Recurrent/Intermittent Vaginal and Rectal Chlamydial Infection Following Treatment: A Prospective Cohort Study Among Female Sexually Transmitted Disease Clinic Patients

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Background. Rectal *Chlamydia trachomatis* (CT) is common among clinic-attending women, but little is known about clearance and health implications of rectal CT.

Methods. At the municipal sexually transmitted disease clinic in Seattle, Washington, in 2017–2018, we enrolled women at high risk for urogenital CT into an 8-week prospective study. Women received standard CT treatment at enrollment. Women self-collected daily rectal and vaginal specimens for nucleic acid amplification tests (NAATs) and completed weekly sexual exposure diaries. We performed CT culture on the enrollment rectal specimen.

Results. We enrolled 50 women; 13 (26%) tested positive for vaginal (n = 11) and/or rectal (n = 11) CT. Sixty percent of women with rectal CT per NAAT were also culture positive. Median time to CT clearance after azithromycin treatment was 8.0 days for vaginal CT and 7.0 days for rectal CT. Eight women with rectal CT at enrollment had at least 1 rectal CT-positive NAAT after clearance of the initial infection; none reported anal sex.

Conclusions. Most NAAT-positive rectal infections were culture positive, suggesting active infection. Time to NAAT clearance of rectal and genital tract CT was similar, and intermittent rectal CT positivity was common in the absence of anal sexual exposure. The cause of recurrent/intermittent rectal CT and the clinical implications of these infections require further study.

Keywords. chlamydia; azithromycin; doxycycline; sexually transmitted disease.

Chlamydia trachomatis (CT) is the most commonly reported infection in the United States, with >1.1 million cases reported among women in 2017 [1]. Untreated urogenital CT can lead to pelvic inflammatory disease, ectopic pregnancy, and tubal factor infertility [2]. Persistent or recurrent urogenital CT—which is associated with an increased risk of these reproductive tract sequelae [3]—is common following treatment, with an estimated 20% of women experiencing repeat urogenital CT within 12 months of initial CT diagnosis [4].

Rectal CT is increasingly becoming recognized as a common infection among women, with an estimated prevalence of rectal CT among clinic-attending women of approximately 6%–9% [5, 6]. However, there are several key gaps in our understanding of

the epidemiology of rectal CT among women. First, it is unclear if prevalent rectal CT among women represents active infection by viable bacteria. Most studies of rectal CT prevalence have utilized nucleic acid amplification tests (NAATs), which do not distinguish between dead and viable CT. Second, the routes by which women acquire rectal CT are a topic of debate [7]. Women can acquire rectal CT via anal sex, but a recent meta-analysis found no association between reporting anal sex and rectal CT [5]. Some investigators have hypothesized that oral acquisition of CT (via penile–oral sex) may lead to rectal infection [8, 9], though this has not been demonstrated in humans. It is also possible that autoinfection of CT from the vagina to the rectum (eg, via wiping “front to back”) may lead to rectal CT [10, 11], but the relative contribution of this and other pathways to rectal CT acquisition remains unknown. Third, we do not know the health implications of rectal CT among women. Rectal CT is typically asymptomatic and the infection itself is not a morbid condition. However, some investigators have hypothesized that women could autoinoculate CT from the rectum to the vagina [8, 9, 12], which may result in reproductive tract sequelae in the absence of vaginal exposure to CT from an infected partner. This has been demonstrated in animals [8], and in women is

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likely facilitated by the close proximity of the vagina and anus. But whether or not this occurs in humans is unclear.

Adding to these unknowns is our lack of understanding of the most appropriate treatment for rectal CT. The Centers for Disease Control and Prevention currently recommends azithromycin or doxycycline for the treatment of CT [2], which reflects an extrapolation of findings from randomized trials of urogenital CT [13]. However, up to 17% of azithromycin-treated rectal infections do not clear after treatment [14], and animal models suggest that azithromycin is less efficacious in the gastrointestinal (GI) tract than in the genital tract [15]. It is possible that women with rectal CT treated with azithromycin may not clear their rectal CT, which could subsequently serve as a source of recurrent urogenital CT via autoinoculation [16].

To begin to address these questions, we conducted a small prospective cohort study of female sexually transmitted disease (STD) clinic patients that aimed to describe the prevalence of active rectal CT, examine time to clearance of vaginal and rectal CT after treatment, and explore the possibility of autoinfection of CT from the rectum to the vagina.

METHODS

Study Design and Population

We conducted an 8-week prospective study of patients attending the Public Health–Seattle & King County (PHSKC) STD Clinic in Seattle, Washington, between September 2017 and August 2018 who were at high risk for vaginal CT. Patients were eligible to participate if they were female sex at birth, ≥ 16 years old, not pregnant, reported sex with a man in the past 12 months, and self-reported 1 of the following: being a contact to a partner with CT or *Neisseria gonorrhoeae* (GC), urogenital symptoms, or a history of CT in the past 12 months, as reported on a clinical intake form. Research staff recruited eligible patients after completion of the intake form but prior to seeing a clinician. Per routine clinical practice, the PHSKC STD Clinic presumptively treats women with a high likelihood of testing positive for CT (described in “Treatment” below); thus, these eligibility criteria were chosen to enroll female patients at high risk for testing positive for CT immediately before they received treatment. Women who enrolled in the study but tested negative for both vaginal and rectal CT at enrollment were subsequently withdrawn after the enrollment visit.

This study was approved by the University of Washington Institutional Review Board. All participants provided written informed consent. Participants were paid \$30 for completing the enrollment visit, and an additional \$180 for completing all study procedures in the cohort study.

Clinical and Behavioral Data Collection and Measures

All patients presenting to the PHSKC STD Clinic for a new problem visit are asked to complete a computerized patient intake form, which queries patients on reason for visit,

symptoms, history of STD, and sexual behaviors. These data are included in the clinic’s electronic medical record (EMR), which also contains information on clinical examination findings, results of laboratory tests, and treatment received. We used the clinic’s EMR data to obtain participants’ clinical information (eg, history of CT, treatment type, and date). Participants also completed a research survey at enrollment that asked about demographics, sexual behaviors in the past 2 and 12 months, and antibiotic use in the past month.

Women enrolled in the 8-week cohort study (ie, those testing positive for vaginal or rectal CT at enrollment) were asked to complete electronic daily diaries each week. Participants received an email with a unique web address to access their diary, which included a personalized calendar based on a participant’s enrollment visit date. Participants indicated on which days in the past week they had condomless oral, vaginal, and anal sex. Participants were also asked to indicate the type of partner with whom they had sex: a new partner, a previous partner who they did not think had an STD (ie, previous partner, no STD), a previous partner who they believed had an STD but was treated (ie, previous partner, treated), and a previous partner who they believed had an STD but was not treated (ie, previous partner, not treated). The research surveys were programmed and automated using Research Electronic Data Capture (REDCap) [17].

Specimen Collection and Testing

At enrollment, participants self-collected 1 rectal specimen and 1 vaginal specimen for NAATs for CT and GC, and 1 additional rectal specimen for CT culture and serotyping. These specimens were collected for research and were in addition to those collected for clinical testing. Participants with vaginal or rectal CT at enrollment self-collected daily vaginal and rectal NAAT specimens for 56 days. Participants were given the option to switch to weekly specimen collection after the first 28 days of the study; 2 participants opted for this weekly collection. Study staff mailed specimen collection kits, instructions, and labels to participants every 2 weeks, and participants were asked to mail collected specimens back weekly, using the prepaid envelopes provided to them. Study staff sent text message reminders to participants when it was time for them to send in their collected specimens.

All vaginal and rectal NAAT specimens were tested for CT and GC using the APTIMA Combo-2 assay performed on the fully automatic Panther System (Hologic, Inc). Enrollment vaginal and rectal NAAT specimens were tested within 5 business days. Prospectively collected NAAT specimens were stored at -80°C and were batch-tested at the conclusion of a participant’s participation. If the final rectal or vaginal specimen tested positive for CT, study staff contacted the participant to alert them of the result and request that they come into the clinic for treatment.

The additional rectal specimen collected at enrollment for CT culture was immediately placed in 2× sucrose phosphate buffer and

frozen at -80°C . For culturing, CT samples were thawed and inoculated onto McCoy cell monolayers. Chlamydial inclusions were detected by labeling the *Chlamydia* with genus-specific monoclonal antibody. Positive cultures grown to high titer were then serotyped using a panel of serogroup- and serovar-specific monoclonal antibodies to determine serovar, as previously described [18, 19].

Treatment

All women were treated for CT at enrollment per routine clinical practice with either azithromycin (1 g orally once) or doxycycline (100 mg orally twice daily for 7 days). Women were instructed to abstain from sex for 7 days. Our clinic presumptively treats patients for CT if they report contact to a partner with CT, GC, or nongonococcal urethritis, or if a clinician in the clinic diagnoses them with mucopurulent cervicitis. Women who were presumptively treated or received treatment for diagnosed CT were also offered treatment packets to provide to their sex partners (ie, patient-delivered partner therapy); each packet contained 1 g azithromycin.

Statistical Analysis

We used Fisher exact test (for proportions) and *t* tests (for means) to describe characteristics of the study population at

enrollment by CT status. We examined median time to clearance after treatment (defined as 2 consecutive days of a negative NAAT result) by anatomic site, among the women who returned at least 1 week of samples. Analyses were completed using Stata version 13 software (StataCorp).

RESULTS

We enrolled 50 cisgender women, of whom 13 (26%) tested positive for vaginal ($n = 11$) or rectal ($n = 11$) CT at enrollment. The median age of all participants was 25, approximately 30% were white non-Hispanic, and 90% had completed high school (Table 1). Women testing positive for CT were younger, less likely to be white non-Hispanic, and more likely to report anal sex in the past 12 months compared to women who did not test positive for CT.

A line listing of the 13 women who tested positive for CT at enrollment is provided in Table 2. Of these 13 women, 9 (69%) had concurrent vaginal and rectal CT, 2 (15%) had isolated vaginal CT, and 2 (15%) had isolated rectal CT. Nine of 11 (82%) women with urogenital CT also had rectal CT. Most of the 11 women with rectal CT did not report anal sex in the past 12 months ($n = 7$) or 2 months ($n = 8$). Of 11 participants

Table 1. Characteristics of Study Participants at the Time of Enrollment, by *Chlamydia trachomatis* Test Result (N = 50)

Characteristic	Total (N = 50)	CT-Positive Enrollees (n = 13)	CT-Negative Enrollees (n = 37)	PValue
Age, y, mean (SD)	27.6 (9.0)	24.5 (4.0)	28.7 (9.9)	.04
Age, y, median (IQR)	25 (22–31)	24 (22–26)	25 (23–32)	
Race/ethnicity				.01
White, non-Hispanic	14 (28.0)	0 (0.0)	14 (37.8)	
Black, non-Hispanic	14 (28.0)	3 (23.1)	11 (29.7)	
Asian, non-Hispanic	9 (18.0)	6 (46.2)	3 (8.1)	
Other, non-Hispanic	4 (8.0)	1 (7.7)	3 (8.1)	
Hispanic	7 (14.0)	2 (15.4)	5 (13.5)	
Highest level of education completed				1.0
Less than high school	5 (10.0)	1 (7.7)	4 (10.8)	
High school or GED	28 (56.0)	8 (61.5)	20 (54.1)	
Bachelor's or associate's degree	13 (26.0)	3 (23.1)	10 (27.0)	
Graduate/professional degree	4 (8.0)	1 (7.7)	3 (8.1)	
History of CT, past 12 mo	16 (32.0)	5 (38.5)	11 (29.7)	.46
No. of sex partners, past 12 mo, median (IQR)	4 (3–8)	4 (2–7)	4 (3–8)	.41
Type of sex, past 12 mo				
Oral (penile–oral)	45 (90.0)	12 (92.3)	33 (89.2)	1.0
Vaginal	48 (96.0)	13 (100.0)	35 (94.6)	1.0
Anal	16 (32.0)	6 (46.2)	10 (27.0)	.3
Concurrent GC diagnosis ^a				
Vaginal	3 (6.0)	1 (7.7)	2 (5.4)	1.0
Rectal	4 (8.0)	1 (7.7)	3 (8.1)	1.0
Treatment received				< .001
Azithromycin	21 (42.0)	11 (84.6)	10 (27.0)	
Doxycycline	3 (6.0)	2 (15.4)	1 (2.7)	
No treatment	26 (52.0)	0 (0.0)	26 (70.3)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: CT, *Chlamydia trachomatis*; GC, *Neisseria gonorrhoeae*; GED, general education development; IQR, interquartile range; SD, standard deviation.

^aAll 3 participants with vaginal GC also tested positive for rectal GC.

Table 2. Line Listing of Sexual Behavior, *Chlamydia trachomatis* (CT) History, Treatment, and Rectal CT Culture Results Among Participants Testing Positive for Rectal or Vaginal CT at Enrollment (n = 13)

ID	CT Test Result at Enrollment		Sexual Exposures, Past 2 mo			Sexual Exposures, Past 12 mo			History of CT, Past 12 mo	Treatment Received	Rectal CT Culture Results ^a	Rectal CT Serovar
	Vaginal	Rectal	Vaginal	Oral	Anal	Vaginal	Oral	Anal				
1	X	X	X	X	...	X	X	...	...	Azithromycin	...	...
2	X	X	X	X	X	X	X	X	...	Doxycycline	Positive	D
3	X	X	X	X	...	X	X	...	...	Azithromycin	Negative	...
4	...	X	X	X	...	X	X	X	X	Azithromycin	Contaminated	...
5	X	X	X	X	X	X	X	X	X	Azithromycin	Positive	F
6	X	X	X	X	...	X	X	...	...	Azithromycin	Positive	F
7	X	...	X	X	X	X	X	X	...	Azithromycin	...	...
8	X	X	X	...	...	X	...	...	...	Azithromycin	Positive	J
9	...	X	X	X	...	X	X	...	...	Azithromycin	Positive	D
10	X	...	X	X	X	X	X	X	X	Doxycycline	...	...
11	X	X	X	X	...	X	X	...	X	Azithromycin	Positive	E
12	X	X	X	...	...	X	X	...	...	Azithromycin	Negative	...
13	X	X	X	X	X	X	X	X	X	Azithromycin	Contaminated	...

Abbreviations: CT, *Chlamydia trachomatis*; ID, patient identifier.

^aAt enrollment.

who tested positive for rectal CT by NAAT at enrollment, 10 provided specimens acceptable for CT culture. Of these, 6 (60%) were positive for CT by culture, 2 (20%) were contaminated, and 2 (20%) were culture negative for CT. The serovars for the 6 positive specimens were D (n = 2), E (n = 1), F (n = 2), and J (n = 1).

The daily vaginal and rectal CT test results and sexual exposures for the 10 women who provided at least 1 week of prospective samples are provided in Figure 1. The median time to clearance after azithromycin treatment for a vaginal infection (n = 7) was 8.0 days (range, 2–15 days) and for rectal infection (n = 8) was 7.0 days (range, 2–16 days). The only participant treated with doxycycline (subject 02) cleared her vaginal infection in 19 days and her rectal infection in 16 days.

Most (9 of 10) participants reported engaging in condomless oral, vaginal, or anal sex on at least 1 day during the 8-week study period (Figure 1). These 9 participants engaged in condomless sex on a median of 8 days during the 8-week study period (range, 2–35 days), and engaged in an average of 1.6 condomless sex acts per day of sexual exposure. No participants reported condomless sex with a partner they knew to have an untreated STD, though 5 participants reported condomless sex with a partner whose STD and treatment status they did not report.

Eight of 9 women with rectal CT at enrollment had at least 1 positive rectal CT rectal test result after clearance of the initial rectal infection; none of these women reported condomless anal sex in the time between clearance of the initial infection and the first subsequent positive rectal CT test. Seven of 8 women with vaginal CT at enrollment had a positive vaginal CT test result after initial clearance; all but 1 reported condomless vaginal sex in the time between treatment of the initial infection and the first subsequent positive vaginal CT test.

DISCUSSION

In this prospective study of 50 female STD clinic patients at high risk for vaginal CT, we found that 22% tested positive for rectal CT by NAAT, and that most women (82%) with vaginal CT had concurrent rectal CT. Sixty percent of rectal specimens that were positive for CT by NAAT at enrollment were also culture positive, indicating the presence of viable bacteria in the rectum. The median time to clearance of CT after azithromycin treatment was similar for vaginal (8.0 days) and rectal (7.0 days) infections. Notably, most (n = 8) participants had at least 1 positive rectal CT–positive test after clearance of their initial infection but none reported anal sex in the time between treatment and their subsequent positive test. The cause of these rectal CT “blips” is unclear, but may be a result of persistent infection, reinfection via autoinoculation, or persistence of nonviable organisms. These results confirm that rectal CT is common among women with vaginal CT, but also underscore the major gaps in our understanding of the natural history of rectal CT among women.

The prevalence of rectal CT (22%) we observed is higher than 2 recent meta-analyses, which noted prevalences of 6% and 9% [5, 6]. This is not surprising, given that we purposefully enrolled women at high risk of vaginal CT, whereas the meta-analyses included studies that enrolled women of varying risk of CT. Of note, our findings are consistent with 2 key observations from prior studies. First, these data support previous observations that rectal CT is not associated with reporting anal sex [5]; in our study, the prevalence of rectal CT was similar among women who did and did not report anal sex in the past 12 months. There are currently no recommendations to screen women routinely for rectal CT, but limiting screening to women

Figure 1. Daily vaginal and rectal *Chlamydia trachomatis* (CT) results and sexual exposure for up to 8 weeks among women testing positive for vaginal or rectal CT at enrollment ($n = 10$). CT test results are from nucleic acid amplification testing. Subjects 01, 10, and 12 did not provide at least 1 week of prospective specimens and are not included. Reported sexual exposure is condomless sex. Subject 02 received doxycycline; all other participants received azithromycin. Abbreviations: CT, *Chlamydia trachomatis*; STD, sexually transmitted disease.

who report anal sex may miss a substantial proportion of rectal CT infections. Second, we noted that the prevalence of rectal CT was high (82%) among women with urogenital CT, a finding consistent with prior studies suggesting that 70% of women with urogenital CT have concurrent rectal CT [5]. Given that nearly 20% of azithromycin-treated rectal CT infections may not be cured [14], it is possible that providing azithromycin to women with urogenital CT may result in a large proportion of women with persistent rectal CT.

Notably, most studies estimating the prevalence of rectal CT among women have employed NAAT, and it is unclear if NAAT-positive results represent the presence of a true infection (ie, viable bacteria), viable organisms that do not infect tissue, or transient passage of nucleic acid from nonviable CT [20]. Our observation that 60% of NAAT-positive rectal CT tests at enrollment were also positive by culture indicated the presence of viable CT and is suggestive of an active infection. Of note, this is the minimum prevalence of an active infection given that 2 specimens were contaminated with commensal bacteria and given the lower sensitivity of CT culture compared to NAAT [21].

We performed serotyping on the cultured rectal specimens to determine if there was 1 particular serovar present in rectal samples. We observed 4 different serovars (D, E, F, and J) among the 6 cultured specimens; these serovars are also commonly found in the female urogenital tract [19]. Unfortunately, we did not conduct culture or serotyping of vaginal specimens, so it is unclear if participants were concurrently infected with the same serovars in both anatomic sites or if these serovars have an affinity for the rectum.

We found that the time to clearance of vaginal and rectal CT infections after azithromycin treatment was relatively short and was similar for the 2 anatomic sites (vaginal, 8.0 days; rectal, 7.0 days). This is somewhat counter to 2 recent prospective studies of nondaily sampling among women with urogenital [22] and urogenital and rectal [23] CT, where approximately 15% of women tested positive for CT by NAAT at 21–23 days after treatment with azithromycin. But our findings are remarkably similar to a 28-day prospective study of CT clearance that employed daily sampling [24]. That study found that the median time to CT clearance was 6.5 days for rectal CT ($n = 7$ men) and 8 days for endocervical CT ($n = 9$ women). We were somewhat surprised that all women with rectal CT cleared their infection, given that azithromycin may be substantially less effective in the rectum (83% efficacy) [14] compared to the urogenital tract (96% efficacy) [13]. Based on that efficacy data, we anticipated that at least 1 woman with rectal CT would not clear CT within the follow-up period. However, it is likely that our sample size was too small to observe a rectal CT azithromycin treatment failure. Of note, only 1 woman who completed the cohort study was treated with doxycycline at enrollment, which prohibited a comparison of the efficacy of the 2 therapies for rectal CT, a topic of considerable debate [25–28].

Unfortunately, the results from this study do not allow us to definitively conclude that autoinfection from the rectum to the vagina occurs. We hypothesized that at least some women would not clear their rectal CT infection, and that these women would subsequently have a repeat vaginal infection in the absence of reported condomless vaginal sex. Although that pattern would not confirm autoinfection from the rectum to the vagina, it would be strongly suggestive of it. However, as described above, no participants experienced persistent rectal CT, and thus the opportunity for autoinoculation to the vagina was limited. Furthermore, the high frequency of condomless vaginal sex would have made it difficult to differentiate between autoinfection from the rectum vs a reinfection after vaginal sex exposure. It is unclear what type of study design would definitively answer this question, but it is possible that enrolling women who have limited opportunity for reinfection [29] may provide more definitive evidence of autoinoculation.

It is noteworthy that 8 women had at least 1 positive rectal CT test after clearance of the initial rectal infection, and that none of these women reported condomless anal sex in the time period between clearance of the initial infection and the first subsequent positive rectal CT test result. Intermittent positive NAATs (or “blips”) have been previously described for urogenital and rectal CT and GC [22, 24, 30, 31], but the source of the intermittent positive test in these studies, including ours, is unclear. We postulate several possible explanations for these rectal CT “blips.” First, it is possible that these positive rectal CT tests were detecting CT from vaginal fluid or from semen after an exposure of condomless vaginal sex. However, in all but 1 case, these intermittent positive rectal CT tests occurred in absence of a positive vaginal CT test on the same day, and in most cases, the most recent vaginal CT-positive test occurred several days prior to the first occurrence of a positive rectal CT test. Second, it is possible that these intermittent positives represent detection of nucleic acid from nonviable bacteria that may be present within the rectum or on participants’ perianal regions. Indeed, a prior prospective study of women with urogenital CT found that most NAAT-positive intermittent positive urogenital CT tests were not confirmed positive by culture [22]. Third, it is possible that the initial rectal CT infection that was detected at enrollment was cleared from the lower GI tract but remained in the upper GI tract, and that this resulted in periodic shedding of bacteria. This has been described in animals [32, 33], but it is not known if CT can establish an infection in the upper GI tract in humans. Finally, it is possible that the pattern of intermittent positives that we observed is indicative of orally acquired CT (via penile–oral sex) whose nucleic acid (or the intact bacterium) is detected after passage through the GI tract, as has been described in animals, though it is not known if this occurs in humans [8, 9, 12].

This study has several strengths. First, participants in this study collected daily paired vaginal and rectal specimens for

up to 8 weeks, which allowed us to examine the day on which a participant cleared her infection and to detect isolated days of subsequent positive CT tests. Second, our comprehensive sexual exposure diary allowed us to examine daily sexual exposure and partner type. Our results should be interpreted in light of several limitations. First, our total sample size ($N = 50$) was small, and data on time to clearance and patterns of autoinfection were only available for 10 women. Second, women self-collected all specimens. Although this is standard of care in our clinic and women were provided with verbal and written instructions from the research team, it is possible that some women mislabeled specimens at home. Third, we only collected specimens for rectal culture at enrollment, and it is unclear if the NAAT-positive tests observed during follow-up were indicative of an active infection. Fourth, women may not have accurately reported sexual exposure, either because of recall bias or social desirability bias. The latter may have been particularly relevant for reporting anal sex, though there were several women who reported anal sex at enrollment, indicating some comfort level with reporting this exposure. Finally, we enrolled a population of women at high risk for CT with frequent sexual exposure who presented at an STD clinic. These women may not be representative of the larger population with diagnosed CT.

In conclusion, we found that the prevalence of rectal CT is high among women at high risk for vaginal CT, that these infections are usually culture positive, and that NAAT rectal CT positivity clears within approximately 1 week of treatment. We also observed that recurrent CT NAAT positives were common among women with rectal CT and occurred in the absence of anal sex. The cause and significance of recurrent rectal NAAT positivity is uncertain, highlighting key gaps in our understanding of the natural history and health implications of rectal CT. We do not fully understand the pathways of rectal CT acquisition or the relative contributions of these pathways, nor do we have data to definitively describe autoinoculation of CT from the rectum to the vagina or if this autoinoculation subsequently leads to adverse health outcomes for women. The answers to these questions will help establish if and in what population of women screening for rectal CT is indicated, which could have a substantial impact on the reproductive health of women.

Notes

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