Microbes and Infection 17 (2015) 745–748

www.elsevier.com/locate/micinf

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

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Received 23 June 2015; accepted 14 September 2015
Available online 30 September 2015

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.

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

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http://dx.doi.org/10.1016/j.micinf.2015.09.019
1286-4579/

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* is associated with TFI, and whether co-infection with *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 periovarian 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 against 650 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

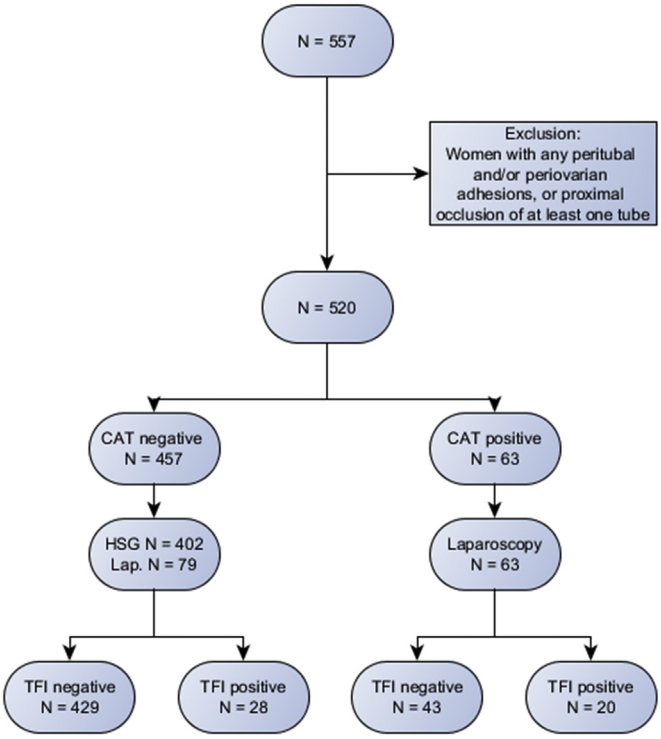

Fig. 1. Flowchart summarizing exclusion criteria and subgroups.