**Waddlia chondrophila** and **Chlamydia trachomatis** antibodies in screening infertile women for tubal pathology

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**Abstract**

Since **Waddlia chondrophila** is closely related to **Chlamydia trachomatis**, we hypothesise that **W. chondrophila** may also be associated with tubal factor infertility (TFI) in women, a major complication of chronic **C. trachomatis** infection. Five hundred twenty serum samples were tested for anti-**Waddlia** antibodies by ELISA. Among the 520 investigated women, a total number of 142 (27.3%) has had laparoscopic diagnosis performed, and were either classified TFI positive or negative. Presence of high titres of **W. chondrophila** antibodies was linked to TFI ($p < 0.0001$; OR: 7.5; 95% CI: 3.3–17). Moreover, antibody positivity to both **W. chondrophila** and **C. trachomatis**-MOMP was strongly associated with TFI ($p < 0.0001$; OR: 21; 95% CI: 3.8–12E1). This association was much stronger than the statistical association of **C. trachomatis**-MOMP antibodies only ($p < 0.0001$; OR: 7.1; 95% CI: 3.7–14), suggesting that co-infection with **W. chondrophila** and **C. trachomatis** may lead to more severe reproductive sequelae and immune responses than single infection with either **Chlamydiales** members.

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**Keywords:** **Waddlia chondrophila; Chlamydia trachomatis; Tubal factor infertility; TFI; Screening**

1. **Introduction**

Damage to the Fallopian tubes, or tubal pathology, is a common cause of infertility in women. Tubal damage is thought to occur when pathogenic microorganisms, such as **Chlamydia trachomatis**, ascend from the lower genital tract and infect the tubes, inducing inflammation [1,2]. This may cause scarring of the Fallopian tubes, resulting in tubal factor infertility (TFI). It has recently been estimated that 45% (28%–62%) of confirmed TFI cases are caused by urogenital **C. trachomatis** infections [3]. The best available standard to diagnose TFI is laparoscopy. However, this invasive procedure is not suitable for screening [4]. In the Netherlands, the first means of screening for TFI is **C. trachomatis** IgG antibody testing (CAT) in serum: antibodies against **C. trachomatis** are detected in up to 80% of women who have TFI [5,6]. Depending on the risk for TFI based on CAT, a patient will undergo additional diagnostics such as hysterosalpingography (HSG) (in low risk CAT negative cases) or laparoscopy (in high risk CAT positive cases), an invasive surgical procedure not without risk of complications [5,6]. A major drawback of **C. trachomatis** serology to detect TFI is its limited positive predictive value of only 50%–60%. This implies that 50%–60% of **C. trachomatis** antibody positive women have TFI, while 40%–50% of **C. trachomatis** antibody positive women...
do not, and thus undergo unnecessary laparoscopies [7,8]. Other less known microorganisms capable of colonising the genital tract of women may also be responsible for the onset of TFI. Recently it has been shown that Waddlia chondrophila, another member of the Chlamydiales order, is capable of causing adverse pregnancy outcomes in ruminants and human [9–11]. W. chondrophila DNA was isolated from upper genital tract tissue and antibodies were detected in the serum of a woman who had a miscarriage. The authors hypothesise that W. chondrophila may potentially mimic fetal antigens suggesting its potential pathogenicity in the upper genital tract [9,12]. Seroprevalence of W. chondrophila has also been associated with ectopic pregnancy in a group of Vietnamese patients [11]. The pathogenicity of W. chondrophila is further supported by the significant growth in various human cell lines including endometrial cells [13]. Moreover, W. chondrophila was shown to exhibit many metabolic activities, such as enzymes for lipid metabolism [14], and express an extended family of OmpA proteins acting as adhesins [15], likely explaining its broader host range. Thus, the aim of this study was to investigate whether W. chondrophila and C. trachomatis may increase the risk for TFI.

For this purpose, we tested serum samples for W. chondrophila and C. trachomatis antibodies from women with and without tubal pathology and looked for a possible association between tubal factor infertility and presence of antibodies against these bacteria.

2. Methods

2.1. TFI definition

TFI positivity is defined as extensive periadnexal adhesions and/or distal occlusion of at least one tube [4]. Severe TFI (sTFI) is defined as bilateral extensive periadnexal adhesions and/or distal occlusion, and is a subgroup of the total TFI positive group.

Women with any peritubal and/or periovian adhesions, or proximal occlusion of at least one tube are considered an intermediate group (neither TFI positive nor TFI negative).

TFI negativity is defined as HSG and/or laparoscopy confirmed TFI negative women.

2.2. Sample collection

Five hundred fifty-seven serum samples were selected, derived from women attending the fertility clinic of the University Medical Center Groningen, the Netherlands, meeting the inclusion criteria (CAT result available, laparoscopic and/or HSG data available). Of the 557 serum samples, 37 were excluded because they were in the intermediate group. Of the 520 serum samples, 457 (87.9%) were CAT negative and 63 (12.1%) were positive (pELISA, Medac Diagnostika mbH, Hamburg, Germany). A total number of 142 (27.3%) women had laparoscopic diagnosis performed and were classified as TFI positive or TFI negative. In total, 402 (77.3%) CAT negative women had HSG performed, 79 CAT negative women had laparoscopy performed (17.3%). Fig. 1 is a flowchart summarising the exclusion criteria and subgroups used for analyses.

2.3. Ethical approval

The act “Medical Research Involving Human Subjects” (WMO, Dutch Law), states that anonymous spare human materials and data may be used for research purposes after patients have been informed about this possible use and they have had the opportunity to object. All patients participating in the present study had not objected and therefore no ethical approval is required (MEC Letter reference: # 10.17.0046).

2.4. Detection of antibodies against W. chondrophila

Enzyme linked immunosorbent assays (ELISA) were used to detect antibodies in serum against W. chondrophila, as described by Lienard et al. [16]. Optical densities (OD) were measured with an ELISA Multiskan ascent reader (Thermo scientific, Zurich, Switzerland) at 492 nm as reference. Experiment was performed in duplicate. Sera from a previous study were included as reference to calculate ROC curves and cut-off levels for positivity, negativity, and grey zone [16]. The cut-off values for seropositivity for the first and second ELISA were 0.164 and 0.073, respectively.

2.5. Statistical analyses

Descriptive statistics were performed and presented as numbers (%) or median (range). Categorical data were
compared between groups using Chi-square test and Fisher's Exact test when appropriate and using the Mann–Whitney test for continuous data. Risk factors were described as Odds ratio (OR) with 95% confidence interval (CI). P-values < 0.05 were considered statistically significant, 0.05 < p < 0.1 was considered a statistical trend. Analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of study population

Median age of the study population was 32 years (18–41 years). Median age for women who underwent HSG was 33 years (21–41 years) and did not differ significantly from median age of women who underwent laparoscopy: 31 years (18–40 years; Mann–Whitney U p = 0.1). In this study, the seroprevalence for C. trachomatis and W. chondrophila is 12.1% and 45.5%, respectively. Table 1 summarises the seroprevalence of C. trachomatis and W. chondrophila in the study population: 48 women had laparoscopy confirmed TFI, of which 10 (20.8%) had bilateral TFI. An additional cut-off for W. chondrophila to test for TFI was determined at an OD of 0.250; if a sample had at least one OD-value > 0.250, the sample was considered highly positive.

3.2. Analyses of antibody distribution

C. trachomatis antibodies were significantly more often present in women suffering from TFI compared to the non TFI group (p < 0.0001; OR: 7.1; 95% CI: 3.7–14), as well as for sTFI compared to the non TFI group (p < 0.0001; OR: 40; 95% CI: 8.2–19E1). No differences in antibody distribution were observed between the non TFI and TFI group for W. chondrophila (p = 0.65), nor for the non TFI and the most severe TFI group for W. chondrophila (p = 1). However, high titres of W. chondrophila antibodies were significantly associated with TFI (p < 0.0001; OR: 7.5; 95% CI: 3.3–17) and sTFI (p < 0.0001; OR: 25; 95% CI: 6.7–95). Seropositivity for both microorganisms was more often present in the TFI group as compared to the non TFI group (p = 0.0007; OR: 5.0; 95% CI: 2.1–12) and more often present in the sTFI group as compared to the non TFI group (p = 0.001; OR: 14.3; 95% CI: 3.8–55). In addition, seropositivity for both microorganisms with a high W. chondrophila cut-off is highly associated with TFI (p < 0.0001; OR: 21; 95% CI: 3.8–12E1) and sTFI (p < 0.0001; OR: 16E1; 95% CI: 24–10E2).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>CT (%)</th>
<th>WC (%)</th>
<th>WC-H (%)</th>
<th>Both (%)</th>
<th>Both-H (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFI +</td>
<td>48 (100)</td>
<td>20 (41.7)</td>
<td>20 (41.7)</td>
<td>11 (22.9)</td>
<td>9 (18.8)</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>sTFI +</td>
<td>10 (20.8)</td>
<td>8 (80.0)</td>
<td>5 (50.0)</td>
<td>5 (50.0)</td>
<td>4 (40.0)</td>
<td>4 (40.0)</td>
</tr>
<tr>
<td>TFI -</td>
<td>472 (100)</td>
<td>43 (9.1)</td>
<td>217 (46.0)</td>
<td>18 (3.8)</td>
<td>21 (4.5)</td>
<td>2 (0.4)</td>
</tr>
</tbody>
</table>

TFI: tubal factor infertility; tTFI: total TFI group; sTFI: severe (bilateral) TFI; +: positive; -: negative; CT: C. trachomatis; WC: W. chondrophila; WC-H: W. chondrophila cut-off 0.25; Both: seropositivity for both C. trachomatis and W. chondrophila; Both-H: seropositivity for both C. trachomatis and W. chondrophila cut-off 0.25.

4. Discussion

This is the first study investigating seroprevalence of W. chondrophila in subfertile women in the Netherlands, and identifying an association of antibodies against W. chondrophila with TFI. We observed a high prevalence in our study population for W. chondrophila (45.5%). A study performed by Baud et al. showed a W. chondrophila seroprevalence of 33% in a group of English women suffering from recurrent miscarriages [9]. They observed an association between contact with animals and positivity for W. chondrophila antibodies, raising the hypothesis of a possible zoonotic potential of the pathogen. However, they were not able to confirm this hypothesis in a later study performed in Switzerland [12]. Although we do not have data on patients living in contact with animals, this may be a possible route of transmission, especially considering the broad range of hosts for W. chondrophila [17].

We did not observe statistical significant differences in antibody distribution for W. chondrophila when comparing the TFI group to the non TFI group. However, high titres of W. chondrophila antibodies are associated with TFI. As expected, we did observe a strong association between C. trachomatis antibodies and tubal pathology. We observed a higher W. chondrophila seroprevalence in the severe TFI group compared to the non TFI group.

An initial C. trachomatis infection may not necessarily induce TFI, however, it has been suggested that reinfection with a Chlamydia spp. like C. pneumoniae may induce a booster immune response, causing more tubal pathology if C. trachomatis is or was also present [18]. It is not unlikely that a similar process may damage the Fallopian tubes after (re) infection with W. chondrophila, but more research is necessary to confirm this hypothesis.

Seroprevalence to both C. trachomatis and W. chondrophila (high titres) was significantly associated with TFI. Only 0.42% of the non TFI controls had antibodies against both microorganisms, while 8.3% of the TFI patients (40% of bilateral TFI patients) carried both antibodies. This result indicates that presence of antibodies directed against both microorganisms is highly specific for TFI, potentially having clinical implications and increasing specificity for serology screening in patients at risk for TFI.

Cross-reactivity with other pathogens seems unlikely as an explanation for our results: earlier studies have clearly shown that W. chondrophila did not react with monoclonal and polyclonal antibodies against other intracellular bacteria, such as C. pneumoniae, which might induce a similar immune response.

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as Rickettsia, Coxiella, Anaplasma, Wolbachia, Para-
chlamydia, or Chlamydia spp. [19–22].

This study has some limitations: first, in the current study
the decision to perform laparoscopy was based on the C.
trachomatis-MOMP antibody response and the clinical pa-
rameters of the patient. This patient selection diminishes
the potential association between W. chondrophila and tubal
pathology as assessed by laparoscopy. Currently, we are including and analysing
a new study population to confirm our results. Second, the W. chondrophila ELISA is not commercially
available currently, so large-scale application is currently not yet possible.

To our knowledge, this is the first study identifying an
association between antibodies against W. chondrophila and TFI.
We have shown that carrying antibodies against C. trachomatis
and W. chondrophila is a very specific marker for TFI. Further
research is warranted to investigate the role of W. chon-
drophila in development of tubal pathology, and the underlying
mechanism at cell and molecular level.

Conflict of interest

The authors declare that they have no conflict of interest.

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