85
Single	215 (72)	61 (69)	154 (72)	
Married	55 (18)	18 (20)	37 (17)	
Divorced	12 (4)	3 (3)	9 (4)	
Widowed	18 (6)	6 (7)	12 (6)	
<b>Parity<sup>§</sup></b>				0.15
0	11 (4)	5 (6)	6 (3)	
1-3	199 (66)	58 (66)	141 (67)	
≥4	75 (25)	24 (27)	51 (24)	
<b>Sexual partners</b>				0.83
1-5	186 (62)	55 (63)	131 (62)	
≥6	100 (33)	28 (32)	72 (34)	
Missing	14 (5)	5 (6)	9 (4)	
<b>Postmenopausal</b>	106 (35)	27 (31)	79 (38)	0.38
<b>CD4 Count (per µL)</b>				0.63
<200	5 (2)	2 (2)	3 (1)	
200-500	83 (28)	27 (31)	56 (26)	
>500	212 (71)	59 (67)	153 (72)	
<b>Detectable viral load</b>	11 (4)	6 (7)	5 (2)	0.12
<b>Currently on <a href="#">antiretroviral therapy</a><sup>RT</sup></b>	300 (100)	88 (100)	213 (100)	--
<b>Length of time on <a href="#">antiretroviral therapy</a><sup>ART</sup>, years [interquartile range]</b>	14 [11 – 15]	14 [9 – 15]	14 [12 – 15]	0.09
<b>History of cervical cancer screening</b>				
Yes	285 (95)	79 (90)	206 (97)	0.02
Pap ≥ASC-US	27 (9)	11 (14)	16 (8)	0.44
VIA positive	3 (1)	1 (1)	2 (1)	1.0
<b>History of cervical excisional procedure</b>	6 (2)	3 (3)	3 (1)	0.18

\*All table entries are number of study subjects (%) unless otherwise noted

<sup>§</sup>Data available for 285 participants

ART: antiretroviral therapy; ASC-US: abnormal squamous cells of undetermined significance; VIA: visual section with acetic acid

**Table 2: Prevalence of CIN2+ (per 100 women living with HIV) who tested positive for high-risk HPV and underwent colposcopy**

HPV type	Number undergoing colposcopy (n)	Number with CIN2+ (n)	Prevalence of CIN2+ (%) [95% CI]
Any high-risk HPV type	82	29	35% [25 – 47]

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HPV 16*	13	4	31% [9 – 61]
HPV 18/45*	19	4	21% [6 – 46]
Other high-risk HPV type*	61	26	43% [30 – 56]
>1 high-risk HPV type	11	5	45% [17 – 77]
*Infection with these sub-types is not mutually exclusive CIN2+: cervical intraepithelial neoplasia grade 2 or higher			

**Table 3: Performance of two-stage screening in detecting CIN2+ among women living with HIV who tested positive for high-risk HPV and underwent colposcopy**

Two-stage screen using different cut-offs		Biopsy result		Two-stage screen characteristics				LR +/- [95% CI]
		CIN2+ (n)	≤ CIN1 (n)	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	PPV (%) [95% CI]	NPV (%) [95% CI]	
Cytology	NILM	11	41	--	--	--	--	
	≥ ASC-US	18	12	62% [42–79]	77% [64–88]	60% [41–77]	79% [65–89]	+ 2.7 [1.1–4.3] -0.5 [0.2–0.7]
	≥ HSIL	9	4	31% [15–51]	92% [82–98]	69% [39 – 91]	71% [59–81]	
Visual inspection with acetic acid (VIA)	normal	12	26	--	--	--	--	
	≥ low-grade impression	17	27	59% [39–76]	49% [35–63]	39% [24–55]	68% [51–83]	+ 1.2 [0.7–1.6] -0.8 [0.4–1.3]
	≥ high-grade impression	4	5	14% [3–32]	91% [79–97]	44% [14–79]	66% [54–76]	
Colposcopy impression	normal	5	26	--	--	--	--	
	≥ low-grade impression	24	27	83% [64–94]	49% [35–63]	47% [33–62]	84% [66–95]	+ 1.6 [1.1–2.1] -0.4 [0.1–0.7]
	≥ high-grade impression	4	5	14% [4–32]	91% [79–97]	44% [14–79]	66% [54–76]	

CIN2+: cervical intraepithelial neoplasia grade 2 or higher; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; NILM: negative for intraepithelial lesion or malignancy; ASC-US: abnormal squamous cells of undetermined significance; HSIL: high-grade squamous intraepithelial lesion

**Table 4: Performance of two-stage screening in detecting CIN2+ among women living with HIV who tested positive for high-risk HPV and underwent colposcopy stratified by HPV type**

Study Arm	CIN 2+ (n)	≤ CIN 1 (n)	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	PPV (%) [95% CI]	NPV (%) [95% CI]
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hrHPV + Cytology	<b>HPV 16/18/45</b>						
	NILM	3	17	--	--	--	--
	≥ ASC-US	5	7	63 (24 – 91)	71 (49 – 87)	42 (15 – 72)	85 (62 – 97)
	≥ HSIL	3	2	38 (9 – 76)	92 (73 – 99)	60 (15 – 95)	81 (62 – 94)
	<b>Other hrHPV</b>						
	NILM	9	26	--	--	--	--
	≥ ASC-US	17	9	65 (44 – 83)	74 (57 – 88)	65 (44 – 83)	74 (57 – 88)
≥ HSIL	8	3	31 (14 – 52)	91 (77 – 98)	73 (39 – 94)	64 (49 – 77)	
hrHPV + VIA	<b>HPV 16/18/45</b>						
	Normal	4	15	--	--	--	--
	≥ low-grade impression	4	9	50 (16 – 84)	63 (41 – 81)	31 (9 – 61)	79 (54 – 64)
	≥ high-grade impression	1	1	13 (0 – 53)	96 (79 – 100)	50 (1 – 99)	77 (58 – 90)
	<b>Other hrHPV</b>						
	Normal	10	14	--	--	--	--
	≥ low-grade impression	16	21	62 (41 – 80)	40 (24 – 58)	43 (27 – 61)	58 (37 – 78)
≥ high-grade impression	4	5	16 (5 – 36)	86 (70 – 95)	44 (14 – 79)	59 (44 – 72)	
hrHPV + Colposcopy	<b>HPV 16/18/45</b>						
	Normal	1	10	--	--	--	--
	≥ low-grade impression	7	14	<del>88+00</del> (47 – 100)	42 (22 – 63)	33 (15 – 57)	91 (59 – 100)
	≥ high-grade impression	2	3	25 (3 – 65)	88 (68 – 97)	78 (58 – 91)	81 (62 – 94)
	<b>Other hrHPV</b>						
	Normal	4	18	--	--	--	--
	≥ low-grade impression	22	17	85 (65 – 96)	51 (34 – 69)	56 (40 – 72)	82 (60 – 95)
≥ high-grade impression	4	3	15 (4 – 35)	91 (77 – 98)	57 (18 – 90)	59 (45 – 72)	

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