Detection of *Chlamydia Trachomatis* infection in women with CIN and invasive carcinoma. Controversial results of different methods

J. Markowska[1], *Prof. M.D.*; **N. Fischer**[2], *M.D. Ph.D.*; **J. B. Warchol**[3], *Prof. M.D.*; **Z. Fischer**[2], *M.D. Ph.D.*

[1]*Chair of Oncology,* [3]*Chair of Radiobiology and Biology, Faculty of Medicine, K. Marcinkowski University School of Medicine;*
[2]*Departments of Gynecology and Obstetrics, Holy Family Clinic, Poznań (Poland)*

Summary

Chlamydia (Ch.) trachomatis infection as a sexually transmitted disease is highly important, but reliable methods of diagnosing it remain to be worked out. We used three methods of detection: an immunoenzymatic technique for detection of *Ch. trachomatis* antigen in endocervical material, in situ PCR, and enzyme-immuno assay for detection of IgG class anti-*Ch. trachomatis* antibodies in serum. We have compared the IS-PCR technique and method of detection of the endocervical antigen. We have not confirmed compatibility of the results obtained in these two methods. Parallel positive results obtained in patient serum and detection of chlamydial DNA by IS-PCR have been accepted to be indicative of persistent infection of *Ch. Trachomatis.*

Key words: Ch. Trachomatis; IS-PCR.

Introduction

Chlamydia (Ch.) are known to be bacteria which resemble viruses in their obligate intracellular life cycle and have an inability to synthesize adenosine triphosphate. In other respects *Chlamydia* are similar to other bacteria containing ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), cell walls, and metabolic enzymes [1]. *Chlamydia* include four species: *Ch. trachomatis, Ch. psittaci, Ch. pneumoniae*, and *Ch. pecorum.* Based on differences in a major outer membrane protein, *Ch. trachomatis* exhibits 15 serotypes (A, B, Ba, C, D-K, L1, L2, and L3). Various serotypes of *Ch. trachomatis* can cause, respectively: A,B,Ba,C-trachoma, L1-L3-venereal lymphogranuloma, D-K-pneumonitis, newborn inclusion conjunctivitis, and genital tract infections. *Ch. trachomatis* is the main cause of nongonococcal urethritis among men but may also cause epididymitis and proctitis. In women it causes cervical infection, urethritis, salpingitis [2]. Cervical inflammations caused by this sexually transmitted infection are suspected to promote CIN and invasive carcinoma.

Ch. trachomatis infection as a sexually transmitted disease is highly important, but reliable methods of diagnosing it remain to be worked out [3].

Patients and Methods

Cervical tissues, endocervical swabs and samples of serum were taken from 135 patients. The patients formed two principal groups, one comprising women who were subjected to surgery due to cervical dysplasia or cancer and the other, a control group of 63 women who were free of such pathology. The first group included eight patients with CIN I, 12 patients with CIN II, 31 patients with CIN III and 21 patients with invasive carcinoma. In the cases of tissue from paraffin blocks prepared by standard histological techniques, the material was tested for the presence of *Ch. trachomatis* using an in situ PCR (IS-PCR) technique. Presence of *Ch. trachomatis* antigen in the endocervical material was documented by an immuno-enzymatic technique (solid phase sandwich immunoassay) and sera of the patients were tested for presence of IgG class anti-*Ch. trachomatis* antibodies. In 10% the tests for presence of *Ch. trachomatis* yielded doubtful results. There were six such disputable results in the control group (10%), one such result in CIN II (9%) and six in CIN III (19%). Therefore, the analyzed patients included finally 122 women: eight in the CIN I group, 11 in the CIN II group, 25 in the CIN III group, 21 in the carcinoma group and 57 women in the control group. The mean age for all the patients was 46.6 years (47.5 in the CIN I group, 40.5 in the CIN II group, 46.6 in the CIN III group, 50.2 in the carcinoma group and 48.4 in the control group).

The McNamara test was used to estimate significance of differences.

IS-PCR

The paraffin-embedded cervical tissues were cut into 0.4 μm sections, mounted on silane-coated glass slides and incubated overnight at 60°C. The sections were dewaxed in xylene, while the residual xylene was extracted in 80, 90, 100% ethanol. The sections were rehydrated in a series of decreasing ethanol concentrations, washed in PBS buffer and transferred into proteinase K buffer (1M Tris-HC1, pH 8.0, 0.5 ml + 0.5M EDTA, pH 8.0, 1 ml + 5M NaC1 1.0 ml) plus proteinase K to a final concentration of μg/ml) for 10 min. The specimens were washed with PBS and air dried.

For amplification of the *Ch. trachomatis*-plasmid sequence, the following PCR primers were used: 5'GGACAAATCG-TATCTCGG-3' (T1, sense primer) and 5'GAAACCAACTC-TACGCTG-3' (T2, antisense primer). If successful, the amplification yielded a 517-BP fragment. The reaction tubes were heated to 94°C for 7 min, which was followed by 40 cycles (95°C for 1 min, 60°C for 1 min, and 72°C for 1.5 min) and the

Revised manuscript accepted for publication October 18, 2000

Table 1. — *Positive negative and disputable results in IS-PCR in the control group, CIN I, CIN II, CIN III and invasive carcinoma.*

	IS-PCR positive results	IS-PCR negative results	IS-PCR disputable results
Control group (63)	19 (30%)	38 (60%)	6 (10%)
CIN I (8)	5 (62.5%)	3 (37.5%)	0
CIN II (12)	8 (66%)	3 (25%)	1 (9%)
CIN III (31)	12 (39%)	13 (42%)	6 (19%)
Carcinoma (21)	11 (52%)	10 (48%)	0
Total (135)	55 (40%)	67 (50%)	13 (10%)

Table 2. — *Positive and negative results in IS-PCR in the control group, CIN I, CIN II, CIN III and invasive carcinoma.*

	IS-PCR positive results	IS-PCR negative results
Control group (57)	19 (33.3%)	38 (66.6%)
CIN I (8)	5 (62.5%)	3 (37.5%)
CIN II (11)	8 (72.9%)	3 (27.3%)
CIN III (25)	12 (48%)	13 (52%)
Carcinoma (21)	11 (52%)	10 (48%)
Total (119)	55 (46.2%)	67 (53.8%)

Table 3. — *Inconsistency of results between the IS-PCR method and detection of endocervical antigen of* Chlamydia trachomatis.

	IS-PCR	Endocervical swabs (–)	Endocervical swabs (+)	Percentage of consistency	Percentage of incompatibility
Control group (49)	(–)	11	19	24 (49%)	25 (51%)
	(+)	6	13		
CIN I (6)	(–)	1	1	4 (66.6%)	2 (33.3%)
	(+)	1	3		
CIN II (9)	(–)	1	1	4 (44.4%)	5 (66.6%)
	(+)	4	3		
CIN III (18)	(–)	5	4	11 (61%)	7 (39%)
	(+)	3	6		
CA (10)	(–)	3	1	8 (80%)	2 (20%)
	(+)	1	5		
Total	(–)	21	26	51 (55.4%)	41 (44.5%)
	(+)	15	30		

final extension at 72°C for 7 min. The standard PCR mix consisted of 11µl 25mM $MgCl_2$ (Boehringer), 10 µl of the buffer 10x (Boehringer), 8.8µl of T1 (0.66 µg/µl), 9.3 µl of T2 (0.62 µg/µl) (TiB MolBiol), 10µM of dNTP (Boehringer), 0.8 µl 5U of Taq DNA polymerase (Boehringer) and 5 µl 10% BSA (Sigma). The final reaction volume was 25 µl.

Digoxygemin labelled DNA fragments were detected with phosphatase labelled antibodies at 1:200 dilution and the anti-phosphatase mix. The incubation time with primary antibody was 1 hour at 37°C, followed by 1 hour at 20°C.

A standard test was used as the negative control.

Solid phase sandwich immunoassay

The rapid immunoassay was used for detection of *Ch. trachomatis* antigen in female endocervical swabs. Specimen *Ch. trachomatis* antigen was extracted from a swab by heating at 80°C with 0.6 ml of extraction reagent, containing about 0.1% sodium azide (10-12 min). The sample was cooled for 5 min at 20°C and placed into the sample window of the *Chlamydia* detecting ELISA kit. The absorbent pad in the sample window contained latex-labelled monoclonal antibody against a genus-specific lipopolysacharide epitope of *Ch. trachomatis*. The extract rehydrated the labelled antibody and the extracted antigen reacted to form a complex. The pad contacted a test strip, which contained a region of immobilized monoclonal anti-*Chlamydia* antibody in the result window. The extract-latex mixture moved by capillary action along the strip. A line in the result window indicated the presence of Chlamydia antigen in the extract.

IgG class anti-*Ch. trachomatis* antibodies in serum

Anti-*Ch. trachomatis antibodies* were detected in serum by enzyme-immuno assay. Serum (100 µl) at 1:20 dilution reacted with the solid phase specific antigen. This antigen bound anti-chlamydial antibodies in the course of 15 min agitation. The pad was incubated for 20 min at 20°C and rinsed. Anti-H-IgG (100 µl) was added. The specific serum antibodies bound the solid phase antigen and anti-H-IgG in the course of 15 min of agitation. This complex reacted with chromogen (50 µl) and gave a color reaction following 10 min of incubation in darkness, at 20°C. The reaction was stopped with 2N H_2SO_4 (50 µl) and intensity of the reaction was measured in a spectroscope (λ=450 nm).

Results

Ch. trachomatis infection was documented in 65.3% of intracervical swabs. Therefore these results were verified using IS-PCR. Using the IS-PCR technique, positive results were obtained in 33.3% of women in the control group, in 62.5% of patients with CIN I, 72.7% of patients with CIN II, 48.0% of patients with CIN III and 52.0% of patients with invasive carcinoma. In general, the two techniques of detecting *Ch. trachomatis* infection yielded compatible results in 55.4% of women. The highest percentage of consistency was in a group of invasive carcinoma patients (80%). The other groups indicated a lower compatibility (49% in the control group, 66.6% in women with CIN I, 44.4% in the group of patients with CIN II, 61% in the group of patients with CIN III). Antibodies reacting with *Ch. trachomatis* were detected in sera of patients but never exceeded the level of 20 AIU/ml. The level of 9 AIU/ml was found to be indicative of infection in the past.

Discussion and Conclusions

We have compared two techniques of detecting infection with *Ch. trachomatis*, the technique aimed at detecting appropriate chlamydial antigens in intracervical swabs and the IS-PCR technique based on detection of DNA of *Ch. trachomatis* in the tissue of the uterine cervix. Since the results of detecting the antigen in endocervical material were positive in more than 65% of patients, we compared them with the results of the IS-PCR approach [4]. We have not confirmed compatibility of the results obtained using the immuno-enzymatic technique and those yielded by the IS-PCR technique. According to David *et al.* [5] detection

of *Ch. trachomatis* in smears presents significant difficulties. False results may stem also from administration of antibiotics [6]. For these reasons, application of such a sensitive assay as IS-PCR seems more appropriate. In addition, the IS-PCR technique provides additional information on either the intra- or extracellular location of *Ch. trachomatis.* Parallel positive results obtained in patient sera and detection of chlamydial DNa by IS-PCR have been accepted to be indicative of persistent *Ch. trachomatis* infection.

References

[1] Smith T. F.: "*Chlamydie*". In: "Clinical and Pathogenic Microbiology". Howard B. J., ed., Mosby 1994, 847.
[2] Mroczkowski T. F.: "Sexually transmitted diseases caused by *Chlamydia trachomatis*". In: "Sexually Transmitted Diseases". Igaku-Shoin, New York, 1990, 1.
[3] Takać, Gorišek B.: "The frequency of *Chlamydia trachomatis* infection in women with intraepithelial neoplasia of uterine cervix". *Eur. J. Gynec. Oncol.*, 1998, XIX, *5*, 492.
[4] Schlott T., Goetz R., Hoppert M. Nagel H., Reimer S., Schumacher-Lutge I. K. *et al.*: "The *in situ* polymerase chain reaction for detection of *Chlamydia trachomatis*". *J. Histochemistry and Cytochemistry,* 1998, *46*, 1017.
[5] David L. M.: "Diagnostic tests and specimens used to screen for *Chlamydia trachomatis* in genitourinary medicine clinics in the United Kingodom". *Int. J. STD-AIDS*, 1999, *10*, 527.
[6] Natomi T., Ikeda Y., Nagayama A.: "Minimum inhibitory and minimal lethal concentration against *Chlamydia trachomatis* dependent on time of addition and the duration of the presence of antibiotics". *Chemotherapy*, 1999, *45*, 242.

Address reprint requests to:
J. MARKOWSKA, M.D.
Chair of Oncology
University of Medical Sciences
Lakowa Str. 1/2
61-878 Poznań (Poland)