Clinical and laboratory study

COMPARISON OF ANTIGEN DETECTION ASSAYS AND SERUM ANTIBODY TESTS FOR DIAGNOSIS OF INFECTION WITH CHLAMYDIA TRACHOMATIS

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ABSTRACT

Objectives. Rapid antigen detection in patients with lower urinary tract infection caused by Chlamydia trachomatis by direct immunofluorescence and an enzyme-linked immunosorbent assay (ELISA) was compared with cell culture as well as polymerase chain reaction (PCR). Furthermore, the performance of two serum antibody ELISAs was compared with an immunoperoxidase test.

Methods. A total of 143 urethral, conjunctival, and pharyngeal swabs were investigated. McCoy cell culture and a molecular assay including PCR served as standard methods. An elementary body direct fluorescent-antibody assay (DFA) was performed on urethral and conjunctival specimens, and an ELISA antigen test was performed on urethral specimens. Serum samples of 49 patients were analyzed for IgA and IgG antibodies.

Results. Antigen ELISA testing of urethral specimens was comparable to culture and PCR concerning specificity and sensitivity. The antibody ELISAs were significantly better in the detection of IgA in comparison with the immunoperoxidase test.

Conclusions. The antigen ELISA was found to be the best rapid diagnostic assay and has sufficient specificity as well as sensitivity. PCR can serve as gold standard equivalent to cell culture. Serum antibody assays are only of value in clinical situations when antigen detection is not possible.

KEY WORDS

Chlamydia trachomatis, PCR, cell culture, antigen detection

INTRODUCTION

Clinical features of chlamydial infection include acute and chronic disorders as urethritis, cervicitis, endometritis, salpingitis, perihepatitis, and pelvic inflammatory disease (1-4). Sensitive, specific and rapid techniques for the diagnosis of infections produced by C. trachomatis are thus of great importance. Ascending endocervical infection is an important cause of infertility and ectopic pregnancy and may promote the transmission of the human immunodeficiency virus (5). Furthermore, C. trachomatis is a frequent cause of newborn and adult keratoconjunctivitis (6,7), and even newborn respiratory tract infection has been reported (8). Its possible role as a triggering agent in rheumatic diseases remains in discussion (9).

Culture on McCoy cell monolayers is considered the standard for detection of infectious elementary

bodies. Results of chlamydial isolation in McCoy cell culture are available after 48 - 72 hours. Molecular assays including PCR have been found to be a good alternative for the detection of C. trachomatis (10-12). However, development of specimens still needs more than four hours. More rapid techniques of chlamydial detection include use of a direct fluorescent-conjugated antibody staining (DFA), and an enzyme-linked immunosorbent assay (ELISA) (13-15). Serum antibody tests have been introduced to facilitate diagnosis in clinical situations without antigen detection, but only elevated titers are indicative of active chlamydial infection (16).

We previously observed that patients with urethritis caused by C. trachomatis have occasionally conjunctivitis at the same time (data not published). In the present study, standard detection of C. trachomatis was done with cell culture and PCR in urethral, pharyngeal and conjunctival specimens to evaluate concomitant infection. DFA was additionally performed on urethral and conjunctival specimens, antigen ELISA was done on urethral specimens. Serum IgA and IgG antibodies were measured with three different test systems to evaluate their performance in comparison with McCoy cell culture as well as PCR.

MATERIALS AND METHODS

Specimens

Seventy-three urethral specimens were collected from 50 women and 23 men with symptoms of urethritis attending the outpatients clinic of the Department of Dermatology of the Graz University Hospital. The mean age of the patients was 26 years (age range, 17 to 72 years). Three dacron-tipped swabs were obtained from the urethra and used in randomized order for DFA, for ELISA and for cell culture and the molecular assay, respectively. The swab for molecular assay and culture was immediately transferred to 1.5 ml of 2-SP medium and stored frozen at -70°C until assayed. Swabs were also obtained from conjunctivae (28 patients) and pharynx (42 patients). Venous blood was collected in 10 ml vials (Sarstedt, Nuembrecht, Germany) from 71 patients with lower urinary tract infection. The sera were centrifuged for 10 minutes at 3000 rpm and stored at -20°C until examination.

Cell culture assay and PCR

Cell culture and PCR were done as recently reported by Kessler et al (12). Shortly, shell vials containing McCoy cell monolayers (ATCC, Rockville, MD, USA) were used for culture. Staining was performed with a direct fluorescent monoclonal antibody stain (Syva, Palo Alto, CA, USA). The presence of one or more typical inclusions was considered a positive result.

For the molecular detection, the Amplicor PCR assay (Roche Molecular Systems, Branchburg, NJ, USA) was used. If the OD_{450} value was greater than 0,25 OD units, the sample was considered positive.

DFA and ELISA-test

For DFA, the Chlamydia trachomatis Direct Specimen Test (Syva, Palo Alto, CA) was used. Slides were prepared immediately after specimen collection. Within 1 h of collection, staining as well as mounting and reading were done in accordance with the manufacturer's advice. The presence of 5 or more chlamydial elementary bodies was needed to be considered positive.

The Chlamydia EIA (Pharmacia, Uppsala, Sweden) was done immediately after specimen collection. Specimens and controls were added to the wells of a microtitration strip within 15 minutes and processed according to the manufacturer's recommendations. Absorbance was measured at 405 nm. Values greater as the cut off value (mean absorbance value for three negative controls + 0.05) were considered positive.

Serum antibodies

The presence of serum antibodies of the IgA and IgG class was tested in 71 patients with indirect IPAzyme chlamydia immunoperoxidase test (Savyon, Beer-Sheva, Israel) with serum dilutions of 1:16 for IgA as well as 1:64 and 1:128 for IgG. In 49 patients, IgA and IgG antibodies were determined additionally with the SeroELISA test (Savyon, Beer-Sheva, Israel) and the rELISA test (medac, Hamburg, Germany). Both tests were performed according to the manufacturer's advice.

The SeroELISA tests employs the L_2 serovar broadly reacting antigen of C. trachomatis. In a first step, the antigenic material was bound in microtiter wells. Specific antibodies, if present in patient serum, bind to the attached antigen forming an antigen-antibody complex. Then, horseradish peroxidase conjugated anti-human IgG (gamma- or alpha-chain specific) was added. The peroxidase conjugated antibody binds to the antibody moiety of the antigen-antibody complex. In a third step, substrate was

added. It reacts with the peroxidase and changes its color to blue. After the enzymatic reaction was stopped, the absorbance was determined at 450 nm. Values greater than the cut off value (0,145 x [absorbance of positive control minus absorbance of negative control] plus absorbance of negative control) are considered positive.

The rELISA test is based on a fragment of the genus specific lipopolysaccharide in the outer membrane of elementary and reticulate bodies. The test principle is similar to the SeroELISA. Instead of horseradish, ABTS substrate is used as chromogen. The absorbance is determined at 405 nm. Each test is run with instrument blank, two negative controls, two positive controls and two borderline controls (human serum containing anti-chlamydial antibodies). Values greater than the cut off (mean of borderline controls) are considered positive.

Statistics

Sensitivities, specificities, prevalences (i.e. pretest odds of disease), odds (=likelihood) ratios for the diagnostic tests and post-test odds for disease were calculated using a 2x2 table and Bayes' theorem for all tests (17). Culture and PCR were taken as gold standard. Significance testing was done by using the chi-square analysis with Yates' correction for small numbers (18).

RESULTS

The positivity rate for lower urinal tract infection caused by Chlamydia trachomatis was 16,4% (12 from 73) defined as positive culture and positive PCR assay. Culture and PCR revealed identical results. The differences in the test performance between culture as well as PCR and ELISA were not significant ($p > 0.05$), whereas DFA differed significantly ($p < 0.05$). DFA had a sensitivity of 25%, and ELISA of 33%. Specifity was 98% in both tests (Table 1). Of 50 female patients 44 were negative for infections with Chlamydia trachomatis. In this group, Ureaplasma urealyticum was found in 10 patients, Candida albicans in 6, Gardnerella vaginalis in 3, double infections in 4, and no germs in 17 patients. Of 23 male patients, 17 were negative for Chlamydia trachomatis. In 5 of these, Ureaplasma urealyticum was detected, 2 were infected by Mycoplasma hominis, and 2 had double infections. In 7 men, no germ could be isolated. Thus, 24 patients (17 women and 7 men) could not be diagnostically cleared.

In conjunctival swabs, one female patient tested positive in both eyes with PCR and negative with culture and DFA. She was considered to have no eye infection, and no urethral germ could be identified. She had chlamydial antibodies of the IgG class, but no IgA antibodies. No pharyngeal infection could be detected with PCR or McCoy culture.

Three different antibody detection systems were compared. Seventy-one sera were tested with the IPAzyme, and 49 of them additionally with the other two ELISAs. Test performances were calculated with cell culture and PCR as reference methods. The results are shown in table 2 for IgA and IgG detection. Concerning IgA antibodies, IPAzyme and SeroELISA as well as IPAzyme and rELISA differed significantly ($p < 0.05$). However, SeroELISA and rELISA gave no significant difference.

In the detection of IgG antibodies, a significant difference between IPAzyme titer of 1:64 and SeroELISA was found ($p < 0.01$), but no statistical difference between IPAzyme titer of 1:64 and rELISA was found. The difference between SeroELISA and rELISA was also significant ($p < 0.02$). There was no statistical difference in the comparison of IPAzyme titre 1:128 with SeroELISA or rELISA, respectively. Taken together, both ELISAs performed better than the immunoperoxidase test. In the detection of IgG antibodies, the SeroELISA proved to be superior to the rELISA. There was no difference between the two ELISAs in the detection of IgA antibodies.

DISCUSSION

Detection of C. trachomatis by cultivation on cell monolayers is still considered the "gold standard". This method has the disadvantage of being a labor-intensive and time-consuming procedure. The difficulties in transportation of live organisms and low copy number in the specimens may additionally complicate detection (19). Therefore, PCR has recently been introduced for detection of C. trachomatis and found to be a good alternative to culture (10-12). For outpatient clinics, cell culture as well as PCR are not feasible. Culture needs at minimum 50 hours, and PCR can be performed within 5 hours. Stand-by tests like DFA and ELISA are easy to perform, but less sensitive and less specific than cell culture and PCR. Using DFA, results can be obtained within one hour after sampling. The antigen ELISA needs three hours. A common standard for antibody

Table 1: Comparison of PCR, DFA and ELISA with cell culture in 73 urethral specimens. McCoy cell culture results are taken as gold standard.

	PCR +	PCR -	DFA +	DFA -	ELISA +	ELISA -
culture +	12	0	3	9	4	8
culture -	0	61	1	60	1	60
sensitivity	100%		25%		33%	
specificity	100%		98%		98%	
PTD	∞	0	2.50	0.13	3.34	0.08

PCR = polymerase chain reaction; DFA= elementary body direct fluorescent-antibody assay; PTD= post test likelihood for disease

detection is not yet established. In this study, performance of commercially available kits was tested to evaluate the value of these tests in the spectrum of diagnostic tools.

Sensitivity and specificity describe the performance of a test. A clinician is interested to reassure or reject a diagnosis. To facilitate this decision, we calculated prevalence (pretest likelihood of disease),

Table 2: Comparison of serum antibody detection by indirect IPAzyme immunoperoxidase test, SeroELISA test and rELISA test with antigen detection by cell culture and PCR in urethral specimens.

	IPAzyme		SeroELISA		rELISA	
	Immunoglobulin A					
	+	-	+	-	+	-
antigen +	1	11	3	4	2	5
antigen-	2	57	5	37	7	35
sensitivity	8%		42%		28%	
specificity	96%		88%		83%	
PTD	0.41	0.19	0.51	0.08	0.24	0.11
	Immunoglobulin G					
	1:64 +	1:64 -	+	-	+	-
antigen +	6	6	2	5	3	4
antigen -	26	33	8	34	19	23
sensitivity	50%		28%		42%	
specificity	55%		80%		54%	
PTD	0.19	0.13	0.21	0.13	0.13	0.18

PCR = polymerase chain reaction;
DFA= elementary body direct fluorescent - antibody assay;
PTD= post test likelihoods for disease.

likelihood (odds) ratios for positive and negative test results and the post-test likelihood of disease (PTD). Prevalence multiplied with likelihood ratios gives post-test likelihoods of disease. PTD is related to a clinical question and is independent from sensitivity and specificity. On the other hand, sensitivity and specificity describe the quality of a test in a laboratory situation. With this approach, the clinical information content of the diagnostic tests is considerably increased. PTD help the clinician to decide whether an infection is caused by C. trachomatis or not (17). PCR and cell culture gave identical results in the investigation of urethral specimens. PCR is therefore a good alternative to culture and should be readily accepted as an equivalent "gold standard".

In comparison with culture and PCR, only ELISA antigen testing proved to be of sufficient sensitivity and specificity. ELISA sensitivity of 33% and DFA sensitivity of 25% were not very high in this study. This might be due to the fact that some patients came to the clinic after various antibiotic treatments initiated by their doctors and the lower sensitivity of these tests compared to the gold standard. The difference between positive PTD and negative PTD was greater in ELISA than in DFA. This may partially be due to test characteristics of the DFA test. It is a subjective method requiring trained staff. There is no possibility of automatization. Subjective influences as well as technical equipment of the fluorescent microscope influence the outcome. ELISA tests are ideal for routine work with larger sample sizes, because automatization is possible, but these tests need more time. Today, a "stand-by" diagnostic service can be offered with DFA, but at the cost of quality. We found ELISA to be more sensitive than DFA. As ELISA can also be performed as a stand-by service, it is the best alternative to PCR in smaller laboratories.

As there were no pharyngeal and conjunctival infections, an evaluation was not possible. Coincidental pharyngeal and conjunctival infections with Chlamydia trachomatis are obviously rare (16).

If antigen detection is not possible, antibody detection may give useful information to the clinician. Recently, new antibody tests have been developed to improve the diagnostic value either by measuring different immunoglobulin classes (20) or by using different antigens. The SeroELISA test is based on the L_2 serovar and employs both the major outer membrane protein (MOMP) and a lipopolysaccharide (LPS) as antigens to detect early group specific antibodies against Chlamydia trachomatis (MOMP) and late genus specific antibodies against Chlamydia psittaci, pneumoniae and trachomatis (LPS). The rELISA uses a recombinant genus specific LPS fragment as antigen and is designed to detect antibodies that arise earlier than anti-MOMP antibodies. These tests can not compete with antigen detection as shown by comparison of corresponding PTD values. Pattern and time course of antibody development do still not allow a diagnosis of acute chlamydial infection with sufficient confidence, although the performance of these newer tests has been improved compared to the IPAzyme test. Thus, despite better discrimination of the antibody ELISAs as shown by PTD values, they are still only of value in chronic disease without the possibility of antigen detection to support clinical management.

In summary, PCR is a good alternative to cell culture. Antigen detection with ELISA remains an alternative for stand-by service in outpatient clinics. Antibody testing maybe useful in clinical situations where the detection of the antigen is not possible, but can not compete with any antigen detection system.

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