The role of chlamydia genus-specific and species-specific IgG antibody testing in predicting tubal disease in subfertile women

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BACKGROUND: We evaluated whether measuring chlamydia genus- and species-specific immunoglobulin (Ig) G antibodies might improve the predictive value of C. trachomatis antibody testing (CAT) in screening for distal tubal pathology (DTP). METHODS: Serum of 313 subfertile women was tested for the presence of species-specific antibodies to C. trachomatis, C. pneumoniae and C. psittaci and genus-specific antibodies to chlamydia lipopolysaccharide (LPS). Only patients who had undergone a laparoscopy with tubal testing, to assess the grade of DTP, were included in this study. RESULTS: The presence of C. trachomatis antibodies was the only independent predictor for DTP. The predictive value of CAT for DTP could not be improved by adding test results of C. pneumoniae or LPS antibody testing. The role of C. psittaci could not be evaluated, due to the absence of C. psittaci-positive patients in our cohort. CONCLUSIONS: In spite of the high interspecies homology, C. pneumoniae does not contribute to the development of DTP. Anti-LPS antibodies, which are considered to be markers for ongoing infections, do not identify C. trachomatis-positive subfertile women who are at highest risk of DTP. The high prevalence of anti-LPS antibodies in C. trachomatis-positive subfertile women may suggest that C. trachomatis remains more active in the upper genital tract than currently is presumed.

Key words: chlamydia/genus-specific antibodies/screening/species-specific antibodies/tubal factor subfertility

Introduction

Chlamydia species which can cause infections in humans are C. pneumoniae, C. psittaci and C. trachomatis. C. pneumoniae is a widespread pathogen that causes respiratory tract infections, and is associated with asthma (Hahn et al., 1991) and cardiovascular disease (Saikku et al., 1988). The prevalence of immunoglobulin (Ig) G antibodies to C. pneumoniae in subfertile women ranges from 56 to 76%, regardless of the cause of subfertility (Freidank et al., 1995) and cardiovascular disease (Saikku et al., 1988). The prevalence of immunoglobulin (Ig) G antibodies to C. pneumoniae in subfertile women ranges from 56 to 76%, regardless of the cause of subfertility (Freidank et al., 1995) and cardiovascular disease (Saikku et al., 1988). The prevalence of immunoglobulin (Ig) G antibodies to C. pneumoniae in healthy males and females (63±74%) (Karvonen et al., 1994; Wong et al., 1999).

C. psittaci is most prevalent in birds (psittacosis), but can also infect humans and result in pneumonia. The prevalence of C. psittaci IgG antibodies in serum depends on population characteristics. A prevalence of 39% is reported in pigeon fanciers, a risk group for acquiring psittacosis (Bourke et al., 1992). In healthy males and females, as well as in patients attending a genitourinary clinic, the prevalence of IgG antibodies to C. psittaci is 0.1% (Moss et al., 1993; Wong et al., 1999).

The role of C. trachomatis in tubal factor subfertility is well established. The prevalence of C. trachomatis IgG antibodies is significantly higher in subfertile women with bilaterally occluded tubes (52–73%) as compared to subfertile women without tubal pathology (17–24%) (Freidank et al., 1995; Gijsen et al., 2001). Healthy (supposedly fertile) female controls have the lowest prevalence (9–10%) (Freidank et al., 1995).

A widely used screening method for tubal factor subfertility is chlamydia antibody testing (CAT) by measuring serum IgG antibodies to C. trachomatis. However, its predictive value is limited, due to false-positive and false-negative test results (Mol et al., 1997). The predictive value of CAT might be improved by using other serological markers for chlamydia-associated tubal damage.

First, it has been questioned whether C. pneumoniae and C. psittaci, following a primary C. trachomatis infection, may play a role in the development of tubal pathology. In a previous study, tubal factor subfertility seemed to be more common in subfertile women with IgG antibodies to both C. trachomatis
and C. pneumoniae (49%) as compared to those with antibodies to C. trachomatis only (30%), but the difference was not statistically significant (Gijsen et al., 2001). Based on the findings of our previous study, we hypothesize that other chlamydia species, besides C. trachomatis, may play a role in the development of tubal factor subfertility.

Furthermore, it has been assumed that repeated exposure to C. trachomatis (by reactivation or reinfection) is an important risk factor for the development of tubal damage (Grayston et al., 1985; Patton et al., 1994). A previous study suggested that anti-lipopolysaccharide (LPS) antibodies are indicators of ongoing chlamydia infections (Tuuminen et al., 2000). Therefore, our second hypothesis is that serum IgG antibodies to chlamydia LPS may be useful (as potential markers of ongoing C. trachomatis infections) in predicting the risk of distal tubal pathology.

In the present study, we used a commercially available micro-immunofluorescence (MIF) test to detect species-specific antibodies to C. trachomatis, C. pneumoniae and C. psittaci. A commercially available enzyme-linked immunosorbent assay (ELISA) was used for the detection of genus-specific antibodies to chlamydia LPS. We evaluated the prevalence of species-specific IgG antibodies to C. trachomatis, C. pneumoniae and C. psittaci respectively, as well as genus-specific IgG antibodies to chlamydia LPS in subfertile women who had undergone a laparoscopy with tubal testing. The serological data were correlated with the presence of distal tubal pathology at laparoscopy.

Materials and methods

The study was performed in subfertile women who entered our clinic between December 1990 and November 2000. As part of the fertility work-up, blood was drawn from all patients at their initial visit for CAT, using a MIF test (Biomerieux, The Netherlands). All spare sera were cryopreserved. Patients with a negative CAT and an otherwise normal fertility work-up underwent a hysterosalpingography (HSG) to evaluate the tubal status. If the HSG showed abnormalities, or if they did not conceive within 6 months after the HSG, a laparoscopy with tubal testing was performed. Patients with a positive CAT underwent a laparoscopy with tubal testing immediately after the fertility work-up. Only patients who had undergone a laparoscopy and tubal testing with Methylene Blue dye were included in the present study. Patients who had undergone previous pelvic surgery (except for an uneventful appendectomy or Caesarean section) were excluded.

For this study, the spare sera of the participating patients were thawed to perform a species-specific MIF test (AniLabsystems, Finland) and a Chlamydia LPS ELISA (Medac, Germany), as described below. In the present study, the MIF test by AniLabsystems was used to detect IgG antibodies to C. trachomatis, instead of the MIF test by Biomerieux, since the test by AniLabsystems was found to predict tubal pathology more accurately than the test by Biomerieux (Land et al., 2003).

Two independent investigators, who were unaware of the CAT results, scored 313 successive laparoscopy reports to assess the grade of distal tubal pathology. In this study, distal tubal pathology was defined as extensive peri-adnexal adhesions and/or distal occlusion of at least one tube (Land et al., 1998). Subfertile women without distal tubal pathology served as controls. The controls had an unexplained subfertility, partners with mild male factor subfertility, or proximal occlusion of at least one tube.

Serological methods

IgG antibodies to C. trachomatis, C. pneumoniae and C. psittaci were detected using the Chlamydia pneumoniae IgG MIF test (AniLabsystems). For this purpose, 10 µl of the serum was diluted eight times in phosphate-buffered saline (PBS) and incubated on the microscope slides dotted with three chlamydia antigens for 30 min at 37°C in a moist chamber. The slides were washed four times with PBS and twice with distilled water and incubated with goat anti-human IgG–fluorescein isothiocyanate conjugate for 30 min at 37°C. Again the slides were washed four times with PBS and twice with distilled water. Mounting fluid was added on the slides, and a cover slip was placed on the slides. Under the microscope the slides were read. All slides were evaluated independently by two readers. In case of disagreement, which was the case in ~10% of all slides, the judgement of a third reader was decisive. For a quantitative determination, serial dilutions in PBS were performed. In the present study, we considered the test results of each chlamydia species as a single test. For C. trachomatis and C. pneumoniae, the cut-off titre for a positive test was set at 32, according to the manufacturer’s instructions. According to the manufacturer’s instructions, LPS was still present on the C. psittaci elementary bodies of the MIF test, while the LPS activity on the C. trachomatis and C. pneumoniae elementary bodies had been reduced. Therefore, the fluorescence on the C. psittaci spot could be due to species-specific anti-C. psittaci antibodies or genus-specific anti-LPS antibodies. We considered the C. psittaci IgG antibodies positive when the IgG titre was ≥2-fold than the titre of IgG antibodies to C. trachomatis or C. pneumoniae, a commonly used definition in literature (Moss et al., 1993; Wong et al., 1999).

IgG antibodies to chlamydia LPS were detected using the Chlamydia IgG rELISA (Medac). For this purpose, sera were diluted 1:100 in PBS and tested in microplates coated with chlamydia-specific recombinant LPS fragments. The plates were incubated for 60 min at 37°C in a humid chamber. The plates were washed three times with 200 µl PBS and tapped dry. To each well, 50 µl of conjugate (goat anti-human IgG, horseradish peroxidase-conjugated) was added and the plates were incubated for 60 min at 37°C. Again the plates were washed three times with 200 µl PBS and tapped dry. To each well, 50 µl of tetramethylbenzidine substrate was added and the plates were incubated for 30 min at 37°C. Finally, 100 µl of 0.5 mol/l sulphuric acid was added to stop the colouring reaction. The optical density of the plates was measured in a spectrophotometer at 450 nm. Threshold indexes were calculated according to the manufacturer’s instructions. The threshold index for a positive test was 1.1.

Statistical methods

Characteristics of women with and without distal tubal pathology were compared using the Mann–Whitney U-test. For comparison of the prevalence of IgG antibodies to C. trachomatis, C. pneumoniae, C. psittaci and LPS in women with and without distal tubal pathology, the χ²-test was used. The association between chlamydia genus- and species-specific antibodies and the presence of distal tubal pathology at laparoscopy was calculated by a logistic regression analysis. The prognostic value of single testing as well as combined testing for distal tubal pathology was determined by calculating sensitivity, specificity, odds ratio (OR) and 95% confidence interval (CI). The bootstrap technique was used to test the difference between OR (Efron and Tibshirani, 1993). P < 0.05 was considered statistically significant.
Results
In 313 subfertile women, chlamydia IgG antibody titres in serum and laparoscopy results were available for analysis. At the start of the fertility work-up, the mean age of the women (30.6 and 31.2 years) and the mean duration of subfertility (2.4 and 2.3 years) did not differ significantly between women with and without distal tubal pathology. In total, there were 59 women (18.8%) who met the definition of distal tubal pathology, whereas 254 women (81.2%) did not have distal tubal pathology at laparoscopy. Of those 254 women without distal tubal pathology, 94.9% had patent tubes and 5.1% had proximal occlusion of at least one tube. Since proximal tubal occlusion is considered not to be associated with chlamydia disease, all 254 women without distal tubal pathology served as controls.

First, we evaluated all four tests (C. trachomatis, C. pneumoniae, C. psittaci and LPS) separately. Table I shows the prevalences of IgG antibodies to C. trachomatis, C. pneumoniae, C. psittaci and LPS in women with and without distal tubal pathology. The prevalence of species-specific IgG antibodies to C. trachomatis was significantly higher in women with distal tubal pathology (54.2%), as compared to women without distal tubal pathology (7.9%). Species-specific antibodies to C. pneumoniae were detected in 83.1% of women who had distal tubal disease, and in 72.8% of women without distal tubal disease. This difference was not statistically significant. No patients met the definition of a positive test due to the prevalence of IgG antibodies. Genus-specific anti-LPS antibodies were detectable in 62.7% of women with tubal pathology, and in 33.9% of women without tubal pathology (P < 0.0001).

Using a logistic regression model, the association between C. trachomatis IgG antibodies and the presence of distal tubal pathology was statistically significant (P < 0.0001). The presence of IgG antibodies to C. pneumoniae (P = 0.6) or LPS (P = 0.8) was not an independent predictor for distal tubal disease.

Secondly, the outcome of combined testing was evaluated. In combined testing, antibodies to C. trachomatis were measured in combination with antibodies to C. pneumoniae and/or LPS. The results are shown in Table II. The OR of C. trachomatis antibody testing was 13.9. The OR increased to 15.4 when both C. trachomatis and C. pneumoniae antibodies were detectable. When both C. trachomatis and LPS antibodies were present, the OR was 13.6. The highest OR (16.6) was reached in patients in whom all three antibodies were present. The increase in OR, when one or two more test results were added, as compared to testing for C. trachomatis antibodies only, was not statistically significant.

Discussion
We evaluated whether potential alternative serological markers for distal tubal pathology might improve the predictive value of C. trachomatis IgG antibodies. In the present study, serological test results were compared with the findings at laparoscopy. Therefore, only women who had undergone a laparoscopy, the reference standard in diagnosing tubal pathology, were included. This inclusion criterion will cause selection bias, which will influence the prevalence of tubal pathology, but which is hard to prevent in clinical studies. In our cohort, the prevalence of tubal pathology will be higher as compared to an unselected population. The prevalence of tubal pathology in our tertiary care population (18.8%), however, is comparable to findings reported from other tertiary care centres (Collins et al., 1995).

The first aim of this study was to evaluate the role of different chlamydia species in the development of distal tubal pathology, since there is a high interspecies homology in various chlamydia antigens, such as chlamydia heat shock protein 60 (CHSP60) (Kikuta et al., 1991) and LPS (Caldwell and Hitchcock, 1984). The potential role of C. pneumoniae or
C. psittaci infections in the development of tubal disease, following a primary C. trachomatis infection, could be explained by a chlamydia genus-specific auto-immune inflammatory response, leading to tissue damage. Such a mechanism has previously been suggested by Wick et al. (2001), who studied the role of C. pneumoniae in the development of atherosclerosis. The immune response to C. pneumoniae, a highly prevalent micro-organism, does not normally result in vascular damage. It is hypothesized that in the presence of a stressor (e.g. hypertension), the vascular endothelial cells express human heat shock protein 60 (hHSP60) on their surface, which may become a target for antibodies initially directed against the highly similar cHSP60. This may lead to destruction of the endothelial cells and the development of atherosclerotic lesions (Wick et al., 2001). Extrapolating this hypothesis to the development of tubal damage, we hypothesize that the basic condition for the development of tubal pathology is a primary (silent) C. trachomatis infection (stressor), leading to the expression of hHSP60 on the tubal epithelium. During an infection, genus-specific antibodies to cHSP60 are produced, which can cross-react with hHSP60 on the epithelial cells in the tubes, leading to epithelial damage and subsequently to tubal pathology. Since cHSP60 is genus-specific, the auto-immune response may be induced by all chlamydia species.

To evaluate the role of different chlamydia species in the development of distal tubal pathology, we first studied the prevalence of antibodies to the three chlamydia species in our cohort. In subfertile women with and without distal tubal pathology, the prevalences of antibodies to C. trachomatis, C. pneumoniae and C. psittaci (Table I) were comparable to the prevalences as reported earlier (Moss et al., 1993; Freidank et al., 1995; Wong et al., 1999; Gijsen et al., 2001).

The association between serum IgG antibodies to C. trachomatis and tubal pathology is commonly known (Punnonen et al., 1979; meta-analysis by Mol et al., 1997), and was confirmed in the present study. A significant additive role of C. pneumoniae in the development of distal tubal pathology could not be confirmed, and, in spite of enlargment of the cohort, the findings of our previous study (Gijsen et al., 2001) could not be confirmed. The role of C. psittaci infections could not be evaluated in the present study.

The second aim of this study was to evaluate genus-specific IgG antibodies to chlamydia LPS, as potential serological markers of repeated exposure to C. trachomatis. LPS is an outer membrane component shared by all three chlamydia species (Caldwell and Hitchcock, 1984), which has antigenic capacities comparable to the major outer membrane protein (MOMP). It has been reported previously that genus-specific anti-LPS antibodies increase rapidly in the early phases of infections, whereas more specific anti-MOMP antibodies are produced at a later stage (Ekman et al., 1993). It is assumed that repeated exposure to C. trachomatis is an important risk factor for the development of tubal damage (Grayston et al., 1985; Patton et al., 1994). Repeated exposure to pathogens causes repeated stimulation of the immune system, and subsequently may cause a continuously high level of anti-LPS antibodies. A study of Tuuminen et al. (2000) supports this hypothesis. Repeated stimulation might be caused by an endogenous reactivation of persistent C. trachomatis microorganisms or by an exogenous reinfection with C. trachomatis.

As shown in Table I, the prevalence of anti-LPS antibodies was significantly higher in women with distal tubal pathology (62.7%) as compared to women without distal tubal pathology (33.9%). Further analysis revealed a remarkable overlap between women with IgG antibodies to C. trachomatis and to LPS; in 92.3% of the women with C. trachomatis antibodies, anti-LPS antibodies were detectable (data not shown). However, the presence of anti-LPS antibodies was no independent predictor for tubal disease. The low OR of the LPS-only test (3.3) might be explained by the high prevalence of C. pneumoniae antibodies in our cohort, causing a positive LPS test in women who do not necessarily have C. trachomatis IgG antibodies and tubal pathology. We did not find a significant additive role of anti-LPS antibodies in predicting the risk of tubal disease.

The high prevalence of anti-LPS antibodies in subfertile women with distal tubal pathology (62.7%) is in agreement with findings in previous studies, in which other markers of ongoing infections were demonstrated in the upper genital tract of subfertile women with late sequelae of C. trachomatis (Gérard et al., 1998; Kinnunen et al., 2002). The high prevalence of anti-LPS antibodies in C. trachomatis-positive subfertile women without distal tubal pathology (33.9%), however, suggests that C. trachomatis may remain more active in the genital tract than is currently presumed. These women may also have viable micro-organisms in the upper genital tract, which may cause minimal tubal epithelial damage or silent endometritis, and may compromise their fertility, despite normal findings at laparoscopy.

In summary, we evaluated whether additional determination of IgG antibodies to C. pneumoniae, C. psittaci and chlamydia LPS might improve the predictive value of C. trachomatis IgG antibody testing in screening for tubal factor subfertility. Nonetheless, in spite of the high interspecies homology, C. pneumoniae does not seem to contribute to the development of distal tubal pathology. The role of C. psittaci cannot be evaluated, due to the absence of C. psittaci-positive patients in our cohort. Although anti-LPS antibodies are considered as markers for chronic inflammation, their presence is not useful in selecting a subset of C. trachomatis-positive subfertile women which is most likely to have tubal damage. The high prevalence of anti-LPS antibodies in our cohort suggests that C. trachomatis may remain more active in the upper genital tract than is currently presumed. C. trachomatis-positive subfertile women with anti-LPS antibodies, but without tubal disease, may have a mild chronic infection, which may compromise their fertility.

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References
Bourke SJ, Carrington D, Frew CE, McSharry CP and Boyd G (1992) A
comparison of the seroepidemiology of chlamydial infection in pigeon fanciers and farmers in the U.K. J Infect 25(Suppl 1),91–98.

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