CLINICAL REPORT

Clinical and Microscopic Signs of Cervicitis and Urethritis: Correlation with *Chlamydia trachomatis* Infection in Female STI Patients

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***Chlamydia trachomatis* is among the most prevalent genital infections and is an important cause of tubal factor infertility. The majority of infected females are asymptomatic. Evidence on the reliability of signs of inflammation used to predict chlamydia in female patients is inconsistent. This study examined associations between criteria routinely used in many Scandinavian sexually transmitted infection (STI) clinics and a positive chlamydia test in a high-prevalence population. Clinical and microscopic signs of cervicitis and urethritis were recorded in 99 women attending due to chlamydia infection in a sexual partner. Mucopurulent cervical discharge, easily induced bleeding from the cervix, and more polymorphonuclear cells than epithelial cells in vaginal wet smear all correlated significantly with a positive *Chlamydia trachomatis* test (odds ratios: 3.4, 4.0 and 4.8, respectively). Increased numbers of polymorphonuclear leucocytes (>30 and ≥5, respectively) in stained cervical and urethral smears were not significantly correlated with chlamydia infection. Hence, routine collection of cervical and urethral smears in female STI patients is questionable. Key words: Chlamydia trachomatis; cervicitis; urethritis; STI.**

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Chlamydia trachomatis infection is the most common bacterial sexually transmitted infection (STI) worldwide. After a peak in 2007, explained in part by earlier missed cases due to the new variant *C. trachomatis* strains, the annually reported number of *C. trachomatis* cases in Sweden is at a high plateau level. Chlamydia infections are asymptomatic in approximately 70% of women and 50% of men (1). If left untreated, this STI can cause complications with serious consequences for women's reproductive health (2). Physicians often wish to use clinical criteria to predict infection before the results of diagnostic tests are available, for several reasons. Firstly, early treatment might reduce the risk of complications and onward transmission. Secondly, in some cases there is concern that the patient may not return for treatment on a later occasion. Thirdly, no laboratory method has a sensitivity of 100%, hence some patients will receive a false-negative result.

The aim of this study was to examine correlations between the macroscopic and microscopic signs of cervicitis and urethritis and a positive *C. trachomatis* test in a high-prevalence population of female STI patients.

METHODS

Participants

The study was conducted in the STI clinic at Sahlgrenska University Hospital, in Gothenburg, which is the second largest city in Sweden, with half a million inhabitants. The inclusion period ran from February 2005 to October 2007. Female patients who attended the clinic due to partner notification of *C. trachomatis* during the study period were included. The only exclusion criterion was menstruation. In Sweden, according to the Communicable Diseases Act, all cases of *C. trachomatis* infection are reported and partner tracing is mandatory.

Demographics, sexual behaviour, method of contraception, previous history of STIs and symptoms were assessed by a questionnaire routinely used in the clinic.

Clinical examination and specimen collection

A genital examination was performed in all patients and the cervix was cleaned of excess mucus with a cotton swab. Samples for microscopy were collected before the specimens for diagnosis of *C. trachomatis* in all cases. Time from the latest micturition was >1 h for all patients.

Visual signs of cervicitis. Mucopurulent cervical discharge was defined as the presence of yellowish mucopus at the cervical orifice. Easily induced bleeding (friability) of the cervix in connection with sampling was registered (blood only on a swab was not registered as bleeding).

Collection of smears for microscopy. Samples were taken from the endocervix by a cotton swab and from the urethra by a plastic loop of 1 µl; these specimens were stained with methylene blue and visually examined through a light microscope (×1000). The cervical smear was regarded as positive when >30 polymorphonuclear leucocytes (PMNL) were seen per high-power field (HPF) in ≥5 HPF, and the urethral smear as positive when ≥5 PMNL were seen per HPF in ≥5 HPF.

For wet smear examination, samples of vaginal discharge were collected and diluted in 10% KOH and 0.9% NaCl, respectively. These vaginal wet smears were examined with a phase-contrast microscope (×400). The number of PMNL in

relation to the number of epithelial cells (PMNL < or > epithelial cells) was recorded, in addition to signs of bacterial vaginosis (BV) and candida. All microscopic examinations were carried out during the patient's visit and recorded before the result of the *C. trachomatis* test was available.

Laboratory methods

The specimens for detection of *C. trachomatis* were sampled by separate standard swabs from the cervix and the distal urethra after the smear from each site. Both swabs were put in the same tube containing BD ProbeTec ET transport medium, which was sent to the Department of Bacteriology at Sahlgrenska University Hospital on the same day. Further analysis was performed by DNA amplification, specifically by strand displacement amplification using the BD ProbeTec ET System C trachomatis Amplified DNA Assay (Becton Dickinson Diagnostic Systems). This assay amplifies a single target region in the CT chromosomal CDA cryptic plasmid with high sensitivity and specificity for diagnosis (3). It detects both wild-type *C. trachomatis* and the mutant strain first described in 2006 (4).

Statistical analysis

All data were analysed using the elrm package under version 2.10.1 of R (The R Foundation for Statistical Computing, Vienna, Austria). Fisher's exact test was used for univariate associations. All tests were two-tailed and statistical significance was set to $p < 0.05$.

Ethics

The study was approved by the regional research ethics committee of Gothenburg.

RESULTS

Sample characteristics

Of the 99 patients included, 53 (53.5%) tested positive for *C. trachomatis*. Demographic data and sample characteristics are listed in Table I. Mean age, number of sexual partners during the previous 6 months and condom use were similar in the *C. trachomatis* positive and negative group. Symptoms of dysuria and vaginal discharge were more often reported by women with chlamydia infection, but there was no significant difference between the groups. BV was diagnosed by Amsel's criteria (5) in 16 patients (16%, 95% confi-

dence interval (CI): 10–25%), 7 of whom were chlamydia positive. Intracellular diplococci were not seen in any of the stained smears. Tests for HIV, syphilis and gonorrhoeae were performed when indicated; all tested patients were negative. No analyses of *Mycoplasma genitalium* were undertaken.

Correlation between inflammatory signs and a positive chlamydia test

Correlations between macroscopic and microscopic signs of inflammation and a positive chlamydia test, odds ratios and *p*-values are shown in Table II. Mucopurulent cervical discharge, cervical bleeding, and the finding of more PMNL than epithelial cells in the vaginal wet smear were all significantly associated with a positive chlamydia test, whereas an increased number of PMNL in the stained smears from cervix and urethra were not. The positive predictive value (PPV) was 0.76 for mucopurulent cervical discharge, 0.80 for friability, and 0.75 for more PMNL than epithelial cells in the vaginal wet smear.

DISCUSSION

This study demonstrated correlations between *C. trachomatis* infection and mucopurulent discharge at the cervical orifice, easily induced bleeding of the same locus, and more PMNL than epithelial cells in the vaginal wet smear. However, there were no correlations between *C. trachomatis* infection and an elevated number of PMNL in stained smears from the cervix and the urethra.

There is no established definition of urethritis and cervicitis in women and a number of criteria for recognizing inflammation in the cervix and urethra have been used in the literature (6). Direct comparison with earlier studies is complicated by varying diagnostic methods for *C. trachomatis* and differences in the prevalence of *C. trachomatis* and other STIs in the studied populations. There is inconsistent evidence for the association between various definitions of cervicitis and urethritis in women and *C. trachomatis* infection (7–9).

According to the STI guidelines published in 2010 by the Center for Disease Control and Prevention (CDC) in the USA, cervicitis is characterized by mucopurulent discharge in the uterine cervix and friability, and >10 PMNL/HPF in vaginal fluid is associated with *C. trachomatis* infection of the cervix. However, an increased number of PMNL in an endocervical Gram stain has not been standardized as a predictive criterion, and urethral smears are not mentioned in connection with *C. trachomatis* infection in women in the CDC guidelines (10).

Marazzo et al. (11) reported that inflammation in the endocervix or the urethra is associated with a higher organism load of *C. trachomatis* and confers a higher

Table I. *Age, sexual behaviour, previous* C. trachomatis *and symptoms*

	Chlamydia positive ($n = 53$)	Chlamydia negative ($n = 46$)
Age (years), mean (range)	24.5 (16–50)	25.8 (18–40)
Sexual partners in the previous 6 months, *n*, mean (range)	2.4 (1–10)	2.7 (1–7)
Previous chlamydia infection, *n* (%)	9/53 (17.0)	15/46 (32.6)
Symptom, *n* (%)		
Dysuria	11/52 (21.2)	5/46 (10.9)
Vaginal discharge	22/52 (42.3)	15/46 (32.6)
No symptoms	26/52 (50.0)	27/46 (58.7)

Table II. *Association of macroscopic and microscopic indicators with a positive test for* C. trachomatis

	Chlamydia positive (*n* = 53) *n* (%)	Chlamydia negative (*n* = 46) *n* (%)	OR (95% CI)	*p*-value
Mucopurulent discharge				
No	37/53 (69.8)	41/46 (89.1)	1	
Yes	16/53 (30.2)	5/46 (10.9)	3.5 (1.1–13.5)	0.026
Cervical bleeding				
No	41/53 (77.4)	43/46 (93.5)	1	
Yes	12/53 (22.6)	3/46 (6.5)	4.1 (1.0–24.5)	0.046
Cervical smear				
PMNL < 30	25/41 (61.0)	26/41 (63.4)	1	
PMNL > 30	16/41 (39.0)	15/41 (36.6)	1.1 (0.4–3.0)	ns
Vaginal wet smear				
PMNL ≤ epithelial cells	14/44 (31.8)	23/33 (69.7)	1	
PMNL > epithelial cells	30/44 (68.2)	10/33 (30.3)	4.8 (1.7–14.8)	0.001
Urethral smear				
PMNL < 5	34/42 (81.0)	36/42 (85.7)	1	
PMNL ≥ 5	8/42 (19.0)	6/42 (14.3)	1.4 (0.4–5.5)	ns

PMNL: polymorphonuclear leucocytes; ns: not significant; OR: odds ratio; CI: confidence interval.

likelihood of a positive diagnostic test for this infection. This statement implies that older studies using diagnostic methods that are less sensitive than nucleic acid amplification tests (NAAT) may have included *C. trachomatis* infected patients with a relatively low bacterial load, no inflammatory changes in the smears and a false-negative result on the *C. trachomatis* test. Data from these studies might produce a stronger correlation between an increased number of PMNL in urethral and cervical smears and *C. trachomatis* (8, 12–14). However, 2 recent studies also reported a correlation between ≥ 5 PMNL in the urethral smear and *C. trachomatis* detected by NAAT in female STI patients (15, 16). In these 2 studies a blunt curette was used for the urethral smears. A study comparing different devices for collecting the urethral smear is needed. Another explanation of the discordance between these studies and our results could be inter- and intra-observer variation in the interpretation of stained smears (17). Differences in the sampling procedure may also be of importance. A limitation of the present study is the lack of sampling for the detection of *M. genitalium*.

It is worth asking whether the results are relevant for female STI patients in general. Younger age and a higher number of sexual partners are known risk factors for *C. trachomatis* infection. In a recent study of female patients in an STI clinic in Oslo, the mean age of women positive for *C. trachomatis* was 25 compared with 26.8 in female patients without any infection, an age distribution similar to the women in the present study (16).

The PPV of diagnostic tests is highly dependent on the prevalence. In our study population, which had a 53% prevalence of *C. trachomatis*, the PPVs of mucopurulent cervical discharge, friability, and more PMNL than epithelial cells in the vaginal wet smear were 0.76, 0.8 and 0.75, respectively. Assuming that the calculated values of sensitivity and specificity are applicable, the estimated PPVs in an STI clinic population with a *C. trachomatis* prevalence of 10% would all be too low to be useful in clinical practice (0.23, 0.27 and 0.20, respectively). However, in high-prevalence populations the value of stained smears is higher.

The high number of *C. trachomatis* cases constitutes a challenge, and demands that our resources are used in the most efficient way. It is time-consuming to collect, process and evaluate the different smears, and sampling from the urethra is known to be inconvenient for many patients. In populations with a higher prevalence of gonorrhoea, stained endocervical and urethral smears contribute to the detection of intracellular diplococci with a reported sensitivity of 40–65% (18). The vaginal wet smear is also valuable in diagnosing BV, candida, and trichomonas infections, and needs no justification in symptomatic female STI patients. In the present study the prevalence of BV was low compared with other studies and did not differ between chlamydia-positive and chlamydia-negative patients.

In conclusion, mucopurulent cervical discharge, friability of the uterine cervix, and more PMNL than epithelial cells in the vaginal wet smear could predict *C. trachomatis* infection in high-prevalence populations. However, predictive algorithms that include indicators such as age and sexual behaviour are desirable. Earlier attempts to create reliable practical guidelines for presumptive diagnosis have failed (19). Modern computerized case records with defined search terms should make it possible to continuously study the association of certain criteria and the occurrence of STIs in larger populations. Nevertheless, according to our results, the clinical significance of an increased number of PMNL in stained cervical smears, and in female urethral smears taken with a plastic loop of 1 µl, is too low to justify further routine use of these smears to predict *C. trachomatis* infection.

ACKNOWLEDGEMENTS

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