LSHTM Research Online

Sturt, Amy S; Webb, Emily L; Phiri, Comfort R; Mudenda, Maina; Mapani, Joyce; Kosloff, Barry; Cheeba, Maina; Shanaube, Kwame; Bwalya, Justin; Kjetland, Eyrun F; +8 more... Francis, Suzanna C; Corstjens, Paul LAM; van Dam, Govert J; van Lieshout, Lisette; Hansingo, Isaiah; Ayles, Helen; Hayes, Richard J; Bustinduy, Amaya L; (2021) Female Genital Schistosomiasis and HIV-1 Incidence in Zambian Women: A Retrospective Cohort Study. Open Forum Infectious Diseases, 8 (7). ofab349-. DOI: https://doi.org/10.1093/ofid/ofab349

Female Genital Schistosomiasis and HIV-1 incidence in Zambian women: a retrospective cohort study

Amy S. Sturt[1], Emily L. Webb[2], Comfort R. Phiri[3], Maina Mudenda[4], Joyce Mapani[4], Barry Kosloff[1,3], Maina Cheeba[3], Kwame Shanaube[3], Justin Bwyala[3], Eyrun F. Kjetland[5,6], Suzanna C Francis[2], Paul L.A.M. Corstjens[8], Govert J. van Dam[7], Lisette van Lieshout[8], Isaiah Hansingo[4], Helen Ayles[1,3], Richard J Hayes[2], Amaya L. Bustinduy[1]

[1]Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, United Kingdom; [2] MRC International Statistics and Epidemiology Group, London School of Hygiene and Tropical Medicine, London, United Kingdom; [3]Zambart, Lusaka, Zambia; [4]Department of Obstetrics and Gynaecology, Livingstone Central Hospital, Livingstone, Zambia; [5]Oslo University Hospital, Oslo, Norway; [6]University of KwaZulu-Natal, Durban, South Africa; [7]Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, The Netherlands; [8]Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands

Running Head: FGS and HIV-1 Incidence

Word Count: 3376/3500

Key Points: FGS has been associated with prevalent HIV-1. In this study, women with FGS had higher rates of HIV-1 seroconversion, however there was no statistical evidence of an association.

24 **Corresponding Author:**

25

26 Dr Amy Sturt

27 London School of Hygiene and Tropical Medicine

28 Department of Clinical Research

29 Keppel Street, Room 358

30 London

31 WC1E 7HT

32 United Kingdom

33 amy.sturt@lshtm.ac.uk

34 +44 2079272642

35

36

37

38   *Background:*

39   Female genital schistosomiasis (FGS) has been associated with prevalent HIV-1. We estimated the

40   incidence of HIV-1 infection in Zambian women with and without FGS.

41   *Methods:*

42   Women (aged 18-31, non-pregnant, sexually active) were invited to participate in this study in

43   January-August 2018 at final follow-up of the HPTN 071 (PopART) Population Cohort. HIV-1

44   negative participants at enrolment (n=492) were included in this analysis with testing to confirm

45   incident HIV-1 performed in HPTN 071 (PopART). Association of incident HIV-1 infection with

46   FGS (*Schistosoma* DNA detected by PCR in any genital specimen) was assessed with exact

47   Poisson regression.

48   *Results:*

49   Incident HIV-1 infections were observed in 4.1% (20/492) participants. Women with FGS were

50   twice as likely to seroconvert as women without FGS but with no statistical evidence for a

51   difference (aRR 2.16, 95%CI[0.21–12.30], p=0.33). Exploratory analysis suggested an association

52   with HIV-1 acquisition among women with ≥2 positive genital PCR specimens (RR 6.02, [0.58–

53   34.96]), p=0.13).

54   *Conclusions:*

55   Despite higher HIV seroconversion rates in women with FGS, there was no statistical evidence of

56   association, possibly due to low power. Further longitudinal studies should investigate this

57   association in a setting with higher schistosomiasis endemicity.

58

59   **Keywords:** HIV incidence, female genital schistosomiasis, polymerase chain reaction, PCR,

60   parasite, *Schistosoma haematobium*

Introduction:

In 2019, an estimated 56 million women were living with female genital schistosomiasis (FGS), a neglected tropical disease that results when eggs from the parasite *Schistosoma (S.) haematobium* are deposited in reproductive tract tissues [1]. Tissue-entrapped eggs incite a cellular response [2], ultimately resulting in FGS-related morbidity, including infertility [3], and distinct cervicovaginal manifestations [4, 5]. In sub-Saharan Africa, there is a geographical association between areas of high *S. haematobium* prevalence and HIV-1 infection [6], and FGS has been associated with prevalent HIV-1 [7] with biological plausibility for a causal relationship [3, 8]. Despite global advances in HIV-1 treatment and prevention, gender related disparities still exist, with particularly heightened risk among young women aged 15-24 years [9]. HIV-1 vulnerability in young women is multifactorial, including biological, behavioural, demographic, social, and structural components [10]. The potential role of FGS as an underreported and preventable co-factor in HIV-1 vulnerability needs further investigation.

Disruption of the protective vaginal and cervical epithelium by FGS-associated lesions may increase HIV-1 susceptibility by providing a portal for viral entry [3, 11]. Additionally, the environment created by *S. haematobium* eggs is more vascular [12], with an increased density of CD4+ lymphocytes [8] compared to non-egg containing tissue. Thus, tissue-entrapped *S. haematobium* eggs create a cellular milieu which may promote HIV-1 infection. Both *S. haematobium and S. mansoni* infection have been associated with prevalent HIV-1 [11]. A cross-sectional study of women with FGS, defined as parasite eggs detected in genital tissue, describes a strong association with prevalent HIV-1 but no evidence of an association between urinary schistosomiasis and HIV-1 [7]. Studies primarily evaluating urinary *S. haematobium* (without

84 universal evaluation for genital involvement) and prevalent HIV-1 have been mixed, with evidence

85 of an association with prevalent HIV-1 in a study of Tanzanian women [13], some evidence of an

86 association with prevalent HIV-1 in a study of Zimbabwean women [14], but with no evidence of

87 an association with prevalent HIV-1 in men and women with urinary *S. haematobium* in Congo

88 [15]. While *S. haematobium* seropositivity in women has been associated with HIV-1 acquisition

89 [16], the association of FGS with incident HIV-1 has not been described.

90

91 FGS diagnosis is challenging and its burden is likely underreported. The presence of parasite eggs

92 or DNA in cervicovaginal tissue is diagnostic of FGS, [17, 18], and, historically, biopsy is used as

93 a reference standard [4, 19]. However, theoretical concerns regarding post-biopsy HIV-1

94 acquisition has limited its acceptance in research settings [17]. Polymerase chain reaction (PCR)

95 on cervicovaginal lavage (CVL) is a less-invasive means of FGS diagnosis, albeit with imperfect

96 sensitivity [17, 18]. Well-defined clinical manifestations have been associated with FGS [5, 20]

97 but are variably correlated with the presence of *S. haematobium* eggs [19, 20] or DNA [17] in

98 genital tissue. The identification of clinical lesions with colposcopy is observer-dependent and

99 subject to low specificity [21]. Homogeneous yellow sandy patches have been associated with

100 herpes simplex virus-2 and human papillomavirus, and abnormal blood vessels may be associated

101 with cervical intraepithelial neoplasia [5]. Urine microscopy and circulating anodic antigen (CAA)

102 can be used to detect active schistosome infection [18, 22], but do not assess involvement of genital

103 tissue. While either *S. haematobium* and *S. mansoni* can cause FGS, the majority of cases are

104 attributed to *S. haematobium* [23, 24] and the current study focuses on *S. haematobium*. We

105 conducted an array of diagnostic tests for *S. haematobium* infection (CAA and urine microscopy)

106 and FGS (portable colposcopy, cervical swabs, vaginal swabs, and cervicovaginal lavage), and

107   have previously demonstrated that self-collected genital swabs had comparable sensitivity to

108   clinic-based, midwife-collected CVL for the detection of *Schistosoma* DNA by real-time PCR

109   [18].

110

111   The longitudinal follow-up of women in the HPTN 071 (PopART) trial in two schistosomiasis-

112   endemic communities in Zambia provided an opportunity for a nested study exploring the

113   association of FGS with HIV-1 incidence.

114

115   **Methods**

116   *Study setting and participants*

117   The cross-sectional bilharzia and HIV (BILHIV) study was nested in HPTN 071 (PopART), a

118   cluster randomized trial assessing the impact of an HIV-1 combination prevention package

119   including "universal testing and treatment" [25]. HIV-1 incidence was measured in a Population

120   Cohort (PC) comprised of one randomly selected adult (18 to 44 years of age) from a random

121   sample of households in each community who provided data and blood samples at baseline, 12,

122   24, and 36 months [25]. Between January and August 2018, after the 36-month HPTN 071

123   (PopART) PC visit, trained community workers conducted home visits to women who had

124   expressed interest in the BILHIV study [18]. Women in Livingstone, Zambia were eligible if they

125   were 18-31 years old, not pregnant, sexually active, and resident in one of the two urban *S.*

126   *haematobium* endemic communities that participated in one of two HPTN 071 (PopART)

127   intervention arms.

128

129 Following written informed consent, the BILHIV study home visit included a questionnaire,

130 genital self-sampling (cervical and vaginal), and a urine specimen, as previously described [18].

131 Within days of self-sampling, non-menstruating participants were invited to attend Livingstone

132 Central Hospital for cervicovaginal lavage (CVL) [18]. Cervicovaginal images were captured with

133 a portable colposcope (MobileODT, Tel Aviv, Israel) and evaluated by one author (EFK) for the

134 presence of any of the four accepted FGS cervicovaginal manifestations: homogenous yellow

135 sandy patches, grainy sandy patches, rubbery papules, and abnormal blood vessels [26]. Women

136 with evidence of schistosome infection by colposcopy [26] or any positive urine or genital

137 diagnostic were treated free of charge with 40 mg/kg praziquantel. Routine testing for sexually

138 transmitted infections (STI) was not performed. Participants with suspected STI were offered

139 syndromic management, as per local guidelines [27].

140

141 ***HIV-1***

142 Laboratory-based fourth-generation HIV-1 testing (Abbott Architect HIV Ag/Ab ComboAssay,

143 Wiesbaden, Germany) was performed for HPTN 071 (PopART) PC participants at each study visit

144 [25]. Additional testing using antigen/antibody screening tests, a discriminatory test, and an HIV-

145 1 RNA test was used to confirm incident HIV-1 infection, as previously described [28].

146

147 ***Circulating Anodic Antigen***

148 CAA levels reflect the burden of live schistosomes and decline after successful treatment with

149 praziquantel [22, 29]. An up-converting reporter particle lateral flow assay for the quantification

150 of CAA in urine was performed at the Leiden University Medical Center (LUMC), as previously

151 described [18, 30]. Analysing the equivalent of 417 μL urine, a CAA value of >0.6 pg/mL was

152 considered positive [22].

153

154 *PCR for detection of Schistosoma **DNA***

155 DNA extraction and PCR was performed at LUMC as previously described, using a custom

156 automated liquid handling station (Hamilton, Switzerland) [20, 31]. DNA was extracted from

157 200μL of specimen (cervical swab, vaginal swab, CVL) with QIAamp spin columns (QIAGEN

158 Benelux; Venlo, The Netherlands). Detection of the schistosome-specific internal-transcribed-

159 spacer-2 (ITS2) target was performed by real-time PCR as previously described [18, 31]. This

160 PCR does not differentiate between *Schistosoma* species. DNA amplification and detection were

161 performed with the CFX96 Real Time PCR Detection System (BioRad, California, USA). The

162 output in threshold cycles (Ct), reflecting the parasite-specific DNA load in the tested sample, was

163 analysed using BioRad CFX software. Parasite DNA loads were categorized by the following pre-

164 specified C_t thresholds: high ($C_t<30$), moderate ($30\leq C_t<35$), low ($35\leq C_t <50$) and negative (no C_t

165 detected), as previously described [32].

166

167 *Patient Consent Statement*

168 The study was approved by the University of Zambia Biomedical Research Ethics Committee

169 (reference 011-08-17), the Zambia National Health Research Authority and the London School of

170 Hygiene and Tropical Medicine Ethics Committee (reference 14506). Permission to conduct the

171 study was given by Livingstone District Health Office and the Livingstone Central Hospital

172 superintendent. Each participant provided written informed consent.

173

174 *FGS Definitions*

175 Comparison groups were defined by the results of four investigations: genital PCR, colposcopy

176 image review, urine CAA, and urine microscopy. Participants were grouped by the outcomes of

177 their diagnostic tests into three mutually exclusive categories. *FGS* was defined as at least one

178 positive genital PCR (cervical swab, vaginal swab or CVL) (Figure 1). *Probable/possible FGS*

179 was defined as the presence of either a positive urine diagnostic (CAA or microscopy) or one of

180 four cervicovaginal manifestations suggestive of FGS on portable colposcopy, or both, with

181 negative genital PCR (Figure 1). FGS *negative* was defined as negative results on all diagnostics.

182

183 **Statistical Methods**

184 Characteristics of study participants were summarized by frequency and percentage. Women

185 living with HIV-1 (WLHIV) at HPTN 071 (PopART) baseline were excluded from further

186 analyses. HIV-1 incidence was calculated as the number of seroconversions per 1000 person-years

187 of follow up. Participants contributed person-time for the calculation of HIV-1 incidence starting

188 with their first HIV-1 test and ending at date of HIV-1 seroconversion for those who seroconverted,

189 or at the date of last follow-up or the end of scheduled follow-up (whichever occurred earliest) for

190 women who did not seroconvert. HIV-1 seroconversion was assumed to occur at the midpoint

191 between the last negative and the first positive HIV-1 test. We assumed that FGS acquisition

192 occurred prior to HPTN 071 (PopART) enrolment [33]. BILHIV study participants were

193 consecutively recruited from the PC, providing the opportunity to compare the rate of incident

194 HIV-1 infection in women with and without FGS, with power determined by the number of HIV-

195 1 seroconversions and FGS prevalence. Data on HIV-1 outcomes were not available until after

196 BILHIV study closure.

197 Associations of risk factors with incident HIV-1 infection were calculated as rate ratios and 95%

198 confidence intervals, estimated using exact Poisson regression in univariable and multivariable

199 analysis. We used a causal conceptual framework to inform our choice of potential confounders.

200 *A priori,* we included age as a confounding variable. Due to loss of precision with further

201 adjustment for potential confounding variables, no additional parameters were included in the

202 multivariable model. To assess the primary exposure of interest, women with FGS (n=26) were

203 compared with an FGS *negative* comparison group comprising those who were negative on all

204 diagnostic investigations (n=218). Participants who were negative on all diagnostic investigations

205 but missing colposcopy images (n=82) were excluded from the primary analysis.

206 To evaluate the association of schistosome infection intensity with HIV-1 seroconversion, two ad

207 hoc exploratory analyses were performed. One compared participants with FGS and a

208 moderate/high *Schistosoma* DNA concentration (Ct <35) with those in the FGS *negative* group.

209 The second compared participants with FGS with ≥2 positive genital PCR specimens with those

210 in the FGS *negative* group. Data were analysed using STATA 15.1 (Stata Corporation, College

211 Station, TX).

212 **Results**

213 A total of 603 eligible women from the HPTN 071 Population Cohort were enrolled in the BILHIV

214 study. WLHIV at HPTN 071 (PopART) trial entry (n=107, 17.7%) were excluded, with 492

215 (82.1%) included in this analysis (Figure 2). Of the included participants, 14% (69/492) did not

216 attend clinic for CVL.

217

218 *Baseline characteristics*

219   The majority of participants had received at least secondary education, were not working, and

220   reported being currently sexually active. A small proportion of women reported current water

221   contact, but more than half reported childhood water contact. Active schistosome infection,

222   defined as either a positive urine microscopy (5.5%, 27/492) or detectable CAA (15.1%, 74/492),

223   was detected in 15.7% (77/492) of participants.

224

225   *HIV incidence*

226   The 492 women without HIV-1 at HPTN 071 (PopART) study entry provided a total of 1,164

227   person-years of follow-up, during which time, 20 (4.1%) incident HIV-1 infections were

228   measured, for an overall rate of 17.2 (95% CI 11.1–26.6) seroconversions per 1000 person-years.

229   HIV-1 incidence rates are shown by baseline characteristics in Table 1.

230

231   HIV-1 incidence rates were 23.6 (14.2 – 39.2) in women aged 18-24 years compared with 9.5 (3.9–

232   22.7) in women aged 25-31, (RR 0.40 [0.15–1.10]), p=0.06 (Table 1)). The HIV-1 seroconversion

233   rate decreased as the household size increased (p=0.007, test for trend) and increased as the number

234   of lifetime sexual partners increased (p=0.01, test for trend). Women self-reporting a history of

235   STI were more likely to seroconvert than women without self-reported STI (RR 5.76 [1.92–17.22],

236   p=0.009) (Table 1). No other sociodemographic or behavioural characteristics were associated

237   with HIV-1 incidence. After adjusting for age, there remained strong evidence that a higher number

238   of people residing in a household (p=0.008, test for trend), a higher number of lifetime sexual

239   partners (p=0.01, test for trend), and self-reported history of STI (aRR 6.05 [2.02–18.12], p=0.008)

240   were associated with HIV-1 seroconversion. Additionally, there was no evidence for an association

241   between urinary schistosome infection (as defined by urine CAA and/or microscopy) and HIV-1

242   seroconversion (Table 2).

243

244   *Association between FGS and HIV-1 seroconversion*

245   FGS was identified in 5.3% of women (26/492), defined as any positive genital PCR (cervical

246   swab 3.5%, [17/492]; vaginal swab 2.4%, [12/492]; or CVL 3.1%, [13/423]). Among women with

247   a negative genital PCR, results from both urine and colposcopy imaging were positive in 4.5%

248   (21/466) of participants and results from either urine or colposcopy imaging were positive in

249   31.1% (145/466). Of the participants with *probable/possible FGS*, 63.8% (106/166) had

250   colposcopy changes in isolation (Figure 1), of whom 62.3% (66/106) had abnormal blood vessels

251   and 37.7% (40/106) had grainy or homogenous yellow sandy patches on colposcopy. There were

252   218 (44.3%) participants who were negative on all diagnostic tests. The rate of HIV-1

253   seroconversion (per 1000 person/year) in women with FGS (31.0 [7.8 – 123.9]) was higher than

254   in the FGS *negative* group (11.3[5.1–25.1]) (Table 2) but without statistical evidence of a

255   difference between these rates in either univariable or multivariable analyses (crude RR 2.75

256   [0.27–15.36], p=0.26; aRR 2.16 (0.21–12.30), p=0.33) (Table 2).

257

258   *Exploratory analyses:* **Schistosoma** *DNA concentrations and disease burden*

259   In the ad hoc exploratory analysis of women (n=13) with FGS and moderate/high *Schistosoma*

260   DNA concentrations the IRR for HIV-1 acquisition after adjusting for age was 4.73 (0.46–27.05),

261   p=0.19) compared to FGS *negative* participants (Table 2). In an ad hoc exploratory analysis of

262   women (n=13) with ≥2 positive genital PCR specimens compared to FGS *negative* participants,

263   the IRR for HIV-1 acquisition after adjusting for age was 6.02 (0.58–34.96, p=0.13) (Table 2). In

264 these groups, n=9 of the women overlapped and the same two participants contributed

265 seroconversions in both groups. There were no HIV-1 seroconversions in participants with one

266 positive genital PCR.

267

268 **Discussion**

269 This study is the first to examine the association of PCR-defined FGS with HIV-1 incidence. We

270 found that women with FGS were twice as likely to seroconvert than women in the comparison

271 group albeit with wide confidence intervals and no statistical evidence for a difference. While

272 barriers to implementation still exist, PCR for FGS diagnosis is reproducible, has high specificity,

273 and can be performed on self-collected genital specimens [18].

274

275 While some cross-sectional studies show an association between schistosomiasis and prevalent

276 HIV-1 infection [13, 23], this association is not universally reported [34, 35]. The association

277 between schistosomiasis and HIV-1 is complex and cross-study comparisons require the

278 consideration of many aspects, including schistosome species (*S. haematobium* versus *S. mansoni*),

279 diagnostic tests used, assessment of genital involvement, and presentation of subgroup analyses

280 e.g. by participant's sex. Our findings, while limited by power, show a point estimate consistent

281 with increased risk of incident HIV-1, but with a wide confidence interval. Recently, two case-

282 control studies nested within longitudinal African cohorts have retrospectively assessed the

283 association between schistosome infection status and HIV-1 seroconversion with conflicting

284 results [16, 36]. A Zambian study showed an increased risk of HIV-1 acquisition in *S.*

285 *haematobium* antibody positive women (aHR=1.4, p<0.05), but not men [16]. Similar to our

286 results, the study from Kenya and Uganda did not show an association between active schistosome

287   infection and HIV-1 seroconversion, including in subgroup analyses by sex, schistosome species,

288   and infection intensity [36]. Notably, however, neither of these nested case-control studies

289   evaluated genital infection status. FGS may enhance HIV-1 vulnerability, with proposed

290   mechanisms including cervicovaginal barrier dysfunction [37], local recruitment or activation of

291   HIV-1 target cells [3], and *Schistosoma*-related alterations in integrin [38] or co-receptor [39]

292   expression.

293

294   Schistosomiasis and FGS are preventable and current WHO control measures recommend

295   praziquantel preventive chemotherapy [40]. However, current control programmes do not

296   universally achieve 75% coverage of school-aged children, representing substantial missed

297   opportunities for prevention [41, 42]. The 2025 AIDS targets place communities at risk for HIV-

298   1 in the centre of societal, system, and service enablers with a call for between sector integration

299   and synergy to advance the HIV-1 response [43]. Programmatic synergy including integrated

300   sexual and reproductive health programmes could leverage and scale-up existing HIV-1 treatment

301   and prevention resources to include FGS screening and treatment programs.

302

303   We carried out two ad hoc exploratory analyses. The intensity of schistosome infection, defined

304   by serum CAA concentration, has been strongly correlated with HIV-1 prevalence [13]. Thus, first,

305   we investigated whether *Schistosoma* DNA concentrations might be associated with HIV-1

306   infection in an analysis of 13 participants with FGS and moderate/high *Schistosoma* DNA

307   concentrations. We found no evidence of an association between FGS and HIV-1 acquisition,

308   albeit with wide confidence intervals. Additionally, participants with moderate to high seminal

309   egg excretion have higher seminal cytokine concentrations than S. haematobium negative

310   participants (Leutscher 2005). Thus secondly, we investigated the association between multiple

311   positive genital PCR specimens as a potential proxy marker of higher FGS burden and HIV-1

312   seroconversion in 13 women with ≥2 positive genital specimens for *Schistosoma* DNA. We found

313   weak evidence of an association between FGS and HIV-1 acquisition, which was less pronounced

314   in the age-adjusted estimates. These findings are hypothesis generating for the association between

315   FGS cervicovaginal disease burden or *Schistosoma* DNA concentrations and HIV-1.

316

317   This study was nested within a large population-based HIV-1 prevention trial and is the first

318   prospective study to document FGS in Zambia, but also had some relevant limitations. Similar to

319   other FGS studies using imperfect available diagnostics, there is the risk of potential diagnostic

320   misclassification, especially in these low-prevalence and low-intensity study settings. We defined

321   FGS by PCR positivity based on its semi-quantitative nature and precedent in FGS diagnosis [17,

322   18, 44]. However, *S. haematobium* eggs in semen from a male sex partner could potentially be

323   detected by PCR of vaginal specimens. We were unable to adjust for potential confounders beyond

324   age and are thus unable to exclude unmeasured and residual confounding. This was related to the

325   low number of HIV-1 seroconversions and FGS cases, which also resulted in a loss of power.

326   Overall, the effect sizes suggest the possibility of a relationship we were not sufficiently powered

327   to detect. While the prevalence of HIV-1 in the study population was high at 17.9%, the two

328   participating communities were enrolled in HPTN 071 (PopART) as intervention sites, potentially

329   reducing the number of HIV-1 seroconversions [25]. The prevalence of urinary *S. haematobium*

330   infection in this study was 5.5% (27/492), lower than anticipated, defined by the World Health

331   Organization as a low prevalence area [45]. In addition, while schistosomiasis is endemic in all of

332   Zambia's 10 provinces [46], and can be found in urban locations , it is generally considered to be

333   a focal disease of rural areas [47], and our study sites were in lower prevalence areas (<10%

334   prevalence) as defined by WHO (cite PC manual). Indeed, a 2013 survey done by the Zambian

335   Ministry of Health reported a wide range of egg-patent infection prevalence in different areas

336   ranging from 3.3 – 73.3% (median 15.0%, mean 23.3%) in school-aged children in Livingstone,

337   highlighting its focal distribution.[18]. For all the above reasons, the presented estimates, obtained

338   in a peri-urban setting, are subject to a high degree of imprecision and may not be generalizable to

339   rural communities. Lastly, vaginal and cervical swabs were self-collected by participants, raising

340   the potential for false negative genital swabs. In future work, this could be addressed by measuring

341   β-globin PCR as a positive control to confirm the presence of human DNA [48]. This study was

342   developed based on a conceptual framework which describes a potentially causal relationship

343   between FGS and HIV-1, with FGS as a potentially preventable and modifiable risk factor. In the

344   literature, albeit in cross-sectional studies, there is evidence for biological plausibility [3, 8, 12]

345   and large effect sizes for the association [7] between FGS and HIV-1. One of our study limitations

346   was the temporality of HIV-1 and FGS diagnostics. HIV-1 seroconversion was measured in HPTN

347   071 (PopART) up to three years prior to participant enrolment in the study and subsequent FGS

348   diagnosis. This sequencing assumes that FGS status and demographic descriptors at the time of

349   genital PCR sampling are similar to those at the time of HPTN 071 (PopART) study entry and/or

350   HIV-1 seroconversion. This assumption is reasonable given that FGS is thought to develop after

351   childhood water exposure [33] and persist into adulthood with chronic genital lesions, often

352   persisting despite treatment with praziquantel [49]. A large, prospective, longitudinal study in

353   areas of higher *S. haematobium* endemicity is needed to evaluate incident HIV-1 infection in

354   women with known *S. haematobium* and FGS status at study baseline. In future work, it will be

355   important to continue to analyse HIV-1 outcomes by both *S. haematobium* infection status and

356   FGS definition (PCR versus clinical findings) to evaluate HIV-1 risk profiles.

357

358   In conclusion, this analysis from a limited number of co-infected women does not show evidence

359   of association between FGS and HIV-1 incidence. The hypothesis generating observations that

360   FGS and in particular higher FGS cervicovaginal disease burden or *Schistosoma* DNA

361   concentrations may be associated with HIV-1 acquisition should be investigated in a larger

362   longitudinal study in a high FGS prevalence area to better explore the role of FGS in HIV-1

363   acquisition.

364
365
366

390 Kantukaleza, and Judith Lungu. We also acknowledge tremendous support in Livingstone from

391 Clement Mwakamui (Zambart) and Tobias Mweene (Zambart). We gratefully acknowledge Eric

392 A.T. Brienen (LUMC) for performing the genital PCR analysis and Claudia J. de Dood (LUMC)

393 and Pytsje T. Hoekstra (LUMC) for performing the CAA analysis.

394

395 **Author Contributions:**

396 Amy S. Sturt – conceptualization, data curation, formal analysis, BILHIV project administration,

397 visualization, original manuscript preparation, manuscript editing and revision

398 Emily L. Webb – conceptualization, data curation, formal analysis, supervision, visualization,

399 original manuscript preparation, manuscript editing and revision

400 Comfort R. Phiri – BILHIV project administration, writing – review and editing

401 Maina Mudenda – investigation, writing – review and editing

402 Joyce Mapani – investigation, writing – review and editing

403 Barry Kosloff – conceptualization, resources, HPTN 071 (PopART) project administration,

404 investigation, writing – review and editing

405 Maina Cheeba – HPTN 071 (PopART) project administration, investigation, writing – review

406 and editing

407 Kwame Shanube – HPTN 071 (PopART) project administration, writing – review and editing

408 Justin Bwalya – HPTN 071 (PopART) project administration, writing – review and editing

409 Eyrun F. Kjetland – investigation, writing – review and editing

410 Suzanna C. Francis – conceptualization, supervision, original manuscript preparation, manuscript

411 editing and revision

412 Paul L.A.M. Corstjens – investigation, writing – review and editing

413 Govert J. van Dam – investigation, writing – review and editing

414 Lisette van Lieshout – investigation, writing – review and editing

415 Isaiah Hansingo – conceptualization, resources, supervision, writing – review and editing

416 Helen Ayles – conceptualization, resources, supervision, writing – review and editing

417 Richard J. Hayes – conceptualization, resources, supervision, formal analysis, writing – review

418 and editing

419 Amaya L. Bustinduy – conceptualization, funding acquisition, supervision, original manuscript

420 preparation, manuscript editing and revision

421

422

423

REFERENCES

1. UNAIDS. No more neglect — Female genital schistosomiasis and HIV — Integrating sexual and reproductive health interventions to improve women's lives, 2019. Available at: https://www.unaids.org/en/resources/documents/2019/female_genital_schistosomiasis_and_hiv Accessed April 30, 2021.

2. Odegaard JI, Hsieh MH. Immune responses to Schistosoma haematobium infection. Parasite Immunol **2014**; 36:428-38.

3. Sturt AS, Webb EL, Francis SC, Hayes RJ, Bustinduy AL. Beyond the barrier: Female Genital Schistosomiasis as a potential risk factor for HIV-1 acquisition. Acta Trop **2020**; 209:105524.

4. Poggensee G, Kiwelu I, Weger V, et al. Female genital schistosomiasis of the lower genital tract: prevalence and disease-associated morbidity in northern Tanzania. The Journal of infectious diseases **2000**; 181:1210-3.

5. Kjetland EF, Ndhlovu PD, Mduluza T, et al. Simple clinical manifestations of genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg **2005**; 72:311-9.

6. Ndeffo Mbah ML, Poolman EM, Drain PK, Coffee MP, van der Werf MJ, Galvani AP. HIV and Schistosoma haematobium prevalences correlate in sub-Saharan Africa. Trop Med Int Health **2013**; 18:1174-9.

7. Kjetland EF, Ndhlovu PD, Gomo E, et al. Association between genital schistosomiasis and HIV in rural Zimbabwean women. AIDS **2006**; 20:593-600.

8. Jourdan PM, Holmen SD, Gundersen SG, Roald B, Kjetland EF. HIV target cells in Schistosoma haematobium-infected female genital mucosa. Am J Trop Med Hyg **2011**; 85:1060-4.

9. UNAIDS. UNAIDS Global AIDS Update, Communities at the Centre 2019, available at: https://www.unaids.org/en/20190716_GR2019_communities [accessed November 2, 2020].

10. Birdthistle I, Tanton C, Tomita A, et al. Recent levels and trends in HIV incidence rates among adolescent girls and young women in ten high-prevalence African countries: a systematic review and meta-analysis. The Lancet Global health **2019**; 7:e1521-e40.

11. Patel P, Rose CE, Kjetland EF, et al. Association of schistosomiasis and HIV infections: A systematic review and meta-analysis. Int J Infect Dis **2021**; 102:544-53.

12. Jourdan PM, Roald B, Poggensee G, Gundersen SG, Kjetland EF. Increased vascularity in cervicovaginal mucosa with Schistosoma haematobium infection. PLoS Negl Trop Dis **2011**; 5:e1170.

13. Downs JA, van Dam GJ, Changalucha JM, et al. Association of Schistosomiasis and HIV infection in Tanzania. Am J Trop Med Hyg **2012**; 87:868-73.

14. Ndhlovu PD, Mduluza T, Kjetland EF, et al. Prevalence of urinary schistosomiasis and HIV in females living in a rural community of Zimbabwe: does age matter? Trans R Soc Trop Med Hyg **2007**; 101:433-8.

15. N'Zoukoudi-N'Doundou MY, Dirat I, Akouala JJ, Penchenier L, Makuwa M, Rey JL. [Bilharziasis and human immunodeficiency virus infection in Congo]. Med Trop (Mars) **1995**; 55:249-51.

16. Wall KM, Kilembe W, Vwalika B, et al. Schistosomiasis is associated with incident HIV transmission and death in Zambia. PLoS Negl Trop Dis **2018**; 12:e0006902.

466   17. Kjetland EF, Hove RJ, Gomo E, et al. Schistosomiasis PCR in vaginal lavage as an indicator of
467   genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg
468   **2009**; 81:1050-5.
469   18. Sturt AS, Webb EL, Phiri CR, et al. Genital self-sampling compared with cervicovaginal lavage
470   for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study. PLoS
471   Negl Trop Dis **2020**; 14:e0008337.
472   19. Leutscher P, Ravaoalimalala VE, Raharisolo C, et al. Clinical findings in female genital
473   schistosomiasis in Madagascar. Trop Med Int Health **1998**; 3:327-32.
474   20. Randrianasolo BS, Jourdan PM, Ravoniarimbinina P, et al. Gynecological manifestations,
475   histopathological findings, and schistosoma-specific polymerase chain reaction results among
476   women with Schistosoma haematobium infection: a cross-sectional study in Madagascar. The
477   Journal of infectious diseases **2015**; 212:275-84.
478   21. Galappaththi-Arachchige HN, Holmen S, Koukounari A, et al. Evaluating diagnostic indicators
479   of urogenital Schistosoma haematobium infection in young women: A cross sectional study in
480   rural South Africa. PloS one **2018**; 13:e0191459.
481   22. Corstjens P, de Dood CJ, Knopp S, et al. Circulating Anodic Antigen (CAA): A Highly Sensitive
482   Diagnostic Biomarker to Detect Active Schistosoma Infections-Improvement and Use during
483   SCORE. Am J Trop Med Hyg **2020**; 103:50-7 doi:10.4269/ajtmh.19-0819.
484   23. Downs JA, Mguta C, Kaatano GM, et al. Urogenital schistosomiasis in women of
485   reproductive age in Tanzania's Lake Victoria region. Am J Trop Med Hyg **2011**; 84:364-9.
486   24. Wright ED, Chiphangwi J, Hutt MS. Schistosomiasis of the female genital tract. A
487   histopathological study of 176 cases from Malawi. Trans R Soc Trop Med Hyg **1982**; 76:822-9.
488   25. Hayes RJ, Donnell D, Floyd S, et al. Effect of Universal Testing and Treatment on HIV
489   Incidence - HPTN 071 (PopART). N Engl J Med **2019**; 381:207-18.
490   26. World Health Organization. Female genital schistosomiasis: a pocket atlas for clinical health-
491   care professionals. World Health Organization. 2015. Available at:
492   http://www.who.int/iris/handle/10665/180863 Accessed April 30, 2021.
493   27. Zambian Ministry of Health. Guidelines for the Etiological and Clinical Management of
494   Sexually Transmitted Infections in Zambia, 2017.
495   28. Eshleman SH, Piwowar-Manning E, Wilson EA, et al. Determination of HIV status and
496   identification of incident HIV infections in a large, community-randomized trial: HPTN 071
497   (PopART). J Int AIDS Soc **2020**; 23:e25452.
498   29. van Lieshout L, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by
499   determination of the circulating antigens CAA and CCA, in particular in individuals with recent
500   or light infections. Acta Trop **2000**; 77:69-80.
501   30. Corstjens PL, De Dood CJ, Kornelis D, et al. Tools for diagnosis, monitoring and screening of
502   Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels.
503   Parasitology **2014**; 141:1841-55.
504   31. Obeng BB, Aryeetey YA, de Dood CJ, et al. Application of a circulating-cathodic-antigen
505   (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of
506   Schistosoma haematobium in urine samples from Ghana. Annals of tropical medicine and
507   parasitology **2008**; 102:625-33.
508   32. Pillay P, Taylor M, Zulu SG, et al. Real-time polymerase chain reaction for detection of
509   Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital

510 Schistosomiasis in primary school girls in KwaZulu Natal, South Africa. Am J Trop Med Hyg **2014**;
511 90:546-52.
512 33. Hegertun IE, Sulheim Gundersen KM, Kleppa E, et al. S. haematobium as a common cause of
513 genital morbidity in girls: a cross-sectional study of children in South Africa. PLoS Negl Trop Dis
514 **2013**; 7:e2104.
515 34. Kapiga S, Hansen CH, Downs JA, et al. The burden of HIV, syphilis and schistosome infection
516 and associated factors among adults in the fishing communities in northwestern Tanzania. Trop
517 Med Int Health **2021**; 26:204-13.
518 35. Sanya RE, Muhangi L, Nampijja M, et al. Schistosoma mansoni and HIV infection in a
519 Ugandan population with high HIV and helminth prevalence. Trop Med Int Health **2015**;
520 20:1201-8.
521 36. Bochner AF, Baeten JM, Secor WE, et al. Associations between schistosomiasis and HIV-1
522 acquisition risk in four prospective cohorts: a nested case-control analysis. J Int AIDS Soc **2020**;
523 23:e25534.
524 37. Feldmeier H, Krantz I, Poggensee G. Female genital schistosomiasis: a neglected risk factor
525 for the transmission of HIV? Trans R Soc Trop Med Hyg **1995**; 89:237.
526 38. Yegorov S, Joag V, Galiwango RM, et al. Schistosoma mansoni treatment reduces HIV entry
527 into cervical CD4+ T cells and induces IFN-I pathways. Nat Commun **2019**; 10:2296.
528 39. Kleppa E, Ramsuran V, Zulu S, et al. Effect of female genital schistosomiasis and anti-
529 schistosomal treatment on monocytes, CD4+ T-cells and CCR5 expression in the female genital
530 tract. PloS one **2014**; 9:e98593.
531 40. World Health Organization. Schistosomiasis. Control and preventive chemotherapy.
532 Available at https://www.who.int/schistosomiasis/strategy/en/. Accessed March 31, 2021.
533 41. World Health Organization. Schistosomiasis and soil-transmitted helminthiasis: number of
534 people treated in 2016. Available at:
535 https://www.who.int/neglected_diseases/resources/who_wer9249/en/. Accessed April 30,
536 2021.
537 42. World Health Organization. Ending the neglect to attain the Sustainable Development Goals
538 A road map for Neglected Tropical Diseases 2021-2030. Available at:
539 https://www.who.int/publications/i/item/9789240010352. Accessed April 30, 2021.
540 43. UNAIDS. Prevailing against pandemics by putting people at the centre - World AIDS Day
541 report 2020. Available at: https://www.unaids.org/en/resources/documents/2020/prevailing-
542 against-pandemics Accessed April 30, 2021.
543 44. Pillay P, van Lieshout L, Taylor M, et al. Cervical cytology as a diagnostic tool for female
544 genital schistosomiasis: Correlation to cervical atypia and Schistosoma polymerase chain
545 reaction. CytoJournal **2016**; 13:10.
546 45. World Health Organization. Preventive chemotherapy in human helminthiasis : coordinated
547 use of anthelminthic drugs in control interventions : a manual for health professionals and
548 programme managers. 2006. Available at:
549 https://apps.who.int/iris/bitstream/handle/10665/43545/9241547103_eng.pdf;jsessionid=564
550 247BFBA426C9B5D72B1048671034E?sequence=1 Accessed April 30, 2021.
551 46. Kalinda C, Chimbari MJ, Mukaratirwa S. Schistosomiasis in Zambia: a systematic review of
552 past and present experiences. Infect Dis Poverty **2018**; 7:41.

553   47. Knopp S, Person B, Ame SM, et al. Elimination of schistosomiasis transmission in Zanzibar:
554   baseline findings before the onset of a randomized intervention trial. PLoS Negl Trop Dis **2013**;
555   7:e2474.
556   48. Polman NJ, Ebisch RMF, Heideman DAM, et al. Performance of human papillomavirus
557   testing on self-collected versus clinician-collected samples for the detection of cervical
558   intraepithelial neoplasia of grade 2 or worse: a randomised, paired screen-positive, non-
559   inferiority trial. Lancet Oncol **2019**; 20:229-38.
560   49. Downs JA, Kabangila R, Verweij JJ, et al. Detectable urogenital schistosome DNA and cervical
561   abnormalities 6 months after single-dose praziquantel in women with Schistosoma
562   haematobium infection. Trop Med Int Health **2013**; 18:1090-6.
563

Table 1 HIV-1 seroconversion rates by baseline BILHIV study characteristic in 492 Zambian women

Socio-behavioural Characteristics		No. (%)	Events	Rate of HIV Seroconversion per 1000 p/y	Crude IRR (95% CI)	p-value
Age in years	18-24	289 (58.7)	15	23.6 (14.2 – 39.2)	Reference	0.06
	25-31	203 (41.3)	5	9.5 (3.9 – 22.7)	0.40 (0.15 – 1.10)	
Marital status	Single	219 (44.5)	12	23.0 (13.1 – 40.5)	Reference	0.35
	Married or Cohabiting	255 (51.8)	7	11.8 (5.6 – 24.7)	0.51 (0.20 – 1.30)	
	Widowed, Divorced, or Separated	18 (3.7)	1	21.2 (3.0 – 150.6)	0.92 (0.12 – 7.10)	
Education (highest level)	None or Any Primary School	137 (27.9)	5	15.7 (6.5 – 37.6)	Reference	0.57
	Any Secondary School	297 (60.4)	11	15.5 (8.6 – 28.0)	0.99 (0.34 – 2.85)	
	Trade, Degree or higher	58 (11.8)	4	29.4 (11.0 – 78.4)	1.88 (0.50 – 7.00)	
District	Community A	260 (52.9)	11	15.5 (8.6 – 28.0)	Reference	0.60
	Community B	232 (47.2)	9	19.8 (10.3 – 38.0)	1.27 (0.53 – 3.07)	
Household members	1-3	141 (28.7)	12	38.4 (21.8 – 67.6)	Reference	0.007$^\phi$
	4-5	201 (40.9)	4	8.2 (3.1 – 21.7)	0.21 (0.07 – 0.66)	
	6+	150 (30.5)	4	11.1 (4.2 – 29.5)	0.29 (0.09 – 0.90)	
Employment status	Not Working	327 (66.5)	13	17.1 (10.0 – 29.5)	Reference	0.99
	Working	165 (33.5)	7	17.3 (8.2 – 36.2)	1.01 (0.40 – 2.52)	

Sexual behaviour characteristics

Age at sexual debut (years)	8-16	197 (40.0)	9	20.6 (10.7 – 39.5)	Reference	0.79
	17-19	220 (44.7)	8	14.9 (7.5 – 29.9)	0.73 (0.28 – 1.88)	
	20-24	75 (15.2)	3	15.7 (5.1 – 48.8)	0.77 (0.21 – 2.83)	
Lifetime sexual partners	1	149 (30.3)	2	5.7 (1.4 – 22.9)	Reference	0.01$^{\phi}$
	2	134 (27.2)	5	15.9 (6.6 – 38.3)	2.78 (0.54 – 14.34)	
	3	103 (20.9)	5	18.7 (7.8 – 45.0)	3.27 (0.63 – 16.85)	
	4+	106 (21.5)	8	34.1 (17.1 – 68.2)	5.95 (1.26 – 28.02)	
Currently sexually active[**,†]	No	63 (12.9)	3	20.1 (6.5 – 62.4)	Reference	0.78
	Yes	427 (87.1)	17	16.9 (10.5 – 27.1)	0.84 (0.25 – 2.86)	
STI history[††]	No	466 (94.9)	16	14.4 (8.8 – 23.5)	Reference	0.009
	Yes	25 (5.1)	4	82.8 (31.1 – 220.5)	5.76 (1.92 – 17.22)	
Condom use with last sex[†††]	No	367 (75.8)	12	13.8 (7.9 – 24.3)	Reference	0.11
	Yes	117 (24.2)	8	29.2 (14.6 – 58.3)	2.11 (0.86 – 5.16)	
Contraceptive Use						
Condoms	No	407 (82.7)	14	14.8 (8.8 – 25.0)	Reference	0.23
	Yes	85 (17.3)	6	27.5 (12.4 – 61.2)	1.86 (0.71 – 4.83)	
OCP	No	440 (89.4)	18	17.3 (10.9 – 27.4)	Reference	0.96
	Yes	52 (10.6)	2	16.6 (4.1 – 66.3)	0.96 (0.22 – 4.14)	
Injectable	No	225 (45.7)	14	22.5 (13.4 – 38.1)	Reference	0.13
	Yes	267 (54.3)	6	11.1 (5.0 – 24.6)	0.49 (0.19 – 1.28)	

26

Implant	No	466 (94.7)	18	16.3 (10.3 – 25.9)	Reference	0.37
	Yes	26 (5.3)	2	34.0 (8.5 – 135.8)	2.10 (0.48 – 8.99)	

566 $^{\phi}$ test for trend p-value
567 **Any sexual activity in the last 6 months
568 †Participants who responded with "no answer" (n=2) are not shown in the table (HIV seroconversions =0)
569 †† STI history was self-reported, participants who responded with "no answer" (n=1) are not shown (HIV seroconversions =0)
570 †††Participants who responded with "no answer" (n=8) are not shown in the table (HIV seroconversions =0)
571
572

573 **Table 2 – HIV-1 incidence by FGS status and schistosomiasis-related factors**

Category		N (%)	Incident HIV Cases	Total PY	Rate per 1000 PY (95% CI)	IRR (95% CI)	p-value	aRR[*]	p-value
FGS Negative[**]		218 (53.2)	6	532.0	11.3 (5.1 – 25.1)	Reference	0.26[#]	Reference	0.33[#]
Probable/possible FGS		166 (40.5)	7	372.2	18.8 (9.0 – 39.5)	1.67 (0.48 – 6.01)		1.73 (0.50 – 6.22)	
FGS		26 (6.3)	2	64.5	31.0 (7.8 – 123.9)	2.75 (0.27 – 15.36)		2.16 (0.21 – 12.30)	
Exploratory Analysis of Participants with FGS									
FGS Negative		218	6	532.0	11.3 (5.1 – 25.1)	Reference	0.09	Reference	0.13
FGS and 2-3 PCR Positive[##]		13	2	24.9	80.4 (20.1 – 321.7)	7.13 (0.70 – 39.89)		6.02 (0.58 – 34.96)	
FGS Negative		218	6	532.0	11.3 (5.1 – 25.1)	Reference	0.15	Reference	0.19
FGS and PCR Ct<35 [†]		13	2	32.6	61.31 (15.33 – 245.14)	5.44 (0.54 – 30.40)		4.73 (0.46 – 27.05)	
Schistosomiasis-related factors[††]									
Urine Microscopy	Negative	465 (94.5)	18	1,102.4	16.3 (10.3 – 25.9)	Reference	0.40	Reference	0.47
	Positive	27 (5.5)	2	61.6	32.5 (8.1 – 129.9)	1.98 (0.46 – 8.58)		1.78 (0.41 – 7.71)	
Urine CAA[‡]	Not detectable	416 (84.9)	16	993.5	16.1 (9.9 – 26.3)	Reference	0.86	Reference	0.78
	Detectable	74 (15.1)	3	166.6	18.0 (5.8 – 55.8)	1.12 (0.33 – 3.84)		1.19 (0.35 – 4.10)	
Active Infection[‡, ‡‡]	Not Present	413 (84.3)	16	985.0	16.2 (10.0 – 26.5)	Reference	0.93	Reference	0.85
	Present	77 (15.7)	3	175.1	17.1 (5.5 – 53.1)	1.05 (0.31 – 3.62)		1.13 (0.33 – 3.88)	

574

575 [**]82 participants who were negative on all diagnostic tests but missing portable colposcopy images were excluded from this analysis

576 [*]Adjusted for age

577 #Test for trend p-value (RR per unit of the exposure variable [FGS *Negative*, *Probable/Possible* FGS and FGS] treated as a continuous
578 variable)
579 ##n=13 excluded (one genital PCR specimen positive)
580 †n=13 excluded (Ct>35)
581 †† n=492, unless otherwise specified
582 ‡n=490, 2 vials arrived at the laboratory empty, HIV-1 seroconversion occurred in (n=1) of these participants
583 ‡‡defined as detectable urine CAA or positive urine microscopy
584
585

586 **Abbreviations:** aRR – adjusted rate ratio, CAA – circulating anodic antigen, Ct – cycle threshold, FGS – female genital schistosomiasis, IRR –
587 incidence rate ratio, PCR – polymerase chain reaction, PY – person-years
588

589 **FIGURE TITLES & LEGENDS**

590 **Figure 1 Title: Female Genital Schistosomiasis categories and Venn diagram illustrating**

591 **results by diagnostic test type**

592 **Figure 1 Legend:**

593 **A**. Female Genital Schistosomiasis diagnostic categories

594 **B.** Participants in the diagnostic categories by test result

595 Participants within the FGS and Probable/Possible FGS categories do not overlap.

596

597 **Figure 2 Title: Study Flow Diagram**

598 **Figure 2 Legend:**

599 **Not visited (n=189)**– the participant was not visited before the study closed for enrolment

600 **Visited but not contacted (n=110)**– a visit was made to the study household, but the participant

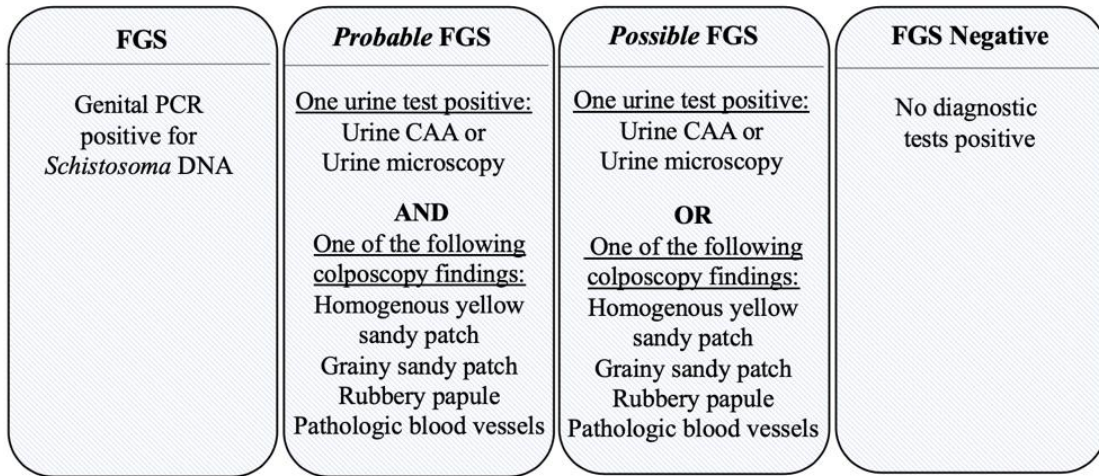601 could not be located (70), had relocated (39), or died (1)

602 **Contacted & not immediately enrolled (n=120)**– **visited but not recruited** (42), out of town

603 (18), declined to participate (60)

604 **Contacted & ineligible (n=41)**– virgin (16), pregnant (17), over 31 (8)

605

606

607 Figure 1

A

FGS	*Probable* FGS	*Possible* FGS	FGS Negative
Genital PCR positive for *Schistosoma* DNA	One urine test positive: Urine CAA or Urine microscopy **AND** One of the following colposcopy findings: Homogenous yellow sandy patch Grainy sandy patch Rubbery papule Pathologic blood vessels	One urine test positive: Urine CAA or Urine microscopy **OR** One of the following colposcopy findings: Homogenous yellow sandy patch Grainy sandy patch Rubbery papule Pathologic blood vessels	No diagnostic tests positive

B

Genital PCR negative and Urine CAA or microscopy positive, n=60

FGS Genital PCR Positive n=26

Possible FGS Urine CAA or Microscopy Positive n=39

Probable FGS Urine & Image Positive n=21

Possible FGS Colposcopy Image Positive n=106

Genital PCR negative with any of four colposcopy findings, n=127
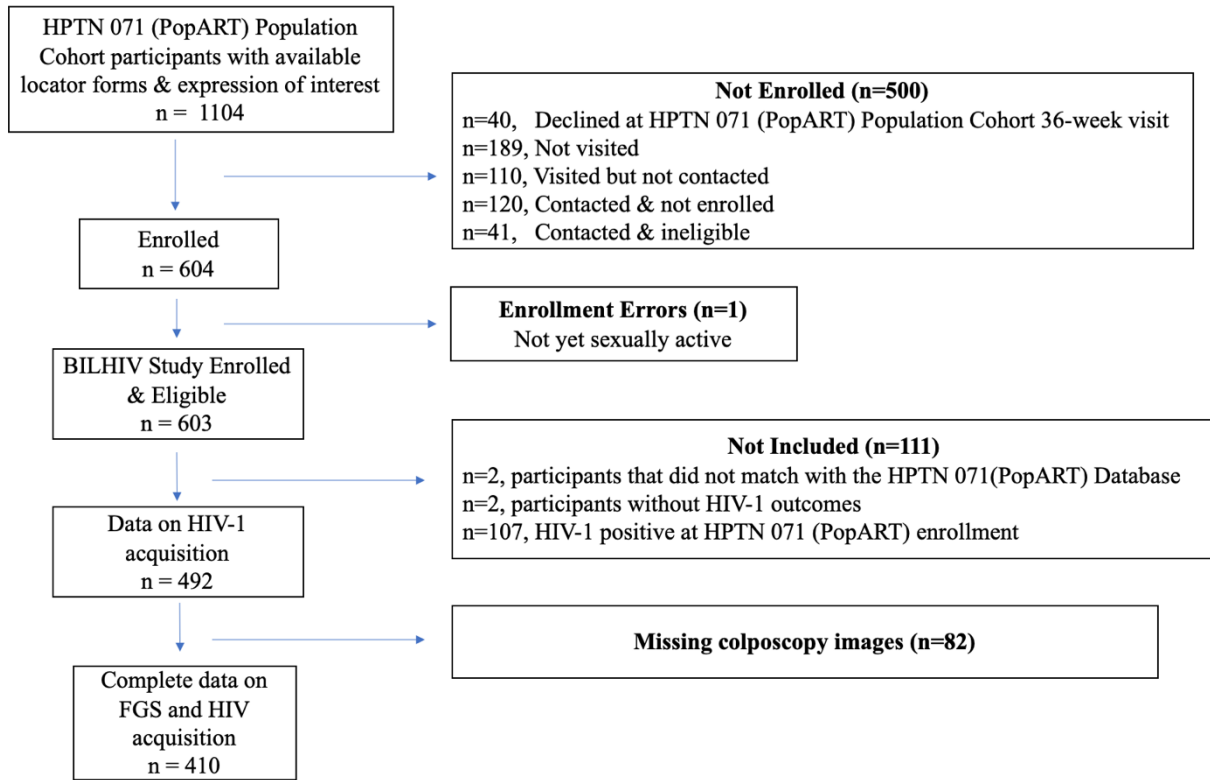
608

609

610   Figure 2
611

Figure 2 flowchart:

HPTN 071 (PopART) Population Cohort participants with available locator forms & expression of interest
n = 1104

→ **Not Enrolled (n=500)**
n=40, Declined at HPTN 071 (PopART) Population Cohort 36-week visit
n=189, Not visited
n=110, Visited but not contacted
n=120, Contacted & not enrolled
n=41, Contacted & ineligible

Enrolled
n = 604

→ **Enrollment Errors (n=1)**
Not yet sexually active

BILHIV Study Enrolled & Eligible
n = 603

→ **Not Included (n=111)**
n=2, participants that did not match with the HPTN 071(PopART) Database
n=2, participants without HIV-1 outcomes
n=107, HIV-1 positive at HPTN 071 (PopART) enrollment

Data on HIV-1 acquisition
n = 492

→ **Missing colposcopy images (n=82)**

Complete data on FGS and HIV acquisition
n = 410

612