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MAJOR ARTICLE

Changes in Inflammatory Cytokine Levels in Rectal Mucosa Associated With *Neisseria gonorrheae* and/or *Chlamydia trachomatis* Infection and Treatment Among Men Who Have Sex With Men in Lima, Peru

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Background. *Neisseria gonorrheae* and *Chlamydia trachomatis* are associated with mucosal inflammation and human immunodeficiency virus 1 (HIV-1) transmission. We assessed levels of inflammatory cytokines in men who have sex with men (MSM) with and without rectal gonorrhea and/or chlamydia in Lima, Peru.

Methods. We screened 605 MSM reporting condomless receptive anal intercourse for rectal *N. gonorrheae*/*C. trachomatis* using nucleic acid testing. We identified 101 cases of gonorrhea and/or chlamydia and randomly selected 50 *N. gonorrheae*/*C. trachomatis* positive cases and matched 52 negative controls. We measured levels of IL-1β, IL-6, IL-8, and TNF-α in rectal secretions. Tests for HIV-1, rectal *N. gonorrheae*/*C. trachomatis*, and mucosal cytokines were repeated after 3 and 6 months. Cytokine levels in cases and uninfected controls were compared using Wilcoxon rank-sum tests and linear regression.

Results. MSM with gonorrhea/chlamydia had elevated levels of all cytokines in rectal mucosa compared with matched controls (all *P* values <.001). Following antibiotic treatment there were no significant differences in cytokine levels at 3- or 6-month follow-up evaluations (all *P* values >.05).

Discussion. Rectal gonorrhea/chlamydia infection is associated with transient mucosal inflammation and cytokine recruitment. Our data provide proof of concept for rectal sexually transmitted infection screening as an HIV prevention strategy for MSM.

Clinical Trials Registration. NCT03010020.

Keywords. HIV-1; HIV-1 prevention; MSM; chlamydia; *Chlamydia trachomatis*; cytokines; gonorrhea; inflammation; *Neisseria gonorrheae*; rectal mucosa.

Infection with sexually transmitted pathogens like *Neisseria gonorrheae* and *Chlamydia trachomatis* are associated with increased vulnerability to human immunodeficiency virus 1 (HIV-1) infection, although the biological factors contributing to HIV transmission through rectal mucosal tissue have not been well defined [1]. Epidemiologic analyses have demonstrated temporal associations between gonococcal and/or chlamydial infection and risk for HIV-1 acquisition [2, 3]. In a cohort of heterosexual women in South Africa, cervical gonorrhea and/or chlamydia infection was associated with a 3-fold increase in risk for HIV-1 acquisition, with gonococcal infection associated with a 7-fold increase in risk, even though 87.7% of cervical sexually transmitted infections (STIs) did not produce any signs of genital discharge or dysuria [4, 5]. Comparable prospective cohort data detailing the impact of rectal STIs on HIV acquisition are not available, although retrospective analyses of men with rectal gonorrhea have found that HIV-1 acquisition risk increases following both isolated and recurrent episodes [6–10]. While the high frequency of HIV seroconversion among men with rectal gonorrhea/chlamydia is likely to be due to a combination of behavioral, biological, and social factors, in vitro studies have suggested a series of mechanisms through which inflammatory cytokines increase the susceptibility of rectal tissue to HIV-1 infection.

Mucosal inflammation triggered by cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), IL-6, and IL-8 disrupts epithelial boundaries, promotes viral replication, recruits vulnerable $CD4^+$ T cells to the site of infection, and induces expression of CCR5 and CXCR4 receptors [11]. While *N. gonorrheae* and *C. trachomatis* do not typically cause ulcerative disease, tissue inflammation does result in physical

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disruption of single-cell layer epithelial barriers in rectal mucosa [12]. At the same time, both symptomatic and asymptomatic infections increase the vulnerability of rectal tissue to HIV-1 infection by recruiting T cells to the site of inflammation and promoting HIV-1 replication in primary resting $CD4^+$ T cells [13]. On a cellular level, *N. gonorrheae*-induced Toll-like receptor 2 (TLR-2) activation promotes nuclear importation and infection by HIV-1 in both naive and memory $CD4^+$ T cells, as well as in Langerhans cells [14]. Chlamydial infection similarly induces secretion of proinflammatory cytokines IL-6 and IL-8, resulting in epithelial cell lysis and release of IL-1β and ongoing local inflammation and T-cell recruitment [15]. While the molecular and cellular pathways linking STI-associated inflammation with vulnerability to HIV-1 have been well characterized, there is limited and conflicting data on the biological effects of gonorrhea/chlamydia infection on the pathways linking mucosal inflammation with HIV-1 infection in complex human models.

Although in vitro models have consistently shown increases in cytokine production following gonorrhea or chlamydia infection, in vivo and clinic-based analyses have shown mixed results. In an experimental human model, urethral inoculation with *N. gonorrheae* resulted in a rapid increase in the levels of cytokines, including IL-1β, IL-6, IL-8, and TNF-α, in urine within 2 hours following infection, followed by a similarly rapid (12–24 hours) reduction in cytokine levels after antibiotic treatment [16]. In contrast, a cross-sectional study of men with urethral chlamydia found higher levels of IL-8, but not other inflammatory cytokines, in urethral swab samples [17]. In another cross-sectional analysis of patients from an Amsterdam STI clinic, inflammatory cytokine levels were actually lower in the rectal mucosa of men with rectal chlamydia compared to STI-negative men who also reported engaging in receptive anal intercourse [18]. Given these conflicting observations, and in the absence of prospective clinical data on STI-associated inflammation in rectal mucosa, it is important to clarify the impact of rectal STIs on mucosal inflammation in rectal tissue. To understand the association between rectal gonorrhea/chlamydia infection and inflammatory cytokine production, and the subsequent response to treatment, we measured levels of inflammatory cytokines in rectal mucosa of a sample of men who have sex with men (MSM) diagnosed with rectal *N. gonorrheae* and/or *C. trachomatis* infection, before and after antibiotic treatment, as compared with a group of *N. gonorrheae*/*C. trachomatis*-negative MSM matched by age and sexual behavior.

METHODS

Participants and Recruitment

We sought to understand the effect of rectal gonorrhea and/or chlamydia on levels of inflammatory cytokines in the rectal mucosa of MSM and transwomen living in Peru. Participants were recruited from community venues in Lima, Peru between July and December 2017 by peer recruiters at Via Libre, a community-based HIV/AIDS service organization. Enrollment was limited to individuals who (1) were ≥18 years old, (2) were assigned male sex at birth, (3) reported condomless receptive anal intercourse in the previous 3 months with a partner with HIV or whose serostatus was unknown, and (4) were HIV-negative according to a fourth-generation rapid HIV test. Although use of HIV preexposure prophylaxis (PrEP) was not an exclusion criteria, no participants were using PrEP at the time of enrollment, likely due to limited availability of the medication in Peru's public health system [19]. At the screening visit, 605 participants underwent testing for HIV, syphilis, and rectal gonorrhea and chlamydia. From 469 HIV-negative participants screened, we identified 101 cases of rectal gonorrhea and/or chlamydia. From this group, we randomly selected 48 *N. gonorrheae*/*C. trachomatis*-infected individuals and 2 individuals with symptomatic proctitis, and matched them with 52 *N. gonorrheae*/*C. trachomatis*-uninfected controls for rectal cytokine monitoring. (The other 51 *N. gonorrheae*/*C. trachomatis*-infected individuals not included in this study were followed for a concurrent analysis of behavioral risk-reduction for individuals with newly diagnosed rectal STIs.) Uninfected controls were matched one-to-one with *N. gonorrheae*/*C. trachomatis*-infected cases according to age (within a range of ± 5 years) and reported number of different partners for receptive anal intercourse during the 30 days prior to screening (categorized as 0, 1–2, 3–5, 6–10, or 11+ partners).

Study Procedures

Participants completed a behavioral survey and underwent physical examination, HIV/STI testing, and rectal mucosal sponge collection at baseline, 3, and 6 months. At each visit, all participants completed a computer-assisted self-interview to collect information on their age, number of insertive and receptive anal intercourse partners in the previous month, as well as partner-specific condom use, HIV serostatus (if known), and use of alcohol and drugs with their last 3 sexual contacts. Participants were screened for rectal *N. gonorrheae*/*C. trachomatis* as well as HIV and underwent physical examination for signs of symptomatic urethritis or proctitis at each visit. Rectal swabs were collected and tested for *N. gonorrheae*/*C. trachomatis* with nucleic acid amplification testing (NAAT) using the Gen-Probe Aptima II assay (Hologic). Participants with clinically symptomatic urethritis or proctitis on physical exam or who tested positive for *N. gonorrheae*/*C. trachomatis* on NAAT testing were treated with ceftriaxone 250 mg intramuscular and azithromycin 1 g orally, according to contemporary Centers for Disease Control and Prevention guidelines [20]. At the baseline visit, blood was collected to test for syphilis by rapid plasma reagin (RPR) assay (RPRnosticon; Biomérieux) with *Treponema pallidum* particle agglutination

assay (TPPA) confirmation (Serodia TPPA; Fujirebio), and serial dilution of positive RPR titers. For the purpose of this analysis, RPR titers ≥16 were considered consistent with untreated syphilis and included in our findings. Participants with untreated syphilis received 1–3 intramuscular injections of benzathine penicillin 2.4 million IU. HIV screening was conducted at each visit with rapid fourth-generation testing (Alere Determine; Abbott). Positive results were referred to the Peruvian Ministry of Health laboratory for confirmation. Individuals with HIV were referred to Via Libre's clinical services or another Ministry of Health-designated HIV treatment site. Participants were compensated 15 Nuevos soles (US $5) for the screening visit, 25 Nuevos soles ($8) for the baseline evaluation, 35 Nuevos soles ($12) for the 3-month follow-up, and 45 Nuevos soles ($15) for the 6-month follow-up.

Rectal Sponge Collection and Cytokine Quantification

Rectal sponge samples were collected at baseline, 3, and 6 months for cytokine analysis. Rectal secretions were collected using 2-cm tip sterile polyvinyl acetate sponges (Merocel; Beaver Visitec) introduced into the rectum via anoscopy and held against the rectal mucosa under direct visualization for 120 seconds [21, 22]. Sponges were stored at −80°C and shipped on dry ice to the University of California, Los Angeles Mucosal Immunology Core Laboratory for processing at the end of the study. Sponges were thawed on ice and sponge tips transferred to a 2-mL Spin-X column (Corning) from which the acetate membrane was removed. Rectal secretions were eluted twice with 250μL of cold elution buffer (PBS containing 0.25% bovine serum albumin, 1% Igepal [Sigma Chemicals], and protease inhibitor cocktail [Sigma Chemicals]) by centrifugation (10 000 rpm for 30 minutes at 4°C). IL-1β, IL-6, IL-8, and TNF-α were then measured using a custom Milliplex high-sensitivity multiplex panel (MilliporeSigma) according to manufacturer's instructions. Selection of cytokines for testing was based on review of the literature to ensure concordance with previous studies assessing similar outcomes in rectal and cervical mucosa [5, 16]. Samples were run in duplicate and repeated if the coefficient of variation was >25%.

Data Analysis

We calculated median (interquartile range) levels of each cytokine at each time point (baseline, 3 months, and 6 months) for cases and controls separately. We used Wilcoxon rank-sum tests to compare each cytokine level separately between cases and controls at each time point. We then constructed linear regression models for each time point, with case versus control status predicting the cytokine level, adjusted for age, baseline syphilis coinfection, and number of receptive anal intercourse partners. All analyses were conducted using Stata 15.0 (StataCorp).

Human Subjects Protections

Written informed consent was obtained from all participants prior to completing any study procedures. The study protocol was reviewed and approved by the Institutional Review Boards of the University of California, Los Angeles, and Asociación Civil Via Libre, and registered on clinicaltrials.gov (NCT03010020) before starting enrollment.

RESULTS

We screened 605 MSM and transwomen who reported at least 1 episode of condomless receptive anal intercourse in the previous 3 months and who had not previously tested positive for HIV (Figure 1). Among 469 HIV-negative participants, we diagnosed 101 cases of rectal gonorrhea and/or chlamydia and enrolled 50 *N. gonorrheae*/*C. trachomatis*-positive cases and 52 *N. gonorrheae*/*C. trachomatis*-negative controls, matched according to age and number of receptive anal intercourse partners during the previous 30-day period (Table 1). Two individuals presented with symptomatic proctitis and were enrolled prior to receiving the results of nucleic acid testing, both of which were negative for *N. gonorrheae*/*C. trachomatis*. All other participants were asymptomatic at enrollment.

At baseline, levels of all inflammatory cytokines were significantly higher in the rectal mucosa of participants with gonorrhea and/or chlamydia compared with STI-negative controls (Table 2, Figure 2, and Supplementary Figure 1). Specifically, median rectal mucosal levels of IL-1β (280.4 vs 66.0 pg/mL), IL-6 (78.1 vs 4.4 pg/mL), IL-8 (1439.7 vs 656.0 pg/mL), and TNF-α (44.0 vs 13.9 pg/mL) were higher among *N. gonorrheae*/*C. trachomatis*-infected participants ($P < .001$ for all comparisons by Wilcoxon rank-sum test). Following antibiotic treatment, there were no significant differences in median levels of IL-1β (133.9 vs 115.1 pg/mL), IL-6 (19.5 vs 17.4 pg/mL), IL-8 (707.5 vs 773.6 pg/mL), and TNF-α (20.4 vs 20.3 pg/mL) in rectal mucosa, suggesting a resolution of STI-associated inflammation in the rectal tissue. Similar patterns of variation in statistical significance were observed when comparing case and control groups in the linear regression model after controlling for age, syphilis coinfection, and number of recent receptive anal intercourse partners. In subgroup analyses, there were no clear differences in cytokine levels between participants with *N. gonorrheae* monoinfection, *C. trachomatis* monoinfection, and *N. gonorrheae*/*C. trachomatis* coinfection, suggesting that *N. gonorrheae* and *C. trachomatis*, both alone and in combination, are associated with localized inflammation.

During the follow-up period, 6 individuals were diagnosed with new HIV-1 infection in the case (n = 5) and control (n = 1) groups after 3 months, and 2 others after 6 months (n = 1 from each group). HIV-1 seroconversion was not associated with an increase in levels of inflammatory cytokines in rectal mucosa [23]. A total of 15 new or persistent cases of rectal *N.*

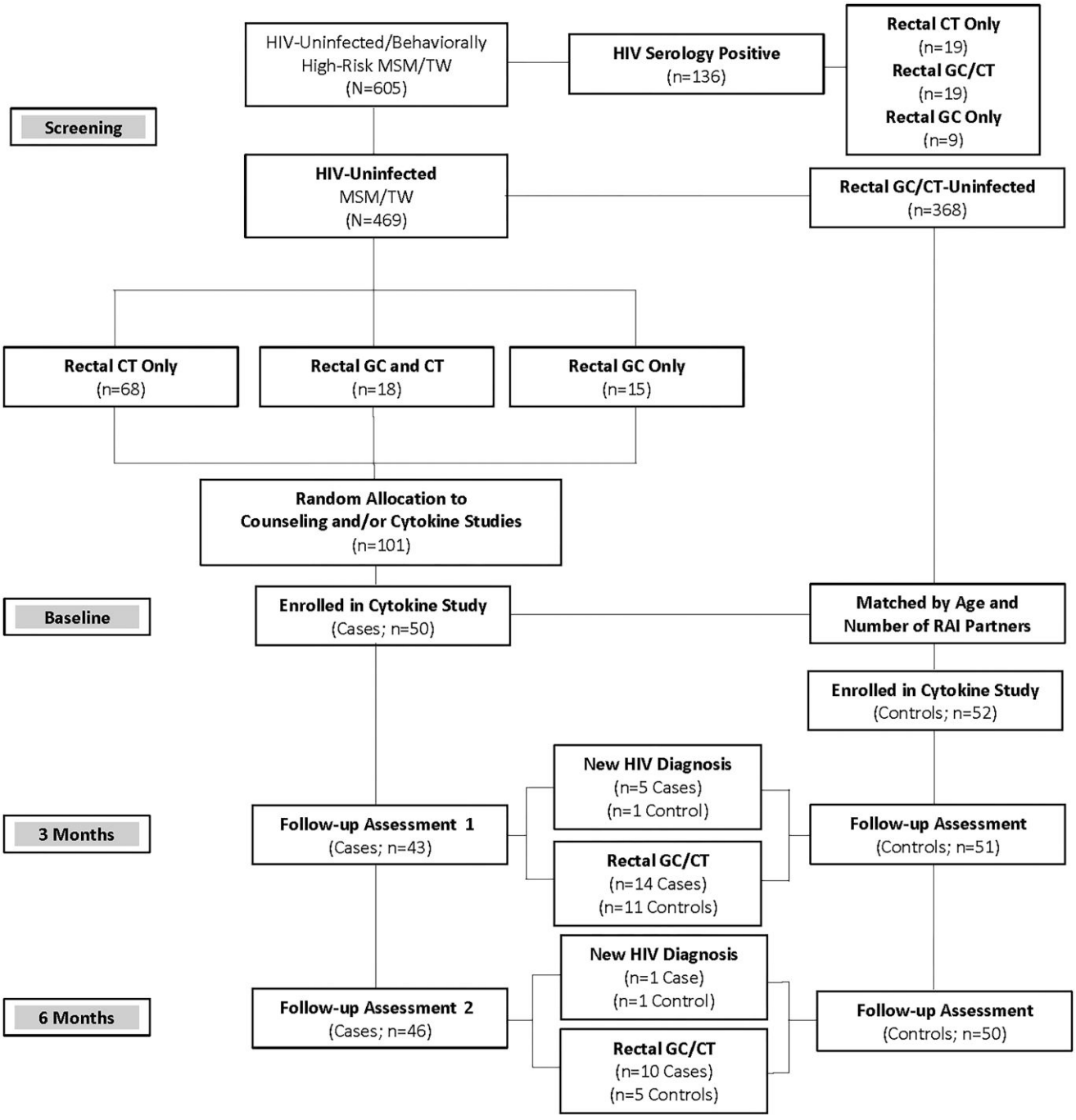

Figure 1. Participant screening, enrollment, and follow-up. Abbreviations: CT, chlamydia; GC, gonorrhea; HIV, human immunodeficiency virus; MSM, men who have sex with men; RAI, receptive anal intercourse; TW, transwomen.

gonorrheae/*C. trachomatis* (11 case participants and 4 controls) were diagnosed at the 3-month follow-up evaluation, and 12 infections (9 cases and 3 controls) at the 6-month visit (Table 3).

DISCUSSION

Our data illustrate the effect of *N. gonorrheae* and/or *C. trachomatis* infection and cure on rectal inflammation, as measured by change in levels of inflammatory cytokines in mucosal tissue. Following an initial rise in IL-1β, IL-6, IL-8, and TNF-α, antibiotic treatment was associated with a resolution in STI-associated inflammation, with no significant differences in cytokine levels observed between case and control groups after 3 and 6 months. Despite the absence of clinically significant proctitis, both gonorrhea and chlamydia were associated with cytokine-mediated inflammation in the rectal mucosa of *N.*

Table 1. Baseline Characteristics of Men Who Have Sex With Men and Transwomen With Rectal *Neisseria gonorrheae/Chlamydia trachomatis* and Matched Controls, Lima, Peru

Characteristic	Rectal *N. gonorrheae/C. trachomatis* Positive Cases (n = 50)	Rectal *N. gonorrheae/C. trachomatis* Negative Controls (n = 52)
Age, y, median (IQR)	23 (21–28)	25 (21–30)
Education		
Incomplete secondary education	1 (2.0)	2 (3.8)
Complete secondary education	23 (46.0)	17 (32.7)
University or technical school	26 (52.0)	33 (63.5)
Gender identity		
Masculine	33 (66.0)	36 (69.2)
Feminine	13 (26.0)	7 (13.5)
Androgenous	4 (8.0)	9 (17.3)
Sexual identity		
Heterosexual	0 (0)	0 (0)
Bisexual	8 (16.0)	8 (15.4)
Homosexual	39 (78.0)	42 (80.8)
Transgender	3 (6.0)	2 (3.8)
Sexual role		
Activo, insertive	0 (0)	0 (0)
Pasivo, receptive	31 (62)	24 (46.2)
Moderno, versatile	19 (38.0)	28 (53.8)
No. of sexual partners in previous 30 d, median (IQR)		
Total	5 (3–10)	5 (3–9)
Number of insertive anal sex partners, all	1 (0–3)	1 (0–3)
Number of insertive anal sex partners, condomless sex	1 (1–4)	1 (1–2)
Number of receptive anal sex partners, all	4 (3–9)	4 (2–7)
Number of receptive anal sex partners, condomless sex	3 (1–5)	3 (1–4)
Baseline STI diagnosis		
Rectal *N. gonorrheae*	10 (20.0)	…
Rectal *C. trachomatis*	32 (64.0)	…
Rectal *N. gonorrheae/C. trachomatis* coinfection[a]	6 (12.0)	…
Syphilis, any RPR titer	10 (20.0)	8 (15.4)
Syphilis, RPR ≥1:8	5 (10.0)	3 (5.8)
AUDIT score ≥8	24 (48.0)	22 (42.3)
Chemsex with 1 or more of last 3 partners	0 (0)	1 (1.9)

Data are No. (%) except where indicated.

Abbreviations: AUDIT, alcohol use disorders identification test; IQR, interquartile range; RPR, rapid plasma regain; STI, sexually transmitted infection.

[a]Two participants diagnosed with symptomatic proctitis during screening were enrolled as cases, but subsequently tested negative for *N. gonorrheae/C. trachomatis* infection.

gonorrheae/C. trachomatis-infected MSM and transwomen (compared with STI-negative controls), with no consistent differences in severity of inflammation noted between the 2 organisms. Although all participants in the study reported recent condomless receptive anal intercourse, the frequency of new HIV diagnoses in the *N. gonorrheae/C. trachomatis*-positive cohort was dramatically higher than in previously published epidemiologic studies, further highlighting the important link between rectal STI acquisition and HIV-1 transmission, and suggesting a potential intervention point for combined HIV/STI prevention programs [24, 25]. Collectively, these findings provide a clinical model to understand biological mechanisms of STI-associated inflammation in mucosal tissue and a framework to guide the use of rectal STI screening as an HIV prevention strategy for at-risk MSM and transwomen.

Our data accentuate the role of subclinical inflammation on risks for HIV transmission during anal intercourse and provide additional support for routine screening for at-risk individuals, regardless of symptomatic presentation [26, 27]. While we found significant elevations in the levels of all cytokines tested (IL-1β, IL-6, IL-8, and TNF-α), none of the participants with laboratory-diagnosed *N. gonorrheae/C. trachomatis* infection had symptomatic proctitis on physical examination. The high frequency of asymptomatic infection emphasizes the importance of routine nucleic acid testing in screening for rectal STIs and further undermines the utility of syndromic management as a public health strategy for STI control in resource-limited settings [28]. Programs designed to diagnose and treat only STIs that come to the attention of a clinician are subject to a series of social, behavioral, and biological gaps in the prevention and treatment cascade that would have resulted in a missed diagnosis for all of the gonorrhea/chlamydia cases diagnosed in our cohort [29]. Periodic NAAT-based rectal STI screening, however, could serve as a platform to ensure regular medical evaluation for at-risk populations of MSM and transwomen to detect asymptomatic infection, identify the individuals whose behavior and/or biological characteristics increase their short-term risk for HIV transmission, and unify HIV prevention with STI control.

Our findings also underline the importance of targeted prevention efforts to identify and address the specific individuals and networks that drive ongoing HIV/STI transmission in their community, instead of uniform approaches to HIV prevention applied to undifferentiated populations of MSM. While both cases and controls in our cohort were selected for their recent sexual risk behavior, 6 of 50 case participants and 2 of 52 controls acquired HIV during the brief, 6-month follow-up period. Although derived from a small sample, the frequency of seroconversions is both higher than reported in prior HIV prevention trials with similar populations and also consistent with other studies of MSM diagnosed with rectal gonorrhea and/or chlamydia [30, 31]. These findings suggest that rectal STI screening could provide a valuable tool to better differentiate members of at-risk populations and identify the individuals and networks at highest immediate risk for HIV acquisition, thereby defining priority targets for prevention interventions [32, 33]. In this context, we suggest that routine screening and diagnosis of rectal gonorrhea/chlamydia could provide an opportunity both to manage the biological factors (through antibiotic treatment) and the behavioral factors (through risk

Table 2. Median Levels of Inflammatory Cytokines in Rectal Mucosa of Men Who Have Sex With Men and Transwomen With Rectal *Neisseria gonorrheae/Chlamydia trachomatis* and Matched Controls Before and After Antibiotic Therapy

	IL-1β, pg/mL[a]			IL-6, pg/mL, Median[b]			IL-8, pg/mL, Median[c]			TNF-α, pg/mL, Median[d]		
Group	Baseline[e]	3 mo[f]	6 mo[f]	Baseline[e]	3 mo[f]	6 mo[f]	Baseline[e]	3 mo[f]	6 mo[f]	Baseline[e]	3 mo[f]	6 mo[f]
Cases, *N. gonorrheae/C. trachomatis* positive (n = 48)	280.4	119.4	171.2	76.5	17.3	14.2	1439.7	633.1	611.7	44.0	19.0	21.6
Controls, *N. gonorrheae/C. trachomatis* negative (n = 53)	66.0	117.9	62.8	4.3	17.0	13.8	656.0	776.1	567.9	13.9	20.0	14.2
Baseline *N. gonorrheae/C. trachomatis* subgroups												
N. gonorrheae only (n = 11)	512.9	148.6	95.8	7.8	2.8	2.4	1356.0	687.6	422.2	40.5	7.8	21.3
C. trachomatis only (n = 31)	243.6	160.3	227.6	94.8	32.6	19.0	1511.0	692.9	192.3	50.2	30.4	21.3
Both *N. gonorrheae/C. trachomatis* (n = 6)	123.1	34.9	19.6	134.3	14.8	10.3	1441.5	669.2	532.7	26.0	16.5	13.2

Adjusted for age, rapid plasma reagin, and number of receptive anal intercourse partners (linear regression model comparing each time point separately).

[a]Cases versus controls, $P = .001$ baseline; $P = .65$ 3 months; $P = .10$ 6 months.

[b]Cases versus controls, $P = .03$ baseline; $P = .90$ 3 months; $P = .92$ 6 months.

[c]Cases versus controls, $P = .002$ baseline; $P = .20$ 3 months; $P = .74$ 6 months.

[d]Cases versus controls, $P = .03$ baseline; $P = .82$ 3 months; $P = .34$ 6 months.

[e]$P < .001$ for comparison by Wilcoxon rank-sum test; each time point analyzed separately.

[f]Nonsignificant for comparison by Wilcoxon rank-sum test.

reduction counseling) that predispose individuals to rectal STI acquisition and increase their risk for HIV transmission.

Our findings contribute important information for understanding the timeline of rectal STI acquisition, inflammation, treatment, and resolution. In our cohort, rectal gonorrhea/chlamydia infection was associated with a transient, measurable increase in levels of IL-1β, IL-6, IL-8, and TNF-α in rectal mucosa that resolved following treatment. The temporary nature of the inflammation suggests that the STI-associated risk for HIV acquisition seen in prior epidemiologic analyses is similarly time limited and results in a transient inflammatory process, rather than a prolonged transformation of the mucosal environment or a permanent increase in biological vulnerability to HIV transmission [6, 7]. These conclusions are muddied by the fact that 5 participants (16.7%) from the case group were diagnosed with recurrent or persistent rectal chlamydia after 3 months, and several new cases of rectal gonorrhea and/or chlamydia were diagnosed in both the case and control groups throughout the follow-up period. It is unclear from our data whether infections that are self-limited, or that resolve without antibiotic treatment, would follow similar patterns of inflammation and recovery, and whether the standard 3-month testing interval recommended for at-risk MSM would be sufficiently frequent to identify and manage rectal STIs and have a measurable effect on HIV acquisition [34–37].

Finally, these findings should be interpreted within and applied to the local social and epidemiologic context of Lima, Peru. Our data suggest that rectal gonorrhea and chlamydia are associated with transient, subclinical inflammation in rectal mucosa that, when accompanied by high-risk sexual behavior like condomless receptive anal intercourse, dramatically increases an individual's short-term risk for HIV acquisition. In these groups, a structured program of rectal STI screening and treatment could provide an opportunity to identify the individuals at greatest short-term risk for HIV/STIs, and to intervene in the sexual networks with a high frequency of sustained pathogen transmission. As these inflammatory changes are likely to be overshadowed by the dramatic reductions in HIV-1 viremia and transmission risk resulting from regular use of antiretroviral therapy and/or PrEP, it may be most useful to understand rectal STI testing as part of a comprehensive HIV prevention strategy that unites behavioral, social, and biological factors [38–40]. While recent efforts like the ImPrEP Demonstration Project have supported PrEP uptake in key populations in Peru, use of PrEP as an HIV prevention method is still considered culturally foreign and practically inaccessible for many Peruvian MSM and transwomen [19, 41, 42]. In this context, diagnosis of rectal gonorrhea/chlamydia infection provides a teachable moment, for both patients and clinicians, to highlight an individual's immediate risk for HIV acquisition and to motivate PrEP initiation and adherence, as well as other strategies to mitigate this risk [43–46].

There are several factors to note in interpreting our findings, including ambiguity surrounding the time of HIV acquisition, the potential effect of persistent and recurrent STIs, and selection bias in cohort recruitment. Due to cost limitations, HIV screening was based on fourth-generation enzyme immunoassay, without RNA testing to identify cases of acute infection. As a result, it is possible that participants diagnosed with HIV-1 infection at the 3-month follow-up visit were actually in the

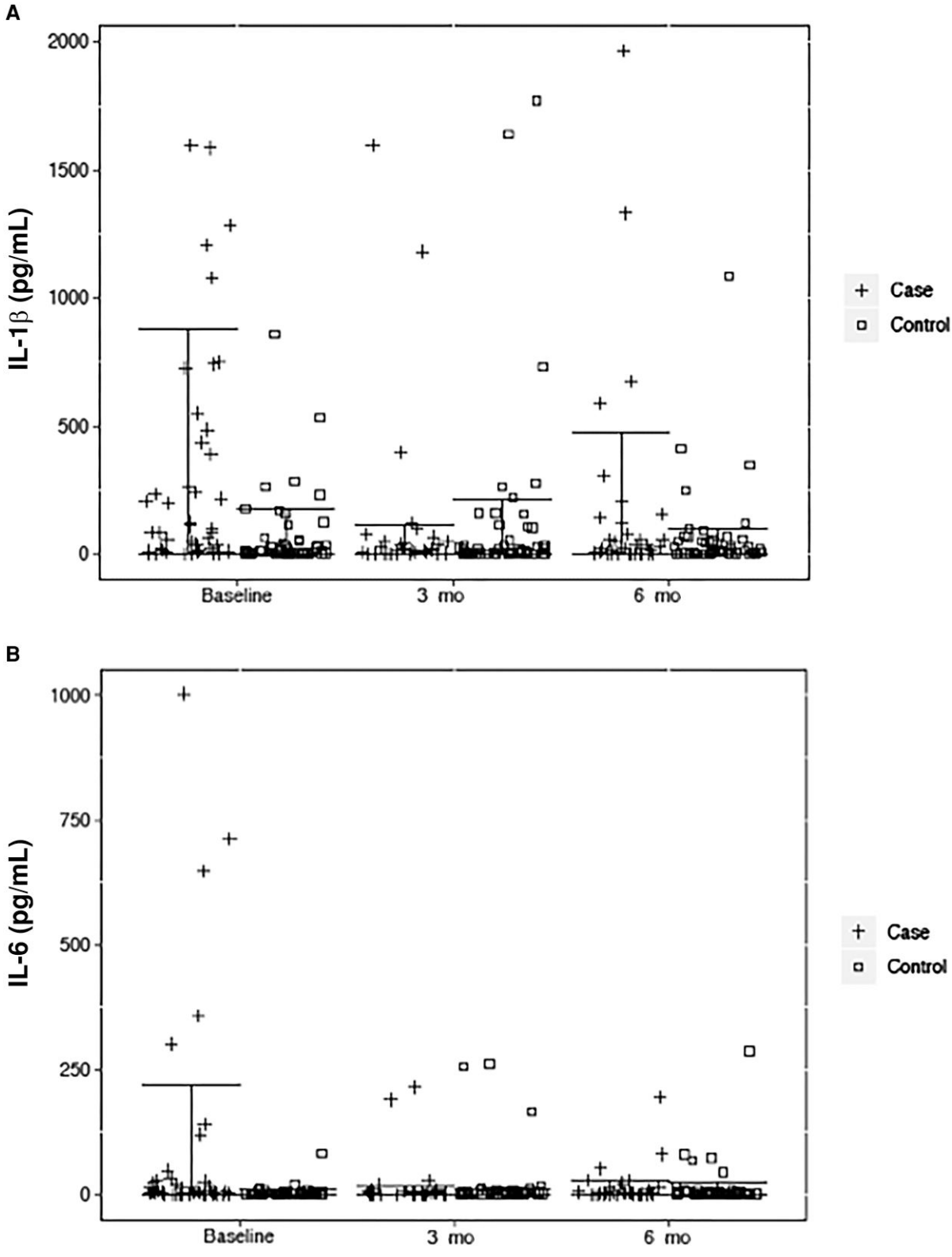

Figure 2. *A*, Initial and posttreatment levels of IL-1β (pg/mL) among MSM with and without rectal gonorrhea/chlamydia infection diagnosed at baseline. *B*, Initial and posttreatment levels of IL-6 (pg/mL) among MSM with and without rectal gonorrhea/chlamydia infection diagnosed at baseline. Abbreviations: IL, interleukin; MSM, men who have sex with men.

Table 3. Frequency of Rectal Gonorrhea and/or Chlamydia Infection at Baseline and Follow-up Evaluation

	Rectal Gonorrhea, Monoinfection		Rectal Chlamydia, Monoinfection		Rectal Gonorrhea and Chlamydia, Dual Infection	
	Cases (n = 50)	Controls (n = 52)	Cases (n = 50)	Controls (n = 52)	Cases (n = 50)	Controls (n = 52)
Baseline	10[a]	…	32	…	6	…
3-mo follow-up	1	1	10[b]	2	0	1
6-mo follow-up	4	0	2[c]	3	3	0

[a]Two participants with symptomatic proctitis at screening were enrolled as cases, but tested negative for rectal *Neisseria gonorrheae*/*Chlamydia trachomatis* infection.

[b]Five cases were diagnosed with persistent/recurrent chlamydial infection at 3-month follow-up.

[c]One case and 1 control were diagnosed with persistent/recurrent chlamydial infection at 6-month follow-up.

process of seroconversion at enrollment, and that mucosal inflammation resulting from rectal *N. gonorrheae*/*C. trachomatis* coinfection did not predispose these individuals to subsequent HIV-1 acquisition [47]. Similarly, the fact that some participants in both groups were diagnosed with rectal gonorrhea/chlamydia during the follow-up period blurs the boundaries between cases and controls and complicates efforts to understand the impact of *N. gonorrheae*/*C. trachomatis* acquisition and clearance on cytokine levels. However, as the majority of cases of gonorrhea and/or chlamydia diagnosed during the follow-up period were found in the case group, it is likely that any potential effect on our data would have been to diminish observed differences in inflammatory cytokines between the infection and treatment periods. Finally, limiting enrollment to individuals who reported recent condomless receptive anal intercourse with a serodiscordant partner limits the generalizability of our findings, as this group may not be representative of the larger communities of MSM and transwomen. However, as the goal of this pilot study was to assess the feasibility and intermediate biological and behavioral outcomes of a rectal STI screening program for combined HIV/STI prevention, our cohort reflects the goal population for our research and provides a clinical model for future integrated screening interventions.

We present data on differences in levels of inflammatory cytokines within the rectal mucosa of HIV-uninfected MSM with and without rectal gonorrhea and/or chlamydia before and after antibiotic treatment. Our findings illustrate how the presence and resolution of rectal gonorrhea/chlamydia infection results in a substantial, transient elevation in inflammatory cytokines within the rectal mucosa, which is independent of clinically symptomatic proctitis, and which is seen in both chlamydial and gonococcal infection. Additional data are needed to identify other factors that may influence cytokine levels (eg, behavioral or biological factors that predispose certain individuals to exuberant cytokine production in response to infection) as well as to determine the clinical significance of specific cytokine levels on mucosal inflammation. While our study was not designed to assess the impact of rectal STIs on HIV acquisition, the frequency of HIV-1 seroconversion in our cohort was dramatically higher than other published studies from similar groups of MSM from Lima, Peru, suggesting that this group represents an important target population for future prevention interventions. Our data support the future use of nucleic acid testing for rectal STIs as a platform for identification of individuals at short-term risk for HIV transmission, and for delivery of integrated biological-behavioral prevention interventions through their sexual networks.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. J. L. C., E. R. S., and R. C. contributed study design. J. L. C., R. C. P., E. R. S., and R. C. collected data. J. A. F. performed laboratory analysis. C. E. O. and W. G. performed statistical analysis. J. L. C. prepared the manuscript. All authors approved the final manuscript.

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Data availability. Data are available upon request from the corresponding author, pending institutional review board approval.

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