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High Pgp3 *Chlamydia trachomatis* seropositivity, pelvic inflammatory disease and infertility among women, National Health and Nutrition Examination Survey, United States, 2013–2016

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Summary: Among women in the National Health and Nutrition Examination Survey, high chlamydia Pgp3 antibody median fluorescence intensity was associated with reported infertility and current chlamydia infection.

Abstract:

Background

Chlamydia trachomatis causes pelvic inflammatory disease (PID) and tubal infertility. Pgp3 antibody (Pgp3Ab) detects prior chlamydial infections. We evaluated for an association of high chlamydial seropositivity with sequelae using a Pgp3Ab multiplex bead array (Pgp3AbMBA).

Methods

We performed chlamydia Pgp3AbMBA on sera from women 18–39 years old participating in the 2013–2016 National Health and Nutrition Examination Survey (NHANES) with urine chlamydia nucleic acid amplification test results. High chlamydial seropositivity was defined as a median fluorescence intensity (MFI $\geq 50,000$; low-positive was MFI > 551 – $<50,000$). Weighted US population high-positive, low-positive, and negative Pgp3Ab chlamydia seroprevalence and 95% confidence intervals (95% CI) were compared for women with chlamydial infection, self-reported PID, and infertility.

Results

Of 2,339 women aged 18–39 years, 1,725 (73.7%) had sera and 1,425 were sexually experienced. Overall, 104 women had high positive Pgp3Ab (5.4% [95% CI 4.0–7.0] of US women); 407 had low positive Pgp3Ab (25.1% [95% CI 21.5–29.0]), and 914 had negative Pgp3Ab (69.5% [95% CI 65.5–73.4]).

Among women with high Pgp3Ab, infertility prevalence was 2.0 (95% CI 1.1–3.7) times higher than among Pgp3Ab-negative women (19.6% [95% CI 10.5–31.7] versus 9.9% [95% CI 7.7–12.4]). For

women with low Pgp3Ab, PID prevalence was 7.9% (95% CI 4.6–12.6) compared to 2.3% (95% CI 1.4–3.6) in negative Pgp3Ab.

Conclusions

High chlamydial Pgp3Ab seropositivity was associated with infertility although small sample size limited evaluation of an association of high seropositivity with PID. In infertile women, Pgp3Ab may be a marker of prior chlamydial infection.

Introduction

Prior to the COVID-19 pandemic, chlamydial infections had consistently been the most commonly reported infectious disease in the United States, with nearly 1.8 million cases reported to the CDC in 2018[1]. About 30% of US women have serologic evidence of *Chlamydia trachomatis* infection[2]. The highest prevalence of chlamydial infection occurs in young women with 4% of women 14–24 years old chlamydia positive by urine nucleic acid amplification test (NAAT)[1]. Following a cervical infection, *C. trachomatis* and other sexually transmitted infections can ascend in the female genital tract and cause reproductive sequelae, including pelvic inflammatory disease (PID), ectopic pregnancy, and tubal factor infertility (TFI). Approximately 10% of untreated chlamydial infections may progress to PID within a year[3], and PID may cause TFI about 11% of the time[4] although currently available diagnostic tests cannot identify women with lower tract disease at risk for progressing to sequelae. Chlamydia NAAT tests can measure acute cervical infection that may lead to sequelae; however, they cannot measure prior infections.

Unlike chlamydial NAATs, chlamydial serologic assays may be used to measure antibodies indicating prior chlamydial infection that may lead to PID and infertility[5]. Using a variety of chlamydial serologic assays, studies have evaluated the association between current and prior chlamydial infection and PID. While some have found an increased risk of PID, other studies have not found a statistically significant association[6-9]. Estimates of the percentage of tubal factor infertility attributable to chlamydia using different serologic assays range from 10–50%[10-12]. Although chlamydia is known to cause PID and infertility, existing knowledge is incomplete about the proportions of PID and infertility caused by chlamydia, and which women with chlamydia are at elevated risk for upper genital tract sequelae primarily due to the poor sensitivity of existing commercially available chlamydial serologic assays[5].

Serological assays can detect antibodies against *C. trachomatis* plasmid gene protein 3 (Pgp3) and may provide further insights on the association between Pgp3 antibody (Pgp3Ab) level and chlamydial sequelae. Detection of Pgp3Abs has shown good (92% at 6 months or less post chlamydial infection) sensitivity for chlamydia compared to NAAT tests[5]. Additionally, the Pgp3Ab multiplex bead array (Pgp3AbMBA) has shown similar[13], or greater[14] ability to detect prior chlamydial infection than the Pgp3 ELISA[2]. Pgp3 may have a role in the inflammatory sequelae of chlamydia. Mice data suggest that Pgp3 may influence *Chlamydia muridarum*'s upper tract ascension with less upper genitourinary tract chlamydial infection when Pgp3 antigen is absent[15]. Immunization with Pgp3 also leads to lower *C. trachomatis* in mouse fallopian tubes[16].

Evidence suggests that higher chlamydia antibody levels are related to increased risk of developing TFI[17, 18]. An assay that could distinguish chlamydial sequelae from uncomplicated chlamydial infection could be used to identify women at risk for developing upper tract sequelae and expand the public health use of chlamydia serology[19]. We took advantage of the wide dynamic range of the MBA assay and used sera from a nationally representative sample of women 18–39 years old to evaluate for associations between a high level of anti-chlamydial Pgp3Ab response, and the reproductive health sequelae of PID and infertility.

Methods

Study design/population

We used data and sera from women 18–39 years old with available urine *C. trachomatis* NAAT results who participated in the 2013–2016 cycles of NHANES. NHANES is a nationally representative survey that has been conducted by the National Center for Health Statistics (NCHS) in the US resident, civilian, non-institutionalized population since 1971[20, 21]. Participants in this survey have undergone a survey interview, physical examination, and were asked to consent to a blood draw and urine specimen

collection. Women with unknown or unavailable laboratory results were excluded. We performed Pgp3AbMBA on the sera as outlined below.

Data sources and methods

Laboratory methods

Prior to testing serum specimens with the Pgp3AbMBA, the positivity cutoff was determined using a receiver operator characteristic (ROC) curve based on a panel of 85 serum samples previously tested by ELISA, MBA and lateral flow assay[13]. The overall positivity cut off for the Pgp3AbMBA was set at a median fluorescence intensity (MFI) of greater than 551. Beads were coupled to Pgp3 antigen as previously described[22]. All reagents, specimens, and plates were brought up to room temperature before testing. Serum was diluted 1:400 in Buffer B (1X phosphate buffered saline [PBS], 0.5% casein, 0.5% polyvinyl alcohol, 0.8% polyvinylpyrrolidone, 0.02% NaN₃, 0.3% Tween 20 and 3 µg/mL of *Escherichia coli* cell extract) and 50 µl of the diluted serum was incubated with Pgp3-coupled beads (1250 per well) for 1.5 hours. Beads were then washed three times with PBST (1X PBS + 0.05% Tween-20) and incubated with 50 ng biotinylated mouse anti-human IgG Fc (Southern Biotech, Birmingham) and 20 ng biotinylated mouse anti-human IgG4 (Southern Biotech, Birmingham, AL) for 45 minutes. After this incubation, beads were again washed three times with PBST and incubated with 250 ng streptavidin phycoerythrin (SA-PE) for 30 minutes, washed three times with PBST and incubated in Buffer A (1X PBS, 0.5% bovine serum albumin [BSA], 0.05% Tween-20 and 0.02% sodium azide) for 30 minutes, washed once more with PBST, and suspended in 100 µl 1X PBS[14]. Beads with antibodies from participant sera were read on the Luminex MAGPIX instrument with the background subtracted.

Variables and definitions

Speculating that the strongest association would be found with the highest level of Pgp3Ab and a) self-reported PID, and/or b) self-reported infertility, we categorized the Pgp3Ab MFI values into three

categories: MFI values of greater than or equal to 50,000 were classified as high-positive (high seropositivity) based on expert opinion, MFI values of greater than 551 to 49,999 were classified as low-positive (low seropositivity), and 551 and lower were considered seronegative. We also performed a secondary analysis that used an alternate definition for a high positivity based on selecting the median value between the 551 cutoff and the highest positive value. With this alternate definition, high seropositivity at the median of the positive values was an MFI of 25,048 or higher, and low seropositivity was defined as an MFI value of 551 to less than 25,048.

We limited our study sample to women who reported ever having had any type of sex including vaginal, oral, or anal sex (sexually experienced). Women with a positive urine chlamydia NAAT at the time of the NHANES exam were classified as having current chlamydia; women who self-reported having been told of a chlamydia diagnosis in the 12 month-period before the NHANES exam were classified as having recent chlamydia although this did not necessarily include women who had current chlamydia or a positive NAAT test at the time of the NHANES exam. PID was defined as a woman reporting ever having been treated for PID. Infertility was defined as a woman reporting an inability to get pregnant over a one-year period despite attempting to get pregnant.

Analytic methods

Sample weights were used to generate US national estimates by multiplying each included participant by the frequency of US women represented by the included participant. Weighted seroprevalences of high-positive, low-positive, and negative results along with 95% confidence intervals (CIs) were calculated overall and by various characteristics by applying Clopper Pearson CIs. We also calculated weighted prevalence ratios for chlamydia sequelae. Median MFI with interquartile ranges (IQR) were calculated for each outcome category. Weighted prevalence or prevalence ratio estimates were suppressed where data presentation criteria were not met for the effective sample size, prevalence, or confidence intervals

per NHANES guidance[23]. Statistical analyses for weighted data were conducted using SAS 9.4 per NHANES guidance[24].

Ethical and IRB review

NHANES respondents provided consent for their specimens to be used in future research during the specimen collection process. Our study protocol was reviewed and approved by the National Center for Health Statistics Ethics Review Board and in accordance with the Helsinki Declaration.

Results

In the 2013–2016 cycles of the NHANES survey, the initial survey response rate ranged from 61–79% among all 16–39 year old women who were approached for inclusion[25] (Figure 1). Of the 10,251 women of all ages with information participating in the survey there were 2,339 women 18–39 years old. Among these 2,339 women, 2,195 (93.8%) had available chlamydia NAAT results.. We limited our analysis to the 1,425 (80.6%) women that had serology results among 1,768 women with chlamydia NAAT results who reported sexual experience. In comparing women with and without available Pgp3 serology results from our sample, women with serology results were older, differed in their racial/ethnic distribution (with a higher percentage of non-Hispanic black women not having serology results), had higher income, more often reported a history of anal sex, and less often had recent chlamydia (data not shown).

High seropositivity, low seropositivity, and seronegativity

Among the 1,425 sexually-experienced women 18–39 years old, the weighted prevalence of high-positive Pgp3 MFI (high seropositivity) was 5.4% (95% CI 4.0–7.0), of low-positive Pgp3 MFI (low seropositivity) was 25.1% (95% CI 21.5–29.0), and of seronegative Pgp3 was 69.5% (95% CI 65.5–73.4) (Table 1). Using our alternate definition of high-positive Pgp3 MFI, the prevalence of high-positive seropositivity was 13.9% (95% CI 11.3–16.8) while low-positive seropositivity was 16.6% (95% CI 13.8–19.7) (Table 2).

A total of 36 women had current chlamydia, 33 had recent chlamydia, 59 reported having ever been treated for PID, and 144 reported having had infertility among the 1,425 women in the sample (Table 1). Among women with reported infertility, 64.2% (95% CI 53.3–74.1) were seronegative and 25.9% (95% CI 16.8–36.9) had low seropositivity. The prevalence estimate for high seropositivity among infertile women could not be reported based on NHANES guidance for reporting confidence intervals. The prevalence estimates for high seropositivity were also unstable for women with current chlamydia, recent chlamydia, and PID.

The prevalence of high seropositivity was higher for non-Hispanic black women compared to non-Hispanic white women, 17.2% (95% CI 11.7–24.0) versus 2.1% (95% CI 0.9–4.2); women with 5–9 lifetime sex partners, 7.2% (95% CI 4.6–10.6) or 10 or more partners, 8.2% (95% CI 5.6–11.6) compared to 1–4 partners, 2.5% (95% CI 1.4–4.1); and women ever having sex with a woman, 10.5% (95% CI 6.8–15.3) versus never having sex with a woman, 4.4% (95% CI 3.0–6.1). The prevalence of high Pgp3Ab seropositivity was lower for women with income to poverty ratio greater than or equal to three times the federal poverty level, 2.4% (95% CI 0.9–4.9) compared to women with income 1.5 times or less than the federal poverty level, 9.8% (95% CI 7.1–13.1); and women college graduates at 1.3% (95% CI 0.3–3.7) compared to women not completing high school, 7.3% (95% CI 4.0–12.0). Women who were older at their first sexual experience also had lower high Pgp3Ab seropositivity prevalence, 0.5% (95% CI 0.0–2.5) for women 20 years and older, 1.2% (95% CI 0.3–3.3) for women 18–19 years,

and 5.8% (95% CI 4.0–8.0) for women 15–17 years, compared to 16.1% (95% CI 11.1–22.3) for women younger than 15 years at first sex.

High seropositivity, chlamydia status, and sequelae of PID and infertility

The prevalence of current chlamydia among women with high seropositivity, 10.7% (95% CI 5.4–18.6) was higher than the prevalence of current chlamydia among seronegative women, 0.4% (95% CI 0.1–1.3) (Table 3). Recent chlamydia prevalence was higher, 5.8% (95% CI 3.7–8.5) versus 0.7% (95% CI 0.1–1.9) comparing women with low seropositivity to seronegative women; as was PID prevalence, 7.9% (95% CI 4.6–12.6) versus 2.3% (95% CI 1.4–3.6); and current chlamydia prevalence, 3.5% (95% CI 1.7–6.4) versus 0.4% (95% CI 0.1–1.3). Using our alternate definition for high seropositivity, comparing women with high seropositivity to those seronegative, we observed a greater prevalence of PID, 8.0% (95% CI 4.0–14.2) versus 2.3% (95% CI 1.4–3.6); recent chlamydia, 7.9% (95% CI 4.7–12.2) versus 0.7% (95% CI 0.1–1.9); and current chlamydia, 7.2% (95% CI 4.4–11.1) versus 0.4% (95% CI 0.1–1.3) (Table 4). The weighted prevalence of infertility did not statistically significantly vary by level of seropositivity based on our primary and alternate high-seropositive definitions.

Women with high positivity had a higher prevalence ratio (PR) of 2.0 (95% CI 1.1–3.7) for infertility comparing high seropositivity to seronegativity (Table 3). For women reporting PID or recent chlamydia, the sample size of women with these characteristics limited our ability to estimate the prevalence and PR of these characteristics comparing high seropositivity to seronegativity. While we could not determine the weighted PR for current chlamydia with precision, the current chlamydia prevalence of 10.7% (95% CI 5.4–18.6) for women with high seropositivity was approximately 25 times higher than the current chlamydia prevalence of 0.4% (95% CI 0.1–1.3) for seronegative women. Using our alternate definition of high seropositivity, the prevalence ratio for infertility for women with high

seropositivity was no longer significant, PR 1.5 (95% CI 0.9–2.5) (Table 4). With our alternate definition, the PR of PID was significantly associated with low seropositivity, 2.9 (95% CI 1.3–6.2).

Seropositivity profiles

Overall, women with current chlamydia had a median MFI of 35,780 [interquartile range (IQR) 7,240–51,480]; women with recent chlamydia had a median MFI of 31,152 [IQR 2,444–49,465]; women with PID had a median MFI of 2,593 [IQR 37–27,156]; and women with infertility had a median MFI of 43 [IQR 19–12,600] (Table 5).

Discussion

In our evaluation of high-positive chlamydial Pgp3AbMBA MFI levels among a nationally representative sample of US women 18–39 years old, we found that the prevalence of infertility among women with high-positive Pgp3Ab results was twice the prevalence among women with negative Pgp3Ab results. PID prevalence also differed by seropositivity level, although our sample size limited an evaluation of an association of PID with high seropositivity. To our knowledge, our study is unique in evaluating quantitative Pgp3AbMBA levels in a nationally representative sample that includes women with infertility.

Two recent studies describe differing chlamydial Pgp3Ab levels in sub-fertile women compared to non-infertile women[17, 26]. Our finding of an association of infertility with high seropositivity is consistent with studies which have found an association of Pgp3Ab and other chlamydial serologic assays with infertility or TFI although this association has not been seen consistently[27] and is not completely understood[28]. Previous studies found that non-Pgp3 chlamydial antibody levels were related to the degree of tubal damage and obstruction[29-31], suggesting the possibility that a higher degree of

chlamydial immune response secondary to higher chlamydial bacterial burden resulted in more reproductive pathology.

While we observed an association of infertility and high seropositivity, the magnitude of the association was small, and the average MFI value among women with infertility was lower than the median level for all other outcome categories except for those with no occurrence of any of the chlamydial outcomes. It is possible that we did not observe higher median Pgp3Ab levels among women with infertility and PID for a few reasons. Firstly, TFI which follows from STIs is only about 25–35% of all reported infertility[32]. Also, because infertility was self-reported and not systematically evaluated, all women with infertility may not have been identified. Finally, although Pgp3Ab can persist for up to 12 years[33], antibody levels may wane as early as weeks to months after an initial chlamydia infection[5], and have been documented to be lower three to 10 years after the initial infection[34]. Similarly for PID, not all PID is caused by chlamydia[35]; thus, we would not expect a high positive or even positive chlamydia serology in all women with PID or infertility. Additionally, PID which can be subclinical a majority of the time, was based on participant self-report and potentially subject to recall bias and misclassification[36].

Although findings from mice studies suggest that Pgp3Abs are related to upper tract disease[15, 16], we found that high-positive Pgp3Abs were more strongly associated with current chlamydia, with higher median Pgp3Ab levels in women with current or recent chlamydia. Thus, Pgp3Ab may not be an ideal marker of chlamydial infection ascending to the upper genital tract and causing tubal damage. Rather Pgp3Ab may better serve as a marker of previous chlamydial infection, as also suggested by Mazraani et al[26]. Unlike most chlamydial serologic assays, Pgp3 ELISA has shown good (92%) sensitivity compared to chlamydia NAAT[5] and fair (72–83%) sensitivity compared to self-report of chlamydial infection[33]. Similar to other studies, we observed higher chlamydial seropositivity in non-Hispanic black as compared to non-Hispanic white women, women with earlier onset of sexual activity, or

women with a higher number of sexual partners[5, 37]. Because these characteristics also represent risk groups for chlamydial infection[38], very high Pgp3Ab serology levels in these risk-groups may again simply indicate a greater risk of having uncomplicated chlamydial infection or recurrent chlamydial infection.

The association between infertility and high Pgp3Ab seropositivity, and PID and low Pgp3Ab seropositivity should be taken in the context of our study design and other limitations beyond those already mentioned. Our study was a cross-sectional analysis in which the temporal relationships between chlamydia and PID or infertility could not be determined and did not adjust for confounders. Because women may not mount an antibody response to chlamydial infection[34], serologic tests may misclassify these women, muting our observed association. Additionally, differences in characteristics between women who did and did not have serum available limit the representativeness of our data.

Despite the limitations of our study design, we did observe an increased prevalence of infertility among women with high Pgp3Ab seropositivity. Better estimates of this association and for women with PID might be obtained by using additional cycles of data to increase sample size or by conducting cohort studies to be sure that chlamydial infection precedes sequelae. A cohort study would also allow for better characterization of the kinetics of the Pgp3Ab response. Future studies should continue the search for serologic or other biomarkers that might predict upper genital tract chlamydial ascension to broaden our understanding of women most at risk for these reproductive sequelae of STIs.

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Table 1:

Characteristics of sexually experienced women 18–39 years with available *Chlamydia trachomatis* serology data, by category of serological Pgp3 multiplex bead array fluorescence intensity result (negative, low-positive [551–49,999], and high-positive [50,000 and above]), National Health and Nutrition Examination Survey (NHANES), 2013–2016

	Total	Negative sample N	Weighted negative prevalence (95% CI)	Low- positive sample N	Weighted low- positive prevalence (95% CI)	High- positive sample N	Weighted high- positive prevalence (95% CI)
Overall	1425	914	69.5 (65.5–73.4)	407	25.1 (21.5–29.0)	104	5.4 (4.0–7.0)
Age group (years)							
18–24	458	297	68.6 (62.8–74.0)	122	24.8 (20.1–30.0)	39	6.6 (4.3–9.6)
25–31	452	299	71.8 (65.9–77.3)	120	23.1 (18.2–28.6)	33	5.1 (3.1–7.9)
32–39	515	318	68.1 (61.4–74.2)	165	27.2 (21.3–33.8)	32	4.7 (2.5–7.9)
Race/ethnicity							
Non-Hispanic white	506	378	77.3 (72.7–81.5)	117	20.5 (16.1–25.5)	11	2.1 (0.9–4.2)
Non-Hispanic black	259	79	29.8 (22.2–38.3)	135	53.0 (44.6–61.3)	45	17.2 (11.7–24.0)

Hispanic	442	295	66.5 (61.9–70.9)	117	26.8 (22.7–31.2)	30	6.7 (4.5–9.6)
Non-Hispanic Asian	138	115	83.1 (75.8–88.9)	19	14.0 (7.9–22.2)	4	* ^a
Other/multi-racial	80	47	64.4 (48.7–78.1)	19	20.8 (10.8–34.4)	14	*
Recent chlamydia							
No	1390	907	70.5 (66.6–74.3)	383	24.1 (20.6–27.9)	100	5.3 (3.9–7.1)
Yes	33	6	*	23	*	4	*
Recent gonorrhea							
No	1417	912	69.6 (65.6–73.5)	401	24.9 (21.3–28.8)	104	5.4 (4.1–7.1)
Yes	6	1	*	5	*	0	*
Recent sexually transmitted disease							
No	1193	799	72.0 (67.7–76.0)	311	22.9 (19.0–27.1)	83	5.1 (3.6–6.9)
Yes	230	114	58.9 (51.4–66.0)	95	34.4 (28.3–40.9)	21	6.7 (3.9–10.8)
Pelvic inflammatory disease							
No	1354	886	70.8 (66.8–74.5)	372	23.9 (20.4–27.8)	96	5.3 (3.9–6.9)
Yes	59	22	*	32	*	5	*
Infertility							

No	1280	837	70.1 (66.0–74.0)	358	25.0 (21.5–28.8)	85	4.9 (3.5–6.5)
Yes	144	76	64.2 (53.3–74.1)	49	25.9 (16.8–36.9)	19	*
Current chlamydia							
Negative	1389	909	70.5 (66.3–74.4)	389	24.6 (21.0–28.6)	91	4.9 (3.6–6.5)
Positive	36	5	*	18	*	13	*
Income to poverty ratio							
<1.5	577	308	57.2 (51.3–62.9)	206	33.0 (27.8–38.6)	63	9.8 (7.1–13.1)
1.5–<3	362	240	69.6 (61.1–77.2)	97	25.7 (19.6–32.7)	25	4.7 (2.3–8.3)
≥3	413	321	80.8 (76.2–84.9)	79	16.9 (13.1–21.2)	13	2.4 (0.9–4.9)
Marital status							
Never married	433	247	63.9 (58.9–68.6)	141	28.3 (24.1–32.8)	45	7.8 (5.2–11.2)
Married/living with partner	721	501	74.1 (68.9–78.8)	184	22.3 (17.5–27.6)	36	3.6 (2.3–5.5)
Divorced/widowed/separated	113	56	54.5 (42.9–65.8)	47	38.2 (28.2–49.1)	10	*
Education							
<High School	190	100	53.3 (45.4–61.1)	74	39.4 (31.5–47.8)	16	7.3 (4.0–12.0)

High school graduate/general education diploma	240	133	59.7 (50.7–68.3)	86	34.3 (26.4–43.0)	21	5.9 (3.3–9.7)
Some college/associates degree	498	299	66.3 (61.2–71.1)	153	26.2 (21.5–31.4)	46	7.5 (4.7–11.1)
≥College graduate	339	272	84.0 (78.0–88.9)	59	14.7 (10.4–19.9)	8	1.3 (0.3–3.7)
Health insurance coverage							
No	345	205	60.6 (53.7–67.2)	108	31.6 (25.6–38.1)	32	7.8 (5.1–11.3)
Yes	1078	708	71.7 (66.7–76.4)	298	23.5 (19.1–28.3)	72	4.8 (3.4–6.7)
Place for routine health care							
No	285	188	69.4 (62.3–75.8)	74	25.4 (19.4–32.2)	23	5.2 (3.0–8.5)
Yes	1140	726	69.6 (65.2–73.7)	333	25.0 (21.0–29.4)	81	5.4 (3.9–7.3)
Type of place for routine health care							
Has office-based provider	1070	695	70.7 (66.6–74.6)	302	24.0 (20.1–28.1)	73	5.3 (3.8–7.2)
Hospital emergency room	66	27	42.0 (28.0–56.9)	31	49.2 (35.1–63.3)	8	*
HIV status							

Negative	1422	913	69.6 (65.5–73.4)	405	25.0 (21.4–28.9)	104	5.4 (4.1–7.0)
Positive	1	0	0	1	100	0	0
Currently pregnant							
No	1168	754	70.1 (65.9–74.0)	335	25.0 (21.3–28.9)	79	5.0 (3.6–6.6)
Yes	81	44	63.2 (50.0–75.0)	27	28.1 (18.1–40.0)	10	*
Ever pregnant							
No	382	302	82.0 (76.6–86.6)	64	15.4 (11.7–19.8)	16	2.6 (1.1–5.3)
Yes	883	500	62.8 (56.9–68.4)	308	30.6 (25.4–36.2)	75	6.6 (4.6–9.1)
Age at first sex (years)							
<15	235	97	48.2 (40.0–56.4)	92	35.7 (27.2–45.0)	46	16.1 (11.1–22.3)
15–17	647	370	63.0 (58.0–67.8)	226	31.2 (26.7–36.0)	51	5.8 (4.0–8.0)
18–19	297	228	80.4 (73.5–86.2)	64	18.4 (12.6–25.3)	5	1.2 (0.3–3.3)
≥20	246	219	91.2 (85.0–95.4)	25	8.3 (4.3–14.1)	2	0.5 (0.0–2.5)
Lifetime number of sex partners							
1–4	705	560	84.8 (80.4–88.5)	121	12.7 (9.1–17.0)	24	2.5 (1.4–4.1)
5–9	359	169	55.7 (48.2–63.0)	152	37.1 (30.3–44.3)	38	7.2 (4.6–10.6)

10+	360	185	57.4 (50.6–64.1)	134	34.3 (28.5–40.5)	41	8.2 (5.6–11.6)
New recent sex partner							
No	1059	690	70.5 (65.9–74.8)	300	24.7 (20.5–29.3)	69	4.8 (3.3–6.7)
Yes	294	174	65.1 (58.4–71.5)	88	26.8 (21.8–32.2)	32	8.1 (4.7–12.7)
Sex with a woman							
No	1207	797	71.6 (67.7–75.2)	337	24.0 (20.4–27.9)	73	4.4 (3.0–6.1)
Yes	218	117	58.9 (50.6–66.9)	70	30.6 (24.0–37.8)	31	10.5 (6.8–15.3)
Sexual identity							
Straight	1226	783	69.3 (65.2–73.1)	356	25.4 (21.6–29.5)	87	5.4 (3.9–7.2)
Lesbian/Gay	25	15	*	9	*	1	*
Bisexual	128	86	73.8 (63.5–82.4)	30	20.8 (12.7–30.9)	12	*
Other	42	28	*	10	*	4	*
Oral sex							
No	192	125	70.7 (61.3–79.0)	56	25.2 (17.9–33.8)	11	*
Yes	1232	788	69.3 (65.3–73.1)	351	25.1 (21.5–29.0)	93	5.6 (4.1–7.3)
Anal sex ever							
No	816	544	73.4 (68.0–78.4)	218	22.1 (17.7–26.9)	54	4.5 (2.9–6.7)

Yes	609	370	65.0 (60.2–69.7)	189	28.6 (24.2–33.2)	50	6.4 (4.6–8.6)
Condomless sex in last year							
Never	273	181	69.8 (61.9–76.9)	65	22.5 (15.8–30.3)	27	7.7 (4.0–13.2)
Less than 50%	234	154	70.1 (63.6–76.1)	60	22.4 (16.9–28.6)	20	7.5 (3.8–13.1)
≥50% and <100%	252	150	65.5 (58.6–71.9)	85	29.3 (22.7–36.7)	17	5.2 (2.8–8.7)
Always	561	357	70.2 (64.6–75.4)	166	25.3 (20.2–30.9)	38	4.5 (2.9–6.5)
Ever had genitourinary cancer							
No	1248	797	69.7 (65.3–73.8)	361	25.0 (21.4–29.0)	90	5.3 (3.8–7.1)
Yes	19	7	*	11	*	1	*

^{a*} Denotes estimates with wide confidence intervals per NHANES guidance; thus estimates are suppressed.

Table 2:

Characteristics of sexually experienced women 18–39 years with available *Chlamydia trachomatis* serology data, by category of serological Pgp3 multiplex bead array fluorescence intensity result (negative, low-positive [551–median of positive results], and high-positive [greater than or equal to the median positive results]), National Health and Nutrition Examination Survey (NHANES), 2013–2016

	Total	Negative sample N	Weighted negative prevalence (95% CI)	Low- positive sample N	Weighted low- positive prevalence (95% CI)	High- positive sample N	Weighted high- positive prevalence (95% CI)
Overall	1425	914	69.5 (65.5–73.4)	255	16.6 (13.8–19.7)	256	13.9 (11.3–16.8)
Age group (years)							
18–24	458	297	68.6 (62.8–74.0)	67	14.4 (10.7–18.9)	94	17.0 (13.4–21.2)
25–31	452	299	71.8 (65.9–77.3)	77	15.9 (11.6–21.2)	76	12.2 (8.7–16.5)
32–39	515	318	68.1 (61.4–74.2)	111	19.0 (13.4–25.7)	86	12.9 (9.6–16.9)
Race/ethnicity							
Non-Hispanic white	506	378	77.3 (72.7–81.5)	86	15.3 (12.1–19.0)	42	7.3 (5.0–10.3)
Non-Hispanic black	259	79	29.8 (22.2–38.3)	66	25.6 (17.6–35.0)	114	44.6 (36.1–53.5)

Hispanic	442	295	66.5 (61.9–70.9)	81	19.0 (14.7–23.9)	66	14.5 (11.0–18.7)
Non-Hispanic Asian	138	115	83.1 (75.8–88.9)	12	9.0 (4.5–15.6)	11	7.9 (4.0–13.7)
Other/multi-racial	80	47	64.4 (48.7–78.1)	10	* ^a	23	26.4 (14.1–42.2)
Recent chlamydia							
No	1390	907	70.5 (66.6–74.3)	247	16.4 (13.6–19.5)	236	13.1 (10.6–15.9)
Yes	33	6	*	7	*	20	*
Recent gonorrhea							
No	1417	912	69.6 (65.6–73.5)	253	16.6 (13.8–19.7)	252	13.7 (11.2–16.6)
Yes	6	1	*	1	*	4	*
Recent sexually transmitted disease							
No	1193	799	72.0 (67.7–76.0)	203	15.6 (12.7–18.9)	191	12.4 (10.2–14.9)
Yes	230	114	58.9 (51.4–66.0)	51	20.9 (15.0–27.8)	65	20.3 (14.1–27.7)
Pelvic inflammatory disease							
No	1354	886	70.8 (66.8–74.5)	236	16.0 (13.4–18.9)	232	13.2 (10.6–16.2)
Yes	59	22	*	16	28.7 (14.1–47.5)	21	*
Infertility							

No	1280	837	70.1 (66.0–74.0)	226	16.6 (13.9–19.5)	217	13.3 (10.5–16.5)
Yes	144	76	64.2 (53.3–74.1)	29	17.0 (10.1–26.0)	39	18.9 (11.7–28.0)
Current chlamydia							
Negative	1389	909	70.5 (66.3–74.4)	247	16.5 (13.6–19.7)	233	13.1 (10.5–16.0)
Positive	36	5	*	8	*	23	*
Income to poverty ratio							
<1.5	577	308	57.2 (51.3–62.9)	126	21.5 (16.8–26.9)	143	21.3 (16.7–26.5)
1.5–<3	362	240	69.6 (61.1–77.2)	58	16.4 (12.0–21.7)	64	14.0 (9.4–19.6)
≥3	413	321	80.8 (76.2–84.9)	57	12.3 (9.2–6.0)	35	6.9 (4.1–10.8)
Marital status							
Never married	433	247	63.9 (58.9–68.6)	71	14.6 (11.4–18.3)	115	21.5 (17.3–26.2)
Married/living with partner	721	501	74.1 (68.9–78.8)	132	16.7 (13.1–20.9)	88	9.2 (6.5–12.5)
Divorced/widowed/separated	113	56	54.5 (42.9–65.8)	31	27.1 (18.0–37.9)	26	18.4 (10.0–29.7)
Education							
<High School	190	100	53.3 (45.4–61.1)	55	30.3 (23.6–37.6)	35	16.5 (10.6–23.8)

High school graduate/general education diploma	240	133	59.7 (50.7–68.3)	45	19.8 (13.0–28.3)	62	20.4 (14.3–27.7)
Some college/associates degree	498	299	66.3 (61.2–71.1)	97	17.8 (14.2–21.8)	102	15.9 (11.8–20.8)
≥College graduate	339	272	84.0 (78.0–88.9)	37	9.5 (5.7–14.5)	30	6.5 (4.0–10.0)
Health insurance coverage							
No	345	205	60.6 (53.7–67.2)	75	23.3 (18.0–29.4)	65	16.0 (11.6–21.3)
Yes	1078	708	71.7 (66.7–76.4)	179	14.9 (11.7–18.7)	191	13.4 (10.5–16.6)
Place for routine health care							
No	285	188	69.4 (62.3–75.8)	45	16.1 (10.4–23.3)	52	14.5 (10.0–20.1)
Yes	1140	726	69.6 (65.2–73.7)	210	16.7(14.0–19.8)	204	13.7 (11.0–16.8)
Type of place for routine health care							
Has office-based provider	1070	695	70.7 (66.6–74.6)	192	16.2 (13.5–19.1)	183	13.1 (10.3–16.3)
Hospital emergency room	66	27	42.0 (28.0–56.9)	18	29.9 (18.6–43.3)	21	28.2 (17.5–41.0)
HIV status							

Negative	1422	913	69.6 (65.5–73.4)	254	16.6 (13.9–19.6)	255	13.8 (11.2–16.7)
Positive	1	0	0	1	100.0	0	0
Currently pregnant							
No	1168	754	70.1 (65.9–74.0)	211	16.7 (13.8–20.0)	203	13.2 (10.6–16.2)
Yes	81	44	63.2 (50.0–75.0)	18	17.6 (9.5–28.5)	19	19.3 (9.2–33.4)
Ever pregnant							
No	382	302	82.0 (76.6–86.6)	40	10.7 (7.2–15.1)	40	7.3 (4.6–10.8)
Yes	883	500	62.8 (56.9–68.4)	194	20.1 (16.0–24.7)	189	17.1 (13.6–21.2)
Age at first sex (years)							
<15	235	97	48.2 (40.0–56.4)	57	22.9 (15.8–31.3)	81	28.9 (21.6–37.2)
15–17	647	370	63.0 (58.0–67.8)	138	20.4 (16.9–24.3)	139	16.5 (12.8–20.8)
18–19	297	228	80.4 (73.5–86.2)	41	12.3 (7.8–18.2)	28	7.3 (4.0–12.0)
≥20	246	219	91.2 (85.0–95.4)	19	6.5 (3.3–11.5)	8	2.3 (0.8–5.0)
Lifetime number of sex partners							
1–4	705	560	84.8 (80.4–88.5)	82	8.7 (5.9–12.3)	63	6.5 (4.8–8.7)
5–9	359	169	55.7 (48.2–63.0)	96	25.1 (19.5–31.3)	94	19.2 (14.6–24.5)

10+	360	185	57.4 (50.6–64.1)	77	21.9 (16.9–27.6)	98	20.7 (15.8–26.3)
New recent sex partner							
No	1059	690	70.5 (65.9–74.8)	191	16.7 (13.3–20.5)	178	12.8 (10.2–15.8)
Yes	294	174	65.1 (58.4–71.5)	48	15.6 (11.6–20.2)	72	19.3 (14.0–25.6)
Sex with a woman							
No	1207	797	71.6 (67.7–75.2)	212	15.9 (13.3–18.7)	198	12.5 (10.1–15.4)
Yes	218	117	58.9 (50.6–66.9)	43	20.5 (14.1–28.1)	58	20.6 (14.8–27.4)
Sexual identity							
Straight	1226	783	69.3 (65.2–73.1)	222	16.8 (13.8–20.0)	221	14.0 (11.4–16.8)
Lesbian/Gay	25	15	*	3	*	7	*
Bisexual	128	86	73.8 (63.5–82.4)	20	14.1 (7.3–23.7)	22	12.1 (6.6–19.9)
Other	42	28	*	9	23.9 (11.4–41.0)	5	*
Oral sex							
No	192	125	70.7 (61.3–79.0)	36	16.4 (11.2–22.7)	31	12.9 (8.5–18.5)
Yes	1232	788	69.3 (65.3–73.1)	219	16.7 (13.8–19.9)	225	14.0 (11.3–17.0)
Anal sex ever							
No	816	544	73.4 (68.0–78.4)	131	13.7 (10.2–17.9)	141	12.9 (9.8–16.5)

Yes	609	370	65.0 (60.2–69.7)	124	19.9 (16.4–23.8)	115	15.0 (11.6–19.0)
Condomless sex in last year							
Never	273	181	69.8 (61.9–76.9)	42	16.1 (9.6–24.7)	50	14.1 (8.6–21.3)
Less than 50%	234	154	70.1 (63.6–76.1)	34	12.3 (7.8–18.1)	46	17.6 (11.3–25.6)
≥50% and <100%	252	150	65.5 (58.6–71.9)	44	16.3 (11.5–22.3)	58	18.2 (13.4–23.8)
Always	561	357	70.2 (64.6–75.4)	113	18.0 (13.8–23.0)	91	11.7 (9.1–14.8)
Ever had genitourinary cancer							
No	1248	797	69.7 (65.3–73.8)	226	16.5 (13.7–19.7)	225	13.8 (11.0–17.0)
Yes	19	7	*	8	*	4	*

^a*Denotes estimates with wide confidence intervals per NHANES guidance; thus estimates are suppressed.

Table 3:

Weighted prevalence of pelvic inflammatory disease, infertility, and chlamydia among women with negative, low-positive (551–49,999), and high-positive (50,000 and above) Pgp3 multiplex bead array median fluorescence intensity results among sexually-experienced women 18–39 years, National Health and Nutrition Examination Survey (NHANES), 2013–2016

Characteristic	Total sample N	Overall prevalence of characteristic	Pgp3 negative sample N	Prevalence of listed characteristic among Pgp3 negative (95% CI)	Pgp3 low-positive sample N	Prevalence of listed characteristic among Pgp3 low-positive (95% CI)	Prevalence ratio of listed characteristic comparing low-positive to negative Pgp3 (95% CI)	Pgp3 high-positive sample N	Prevalence of listed characteristic among Pgp3 high-positive (95% CI)	Prevalence ratio of listed characteristic comparing high-positive to negative Pgp3 (95% CI)
PID	59	3.8 (2.7–5.2)	22	2.3 (1.4–3.6)	32	7.9 (4.6–12.6)	* ^a	5	*	*
Infertility	144	10.7 (9.0–12.5)	76	9.9 (7.7–12.4)	49	11.0 (7.5–15.5)	1.1 (0.7–1.7)	19	19.6 (10.5–31.7)	2.0 (1.1–3.7)
Recent chlamydia	33	2.1 (1.4–3.0)	6	0.7 (0.1–1.9)	23	5.8 (3.7–8.5)	*	4	*	*
Current chlamydia	36	1.7 (1.1–2.6)	5	0.4 (0.1–1.3)	18	3.5 (1.7–6.4)	*	13	10.7 (5.4–18.6)	*

^a* Denotes estimates with wide confidence intervals per NHANES guidance; thus estimates are suppressed.

Table 4:

Weighted prevalence of pelvic inflammatory disease, infertility, and chlamydia among women with negative, low-positive (551–median of positive results), and high-positive (greater than or equal to the median of positive results) Pgp3 multiplex bead array median fluorescence intensity results among sexually-experienced women 18–39 years, National Health and Nutrition Examination Survey (NHANES), 2013–2016

Characteristic	Total sample N	Overall prevalence of characteristic	Pgp3 negative sample N	Prevalence of listed characteristic among Pgp3 negative (95% CI)	Pgp3 low-positive sample N	Prevalence of listed characteristic among Pgp3 low-positive (95% CI)	Prevalence ratio of listed characteristic comparing low-positive to negative Pgp3 (95% CI)	Pgp3 high-positive sample N	Prevalence of listed characteristic among Pgp3 high-positive (95% CI)	Prevalence ratio of listed characteristic comparing high-positive to negative Pgp3 (95% CI)
PID	59	3.8 (2.7–5.2)	22	2.3 (1.4–3.6)	16	* ^a	2.9 (1.3–6.2)	21	8.0 (4.0–14.2)	*
Infertility	144	10.7 (9.0–12.5)	76	9.9 (7.7–12.4)	29	10.9 (6.7–16.4)	1.1 (0.7–1.7)	39	14.5 (9.5–20.8)	1.5 (0.9–2.5)
Recent chlamydia	33	2.1 (1.4–3.0)	6	0.7 (0.1–1.9)	7	*	*	20	7.9 (4.7–12.2)	*
Current chlamydia	36	1.7 (1.1–2.6)	5	0.4 (0.1–1.3)	8	*	*	23	7.2 (4.4–11.1)	*

^a* Denotes estimates with wide confidence intervals per NHANES; thus estimates are suppressed.

Table 5:

Weighted median and interquartile range of Pgp3 multiplex bead array chlamydia median fluorescence intensity for women 18–39 years with various outcomes, National Health and Nutrition Examination Survey, 2013–2016

Weighted median [interquartile range] Pgp3 multiplex bead array median fluorescence intensity	Chlamydia positive	Recent chlamydia positive	Pelvic inflammatory disease positive	Infertility positive	Sample N
35,780 [7,240–51,480]	Yes				36
31,152 [2,444–49,465]		Yes			33
2,593 [37–27,156]			Yes		59
43 [19–12,600]				Yes	144
29 [18–1,021]	No	No	No	No	1166

Figure 1 legend:

Women included in final sample of 1,425 women aged 18–39 years with available chlamydia nucleic acid amplification test result, reported sexual experience, and available chlamydia serology result, National Health and Nutrition Examination Survey 2013–2016

Figure 1:

