Human Reproduction Vol.16, No.10 pp. 2206–2210, 2001

Combined screening for *Chlamydia trachomatis* and squamous intra-epithelial lesions using a single liquid-based cervical sample

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BACKGROUND: Cervicitis is believed to alter cytological interpretation and could compromise a combined screening for *Chlamydia trachomatis* (CT) cervicitis and squamous intra-epithelial lesions (SIL). Liquid-based cytological methods have been shown to limit obscuring factors and permit the detection of infectious agents by DNA amplification techniques. The aim of this study was to evaluate a combined screening for SIL and CT cervicitis with a single liquid-based cervical sample. METHODS: Two cervical samples were obtained from each of 590 women considered at high risk for CT. The modified Ligase chain reaction (LCR) procedure for CT detection using specimens collected in the AutoCyte's preservative fluid was compared with the conventional Abbott LCx method using cervical swabs. We have also compared the percentage of inflammatory specimens and adequacy of cellular material in the populations of CT+ and CT- women. RESULTS: The results show total agreement for 588 of 590 cervical samples using the two LCR protocols (Kappa = 0.96; 95% confidence interval: 0.91–1.00). The quality of cervical cytology was not compromised by CT cervicitis. CONCLUSIONS: We demonstrated the feasibility of combined screening for CT and SIL with a single liquid-based cervical sample.

Key words: cervical screening/*Chlamydia trachomatis*/DNA amplification techniques/liquid-based cervical sample/squamous intra-epithelial lesion

Introduction

Chlamydia trachomatis (CT) and Human papillomavirus (HPV) infections are the two commonest sexually transmitted infections world-wide. They are often asymptomatic and responsible for tubal factor infertility and precancerous cervical lesions respectively. Combined screening for CT cervicitis and squamous intra-epithelial lesions (SIL) could be justified in a high risk population for CT infection given that these infections share common risk factors. However, cervicitis is believed to alter cytological interpretation and the current recommendation is deferral of cytology to allow for treatment.

Methods using liquid-based cytology have the potential to reduce the number of unsatisfactory specimens by the elimination of obscuring factors such as red blood cells and leukocytes. Moreover, additional tests can be carried out from the same residual liquid-based sample, like HPV detection (Kunz *et al*., 1998; Vassilakos *et al*., 1998a).

We sought to evaluate firstly the possibility of additional screening for CT cervicitis from the same liquid-based sample used for cervical cancer detection and, secondly, to evaluate whether the quality of cervical cytology was modified by CT status.

For CT screening, we compared a modified procedure of the LCR technique using a cervical specimen collected in preservative medium with the conventional LCR technique using endocervical swabs. Specimen adequacy, depending on CT status, was compared according to the Bethesda classification system (Kurman *et al*., 1994).

Materials and methods

Patients

Five hundred and ninety non-consecutive women consulting at the Gynaecological Outpatient Clinic at the University of Geneva Hospitals between January 1998 and April 1999 were enrolled in the study. Women were seen in different units of the Outpatient Clinic, namely family planning, adolescent, and general gynaecological consultations, and classified into high risk (n = 520) or low risk (n = 70) groups for CT infection.

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The high risk group was defined as sexually active asymptomatic adolescents, women with clinical suspicion of pelvic inflammatory disease, a sexually transmitted disease, and those demonstrating high risk sexual behaviour including first sexual intercourse at early age, multiple partners, unprotected intercourse, and multiple termination of pregnancy. The low risk group was defined as women consulting for an annual gynaecological check up without any of the above-mentioned high risk criteria.

Patients who received antibiotic therapy within the month before consultation were excluded from the study.

Clinical procedure

Two cervical samples per woman were obtained. The first sample for CT detection was collected using an endocervical swab (Abbott LCx Specimen Collection Kit®; Abbott Laboratories, Abbott Park, IL, USA). Samples were sent daily to the hospital central bacteriology laboratory for processing according to the manufacturer's recommendations.

The second sample was obtained using a Cervex-brush® (Rovers, Oss, The Netherlands) and, in the case of cervical stenosis, a Cytobrush® (Medscand, Geneva, Switzerland). The brush head was detached and immersed in the collection vial containing the CytoRich® preservative medium (Tripath Imaging Inc., Burlington, NC, USA). Specimens were maintained at room temperature and submitted once weekly to the laboratory (Centre for Cytology and Clinical Pathology, Cytopath, Geneva, Switzerland) for cytological diagnosis and CT DNA testing.

Cytological procedure

Sampling using the Cytobrush® or Cervex-brush® allows for retrieval of about 500 000 epithelial cells. Specimens were prepared for cytology using the AutoCyte® PREP manual technique as described previously (Vassilakos *et al*., 1999).

During this preparation phase, specimens underwent vortexing, centrifugal density sedimentation and resuspension. Cells were distributed in two circles onto cationically-coated slides. The median epithelial cell number for both circles is 115 000 cells. Thin-layer preparations were stained using the Papanicolaou method and the inflammatory character of the preparation was studied. The specimen adequacy of cellular material was classified according to the Bethesda criteria (Kurman *et al*., 1994). The residual cellular material was made available for CT testing.

Microbiological procedure

Conventional endocervical swabs were processed at the central bacteriology laboratory within 4 days of reception using the conventional Abbott LCx method according to the manufacturer's recommendations.

Residual material from the liquid-based sample after preparation of thin-layer slides for cytology was analysed for CT detection at the laboratory (Institut Bio-Analytique). The liquid medium contains 20% ethanol, ensuring cell fixation and allowing for analysis for up to 10 days.

Using the modified LCR procedure for the detection of CT, cells were washed and resuspended in Abbott LCx resuspension buffer before processing according to the manufacturer's instructions.

Data analysis

Results for CT detection were independently analysed in each laboratory and were compared at the end of the study. Data were collected using EXCEL and statistical analysis was carried out with Epi Info version 6 (CDC, Atlanta, GA, USA). The agreement between the two DNA amplification procedures was assessed by the Kappa coefficient. A Kappa value >80% was considered as indicating excellent concordance (Seigel *et al*., 1992). Statistical significance of differences in proportions were computed using Fisher's exact test or χ^2 test. A *P* value <0.05 was considered as statistically significant.

Table I. Results of the modified procedure of LCR using liquid-based samples as compared with conventional LCR using cervical swabs

Conventional LCR using cervical swabs	Modified LCR procedure using liquid-based cervical sample	
	Negative	Positive
Negative	560	1[a]
Positive	1[b]	28

[a]Uncertain result (fluctuating values < threshold).
[b]Weakly positive result (<threshold).

Results

A total of 590 selected women were included in the study. Thirty tested positive for CT, whilst 560 were negative.

Comparison between the LCR–DNA amplification procedures showed excellent concordance with a Kappa = 0.96; 95% confidence interval (CI): 0.91–1.00. Results demonstrated total agreement for 588 of 590 cervical samples (Table I). The two discordant results were one which tested negative by conventional LCR test and uncertain by modified LCR test, and the other which was positive by conventional LCR test and weakly positive by modified LCR test.

Cytological results in the two populations showing inflammatory smears, specimen adequacy and cytological diagnosis are presented in Table II. Inflammatory specimens were more frequently encountered in CT+ women but this difference was not statistically significant [*P* = 0.07 (NS), Fisher's exact test]. There were no significant differences between the two populations for specimen adequacy [*P* = 0.83 (NS), χ^2 test]. The percentage of satisfactory specimens was similar (86.7% CT+ women versus 89.3% CT- women). Similarly, satisfactory but limited specimens were not influenced significantly by CT cervicitis (13.3% versus 10.3%). Likewise, cytological results were not affected by CT status [*P* = 0.77 (NS), χ^2 test].

Overall CT prevalence in our study was 5.1%. Prevalence in high risk women was 5.9 versus 1.4% in low risk women. The CT prevalence was inversely associated with age, at least between 20–35 years of age (Table III). The highest prevalence of 9.4% was noted in women between 20 and 24 years of age. The overall prevalence was 7.7% for women up to 25 years of age who represented 73% of the CT+ population. In women younger than 30 years of age, this prevalence was 6.7% and corresponded to 90% of the CT+ population. Screening failed to identify any CT+ women older than 35 years.

Table II. Cytological results in the *Chlamydia trachomatis* positive and negative populations

		C. trachomatis positive (n = 30)	*C. trachomatis* negative (n = 560)
Inflammatory specimens	(n = 186)	14 (46.7%)	172 (30.7%)
Specimen adequacy (n = 590)			
satisfactory	n = 526 (89.2%)	26 (86.7%)	500 (89.3%)
satisfactory but limited:	n = 62 (10.5%)	4 (13.3%)	58 (10.3%)
by absence of endocervical component	57 (9.7%)	4 (13.3%)	53 (9.5%)
by obscuring factors	5 (0.8%)	0	5 (0.9%)
unsatisfactory	n = 2 (0.3%)	0	2 (0.4%)
Bethesda cytological diagnosis (n = 590)			
normal	n = 528	27 (90%)	501 (89.5%)
ASCUS[a]	n = 13	0	13 (2.3%)
LGSIL[b]	n = 45	3 (10%)	42 (7.5%)
HGSIL[c]	n = 4	0	4 (0.7%)

[a]Atypical squamous cells of undetermined significance.
[b]Low grade squamous intra-epithelial lesion.
[c]High grade squamous intra-epithelial lesion.

Table III. High risk and low risk groups combined and stratified by age for number of women screened for *Chlamydia trachomatis*

Age (years)	Screened	Positive	Prevalence (%)
⩽19	160	10	6.3
20–24	127	12	9.4
25–29	112	5	4.5
30–34	93	3	3.2
⩾35	98	0	–

Discussion

The combined screening of CT cervicitis and SIL with a single cervical sample has already been studied (Rantala and Kivinen, 1998; Banuelos Panuco *et al*., 2000). The first method for CT screening was based on the cytological detection of chlamydial intracytoplasmic inclusions using the Papanicolaou staining technique (Banuelos Panuco *et al*., 2000). However, cytological detection of CT in cervical smears remained unsatisfactory due to the difficulty of differentiating real nebular chlamydial inclusions from other intracytoplasmic inclusions. Because of its low specificity and high interobserver variation, this method has now been abandoned. Rantala *et al.* developed a fluorescent antibody staining method to detect CT elementary bodies in Papanicolaou-stained cervical smears without disturbing subsequent cytological morphology (Rantala and Kivinen, 1998). However, the use of immunofluorescence for CT detection is delicate and subjective. This method for CT detection is also less sensitive than amplified DNA techniques which are presently considered to be the method of choice (Schachter *et al*., 1994; Warford *et al*., 1999). On the other hand, detection of amplicons is automated for the DNA amplification techniques and may be less operator-dependent than tissue cell culture and direct immunofluorescence. Using the modified LCR procedure with the residual material from the CytoRich® preservative fluid, we have demonstrated excellent agreement in our study between the two procedures for CT detection (Kappa = 0.96, 95% CI: 0.91–1.00). The LCR procedure also proves its effectiveness when compared to the conventional procedure, with the added advantage of deferred analysis of up to 10 days. Indeed, DNA amplification techniques have the advantage that the viability of the organism is not a prerequisite for the identification of bacterial DNA. The possibility of conserving cervical samples in liquid medium at room temperature could facilitate the procedure in screening programmes. An additional advantage of using DNA amplification techniques is that they allow for the simultaneous detection of more than one sexually transmitted agent such as HPV and CT and, in the near future, gonococcus, using a single specimen. Presently, liquid-based cytology offers the possibility of testing for HPV and CT using DNA amplification techniques. This confirms the feasibility of combined screening using a single specimen.

Cervicitis is believed to alter cytological interpretation and the current clinical recommendation is deferral of cytology to allow for treatment. We did not confirm this notion in this study. Inflammation did not obscure significantly the cell preparation as defined by the Bethesda criteria for specimen adequacy. In spite of a non-significant increase of inflammatory specimens in our CT+ population, we found that the CT cervicitis did not affect the specimen adequacy. This is due to the well-recognized effect of liquid-based cytology, which improves specimen adequacy with concurrent reduction in both unsatisfactory and satisfactory but limited preparations (Vassilakos *et al*., 1998b, 1999). The specimen adequacy classified according to Bethesda criteria in our high risk population is similar to the results published in Geneva for the general population: satisfactory specimens 87.5%, satisfactory but limited obscuring factors 1.23% and unsatisfactory specimens 0.15% (Vassilakos *et al*., 1998b, 1999). The results of our study do not confirm the necessity of cervical cytology deferral in the presence of cervicitis. The danger of deferral is high patient default once the genital infection has

been treated. Ross *et al.* reported a 15% default rate in genitourinary medicine clinic attendees and this rate increased in women younger than 25 years (Ross *et al*., 1995). Non-adherence to screening programmes was found to be the most important modifiable antecedent to invasive cervical carcinoma (Sung *et al*., 2000).

Chlamydial infections meet the general prerequisite for disease prevention by screening since they are highly prevalent, associated with significant morbidity, can be diagnosed, and are treatable (Paavonen, 1997). They also have serious sequelae, including pelvic inflammatory disease, infertility, and ectopic pregnancy. All these conditions, except for infertility, have been shown to be preventable if CT is treated in its asymptomatic phase (Scholes *et al.,* 1996; Egger *et al.,* 1998; Kamwendo *et al.,* 2000). During the last decade, many studies with cost-benefit and cost-effectiveness analyses have confirmed the interest of screening for CT (Humphreys *et al.,* 1992; Genc and Mardh, 1996; Paavonen, 1997; Howell *et al.,* 1998). In women undergoing routine pelvic examination, LCR of cervical specimens would prevent most diseases and provide the highest cost savings (Howell *et al.,* 1998). Genc and Mardh showed that when the prevalence of chlamydial infection exceeded 6%, screening of women with DNA amplification assays of endocervical swabs combined with the single dose azithromycin treatment of positive patients is the most cost-effective strategy (Genc and Mardh, 1996). In the high risk CT study population, we report a prevalence of 6.7% in women under 30 years of age, accounting for 90% of all those who screened positive for CT. However, such a screening strategy remains to be validated in the Swiss medical care system.

Chlamydia cervicitis has been suspected to be a risk factor for SIL and cervical cancer on the basis of case-control comparisons of serological tests (Schachter *et al.*, 1982; Hsieh *et al.*, 1999) but the risk of cervical cancer associated with chlamydia seropositivity has been low (OR = 2) after adjusting for HPV infection (de Sanjose *et al.*, 1994; Koskela *et al.*, 2000). Koutsky showed in a cohort study that the development of high grade SIL was associated with antibodies to CT (adjusted relative risk = 2.4) but not with CT culture from the cervix (adjusted relative risk = 1.1) (Koutsky *et al.*, 1992).

The respective roles of chlamydia and HPV in the genesis of the dysplasias were not studied because of the small study population size. Given the prevalence of CT and HPV, a sample size of 6850 women would be required to demonstrate a relative risk of 2 in CT+ women, independently of HPV status. A wider-based screening programme combining HPV and chlamydia using liquid-based cytology could possibly permit an improved understanding of the role of CT in cervical cancer.

Combined screening for CT cervicitis and SIL can only be justified by a high prevalence of CT infection and a good adherence of the population to cervical cancer screening programmes. Data from the Swiss Cancer Register in 1997 showed that 71.7% of women have had at least one cervical cytology examination between 20 and 29 years of age (Wanner *et al.*, 2000). Another survey with 1993 data from the pathology laboratories in Geneva (unpublished) showed an annual cover rate of 55% for women in the same age group. Over 95% of cervical cytology performed in Geneva are currently carried out using liquid-based cervical sampling. Based on this fact, it seems judicious to propose a combined screening to women at high risk of CT and under 30 years of age when presenting for a cervical cytology. This procedure represents only one of the many options to improve the detection of CT, but the cost-effectiveness of such a screening strategy remains to be calculated.

Acknowledgements

We would like to thank Dr Jeanine Henry-Suchet for her critical reading of the manuscript and Dr Michel Boulvain for his assistance in statistical analysis of the data.

References

Banuelos Panuco, C.A., Deleon, R.I., Hernandez Mendez, J.T. *et al.* (2000) Detection of Chlamydia trachomatis in pregnant women by the Papanicolaou technique, enzyme immunoassay and polymerase chain reaction. *Acta Cytol.*, **44**, 114–123.

de Sanjose, S., Munoz, N., Bosch, F.X. *et al.* (1994) Sexually transmitted agents and cervical neoplasia in Colombia and Spain. *Int. J. Cancer*, **56**, 358–363.

Egger, M., Low, N., Smith, G.D. *et al.* (1998) Screening for chlamydial infections and the risk of ectopic pregnancy in a county in Sweden: ecological analysis. *Brit. Med. J.*, **316**, 1776–1780.

Genc, M. and Mardh, A. (1996) A cost-effectiveness analysis of screening and treatment for Chlamydia trachomatis infection in asymptomatic women. *Ann. Intern. Med.*, **124**, 1–7.

Howell, M.R., Quinn, T.C., Brathwaite, W. *et al.* (1998) Screening women for chlamydia trachomatis in family planning clinics: the cost-effectiveness of DNA amplification assays. *Sex. Transm. Dis.*, **25**, 108–117.

Hsieh, C.Y., You, S.L., Kao, C.L. *et al.* (1999) Reproductive and infectious risk factors for invasive cervical cancer in Taiwan. *Anticancer Res.*, **19**, 4495–4500.

Humphreys, J.T., Henneberry, J.F., Rickard, R.S. *et al.* (1992) Cost-benefit analysis of selective screening criteria for Chlamydia trachomatis infection in women attending Colorado family planning clinics. *Sex. Transm. Dis.*, **19**, 47–53.

Kamwendo, F., Forslin, L., Bodin, L. *et al.* (2000) Epidemiology of ectopic pregnancy during a 28 year period and the role of pelvic inflammatory disease. *Sex. Transm. Infect.*, **76**, 28–32.

Koskela, P., Anttila, T., Bjorge, T. *et al.* (2000) Chlamydia trachomatis infection as a risk factor for invasive cervical cancer. *Int. J. Cancer*, **85**, 35–39.

Koutsky, L.A., Holmes, K.K., Critchlow, C.W. *et al.* (1992) A cohort study of the risk of cervical intra-epithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N. Engl. J. Med.*, **327**, 1272–1278.

Kunz, J., Rondez, R., Yoshizaki, C. *et al.* (1998) Comparison of conventional PAP smears with thin layer specimen (liquid-based PAP test) and correlation with cytopathological findings with HPV status using the hybrid capture system. *Schweiz. Rundsch. Med. Prax.*, **87**, 1434–1440.

Kurman, R.J., Henson, D.E., Herbst, A.L. *et al.* (1994) Interim guidelines for management of abnormal cervical cytology. The 1992 National Cancer Institute Workshop. *J. Am. Med. Assoc.*, **271**, 1866–1869.

Paavonen, J. (1997) Is screening for Chlamydia trachomatis infection cost effective? *Genitourin. Med.*, **73**, 103–104.

Rantala, I. and Kivinen, S. (1998) Demonstration of Chlamydia trachomatis in Papanicolaou-stained gynecological smears. *Eur. J. Clin. Microbiol. Infect. Dis.*, **17**, 46–48.

Ross, J.D., McIver, A., Blakely, A. *et al.* (1995) Why do patients default from follow-up at a genitourinary clinic?: a multivariate analysis [see comments]. *Genitourin. Med.*, **71**, 393–395.

Schachter, J., Hill, E.C., King, E.B. *et al.* (1982) Chlamydia trachomatis and cervical neoplasia. *J. Am. Med. Assoc.*, **248**, 2134–2138.

Schachter, J., Stamm, W.E., Quinn, T.C. *et al.* (1994) Ligase chain reaction to detect Chlamydia trachomatis infection of the cervix. *J. Clin. Microbiol.*, **32**, 2540–2543.

Scholes, D., Stergachis, A., Heidrich, F.E. *et al.* (1996) Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. *N. Engl. J. Med.*, **334**, 1362–1366.

Seigel, D.G., Podgor, M.J. and Remaley, N.A. (1992) Acceptable values of kappa for comparison of two groups. *Am J. Epidemiol.*, **135**, 571–578.

Sung, H.Y., Kearney, K.A., Miller, M. *et al.* (2000) Papanicolaou smear history and diagnosis of invasive cervical carcinoma among members of a large prepaid health plan. *Cancer*, **88**, 2283–2289.

Vassilakos, P., de Marval, F., Munoz, M. *et al.* (1998a) Human papillomavirus (HPV) DNA assay as an adjunct to liquid-based Pap test in the diagnostic triage of women with an abnormal Pap smear. *Int. J. Gynaecol. Obstet.*, **61**, 45–50.

Vassilakos, P., Griffin, S., Megevand, E. and Campana, A. (1998b) CytoRich liquid-based cervical cytologic test. Screening results in a routine cytopathology service. *Acta Cytol.*, **42**, 198–202.

Vassilakos, P., Saurel, J., and Rondez, R. (1999) Direct-to-vial use of the AutoCyte PREP liquid-based preparation for cervical-vaginal specimens in three European laboratories. *Acta Cytol.*, **43**, 65–68.

Wanner, P., Raymond, L. and Bouchardy, C. (2000) Taux de participation au dépistage du cancer du col utérin d'après l'Enquête Suisse de santé conduite en 1997. *Bulletin Suisse du Cancer*, **2**, 86–89.

Warford, A., Chernesky, M. and Peterson, E. (1999) Laboratory diagnosis of Chlamydia trachomatis infections. *CUMITECH*, **19A**, 1–18.

Received on March 29, 2001; accepted on July 6, 2001