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High Rates of Anorectal Chlamydia in Women: Cross-sectional Study in General Practice

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ABSTRACT

Background. Genital and anorectal *Chlamydia trachomatis* frequently present together in sexually transmitted infection clinics.

Aim. This study aimed to investigate the prevalence of co-occurrent genital and anorectal chlamydia infection, and to study whether sexual behavior is associated with anorectal infection.

Design and Setting. A cross-sectional study in general practices in the north of the Netherlands.

Methods. Women attending general practice with an indication for genital chlamydia testing were included and asked to complete a structured questionnaire on sexual behavior. Anorectal infection prevalence was compared according to testing indications: standard vs experimental (i.e. based on questionnaire answers). Variables associated with anorectal chlamydia were analyzed by univariate and multivariate logistic regression analyses.

Results. Data could be analyzed for 497 of 515 included women. Overall, 17.8% (87/490) were positive for *C. trachomatis*; of these, 72.4% (63/87) had co-occurrent genital and anorectal infection, 13.8% (12/87) had genital infection only, and 12.6% (11/87) had anorectal infection only. Rectal infection was missed in 69.3% of cases using the standard indication alone, while adding the sexual history still missed 20.0%. Age was the only variable significantly associated with anorectal infection.

Conclusions. The prevalence of anorectal disease is high among women who visit their general practitioner with an indication for genital *C. trachomatis* testing. Many anorectal infections are missed despite taking comprehensive sexual histories, meaning that standard treatment of genital infection with azithromycin may result in rectal persistence. Performing anorectal testing in all women with an indication for genital *C. trachomatis* testing is therefore recommended.

Keywords

Chlamydia trachomatis, Sexually Transmitted Diseases, Sexual behavior, Women, General Practice

How this fits in

- *Chlamydia trachomatis* infection continues to be prevalent in women despite screening programs and awareness among care providers and patients.
- This could reflect anorectal persistence, with a high rate of infection at both genital and anorectal sites reported in high-risk populations.
- We add data from general practice showing a high rate of missed co-occurrent genital and anorectal chlamydia infection with standard diagnosis and treatment.
- One advisable strategy is to perform additional anorectal testing for *C. trachomatis* in all women with an indication for genital testing.

INTRODUCTION

The prevalence of *Chlamydia trachomatis* (CT) is high and continuing to rise worldwide.¹ In an effort to understand why this epidemic has not been controlled, the possible occurrence of undetected and untreated anorectal CT infection has gained attention. Testing guidelines for sexually transmitted infection (STI) advocate rectal CT screening for women who visit a health care facility with a history of anal intercourse or anal symptoms.^{2,3,4,5,6} However, anorectal CT is frequently diagnosed without such a history, as shown in studies of women attending STI clinics or hospitals.^{7,8,9} One study from an STI clinic in the Netherlands reported a high rate of genital and anorectal CT co-occurrence even with no indication for anorectal testing.⁹ The authors therefore concluded that testing based on classical indication is no longer appropriate.⁹ Once identified, rectal infection should be treated with doxycycline because standard treatment, with azithromycin, which is used for genital CT in the Netherlands, may fail to clear rectal infection and promote recurrent genital infection by auto-inoculation.⁹ BASHH and NHS guidelines recommend doxycycline as first line treatment for uncomplicated CT infections.^{10,11} Another problem with anorectal testing by indication is that women might interpret the question “did you have anal sex?” as meaning anal penetration by the penis, whereas CT may be transmitted to the rectum by other anal contact. To date, studies of

anorectal CT in women have been carried out in STI clinics or hospitals where there is typically a high-risk of STI.⁷ To our knowledge, there are no data in primary care populations where women opting for an STI test are generally at lower risk.⁷

In this study, we aimed to discover the prevalence of both genital and anorectal CT in women with an indication for genital CT testing when visiting a general practitioner (GP) about an STI. We also aimed to determine if anorectal infection (co-occurrence) could be predicted from an in-depth questionnaire on sexual history.

METHODS

Study Design, Patients, and Setting

This cross-sectional study was conducted in seven general practices with a GP-led STI consultation facility in the north of the Netherlands. These practices delivered protocol-based care following Dutch GP guidelines, for patients with STI-related signs, symptoms, or questions. We have no national asymptomatic screening program for CT. Between September 2017 and October 2019, consecutive women aged ≥ 18 years with an indication for genital CT testing were informed about the study by a nurse or GP and asked to participate. The following indications were used: multiple sexual partners in the last 6 months, unprotected sex, a sexual partner with STI, vaginal symptoms, and fear of having an STI. Women were excluded if they refused anorectal testing or were unable or unwilling to provide an adequate sexual history.

Data Collection

A sexual history was obtained using a structured questionnaire administered by the nurse or GP (Supplementary Figure 1). Standard questions were first asked about anal sex and symptoms. Thereafter, extended questions were asked about anal contact with and without penetration, condom use for anal sex, anal contact with fingers, use of sex toys, and oral contact with either the

patient's genitals or anus.

Genital and anorectal samples were taken for CT testing after receiving informed consent from the patient. Self-collection at home was allowed, for which we gave clear written and verbal instructions on how to take the samples (e.g., to prevent cross-contamination). The swabs were sent to a laboratory (Certe, Groningen) for CT testing by real-time PCR. DNA was isolated from samples, using MagNAPure 96 Roche Diagnostics Germany, according to the manufacturer's protocol, and was tested using the Presto CT-NG assay (Goffin Molecular Technologies). The PCR cycle threshold (Ct) value was recorded for all positive samples, with lower Ct values indicating larger amounts of DNA (i.e., inversely proportional).

To facilitate comparison with other studies, patients were grouped into three age categories and the indication for testing based on data from the sexual behavior questionnaire. We considered "standard" indications for anorectal testing as self-report of anal symptoms and/or anal sex, and we considered "experimental" indications for anorectal testing to be any positive answer to at least one relevant question from among all standard and extended questions on anal contact.

Demographic data and questionnaire responses were recorded on study forms by the general practitioners and nurses of the general practice and sent to the study center after anonymization. All data were imported in an Excel database and independently checked for input errors. Patients were treated according to the Dutch guideline on STI in general practice, based on their test results (azithromycin for genital CT and doxycycline for anorectal or double infection).

Sample Size

We expected 10% of the genital CT tests to be positive based on results from a previous study¹² and assumed that, among these, 50% of subsequent anorectal tests would be positive. Therefore, given a population of 500 tested women, we expected genital CT to be present in 50 (95% confidence interval [CI], 37–63), with anorectal CT present in 25 of these ($25/500 = 5\%$; 95% CI, 3%–7%). This was considered sufficiently accurate for the study aims.

Statistical Analysis

The two standard and experimental indication categories were analyzed for their ability to predict anorectal CT infection. Missed anal infections were categorized as “missed by standard indication” (no anal sex or anal symptoms, but a positive anorectal test) and “missed by experimental indication” (no anal sex or symptoms, no positive response to questions in the extended history, but a positive anorectal test). To describe the anatomic distribution of CT-positive cases, patients were grouped into non-overlapping categories (genital only, anorectal only, or anorectal and genital). Descriptive data are presented as medians, ranges, and interquartile ranges (IQRs). Univariate and multivariate logistic regression were used to identify variables associated with anorectal CT. Univariate analysis included age, standard indication, experimental indication, and each questionnaire item separately. Variables with a significant association ($p < 0.05$) were then included in a multivariate logistic regression model. A stepwise backward-elimination selection strategy was followed to arrive at a model that included only the predictors with $p < 0.05$, reporting their odds ratios (ORs) and 95% CIs. In a post-hoc analysis, we explored the correlation between anorectal and genital Ct values. All analyses were performed using IBM SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Participants and Descriptive Data

In total, 515 patients from 7 general practices were included by 16 practice nurses and 4 GPs. Only a few eligible women refused to participate, but neither the actual number nor the reason was recorded (anecdotally, most felt uncomfortable having an anorectal swab taken). In addition, we excluded data for 17 patients because their rectal tests were taken 2–9 days after the genital test and excluded retest data from 1 patient after a previous positive test. This left 497 patients for the analyses. Both anorectal and genital CT test results were missing in 7 patients (most likely because

participants had not sent their self-collected samples to the laboratory), only the anorectal test was missing in 3 cases, and only the genital test was missing in another 3 cases. Therefore, both rectal and genital CT test results were available for 484 participants. Fewer than 1.5% of the questionnaires had missing values, so we did not perform imputation for missing data.

Table 1 shows the distribution of patients by age and indication for rectal CT testing, together with the prevalence of CT by site. The median participant age was 25 years (range, 18–72; IQR, 22–30) and the overall prevalence of CT (urogenital and/or anorectal) was 17.8% (87/490). Of the CT positives, 72.4% (63/87) had a double infection, 13.8% (12/87) had a genital mono-infection, and 12.6% (11/87) had an anorectal mono-infection. In total, 42.5% and 83.3% had an indication for anorectal testing according to the standard and experimental indications, respectively. In the experimental indication group, 14.8% (60/406) tested positive, whereas 18.5% (15/81) of the remaining women tested positive (OR: 0.76; 95% CI 0.41–1.42; $p = 0.40$). Of the positive cases, 69.3% (52/75) were in the group with no indication for anorectal testing according to the standard guidelines and 20.0% (15/75) were still missed.

In the group defined by standard, anal symptoms were reported by 20.0% of patients and anal sex by 28.2%, with both reported in 5.7% (Table 2). The most common anal/bowel symptoms were itching ($n = 43$), bleeding ($n = 38$), pain ($n = 23$), hemorrhoids ($n = 16$), burning sensation ($n = 12$), and discharge ($n = 11$). Some patients also reported redness ($n = 6$), irritable bowel syndrome ($n = 6$), diarrhea ($n = 5$), constipation ($n = 5$), and anal fissure ($n = 5$), and there were single reports of swollen anus, dry skin, proctitis, irritation, ulceration, and irregular stool. Symptoms also frequently occurred together. According to the answers on the structured sexual history questionnaire, anal sex was reported without penetration by 47.7% and with penetration by 21.1%. Anal contact with fingers was reported by 38.4% and with toys by 7.1%. Oral contact with the genitals was reported by 72.6% and oral contact with the anus by 18.5%.

Variables Associated with Anorectal CT Infection

Women with a standard indication for anorectal testing had significantly less anorectal CT. Participants reporting anal sex with penetration, anal contact with fingers, or oral-anal contact had significantly lower anorectal CT rates than those answering “no or do not know” on these sexual history questions (see Table 3). Age was significantly associated with anorectal CT, with infection more common in younger patients. Multivariate logistic regression including these four variables resulted in a significantly independent association only for age: OR 0.40 (95% CI, 0.29–0.53; $p = 0.002$) for the age 22–28 years group and OR 0.18 (95% CI, 0.12–0.27; $p < 0.001$) for age >28 years group when compared to the age ≤ 21 years group.

Anorectal and Genital Bacterial Load by Ct Values

Ct values were available for 86 PCR-positive cases. Genital and anorectal Ct values were missing for one woman with a positive PCR test. The median Ct values for positive PCR tests were 24.4 (range, 18.1–40.0; IQR, 22.8–26.9) and 30.2 (range, 19.7–37.5; IQR, 25.0–33.9) in the genital and anorectal samples, respectively. Ct values did not differ by age (data not shown). Of the 62 patients with double infection and Ct values for both test sites, the genital Ct value was lower (indicating a higher bacterial load) in 50 (80.6%). Figure 1 shows the distribution of Ct values.

DISCUSSION

Summary

We found a high prevalence of urogenital and anorectal CT among women visiting their GP with STI-related symptoms or questions and for whom a urogenital CT test was indicated, with almost three-quarters having infection at both sites. Younger age was associated with higher rates of anorectal CT, and self-reported anal sex or symptoms (i.e., the basis for standard anorectal testing) was associated with less anorectal CT. After multivariate logistic regression, only age retained a significant association, and this was not affected by expanding the standard indication for testing to include questions on sexual behavior. The proposed experimental indication also missed a fifth of

the anorectal infections, despite most of the study population being in this group.

Strengths and Limitations

The results of ten urogenital and ten rectal CT tests were missing for samples collected at home. Although we could not contact participants to ask why, loss of interest or fear of taking a sample are plausible reasons. Participation in research may also have led to patients reporting more anal symptoms than would normally be the case, leading to an artificially higher prevalence than normally found in clinics. Moreover, we only asked about condom use if anal sex was reported, including no questions about prior STI or contact notification. Finally, the study was not powered to detect associations between determinants and actual anorectal CT.

The number of variables in the logistic regression analysis was set to a minimum of ten cases per independent variable added to the model. Although this is a standard approach, analyses of variables with a low prevalence (e.g., anal contact with toys) will produce results that have limited power and must be interpreted with caution. However, our exploratory results support those reported by van Liere,⁹ indicating that many anorectal infections will be missed when testing based on sexual technique alone.

Despite our best efforts to prevent cross-contamination of anorectal and genital samples, we cannot be certain that self-collected samples were not contaminated. CT levels were certainly lower for anorectal samples than for genital samples (higher Ct values), but direct comparison is not possible given how much the different sample sites affect bacterial load.¹³ A significant proportion of the anorectal tests also had Ct values consistent with at least moderate DNA loads, arguing against contamination as the only explanation for its presence on rectal swabs. Furthermore, we do not know whether high Ct values indicate contamination or inactive infection with low transmissibility. These possibilities should be addressed in future research.

Major strengths of this study are that it was carried out in general practice, with a large sample, and with almost all eligible women. These features contribute to the generalizability of our results to

primary care settings in the Netherlands. Furthermore, we asked detailed questions on sexual behavior that may be associated with anorectal CT. This strengthens our conclusion that anorectal CT cannot reliably be predicted in women based on sexual behavior or symptoms alone.

Comparison with Existing Literature

The prevalence of CT in our study was higher than previously reported in Dutch primary care (10%–11%),⁹ being closer to that reported in sexual health centers (15%).¹² In England, a CT screening program reported a positivity rate of 10% between 2018 and 2019.¹⁴ Our assumption that women visiting their GP for STI-related symptoms or questions would have a lower risk of STI than women visiting STI clinics might not be correct. We only included practices where a structured STI consultation was done by a practice nurse, possibly leading to bias because they may have more frequent consultations for STI than other practices. Although this could reflect location, such as areas with high-risk populations, only three of the participating practices were located in the inner city of Groningen and the other four were located in more rural areas. The percentage of anorectal infections that we missed when testing based on the standard indication (69.3%) was comparable to that in an STI clinic (70.9%).⁹ standard guidelines do not detail specific anal symptoms, and compared with previous research in an STI clinic, we found that symptoms were more varied.⁹ However, a relationship with CT infection was not plausible for all of these symptoms.

Implications for Clinical Practice and Research

A comprehensive sexual history does not help to identify women who require anorectal testing, and as such, cannot be recommended. Instead, we recommend improving case identification by offering anorectal tests to all women with an indication for genital CT testing. An alternative may be to treat all positive urogenital CT tests with doxycycline, as is already recommended in BASHH and NHS guidelines, and omit rectal testing entirely. That is a cheaper strategy than double testing, but it risks leaving anorectal mono-infection untreated. We also need to understand how such an approach affects the prevalence of CT.

FUNDING

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ETHICAL APPROVAL

The Medical Ethics Committee of the University Medical Center Groningen approved the study (METc 2017.163).

Competing interests

None

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Table 1. Population Characteristics and CT Prevalence by Indication for Rectal Testing

	Standard indication	Experimental indication	Total
	N = 211*	N = 414**	N = 497
	% (n/N)	% (n/N)	% (n/N)
Age			
≤21years	15.6 (33/211)	20.0 (83/414)	21.7 (108/497)
22–28 years	44.1 (93/211)	46.4 (192/414)	45.1 (224/497)
>28 years	40.3 (85/211)	33.6 (139/414)	33.2 (165/497)
Chlamydia prevalence †			
Any site	12.0 (25/208)	16.7 (68/408)	17.8 (87/490)
Urogenital	10.1 (21/207)	14.5 (59/406)	15.4 (75/487)
Anorectal	11.1 (23/208)	14.8 (60/406)	15.4 (75/487)

* 1 missing indication due to missing questionnaire data

** 3 missing experimental indication due to missing questionnaire data

† Denominators were adjusted to the number of participants tested at each site (any, urogenital, or rectal). Both tests were missing for 7, of which 3 had a urogenital test only (all negative) and 3 had a rectal test only (one was positive).

Abbreviations: CT, *Chlamydia trachomatis*.

Table 3. Anorectal Chlamydia and Association with Different Variables

Variables	N	Rectal CT (%)	Univariate analysis OR (95 %CI)	P	Multivariate analysis OR (95%CI)	P
Standard indication for rectal testing	208	(11.1)	0.54 (0.32–0.92)	0.023		
Experimental indication for rectal testing	406	(14.8)	0.76 (0.41–1.42)	0.40		
Anal symptoms	98	(12.2)	0.72 (0.37–1.39)	0.35		
Anal sex	138	(10.9)	0.59 (0.32–1.07)	0.10		
Anal sex without penetration	230	(12.6)	0.67 (0.41–1.12)	0.13		
Anal sex with penetration	103	(7.8)	0.40 (0.19–0.86)	0.020	0.64 (0.28–1.47)	0.29
Anal contact with fingers	185	(9.2)	0.43 (0.24–0.76)	0.003	0.58 (0.31–1.11)	0.10
Anal contact with toys	34	(5.9)	0.33 (0.08–1.42)	0.14		
Oral contact with genitals	352	(15.6)	1.09 (0.62–1.92)	0.89		
Oral contact with anus	91	(7.7)	0.40 (0.18–0.91)	0.024	0.69 (0.29–1.66)	0.41
Age*						
≤21	104	(30.8)				
22–28	220	(14.5)	0.38 (0.22–0.67)	0.001	0.40 (0.23–0.71)	0.002
>28	163	(6.7)	0.16 (0.08–0.34)	<0.001	0.20 (0.09–0.41)	<0.001

* Age ≤ 21 was the reference category.

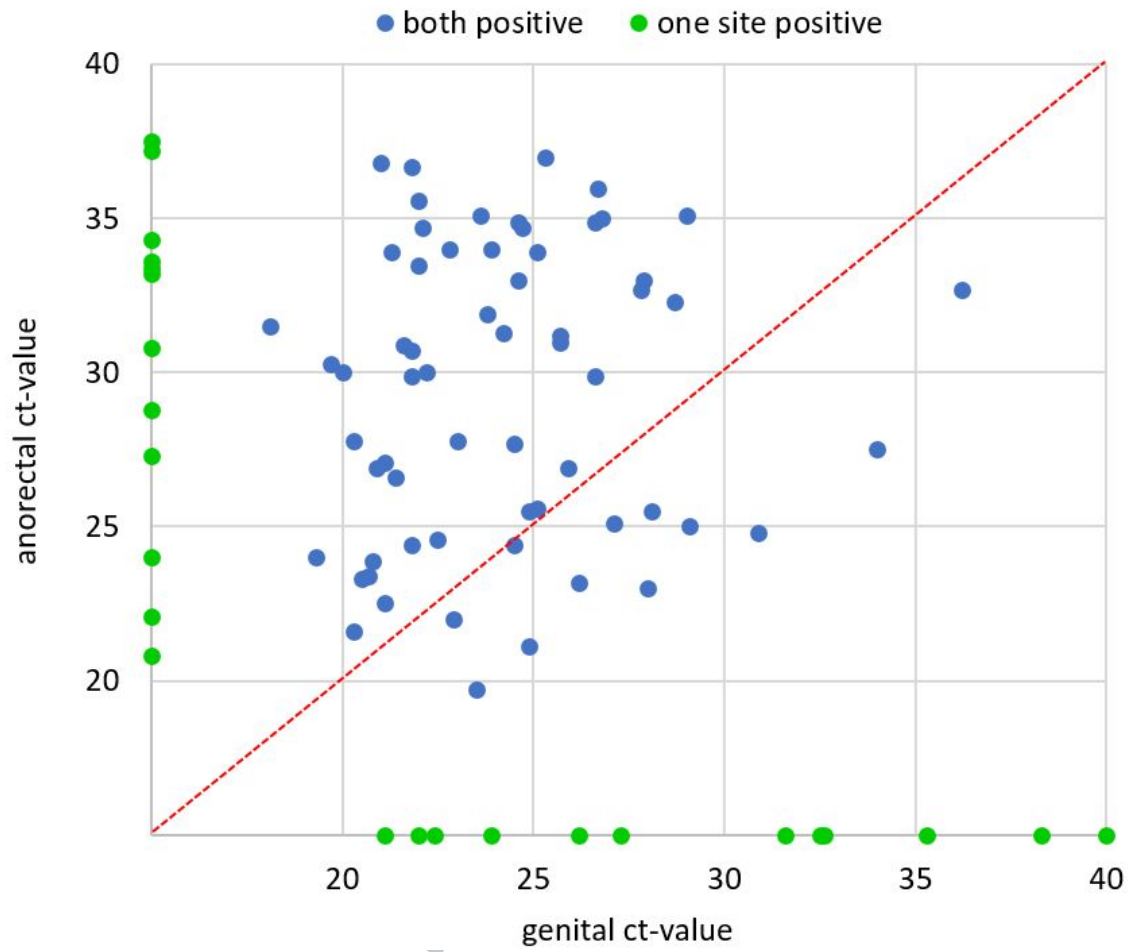
N is the total number positive for the variable, excluding 10 women without a rectal CT test. The rectal CT positive (%) was calculated by excluding women with a missing answer (0–5 per question). Women answering “don’t know” were added to the “no” category when calculating odds ratios and Fisher’s exact p-values.

Significant P-values ≤0.05 are printed in bold.

Only possible risk-factor variables (below the double line) were analysed with logistic regression if significantly associated with rectal CT in the univariate analysis.

Abbreviations: CI, confidence interval; CT, *Chlamydia trachomatis*; OR, odds ratio.

Figure 1. Distribution of Cycle Threshold Values of Samples Positive in the CT PCR Test



Abbreviations: CI, confidence interval; ct, cycle threshold; CT, *Chlamydia trachomatis*.

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