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What Can Serology Tell Us About the Burden of Infertility in Women Caused by Chlamydia?

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Abstract

Chlamydia trachomatis (CT) causes pelvic inflammatory disease, which may result in tubal factor infertility (TFI) in women. Serologic assays may be used to determine the proportion of women with and without TFI who have had previous CT infection and to generate estimates of infertility attributable to chlamydia. Unfortunately, most existing CT serologic assays are challenged by low sensitivity and, sometimes, specificity for prior CT infection; however, they are currently the only available tests available to detect prior CT infection. Modeling methods such as finite mixture modeling may be a useful adjunct to quantitative serologic data to obtain better estimates of CT-related infertility. In this article, we review CT serological assays, including the use of antigens preferentially expressed during upper genital tract infection, and suggest future research directions. These methodologic improvements, coupled with creation of new biomarkers for previous CT infection, should improve our understanding of chlamydia's contribution to female infertility.

Keywords

Infertility; chlamydia; serology; antibody; diagnosis

Chlamydia trachomatis (CT) is the most common sexually transmitted bacterium in the world [1]. In CT-infected women who go untreated, approximately 17% develop pelvic inflammatory disease (PID), inflammation of upper genital tract structures, and this may

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result in chronic sequelae, including ectopic pregnancy and tubal factor infertility (TFI) [2]. In the United Kingdom, it has been estimated that CT causes approximately 35% of PID cases in women <25 years of age and 20% in aged 16–44 years [2, 3]. Other PID-associated pathogens include *Neisseria gonorrhoeae, Mycoplasma genitalium*, and bacterial vaginosis–associated bacteria [4]. The majority of symptomatic PID cases have no pathogen identified [2, 4, 5]. Furthermore, many PID cases remain undiagnosed due to atypical or absent symptoms [2-4]. There is good evidence from a longitudinal cohort study of 1884 women with PID that the persistence of fallopian tube pathology (adhesions/scarring) can cause infertility in women—TFI [5]. Only women with PID who have macroscopic fallopian tube inflammation (salpingitis) are at risk of TFI, which is present in some but not all diagnosed cases of PID [2, 5]. A dose-response relationship has been observed with more severe macroscopic salpingitis and more PID episodes leading to a greater risk for adverse reproductive outcomes, especially for CT infection [2, 5, 6]. There is also evidence that upper genital tract CT infection may also impair fertility in the absence of tubal pathology; this is reviewed elsewhere in this supplement by Horner et al [7-9].

DETERMINING THE ATTRIBUTABLE FRACTION OF INFERTILITY DUE TO CT

CT infection may resolve spontaneously (ie, immune-mediated clearance without treatment) with an estimated average duration of asymptomatic infection of 16 months in women [2, 10]. Most women present for infertility evaluation after age 30 years, likely several years after their inciting infection, given that most CT infections occur in women aged <25 years [11, 12]; thus their inciting infection would no longer be detected by nucleic acid amplification testing or other tests for active urogenital infection [2, 13, 14]. At this time, serological evaluation for serum antibodies to CT, which can be maintained in sera for >12 years after infection [15, 16], is the only available means for determining prior CT infection. While serology can be used to examine the strength of the association of CT infection with infertility in women, it is confounded by association of CT infection with other infections that cause PID and is thus not in itself proof of causality [2, 4, 14, 17]. However, there is good evidence that upper genital tract CT infection can elicit a significant inflammatory response, which may result in TFI [2, 18]. This review examines the potential for using serology to estimate the attributable fraction of infertility that is due to CT [2, 17].

TRADITIONAL CT SEROLOGICAL ASSAYS

CT microimmunofluorescence (MIF), once considered the “gold standard” assay for CT serological evaluation, uses CT elementary bodies (EBs), which makes it labor-intensive. Reading of MIF results is subjective, which can negatively impact its sensitivity and specificity. The whole immunofluorescence (WIF) assay uses both CT EBs and reticulate bodies, with the majority of CT antigens being expressed [19]. Not only does it suffer from similar methodological issues as MIF, it is also not commercially available. Furthermore, because they use the whole CT organism, there is potential for specificity to be further impacted by antibodies that cross-react with proteins of *Chlamydia pneumoniae*, another *Chlamydia* species that is a common respiratory tract pathogen in humans and whose

genomes contain many similar genes as that of CT [19, 20]. Enzyme-linked immunosorbent assays (ELISAs) using CT peptides subsequently replaced MIF because they were less labor-intensive, did not require subjective interpretation of results, and could use peptides of CT proteins that would not elicit cross-reactive antibodies. The primary CT protein used for peptides in CT ELISAs is the major outer membrane protein (MOMP). There are 3 commercially available CT MOMP peptide-based ELISAs that have been used in evaluating CT-associated infertility: Medac pELISA, Labsystems CT enzyme immunoassay, and Savyon Sero-CT. The sensitivity of these ELISAs for detecting laparoscopy-confirmed TFI in infertile women ranges from 24% to 57%, lower than that for detecting women with uncomplicated urogenital infection (68%–75%) [21-23]. The low sensitivity reflects that there are other TFI etiologies besides CT, that some CT-infected humans may not mount strong antibody responses to MOMP peptides, and that antibody responses to MOMP peptides may be short-lived [16]. These studies were published >15 years ago.

In the last 10 years, a CT ELISA based on a mixture of CT EB serovars has been studied as a serological tool for evaluating CT outcomes. One study demonstrated that the CT EB ELISA had a higher sensitivity than the MOMP-based Medac pELISA for detecting CT antibodies in women with current uncomplicated urogenital CT infection (90% vs 73%, respectively) [24]. This EB ELISA measured immunoglobulin G (IgG) subclass-specific responses IgG1 and IgG3 (instead of total IgG, which causes high background reactivity) and responses did not appear to be confounded by *C. pneumoniae* cross-reactivity; the longer-lived IgG1 response was stable at 6 months after initial measurement. The CT EB ELISA was then used to evaluate population attributable fraction (PAF) (ie, the fraction of all cases) of TFI due to CT in a sample of US women (2012–2015) with TFI (cases) and women with patent tubes who had infertility due to non-TFI etiologies (controls) [25]. The TFI diagnosis for primary analyses was based on hysterosalpingogram findings; however, there was a subanalysis evaluating women in which laparoscopy was used to diagnose TFI. The primary analysis did not demonstrate an independent association between CT seropositivity and TFI, and the PAF of TFI due to CT ranged from 11% among non-black women to 15% among black women. This PAF estimate was much lower than 2 previous modeling estimates: One used serology that reported 45% of TFI was due to CT in infertile women from the Netherlands (1991–2002), and the other estimated 29% using multiparameter evidence synthesis of all available data from the United Kingdom in the early 2000s [2, 17, 23]. One potential explanation for the lack of association with TFI and low CT PAF estimates in the US cohort was that there was a high CT seropositivity among infertile women with patent tubes, which was particularly striking among black participants (80%), indicating that EB ELISA seropositivity is likely not able to distinguish prior uncomplicated CT infection from CT infection leading to infertility [23, 25]. The subanalysis using laparoscopy-confirmed TFI did show an association with CT seropositivity in non-black participants; however, PAF estimates were still low. Because the study did not evaluate fertile women, it is highly likely that some non-TFI infertile controls had infertility due to CT infection, which could have affected both the association and PAF measures. Hysterosalpingography is imperfect at identifying infertile women with TFI, with a sensitivity and specificity of 53% and 87% for any tubal pathology [26]. There is also

evidence that infertility caused by CT may not be always be due to TFI [7-9]. This is reviewed by Horner et al elsewhere in this supplement [7].

Thus, traditional CT serological assays likely have limited utility in evaluating the contribution of CT to infertility either because of low sensitivity and/or inability to distinguish uncomplicated vs complicated infection. Furthermore, earlier study designs that did not include a fertile group also probably had limited ability to evaluate PAF of infertility due to CT, particularly when laparoscopy was not used for evaluating TFI. Finally, derivation of the CT PAF from case-control studies assumes the association determined using serology is causal [27]. As mentioned above, there are other sexually transmitted pathogens that cause PID besides CT (none which have good serological assays available) and thus, estimates of CT PAF for TFI could be confounded by exposure to other infectious etiologies of TFI [2, 14].

ELISAS BASED ON RECOMBINANT CT PROTEINS

Immunoproteomic approaches have been utilized to identify CT proteins that may be more specific for TFI and thus could serve as biomarkers for CT-associated TFI evaluation. A previous study used a CT proteome array and compared antibody profiles in women with TFI, fertile women, and women with current CT infection [28]. Of 908 CT antigens studied, 13 were recognized by more than half of women with TFI, fertility, or current CT infection; of these antigens, the one recognized at the highest frequency (93%) was the plasmid-encoded pCT03 protein (CT Pgp3). There were 4 CT antigens that distinguished TFI vs fertility with 63% sensitivity and 100% specificity but still were recognized by women with current CT infection: heat shock protein 60 kD (HSP60), CT376, CT557, and CT443 (CT OmcB). CT443 was recognized in the TFI group at the highest frequency (88%) and intensity.

Studies have investigated performance of ELISAs incorporating CT OmcB, HSP, or Pgp3. In a recent study evaluating CT OmcB ELISA performance in women with current uncomplicated CT infection, the sensitivity was 79.3%, comparable to earlier MOMP-peptide based ELISAs; the assay had a high specificity [29]. However, the assay likely has low utility for CT TFI evaluation because the magnitude of the OmcB IgG1 antibody response declined significantly within 6 months after infection treatment [29]. Antibodies to HSP60 have been associated with CT TFI in the majority of studies undertaken, but the association is weak [28, 30-33]. HSPs are among the most conserved proteins in phylogeny, and although CT HSP60 is an immunodominant CT antigen, antibodies to other microorganism and human HSP60 will cross-react with CT HSP60 assays [18, 34]. Two Pgp3 ELISAs, an indirect and a double antigen, have been evaluated using sera from CT nucleic acid amplification test–positive women and controls who were likely CT naive [15, 35]. Pgp3 has not been found in human *C. pneumoniae* isolates and antibody to Pgp3 does not cross-react with *C. pneumoniae* proteins [15, 35]. The respective sensitivities for the indirect and double-antigen Pgp3 assays to detect previously diagnosed CT infection were 73.8% (95% confidence interval [CI], 66.5%–79.9%) and 82.9% (95% CI, 77.0%–88.8%), with specificities of 97.6% (95% CI, 96.2%–98.6%) and 97.8% (95% CI, 96.5%–99.1%) [15, 35]. Although the magnitude of the antibody response declines over time since

infection, a loss in sensitivity was only observed with the indirect ELISA but not the double-antigen assay [16, 36]. More recently, higher Pgp3 absorbance using an indirect ELISA was reported to be associated with CT TFI compared to pregnant controls in Australian women and chlamydial subfertile women in Samoa compared with fertile or subfertile for other reasons, but not when compared to women with current CT infection in either population [37]. The study concluded that although the Pgp3 assay is effective for seroepidemiological analysis of burden of CT infection, it could not be used for evaluating CT-associated infertility. Thus, although the double antigen Pgp3 assay is more sensitive than the indirect ELISA and less likely to lose sensitivity over time since infection, it is also not likely to be useful in accurately determining the PAF of TFI attributable to CT as it cannot distinguish between uncomplicated CT infection vs infection resulting in TFI.

Antibodies to several other proteins/antigens have been associated with CT infection sequelae in women, and there is evidence that some are preferentially recognized in women who have had marked inflammatory response as a result of upper genital tract CT infection. Huston et al observed no association with antibodies to recombinant serine protease HtrA (involved in CT persistence in vitro) based on a western blot, but Rantsi et al reported an association of HtrA and TroA (the latter also involved in CT persistence in vitro) with CT-related TFI based on an ELISA [38, 39]. It thus remains to be confirmed that an ELISA using CT antigen(s) can be developed and applied to accurately determine the PAF of TFI attributable to CT.

FINITE MIXTURE MODELING INCORPORATING ANTIBODY TITERS

An alternative and potentially complimentary approach to developing a CT antigen ELISA that is sensitive and specific for CT-associated TFI is to use finite mixture modeling. This uses antibody titer distribution to estimate the population excess fraction (PEF) of infertility attributable to CT, which is essentially similar to PAF [27]. Finite mixture models are used when a distribution (in this case, distribution of serum antibody titers) is considered to be a mixture of several components (eg, “positives” and “negatives”) and where there is an interest in estimating the proportion of serum samples in diseased and healthy populations for each component [27, 40]. It is based on the principle that women at higher risk of reproductive damage following CT infection are more likely to have higher CT loads with a greater inflammatory response and be CT seropositive and are more likely to have particularly higher titers than CT antibody-positive controls [14, 18, 41-45]. This approach cannot be used to diagnose CT TFI [14, 44].

By attributing the causal mechanisms for TFI to differences between cases and controls in specific components of titer distributions, rather than differences in overall seropositivity, the mixture modeling approach reduces the extent to which PEF estimates are vulnerable to confounding, although it does not eliminate it [14]. Ades et al applied finite mixture modeling using CT WIF antibody titer to 434 case women with laparoscopy-confirmed TFI and 573 women with non-TFI infertility [14, 44]. A 4-component overlapping antibody titer distribution was observed (CT-negative and CT+, CT++, or CT+++). The CT+++ distribution (ie, the highest titers), occurred only in TFI cases, suggesting that this did indeed represent a causal mechanism, which would be consistent with scarring and adhesions

being more likely to persist when a greater inflammatory response occurs. This CT+++ distribution represented 28% of cases. This was considered the lower bound estimate for CT PEF for TFI. The CT-negative distribution represented CT-unexposed women. The next highest distribution, CT+, was believed to represent those exposed at the lower genital tract only. Uncomplicated urogenital CT infection in women is often asymptomatic and associated with a lower CT load, which is a weaker antigenic stimulus for generating CT antibodies [14, 41]. The next highest distribution, CT++, was also observed in both cases (29.4%) and controls (6.5%). Ades et al speculated that these women without TFI might represent women with previous upper genital tract infection in whom inflammation resolved, either following treatment or spontaneously, without causing tubal damage [14]. However, as a recent lower genital tract infection is associated with a higher antibody titer and repeated uncomplicated urogenital can maintain such a titer, it is also possible that this distribution could also include women with recent lower genital tract CT infections or repeat infections in both cases and controls [14, 16, 36, 37]. A further potential confounder is that the excess CT++ seen in cases could simply be due to increased exposure to CT among women with TFI that was in fact caused by other sexually transmitted pathogens [14]. Understanding what causes this excess is further complemented by the recent hypothesis from Horner et al, who argue that it is also likely that previous upper genital tract CT infection as a result of epigenetic epithelial-to-mesenchymal transition may pre-dispose women to developing PID from other microorganisms [7]. Including the excess CT++ TFI women would give an upper bound estimate of 46.8% for the PEF attributable to CT for TFI, when both CT+++ and CT++ components are considered, since it ascribes the entire excess CT++ in cases to a causal mechanism rather than being partly or wholly the result of confounding [14]. The methodological advantages of using finite mixture modeling were highlighted by comparing the PEF using this approach with that using the standard PEF formula, which determined the PEF to be between 65.4% and 71.3% depending on the estimated proportion of the population ever infected with CT [14].

The WIF assay is not available commercially and is technically both time consuming and demanding, and therefore not suitable for widespread use. Another approach would be to investigate whether finite mixture modeling can be applied to antibody titer distribution of multiple individual CT antigens or combinations of antigens associated with both upper genital tract and lower genital tract infection including Pgp3, in order to estimate the CT PEF for infertility. This would also potentially increase our understanding of how repeat infection and effect of time since treatment or spontaneous clearance effects antibody titer distributions in women with and without TFI and how this impacts on estimating the CT PEF for infertility [14, 40]. It is certainly possible that such an approach might identify additional antibody distributions consistent with current and/or repeat lower genital tract infection. The ELISA format only has a limited quantitative absorbance range and may not be suitable to use with finite mixture modeling. The Luminex fluorescent bead-based assay, in which the median fluorescence intensity has a large dynamic range, or an immunoblot assay, if measured optically, merit further investigation [28, 31-33, 38, 46].

SUMMARY AND FUTURE DIRECTIONS

Using a CT seropositivity measure determined by current CT serological assays is insufficient to obtain an accurate estimate of PAF/PEF for CT-associated TFI. CT antibody assays for antigens preferentially expressed during upper genital tract infection are beginning to be evaluated. However, accurately determining such an association may be confounded by several factors, including other infections that may cause TFI, not all *Chlamydia* antigens being CT specific, and the possibility that antibodies to some of these CT antigens may also be associated with uncomplicated lower genital tract infection. Finite mixture modeling, which uses antibody titer distribution to estimate PEF of TFI attributable to CT, offers a potential future solution as it reduces the extent to which PAF/PEF estimates are vulnerable to confounding, although it does not eliminate it. While the WIF assay has been used to demonstrate how finite mixture modeling can be used to estimate PAF/PEF for TFI attributable to CT, it also has limitations. Future studies investigating CT antigen(s) associated with upper and lower genital tract infection, including Pgp3, should use assays capable of providing a quantitative antibody response measure. Although hysterosalpingography is inferior to laparoscopy for determining TFI status, accurate estimates of hysterosalpingography performance could enable PAF/PEF estimates to be adjusted for this uncertainty. Finally, there is evidence that some women with macroscopically normal fallopian tubes may be infertile following upper genital tract CT infection, and the underlying mechanism is uncertain. Including fertile women in future case-control studies would therefore be important when estimating PAF/PEF of TFI attributable to CT.

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