Prevalence, incidence and characteristics associated with asymptomatic urethral inflammation in men who have sex with women

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Abstract

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Background: Despite the potential to increase risk for acquisition of HIV and other sexually transmitted pathogens, the frequency with which men experience asymptomatic urethral inflammation is unknown. To better understand the full spectrum of perturbations to the male urethra, we aimed to determine the prevalence, incidence, and risk factors for asymptomatic urethral inflammation in men who have sex with women (MSW) attending a sexually transmitted infections clinic in Seattle.

Methods: We performed a secondary analysis of data collected during a cohort study designed to investigate the contribution of the male urethral microbiota to non-gonococcal urethritis (NGU). At enrollment, participants underwent a physical exam with assessment for urethral discharge and collection of urethral swab and first void urine specimens. Participants also completed a routine computer assisted interview (CASI) that collected information on STI history, sexual history, urogenital symptoms, and sociobehavioral data. Urethral discharge was transferred to a slide which was Gram-stained to assess polymorphonuclear (PMN) cell count and the presence of diplococci indicating *Neisseria gonorrhoeae* (GC) infection. First void urine specimens were tested for GC, *Chlamydia trachomatis* (CT), and *Mycoplasma genitalium* (MG) using Aptima Assays (Hologic, Inc., San Diego, CA). Participants also completed a series of follow-up visits at four-week intervals and underwent the same study procedures as at the

enrollment visit. We estimated the prevalence of asymptomatic urethral inflammation at enrollment in a cross-sectional study and identified characteristics associated with asymptomatic urethral inflammation in a case control study. Cases were defined as ≥5 polymorphonuclear cells on microscopic examination of a Gram-stained urethral smear without reported urethral symptoms and without urethral discharge on genital exam. Controls were men who had no urethral inflammation, no urethral symptoms, and no urethral discharge on genital exam. We used a cohort study design to estimate the incidence of asymptomatic urethral inflammation over time and identify characteristics associated with the incidence of this condition.

Results: Twenty-one of the 317 MSW had asymptomatic urethral inflammation at enrollment (6.6%; 95% CI 4.1%-9.9%). Relative to controls (n=134), cases (n=21) were significantly more likely to have a lifetime history of NGU (20% vs. 5% p=0.04). Cases were also somewhat more likely than controls to have a lifetime history of *Chlamydia trachomatis* (CT) (29% vs. 16% p=0.19), somewhat more likely than controls to have ≥ 2 sex partners in the past two months (74% vs. 52%, p=0.09), and somewhat less likely to be non-Hispanic white (38% vs. 52%, p=0.26), although these differences were not statistically significant. Men met criteria for asymptomatic urethral inflammation at 121 of 1444 eligible follow-up visits over 106.6 person-years at risk for an estimated incidence rate of 1.14 cases per person-year (95% CI: 0.94-1.4). Non-Hispanic Black MSW [aRR 1.84 (1.24-2.72), p=0.002), those who reported ≥ 2 sex partners in the two months prior to enrollment [aRR 1.62 (1.06-2.47), p=0.02], and those who had NGU at enrollment [aRR 1.64 (1.08-2.50), p=0.02] were significantly more likely to experience an incident episode of asymptomatic urethral inflammation. In contrast, having CT at enrollment was not associated with asymptomatic urethral inflammation [aRR 0.75 (0.44-1.27), p=0.28].

Discussion: In these MSW attending an urban sexual health clinic, the prevalence of asymptomatic urethral inflammation was low but incidence was high. Significant associations

with non-Hispanic Black race/ethnicity, NGU at enrollment, and ≥2 partners in the past two months but not prior CT infection suggest that persistent inflammation may occur frequently in men subsequent to non-chlamydial urethral infections. Whether asymptomatic urethral inflammation is associated with adverse health consequences is unknown and should be investigated.

BACKGROUND AND SIGNIFICANCE:

Inflammation of the male urethra is a defining characteristic of the condition termed urethritis. Men with urethritis experience symptoms including discharge, dysuria, or itching at the tip of the urethra. On physical exam, urethritis typically presents as urethral discharge[1]. The pathognomonic microscopic finding confirming urethritis is an increased number of polymorphonuclear leukocytes (PMN) on Gram-stained urethral smear[1]. However, clinical definitions of urethritis often rely on the presence of urethral symptoms as this is what typically motivates a clinic visit.

Urethritis is most commonly caused by sexually transmitted infections (STIs)[2]. However, many cases have no known etiology. The rates of gonococcal and chlamydial urethritis, the two most commonly identified infectious causes, are increasing in the United States and in Europe[3]. This increase is most significant in populations that have traditionally had less access to health care, including non-Hispanic Black persons[3]. Infectious causes of urethritis are split into two categories: gonococcal urethritis (GU) and nongonococcal urethritis (NGU) based on the presence or absence of *Neisseria gonorrhoeae*[1] *(*GC). Due to the degree of overlap in clinical presentation between men with GU and NGU, diagnosis cannot accurately hinge solely on clinical grounds and instead relies on diagnostic testing. Although Gram-staining of urethral discharge identifies cases of GU with impressive sensitivity (95%) and specificity (97%) when Gram-negative intracellular diplococci are visualized by a trained examiner,[4] microscopy is rarely performed in clinic settings. The primary method of diagnosing urethral gonorrhea is a positive nucleic acid amplification test (NAAT).

Once GU can be excluded, a myriad of other infectious causes remain for NGU. In men with urethritis attending STI clinics across the US, the prevalence of CT was 24.8%, *Mycoplasma genitalium* (MG) was 28.7%, and *Trichomonas vaginalis* was 6.9%[5]. Other known causes of

NGU include herpes simplex virus[6], adenovirus[7] and *Neisseria meningitidis*[8]. More rarely, even *Treponema pallidum*, the causative agent of syphilis, can cause NGU[9]. Still other infectious agents are under investigation for their suspected roles in NGU including *Moraxella catarrhalis,*[10] *Haemophilus Influenzae,*[11] and *Mycoplasma penetrans*[11].

NGU can occur frequently in men at high risk of STIs. One study reported an incidence of NGU of one episode every 2 years in men who have sex with women (MSW) who presented to an STI clinic in Seattle[12]. Interestingly, only 21% of these incident cases presented with symptoms[12]. Such a small percentage of men with NGU presenting with symptoms suggests that asymptomatic urethral inflammation without clinical findings may be just as prevalent as NGU. However, given the decreasing access to microscopy and Gram-staining capabilities along with NAAT testing becoming the standard of care in most clinical settings, little empiric data are available on the frequency with which men experience asymptomatic urethral inflammation.

The etiology of urethral inflammation in the absence of symptoms or clinical findings is unknown. It is possible that the first infection with a specific urethral organism leads to some naturally acquired protective immunity. On re-infection, this protective immunity could reduce organism burden and speed resolution of the second infection resulting in a minimal immune response with a limited or non-existent symptomatic profile the next time a person is infected. This 'protective immunity' theory was demonstrated in murine models[13] and may play a role in humans as well. Another possible etiology is that a prior urethral infection initiated a local immune response which persisted despite the clearance of the original infecting organism. A similar phenomenon is responsible for reactive arthritis where patients suffer from sterile inflammatory arthritis following an extra-articular infection and antecedent genitourinary infections, such as CT and GC, are known to cause reactive arthritis[14,15]. Asymptomatic urethral inflammation often goes unnoticed because most STI clinics only collect urethral swabs on men

with symptoms or clinical findings consistent with urethritis, resulting in an extremely limited understanding of this condition. Determining how common this condition is and identifying characteristics associated with it would enable a better understanding of the full spectrum of perturbations to the urethral environment, including those that are asymptomatic. The current study leverages a previously conducted cohort study where men provided urethral swab specimens at study visits over time regardless of symptoms or clinical findings.[16] We sought to estimate the prevalence and incidence of asymptomatic urethral inflammation and identify risk factors associated with this condition in this cohort.

METHODS:

Study Design: We performed a secondary analysis of data collected during a cohort study that was designed to investigate the contribution of the male urethral microbiota to NGU in MSW. We first utilized a cross-sectional study design to estimate the prevalence of asymptomatic urethral inflammation at enrollment. Next, we identified characteristics associated with asymptomatic urethral inflammation in a case control study, comparing cases of asymptomatic urethral inflammation to men who had no urethral inflammation, no urethral symptoms, and no urethral discharge on genital exam (controls). Finally, we used a cohort study design to estimate the incidence of asymptomatic urethral inflammation over time and identify characteristics associated with the incidence of this condition.

Study Setting and Study Population: MSW attending the Public Health – Seattle & King County (PHSKC) sexual health clinic in Seattle, WA were enrolled from August 2014 to July 2018. Cisgender male sexual health clinic attendees ≥ 16 years of age with at least one episode of anal, oral, or vaginal intercourse and exclusively female partners in the past 60 days were eligible. Participants were excluded if they had a history of HIV, evidence of a gonococcal infection as demonstrated by intracellular diplococci on a Gram-stained urethral smear or NAAT

test, recent exposure to a person with a gonococcal infection, or antibiotic use in the 30 days prior to enrollment. Additional exclusion criteria included an inability to complete follow-up appointments or speak English.

Data Collection: At enrollment, participants underwent a physical exam with assessment for urethral discharge and collection of urethral swab and first void urine specimens. All clinic attendees completed a routine clinic-specific computer assisted interview (CASI) that collected information on STI history, sexual history, and urogenital symptoms. Study participants completed an additional CASI collecting more detailed sociobehavioral data including condom use. Urethral discharge was transferred to a slide which was Gram-stained to assess PMN cell count and determine whether intracellular diplococci were present. First void urine specimens were tested for GC, CT, and MG using Aptima Assays (Hologic, Inc., San Diego, CA). Participants with NGU at enrollment were empirically treated for NGU during the enrollment visit (azithromycin 1g in a single dose or doxycycline 100mg twice daily for 7 days). Participants also completed a series of follow-up visits at four-week intervals. At each follow-up visit, participants underwent the same clinical exam and specimen collection procedures as at the enrollment visit and completed a study specific CASI collecting information on sexual behavior since the previous visit. Participants without NGU at enrollment completed six follow-up visits after the enrollment visit for a total of seven visits. Participants with NGU at enrollment were treated and returned to clinic four weeks after the enrollment visit with the goal of having all men begin follow-up without NGU. With this additional visit, participants with NGU at enrollment had a total of eight visits.

Data Analysis

Cross-Sectional Study: Asymptomatic urethral inflammation was defined as ≥ 5 PMNs/hpf over three fields on a Gram- stained slide in the absence of patient reported urethral symptoms and

with no urethral discharge on genital exam. The prevalence of asymptomatic urethral inflammation in this population was estimated by dividing the total number of cases at enrollment by the number of men who completed an enrollment visit. An accompanying Fisher Exact (Clopper-Pearson) 95% confidence interval (CI) was calculated.

Case Control Study: Cases were men with asymptomatic urethral inflammation at enrollment, defined as above. Controls were men with < 5 PMNs/hpf without patient reported urethral symptoms and without urethral discharge on genital exam. Characteristics of cases and controls were compared using a Fisher's exact test to determine statistical significance for categorical characteristics. The Student's t-test and Wilcoxon tests were used to determine statistical significance for characteristics measured on a continuous scale. Given the small number of cases of asymptomatic urethral inflammation, a multivariable model was not developed.

Cohort Study: Incident case visits were defined as study visits where asymptomatic urethral inflammation was detected that were preceded by a study visit without asymptomatic urethral inflammation. Cases at the enrollment visit were excluded. Time that elapsed since the prior clinic visit where a participant was not diagnosed with asymptomatic urethral inflammation contributed to follow-up time at risk for asymptomatic urethral inflammation. A Fisher Exact (Clopper-Pearson) 95% confidence interval was calculated for the incidence estimate.

Generalized estimating equations (GEE) with a log link, Poisson family, an exchangeable correlation structure, and robust variances were employed to develop univariable and multivariable models describing characteristics associated with incident asymptomatic urethral inflammation. Having CT at enrollment was included in the initial multivariable model *a priori,* given our original hypothesis that previous CT infection stimulates an ongoing immune response. Additional characteristics that were associated with incident asymptomatic urethral

inflammation in the univariable model at $p<0.20$ were also assessed using backward stepwise selection. Variables that were not significant at $p<0.05$ or that did not affect the estimates for other variables upon removal by more than 10% were excluded from the final model.

R Statistical Software version 3.6.2 and Stata/BE 17.0 were used for statistical analyses. This secondary analysis of existing de-identified data was exempt from Institutional Review Board (IRB) review.

RESULTS:

Cross-Sectional Study:

Overall, 317 MSW aged 17-71 years completed an enrollment visit; the median age was 32 years (Table 1). Twenty-one of the 317 MSW had asymptomatic urethral inflammation at enrollment (6.6%; 95% CI 4.1%-9.9%). Most (46%) reported non-Hispanic white race while 22% reported non-Hispanic Black race. Sixty-four percent of men completed two or more years of education beyond high school and 58% had ≥ 2 partners in the past two months. Nearly all men engaged in vaginal (97%) and insertive oral sex (98%) in the past two months, whereas 19% of men engaged in insertive anal sex in the past two months. Microscopy identified 44% of men as having ≥ 5 PMNs/hpf and 37% met criteria for NGU. Twelve percent of men had a history of NGU, 22% had a history of CT, and 11% had a history of GC. Eleven percent of men tested positive for CT, 11% tested positive for MG.

Case-Control Study:

Twenty-one men met criteria for asymptomatic urethral inflammation and 134 men met criteria for the comparator group of men without inflammation, without urethral symptoms, and without visible discharge (Table 2). Relative to controls, cases were significantly more likely to have a lifetime history of NGU (20% vs. 5% p=0.04). Cases were somewhat more likely than controls to have a lifetime history of CT (29% vs. 16% p=0.19), and somewhat more likely than controls to have ≥ 2 sex partners in the past two months (74% vs. 52%, p=0.09), although these latter two were not statistically significant. In contrast, age was not associated with prevalent asymptomatic urethral inflammation with cases and controls having similar distributions across groups. Race, education, history of GC, number of lifetime sex partners, sexual behaviors in the past two months, consistent condom use with the most recent partner, CT, and MG were also not associated with prevalent asymptomatic urethral inflammation.

Cohort Study:

Men met criteria for asymptomatic urethral inflammation at 121 of 1444 eligible follow-up visits over 106.6 person-years of time at risk for an estimated incidence rate of 1.14 cases per person-year (95% CI: 0.94-1.4).

Univariable analysis: Non-Hispanic Black race [IRR 1.76 (1.14-2.72), p=0.01], lifetime history of GC [IRR 1.86 (1.02-3.40), p=0.04], ≥ 2 sex partners in the two months prior to enrollment [IRR 1.60 (1.05-2.44), p=0.03], ≥ 10 PMNs/hpf on gram-stained slide of a urethral smear collected at enrollment [IRR 1.86 (1.23-2.83), p=0.01], and NGU status at enrollment [IRR 1.75 (1.19-2.57), p=0.01] were all associated with increased risk of incident asymptomatic urethral inflammation (Table 3). Clinician observed discharge at enrollment [IRR 1.42 (0.97-2.09), p=0.07], only engaging in insertive oral sex in the past two months [IRR 1.79 (0.82-3.89), p=0.15], and 5-9 PMNs/hpf on gram-stained slide of a urethral smear collected at enrollment [IRR 1.61 (0.92-

2.84), p=0.10] were also associated with increased risk of asymptomatic urethral inflammation, but these associations were not statistically significant. The IRR for history of vaginal sex only in the past two months could not be estimated because few participants reported this and there were no cases in that group.

Multivariable analysis. After manual backward stepwise selection, three characteristics were significantly associated with asymptomatic urethral inflammation (Table 4). Non-Hispanic Black MSW [aIRR 1.84 (1.24-2.72), p=0.002), those who reported ≥ 2 sex partners in the two months prior to enrollment [aIRR 1.62 (1.06-2.47), p=0.02], and those who had NGU at enrollment [aIRR 1.64 (1.08-2.50), p=0.02] were significantly more likely to experience an incident episode of asymptomatic urethral inflammation. In contrast, having CT at enrollment was not associated with asymptomatic urethral inflammation [aIRR 0.75 (0.44-1.27), p=0.28]. Additional adjustment for having only insertive oral sex in the past two months or lifetime history of GC did not change any of the estimates by >10% and these characteristics were not included in the final model.

DISCUSSION:

In this group of MSW attending an urban sexual health clinic, the prevalence of asymptomatic urethral inflammation was low (6.6%) and MSW reporting a history of NGU were more likely to have asymptomatic urethral inflammation at enrollment. Prevalent asymptomatic urethral inflammation was also more common in MSW who reported a history of CT and ≥2 partners in the past two months but not statistically significantly so. In contrast, the incidence of asymptomatic urethral inflammation was high, with 1.14 cases per person-year. Non-Hispanic Black race/ethnicity, NGU at enrollment, and ≥2 partners in the past two months were associated with higher risk of incident asymptomatic urethral inflammation. However, contrary to

our initial hypothesis, prior CT infection (either history or infection at enrollment) was not significantly associated with either prevalent or incident asymptomatic urethral inflammation.

To our knowledge, there are no prior estimates of the prevalence of asymptomatic urethral inflammation to directly compare the 6.6% prevalence that we observed. Although a cross-sectional study estimated the prevalence of asymptomatic urethritis in men in an emergency department waiting room as 16%[17], this study included men with evidence of discharge on genital exam in the definition of asymptomatic urethritis. In that same study, if men with evidence of discharge on genital exam had been excluded, the prevalence estimate would have been 9% (236 men included in the study, 38 with asymptomatic urethritis, 16 of those 38 men with evidence of discharge on genital exam), which is closer to the 6.6% prevalence that we observed. In that cross-sectional study, history of urethral insults, including NGU and CT infections, were associated with prevalent asymptomatic urethral inflammation, but history of GC was not.

To our knowledge, only one other study has assessed characteristics associated with prevalent asymptomatic urethral inflammation. A case-control study performed in the United Kingdom found that men with prevalent asymptomatic non-chlamydial non-gonococcal urethritis (NCNGU) reported less consistent condom use and were more likely to have been tested previously for STIs than men without urethritis. The UK study also demonstrated that men with prevalent asymptomatic NCNGU reported similar clinical, demographic, and sexual risk characteristics as men with symptomatic NGU[18]. While we observed no association with consistent condom use, we did observe associations with a characteristic typically associated with NGU (prior history of STI). Although not statistically significant, our finding that more than two sex partners in the past two months was associated with prevalent asymptomatic urethral inflammation suggests that increased number of exposures to diverse vaginal microbiota from different female partners may play a role in the development of urethral inflammation. Bacterial

vaginosis (BV), a condition caused by a disturbance in the vaginal microbiota, displays a similar epidemiological profile to STIs, including early sexual debut, recent intercourse, multiple partners, and unprotected sex [19]. BV associated bacteria are likekly transmitted through sexual contact. These bacteria have been investigated as potential causes of urethritis with inconsistent results[20–22]. Furthermore, it has been shown that sexual activity can influence the vaginal microbiota of young women[23]. It is also possible that exposure to the vaginal microbiota could cause inflammation of the male urethral tract through similar pathophysiology.

The incidence of asymptomatic urethral inflammation was 1.14 cases per person-year. To our knowledge, the literature includes no prior estimates of incident asymptomatic urethral inflammation for comparison. However, this is approximately double the estimated incidence of NGU in this cohort (56 per 100 person-years),[12] which was contrary to our initial hypothesis that the incidence of asymptomatic urethral inflammation would be about the same as that of NGU. Further investigation into the clinical relevance of asymptomatic urethral inflammation would clarify the importance of this finding.

Non-Hispanic Black race, NGU at enrollment, and ≥2 partners in the past two months were associated with higher risk of incident asymptomatic urethral inflammation. One factor driving the association between non-Hispanic Black race and incident asymptomatic urethral inflammation may be the higher prevalence of past urethral infections in non-Hispanic Black participants than other men. Our finding that the number of partners in the past two months was associated with incident asymptomatic urethral inflammation provides evidence supporting a possible disturbance of the urethral microbiota following sexual contact like that seen with BV in females. Characteristics associated with increased risk of incident asymptomatic urethral inflammation were similar to those associated with incident NGU in this same cohort. Low education (less than high school), history of CT, history of NGU, and NGU at enrollment were associated with incident NGU but Black race was only associated with non-CT/non-MG NGU

and symptomatic NGU[12]. Education has also been associated with other STIs including chlamydia[24,25]. Multiple prior studies have reported an association between Black race and STIs[26–28], although the racial disparities in STI rates likely reflect environmental, institutional, and contextual differences between races and not differences in risk behaviors[29]. Black young adults remain at high risk of STIs even when engaging in low-risk behaviors, whereas white young adults' risk of STIs increase as they increasingly engage in risky behaviors. Our study did not identify any significant associations between education level, history of CT, history of NGU, or consistent condom use with the most recent partner and incidence of asymptomatic urethral inflammation. These findings suggest that incident asymptomatic urethral inflammation shares some but not all risk factors with NGU and other STIs.

The lack of a significant association between history of CT or recent CT infection (e.g., CT detected at enrollment) with either incidence or prevalence of asymptomatic urethral inflammation was contrary to our original hypothesis. However, there was some evidence suggesting that prior urethral infection may play a role. Previous episodes of NGU were strongly associated with prevalent (history of NGU) and incident (NGU at enrollment) asymptomatic urethral inflammation. Additionally, history of CT and history of GC were more common among men with prevalent and incident asymptomatic urethral inflammation, respectively, although these latter relationships were not statistically significant in multivariable analyses. It is not possible to tell whether the lack of a significant relationship was due to misclassification, bias, low statistical power, or the absence of a true relationship. These relationships somewhat support the protective immunity hypothesis that the immune system may deal with repeat infectious insults more effectively, dampening the associated symptomatic profile.

Our study found that the prevalence of asymptomatic urethral inflammation was somewhat low at 6.6% but the incidence was substantially higher at 1.14 cases per person-year. These findings indicate that there are possibly many men in the general population experiencing

undetected cases of asymptomatic urethral inflammation. The paradoxical lower prevalence and relatively higher incidence findings are likely due to a short duration of asymptomatic urethral inflammation when it occurs. Another paradoxical relationship between prevalence and incidence is evident with human papillomavirus (HPV). In the US, the prevalence of HPV is high at 42.5 million cases per year but the incidence is relatively low at 13 million cases per year[31]. In contrast to the likely short duration of asymptomatic urethral inflammation, the relationship between prevalence and incidence for HPV is due to the long duration of persistent HPV infections.

There are several strengths to this study. First, our study was novel in that we were able to estimate prevalence, incidence, and characteristics associated with the frequency of asymptomatic urethral inflammation. Second, the planned follow-up visits with collection of urethral swabs from all participants at each visit allowed us to assess asymptomatic urethral inflammation, which is not typically detected. In most settings, urethral swabs are only collected from men with symptoms of urethritis and therefore detecting asymptomatic urethral inflammation with increased PMNs would be impossible. Third, our study tested for MG infections at enrollment which allowed consideration of MG as a risk factor for prevalent and incident asymptomatic urethral inflammation. Fourth, participants completed 1444 visits over 6 to 8 months contributing 106.6 person-years of follow-up time. At each enrollment visit, our team captured a range of data including examination findings, other clinical characteristics, allowing us to assess associations with asymptomatic urethral inflammation.

In addition to the strengths, there are also several limitations to the current study. First, there may have been selection bias in the original parent study considering 62% of STI clinic attendees declined participation. Second, interobserver variation between microscopists performing microscopic urethral smear analyses may have affected PMN counts. However, any influence of this was likely small as there were only two clinicians performing urethral smear

microscopy. Third, the retrospective study design limited characteristics to those collected by the study team. Future studies may obtain more detailed characteristics such as the timing of past urethral episodes, such as CT or NGU, which would allow separating out recent infections from distant infections. Fourth, the current study only assessed the association of enrollment characteristics with incident asymptomatic urethral inflammation. Assessment of time varying characteristics collected at each follow-up visit, such as recent number of partners or sexual behaviors, may have provided more precise insights into factors that initiated urethral inflammation in the absence of urethral symptoms or discharge. Finally, the results of this study may not be generalizable to community settings beyond the high risk STI clinic attendee population. The study population included only men who reported to the STI clinic. Many men in the study reported to the clinic for urethral symptoms who thus would not meet criteria for the outcome. The prevalence and incidence of asymptomatic urethral inflammation in the general population may be higher than what we observed because the general population would not have such a high proportion of men with urethral symptoms. Alternatively, STI clinic attendees are at generally higher risk for STIs and STI related syndromes; therefore it is also possible that the prevalence and incidence of asymptomatic urethral inflammation in the general population is lower.

These findings provide one of the few estimates of the prevalence of asymptomatic urethral inflammation and an initial estimate of the incidence of this condition. Although the prevalence was somewhat low, the incidence over a 6-month time-period was high. Characteristics associated with prevalent asymptomatic urethral inflammation were similar but not identical to those associated with new episodes of asymptomatic urethral inflammation. However, the association between a higher number of sex partners and prior infections with both prevalent and incident asymptomatic urethral inflammation and higher risk in populations with historically less access to care suggest that persistent inflammation may occur frequently in men

subsequent to a urethral infection. Future studies with larger sample sizes should investigate the duration of the immune response to CT and other NGU infections, the sociostructural factors leading to increased incidence of asymptomatic urethral inflammation in Black men, and the health consequences of urethral inflammation.

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Table 1: Enrollment characteristics of men who have sex with women (MSW) attending an urban sexually transmitted infections (STI) clinic between August 2014 and July 2018.

Characteristic	Study Population N=317 n	%
Age		
Median Age [Range]	32 [17-71]	
17-24	45	14%
25-34	158	50%
35+	114	36%
Race/ethnicity		
non-Hispanic White	146	46%
non-Hispanic Black	71	22%
Hispanic	36	11%
Other, Multiple Races, Unknown	64	20%
Education		
Completed High School or Less	111	36%
2-year College Completion (Associates Degree) or Beyond	200	64%
STI Lifetime History		
History of CT	64	22%
History of GC	32	11%
History of NGU	37	12%
Number of Partners in Lifetime		
1-4	10	3%
5-9	46	15%
10-24	93	30%
25-49	75	25%
50+	82	27%
Number of Partners Past Two Months		
0-1	125	42%

2+	175	58%
Sexual Behaviors in Last 2 Months		
Any Vaginal Sex	303	97%
Any Insertive Anal Sex	59	19%
Any Insertive Oral Sex	302	98%
Avg PMNs/hpf on urethral Gram-stain		
0-4	179	56%
5-9	37	12%
10+	101	32%
Consistent Condom Use Most Recent Sex Partner		
Never	124	40%
Sometimes	79	26%
Usually	36	12%
Always	68	22%
STI Testing Result		
CT Positive	35	11%
MG Positive	35	11%
NGU at Enrollment	117	37%

Abbreviations: SD = standard deviation; STI = sexually transmitted infections; CT = *Chlamydia trachomatis*; MG = *Mycoplasma genitalium*; NGU = non-gonococcal urethritis; PMNs = polymorphonuclear cells; hpf = high powered field;

Table 2: Enrollment characteristics of cases of prevalent asymptomatic urethral inflammation (n=21) and controls (n=134) among men who have sex with women (MSW) attending an urban sexually transmitted infections (STI) clinic between August 2014 and July 2018

Characteristic	Men with Asymptomatic Urethral Inflammation* N=21 n	%	Men without Urethral Inflammation, Urethral Symptoms, or Urethral Discharge N=134† n	%	p-value§
Age					
Median Age [Range]	30 [18-61]		32 [19-71]		0.63
17-24	3	14%	19	14%	0.81
25-34	11	52%	61	46%	
35+	7	33%	54	40%	
Race/ethnicity					
non-Hispanic White	8	38%	70	52%	0.26
non-Hispanic Black	5	24%	19	14%	
Hispanic	1	5%	17	13%	
Other, Multiple Races, Unknown	7	33%	28	21%	
Education					
Completed High School or Less	8	40%	38	29%	0.31
2-year College Completion or Beyond	12	60%	93	71%	
STI Lifetime History					
History of CT	5	29%	21	16%	0.19
History of GC	3	15%	10	8%	0.38
History of NGU	4	20%	7	5%	**0.04**
Number of Partners in Past Two Months					
0-1	5	26%	62	48%	0.09
2+	14	74%	66	52%	

Sexual Behaviors in Last 2 Months					
Vaginal Sex	19	90%	127	96%	0.25
Insertive Anal Sex	6	29%	27	20%	0.40
Insertive Oral Sex	20	100%	130	98%	1.00
Avg PMNs/hpf on urethral Gram-stain					
0-4	0	0%	134	100%	
5-9	11	52%	0	0%	
>=10	10	48%	0	0%	
Consistent Condom Use Most Recent Sex Partner					
Never	9	45%	54	41%	0.48
Sometimes	3	15%	30	23%	
Usually	4	20%	13	10%	
Always	4	20%	36	27%	
STI Testing Result					
CT Positive	1	5%	1	1%	0.25
MG Positive	1	5%	5	4%	0.59

Abbreviations: SD = standard deviation; STI = sexually transmitted infections; CT = *Chlamydia trachomatis*; MG = *Mycoplasma genitalium*; NGU = non-gonococcal urethritis; PMNs = polymorphonuclear cells; hpf = high powered field;

* Asymptomatic urethral inflammation was defined as ≥ 5 PMNs (polymorphonuclear cells) per hpf (high-powered field) over three fields on a Gram-stained slide without patient reported urethral symptoms and without urethral discharge on genital exam.

† Control status was defined as <5 PMNs (polymorphonuclear cells) per hpf (high-powered field) over three fields on a Gram-stained slide without patient reported urethral symptoms or urethral discharge on genital exam.

§ A t-test was performed to assess for differences in means between groups. A Wilcoxon test was performed to assess for differences in medians between groups. Fisher's exact test was used to determine statistical significance of categorical comparisons.

Table 3: Characteristics associated with incident asymptomatic urethral inflammation on univariable analysis among 317 men who have sex with women (MSW) attending an urban sexually transmitted infections (STI) clinic between August 2014 and July 2018 with 106.6 person-years of follow-up time.

Characteristic	N Incident Cases*	Crude IRR (95% CI)	p-value
Age			
17-24	16	Ref	
25-34	62	1.13 (0.62-2.06)	0.69
35+	43	0.89 (0.47-1.67)	0.71
Race/ethnicity			
non-Hispanic White	56	Ref	
non-Hispanic Black	39	1.76 (1.14-2.72)	**0.01**
Hispanic	8	0.63 (0.29-1.37)	0.24
Other, Multiple Races, Unknown	18	0.75 (0.43-1.34)	0.33
Education			
Completed High School or Less	45	Ref	
2-year College or Beyond	74	0.84 (0.56-1.27)	0.41
STI Lifetime History			
History of CT	29	1.26 (0.82-1.93)	0.29
History of GC	17	1.86 (1.02-3.40)	**0.04**
History of NGU	16	1.30 (0.73-2.34)	0.38
Number of Partners Past Two Months			
0-1	35	Ref	
≥2	78	1.60 (1.05-2.44)	**0.03**
Sexual Behaviors in Last 2 Months†			
Only Vaginal Sex	0	-	-
Any Insertive Anal Sex	23	1.00 (0.60-1.67)	1.00
Only Insertive Oral Sex	6	1.79 (0.82-3.89)	0.15

Avg PMNs/hpf on urethral Gram-stain at enrollment			
0-4	53	Ref	
5-9	17	1.61 (0.92-2.84)	0.10
≥10	51	1.86 (1.23-2.83)	**0.01**
Clinician Observed Discharge at Enrollment			
No clinician Observed Discharge	62	Ref	
Clinician Observed Discharge	59	1.42 (0.97-2.09)	0.07
Urethral Symptoms at Enrollment			
No Urethral Symptoms	64	Ref	
Urethral Symptoms	57	1.23 (0.84-1.81)	0.29
Consistent Condom Use Most Recent Sex Partner			
Never	47	Ref	
Sometimes	27	1.02 (0.62-1.67)	0.94
Usually	15	1.08 (0.57-2.05)	0.81
Always	21	0.94 (0.53-1.65)	0.82
STI Testing Result At Enrollment Visit			
CT Negative	104	Ref	
CT Positive	17	1.19 (0.70-2.02)	0.53
MG Negative	112	Ref	
MG Positive	9	0.69 (0.38-1.24)	0.21
NGU Status at Enrollment			
Men without NGU	61	Ref	
Men with NGU	60	1.75 (1.19-2.57)	**0.01**

Abbreviations: STI = sexually transmitted infections; CT = *Chlamydia trachomatis*; MG = *Mycoplasma genitalium*; NGU = non-gonococcal urethritis; PMNs = polymorphonuclear cells; hpf = high powered field; IRR = incident risk ratio; Ref = referent group

* Asymptomatic urethral inflammation was defined as ≥ 5 PMNs (polymorphonuclear cells) per hpf (high-powered field) over three fields on a Gram-stained slide without patient reported urethral symptoms and without discharge on genital exam.

†The IRR for history of vaginal sex only in the past two months could not be estimated because there were few participants who reported this and there were no cases in that group. The referent category for any insertive anal sex was other participants with no history of insertive anal sex in the past two months. The referent category for only oral sex was participants who reported either insertive anal or vaginal sex in the past two months.

Table 4: Characteristics associated with incident asymptomatic urethral inflammation on multivariable analysis among 317 men who have sex with women (MSW) attending an urban sexually transmitted infections (STI) clinic between August 2014 and July 2018 with 106.6 person-years of follow-up time.

Characteristic	Adj IRR	p-value
Race/Ethnicity		
Hispanic, non-Hispanic White, Other, Multiple Races, Unknown	Ref	
Non-Hispanic Black	1.84 (1.24-2.72)	0.002
Number of Partners Past Two Months		
0-1	Ref	
≥2	1.62 (1.06-2.47)	0.02
CT Result at Enrollment		
Positive	Ref	
Negative	0.75 (0.44-1.27)	0.28
NGU Status at Enrollment		
Men without NGU	Ref	
Men with NGU	1.64 (1.08-2.50)	0.02

Abbreviations: STI = sexually transmitted infections; CT = *Chlamydia trachomatis*; NGU = non-gonococcal urethritis; IRR = incident risk ratio; Ref = referent group

* Asymptomatic urethral inflammation was defined as ≥ 5 PMNs (polymorphonuclear cells) per hpf (high-powered field) over three fields on a Gram-stained slide without patient reported urethral symptoms and without discharge on genital exam.