High *Mycoplasma genitalium* Organism Burden Is Associated with Shedding of HIV-1 DNA from the Cervix

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We assessed the relationship between infection with *Mycoplasma genitalium*, an emerging sexually transmitted pathogen, and cervical shedding of human immunodeficiency virus (HIV)–1 DNA among 303 HIV-1–positive Kenyan women. HIV-1 shedding was detected by qualitative polymerase chain reaction (PCR) in 154 women (51%); *M. genitalium* was detected by qualitative PCR in 52 (17%), and organism burden was determined by quantitative PCR. Women with high *M. genitalium* organism burdens (more than the median of 3195 genomes/mL) were 3-fold more likely to shed HIV-1 DNA than were *M. genitalium*–negative women (adjusted OR, 2.9 [95% confidence interval, 1.1–7.6]), yet this did not appear to be mediated by traditional measures of cervical inflammation (elevated polymorphonuclear leukocyte count).

Sexually transmitted infections (STIs) are thought to increase the infectiousness of HIV-1–seropositive individuals by recruiting HIV-1–infected lymphocytes and macrophages to genital mucosal sites and by disrupting epithelial integrity [1]. Inflammatory syndromes such as vaginitis, cervicitis, and urethritis are strongly associated with increased HIV-1 load in genital secretions [2–4], and treatment of these syndromes decreases genital viral shedding [5]. *Mycoplasma genitalium,* an emerging sexually transmitted pathogen, has been associated with nongonococcal urethritis in men [6] and with cervicitis [7] and endometritis [8] in women. We evaluated whether *M. genitalium* was associated with HIV-1 shedding in cervical secretions of HIV-1–infected women.

Methods. Between December 1994 and April 1996, 318 HIV-1–seropositive women attending an STI clinic in Mombasa, Kenya, were enrolled in a cross-sectional study to identify factors associated with genital shedding of HIV-1 DNA, as described elsewhere [3]. Participants provided informed consent, and study procedures were approved by the University of Washington and University of Nairobi institutional review boards.

Clinical examinations were conducted by one investigator (S.B.M.). Women provided a blood specimen and underwent a speculum pelvic examination. Dacron swabs of cervical secretions were collected, placed in dry cryovials or 1 mL of freezing medium (70% RPMI 1640, 20% fetal calf serum, and 10% DMSO, with added penicillin, streptomycin, and amphotericin B) and subsequently placed on ice. On receipt in the laboratory, swabs were frozen, either at −70°C or in liquid nitrogen. Abnormal cervical discharge was defined as cloudy/white, yellow/green, brown, or bloody exudates. Elevated polymorphonuclear leukocyte (PMN) count was defined as ⩾30 PMNs/high-power field of gram-stained cervical secretions. Women with signs or symptoms of STIs were treated according to Kenya's national treatment guidelines.

HIV-1 seropositivity was confirmed using 2 ELISAs (Detect from BioChem ImmunoSystems and Recombigen from Cambridge Biotech). CD4 lymphocytes were enumerated using Cytosphere (Coulter). *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were originally identified by culture and antigen detection, respectively [3]. For the present study, we retested archived cervical swabs for *N. gonorrhoeae* and *C. trachomatis* by polymerase chain reaction (PCR) (Amplicor CT-NG multiplex assay; Roche Diagnostics).

Cervical swabs were shipped to the University of Washington and were tested for HIV-1 DNA by nested PCR amplification of the *gag* gene [9]. Of the original 318 women enrolled, 303 had archived specimens available for *M. genitalium* testing. These included 185 (61%) stored in dry cryovials (rehydrated with 1 mL of 2SP buffer [0.2 mol/L sucrose in 0.02 mol/L phosphate buffer]) and 118 (39%) stored in freezing medium. *M. genitalium* was detected by PCR [10] after preparation by use of the MasterPure DNA purification kit (Epicentre). All PCR-positive specimens underwent a second *M. genitalium* quantitative

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Table 1. Characteristics associated with *Mycoplasma genitalium* infection among 303 HIV-1–positive women.

Characteristic	*M. genitalium* negative ($n = 251$)	*M. genitalium* positive			
		Low burden[a] ($n = 26$)	*P* for low burden[b]	High burden[c] ($n = 26$)	*P* for high burden[b]
Demographic and behavioral characteristics					
Age <28 years	100 (40)	13 (50)	.40	20 (77)	.001
Zero sex partners in the past week	88 (35)	6 (23)	.28	2 (8)	.004
Condom used in the past week	66 (26)	13 (50)	.02	12 (46)	.04
Last sexual intercourse ⩽7 days ago	162 (65)	20 (77)	.28	24 (92)	.004
Hormonal contraceptive use (current)	78 (33)	6 (26)	.64	9 (38)	.65
Cervical pathogens					
Chlamydia trachomatis	14 (5)	2 (8)	.65	5 (19)	.02
Neisseria gonorrhoeae	25 (10)	3 (11)	.74	2 (8)	1.0
Clinical characteristics					
Abnormal cervical exudates[d]	67 (27)	8 (31)	.67	15 (58)	.001
Elevated cervical PMN count (⩾30/HPF)	68 (28)	7 (27)	.95	10 (39)	.24
Easily induced cervical bleeding	43 (17)	3 (12)	.59	6 (23)	.43
HIV disease					
Cervical shedding of HIV-1 DNA	125 (50)	12 (46)	.84	18 (69)	.06
CD4 cell count, median (range), cells/μL	427 (29–1402)	464 (58–934)	.79[e]	402 (29–1343)	.95[e]

NOTE. Data are no. (%) of participants, unless otherwise indicated. HPF, high-power field; PMN, polymorphonuclear leukocyte.

[a] *M. genitalium* organism burden <3195 genomes/mL.

[b] Fisher's exact test for comparison with *M. genitalium*–negative women, unless otherwise indicated.

[c] *M. genitalium* organism burden ⩾3195 genomes/mL.

[d] Abnormal cervical discharge (cloudy/white, yellow/green, brown, or bloody exudates).

[e] Wilcoxon rank sum test for comparison with *M. genitalium*–negative women.

PCR assay using primers MG16–45F and MG16–447R [11], performed on a LightCycler 2.0 real-time PCR instrument (Roche Applied Science) under the following conditions: 95°C for 10 min; 45 cycles of 95°C for 10 s, 60°C for 5 s, and 72°C 18 s; a single acquisition at 83°C; and a melting program to 55°C for 30 s. PCR products were detected with SYBR Green (Roche Applied Science). This assay is specific for the 16S rDNA of *M. genitalium* and has an analytical sensitivity of 10 genomes/reaction tube. Specimens that were initially positive but that were subsequently negative by the quantitative assay ($n = 5$) were classified as <200 genomes/mL.

We assessed the relationship between cervical HIV-1 DNA shedding and *M. genitalium* infection, defined as present/not present and by quantitative organism burden (dichotomized at the median). For univariate analyses, we used Fisher's exact test for categorical comparisons and the Wilcoxon rank-sum test for continuous factors. In multivariate logistic regression analysis, we used manual forward selection. Factors that were associated with the detection of *M. genitalium* in the present analysis or with HIV-1 shedding in previous analyses [3] were considered for inclusion in the multivariate model. Factors were retained if they were significantly associated with HIV shedding at $P < .05$ or if their inclusion changed the odds ratio (OR) for *M. genitalium* by ⩾10%. All analyses were conducted using Stata (version 8.0; StataCorp).

Results. The median age of these 303 HIV-1–infected women was 28 years (range, 17–46 years), and 72% reported commercial sex work. The median CD4 cell count was 431 cells/μL (range, 29–1402 cells/μL), and 191 women (63%) had CD4 cell counts <500 cells/μL. *C. trachomatis* was detected in 21 women (7%), and *N. gonorrhoeae* was detected in 30 (10%).

M. genitalium was detected in 52 women (17%). There were no significant differences in *M. genitalium* prevalence by specimen storage conditions (i.e., dry vs. freezing medium). The median organism burden was 3195 genomes/mL, with a broad range (<200 to 5.94×10^6 genomes/mL). Women with *M. genitalium* organism burdens above the median ($n = 26$ [9%]) were significantly younger, had more sex partners, were more likely to report recent condom use, and had a shorter interval between their last sexual intercourse and the study visit than *M. genitalium*–negative women (table 1). High *M. genitalium* organism burden was also significantly associated with *C. trachomatis* infection and abnormal cervical exudates but was not significantly associated with elevated cervical PMN counts (OR, 1.6 [95% confidence interval {CI}, 0.7–3.8]). The difference seen for abnormal cervical exudates was primarily due to a higher prevalence of white/cloudy exudates among those with high *M. genitalium* organism burdens, compared with that among *M. genitalium*–negative women (20% vs. 7%; $P = .004$), with smaller differences in the prevalence of yellow/green exudates (19% vs.

Table 2. Multivariate analysis of the association between *Mycoplasma genitalium* infection and cervical shedding of HIV-1 DNA among 303 HIV-1–positive women.

Characteristic	OR[a] (95% CI)
M. genitalium	
Negative	1.0
Positive, low burden[b]	1.0 (0.4–2.3)
Positive, high burden[c]	2.9 (1.1–7.6)
Neisseria gonorrhoeae	
Negative	1.0
Positive	1.9 (0.8–4.5)
Chlamydia trachomatis	
Negative	1.0
Positive	0.6 (0.2–1.6)
CD4 cell count	
Per 100 cell/μL decrease	1.3 (1.2–1.5)
Contraceptive use	
Not using hormonal contraception	1.0
Using depot medroxyprogesterone acetate	2.9 (1.5–5.8)
Using oral contraceptives	4.5 (1.9–10.7)

NOTE. CI, confidence interval; OR, odds ratio.

[a] Adjusted for all variables in the table. Further adjustment for age, storage conditions of the cervical swab, recent condom use, no. of sex partners in the past week, and time since last intercourse did not appreciably change the estimates.

[b] *M. genitalium* organism burden <3195 genomes/mL.

[c] *M. genitalium* organism burden ⩾3195 genomes/mL.

10%; $P = .15$). Women with low *M. genitalium* organism burdens (below the median; $n = 26$) were generally similar to *M. genitalium*–negative women, with the exception of more frequent condom use in the past week.

Cervical shedding of HIV-1 DNA was detected in 154 women (51%). Overall, there was little difference in cervical HIV-1 shedding between the women with and those without *M. genitalium* infection (19% vs. 15%; $P = .31$). However, cervical shedding of HIV-1 DNA was moderately associated with high *M. genitalium* organism burden ($P = .06$) (table 1). In multivariate analysis, women with high *M. genitalium* organism burdens were significantly more likely to have HIV-1 DNA detected in the cervix than were women without *M. genitalium* (adjusted OR, 2.9 [95% CI, 1.1–7.6]; $P = .03$), adjusting for infection with *N. gonorrhoeae* or *C. trachomatis,* CD4 cell count, and hormonal contraception (table 2). This relationship was unchanged when we substituted gonorrhea culture and chlamydia antigen-detection test results for PCR results. Further adjustment for abnormal cervical exudates did not appreciably change these results (adjusted OR, 2.8 [95% CI, 1.04–7.30]). In contrast, low *M. genitalium* organism burden was not associated with cervical HIV-1 shedding.

Discussion. To our knowledge, this represents the first report of a link between *M. genitalium* and genital HIV-1 shedding. Among these Kenyan women, high *M. genitalium* organism burden was strongly and independently associated with cervical HIV-1 shedding. In contrast, women with low *M. genitalium* organism burdens were similar to *M. genitalium*–negative women and had no increased HIV-1 shedding.

The prevalence of *M. genitalium* (17%) was almost 3 times higher than that of *C. trachomatis* (7%) and almost twice as high as that of *N. gonorrhoeae* (10%). In another Kenyan study, incident *M. genitalium* infection was more common among HIV-1–positive women than HIV-1–negative women [12], likely reflecting high-risk behavior and/or immunosuppression that may facilitate *M. genitalium* acquisition.

High *M. genitalium* organism burdens were present in 9% of these HIV-1–positive women and were associated with risk behaviors in addition to cervical HIV-1 shedding. Previous studies have shown that *M. genitalium* infection is more common among younger individuals with higher-risk sexual behaviors [7, 12, 13]. High organism burdens may be characteristic of recent infection, whereas lower burdens may reflect clearing infection once specific immunity is induced. However, antigenic variation may allow *M. genitalium* to escape detection by the immune system [14]. Thus, high organism burdens may alternatively represent *M. genitalium* infections of longer duration. We had no information on duration of infection, but other studies have shown that *M. genitalium* can persist for >2 years [12]. Prospective evaluations are needed to identify longitudinal correlates of organism burden. The hypothesis that HIV-induced immunosuppression contributes to *M. genitalium* organism burden was not supported, given the small difference in median CD4 cell count observed between the women with high and those with low *M. genitalium* organism burdens.

In the previous report from this study, elevated cervical PMN counts were strongly associated with cervical HIV-1 DNA shedding. Therefore, it was somewhat perplexing that high *M. genitalium* organism burden was strongly associated with HIV-1 shedding yet only marginally associated with this marker of acute inflammation. The association between *M. genitalium* and abnormal cervical exudates, rather than elevated PMN counts, suggests that a more chronic inflammatory response may occur. Chronic *M. genitalium* infection could potentially increase plasma HIV-1 load and thereby increase cervical HIV-1 levels [4], but we lacked the data on blood plasma HIV-1 concentrations needed to evaluate this. Alternatively, *M. genitalium* may stimulate cytokine and chemokine responses that recruit infected lymphocytes and macrophages to the genital tract in the absence of PMNs.

We measured only the presence or absence of HIV-1 DNA, and quantitative measurement of RNA and DNA might have been more informative. However, it is unknown whether HIV-1 RNA (reflecting cell-free virions) or HIV-1 DNA (reflecting cell-associated virus) is more infectious [1]. Prospective natural history studies monitoring organism burden over time are required to confirm a causal relationship between *M. genitalium* infection and HIV-1 shedding. The nature and type of inflammatory cells

induced during *M. genitalium* infection also requires further investigation.

The relationship between high organism burden and HIV-1 shedding suggests that treatment may be merited for HIV-1–positive women infected with *M. genitalium*. Although treatment guidelines for *M. genitalium* infection have not yet been developed, azithromycin may be more effective than doxycycline [15]. Given the high prevalence of *M. genitalium* infection in this population and the absence of traditional signs of cervical inflammation among half of *M. genitalium*–infected women, screening high-risk HIV-1–seropositive women for *M. genitalium* in addition to treating cervicitis may be advisable. To accomplish this, affordable point-of-care tests for detecting *M. genitalium* must be developed.

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