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Chlamydia trachomatis-associated tubal factor subfertility: immunogenetic aspects and serological screening

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***Chlamydia (C.) trachomatis* female genital tract infections usually remain asymptomatic and untreated. Therefore, an adequate immune response, rather than antibiotic treatment, is essential to clear the pathogen. Most women will effectively clear *C. trachomatis* infections, but some will have persistent *C. trachomatis* infections, which may ascend to the upper genital tract and increase the risk of tubal factor subfertility. Pattern recognition receptors (PRRs) of the toll-like receptor (TLR) and nucleotide-binding oligomerization domain (NOD) families recognize *C. trachomatis* and initiate the immune response. Host immune factors are determinants of the course of *C. trachomatis* infections. Genetic variations in *TLR* and *NOD* genes may affect receptor function, leading to inadequate recognition of *C. trachomatis*, an inadequate immune response, and consequently an increased risk of persistence and late sequelae. For the risk assessment of tubal pathology in subfertile women, *C. trachomatis* immunoglobulin (Ig) G antibody testing (CAT) in serum is widely used. A positive CAT is indicative of a previous infection but not of a persistent infection. Measuring serological markers of persistence, of which C-reactive protein (CRP) seems promising, in CAT-positive women may identify a subgroup of subfertile women with persistent *C. trachomatis* infections and the highest risk of tubal pathology.**

Key words: *Chlamydia trachomatis*/immunogenetics/serological markers/tubal factor subfertility

Introduction

A large inter-patient variability exists in the course and outcome of a *C. trachomatis* infection. Some women clear the infection adequately without developing tissue damage, whereas others get a persistent infection, which increases the risk of tubal damage and tubal factor subfertility. The course and outcome of infectious diseases are generally determined by virulence factors of the pathogen, environmental factors and host immune factors. Regarding *C. trachomatis* female genital tract infections, pattern recognition receptors (PRRs) of the innate immune system are suggested to be involved in clearance of the infection. Genetic variations in PPRs may contribute to persistence, thereby increasing the risk of tubal pathology.

A better understanding of the role of persistent *C. trachomatis* infections in tubal factor subfertility may be useful in optimizing the fertility work-up by incorporating screening tests for persistent *C. trachomatis* infections, aiming to accurately estimate the risk of persistence and identify those women who are at highest risk of tubal pathology.

This review will address the following items:

(i) The normal immune response to infections.
(ii) The course of a *C. trachomatis* infection: recognition of the pathogen.
(iii) Screening for *C. trachomatis*-associated tubal factor subfertility.
(iv) Summary and future perspectives.

The normal immune response to infections

Innate immune system

The innate immune system is a general, non-specific system, which is the first line of defence against pathogens that are unknown to the host. Key elements of the innate immune system are macrophages, neutrophils, dendritic cells and natural killer (NK) cells. Several studies have suggested that besides the above-mentioned immune cells, epithelial cells play an important role in the early immune response to infections (Rasmussen *et al*., 1997; Quayle, 2002; Stephens, 2003).

Both epithelial cells and circulating cells of the innate immune system possess cell-surface-bound or intracellular PPRs. The two most important families of PRRs are the toll-like receptor (TLR) family and the nucleotide-binding oligomerization domain (NOD) proteins. PRRs recognize and bind pathogen-associated molecular

patterns (PAMPs), which are components on and in foreign organisms. Binding of a PRR to its PAMP initiates several intracellular reactions, including a signal transduction cascade with nuclear factor (NF)-κB as the end product. NF-κB is able to bind to specific DNA sequences in the nucleus, thereby enhancing the production of pro-inflammatory cytokines. Some PRRs, such as cluster of differentiation 14 (CD14), (partly) exist in a soluble extracellular form and act as a co-receptor. Initiation of the innate immune response then occurs by binding of an extracellular PAMP–PRR complex to a transmembrane PRR. Because different PRRs recognize different PAMPs, the PRR system provides a complex and flexible initiation of the innate immune response. Figure 1 shows the initiation of the innate immune response by PAMP–PRR complexes.

When a pathogen enters the body, epithelial cells are the first line of defence. The epithelial PRRs bind to the pathogen, and the epithelial cells start to secrete chemokines (which attract circulating cells of the innate immune system to the site of infection) and other pro-inflammatory cytokines. When the circulating cells of the innate immune system arrive at the site of infection, their PRRs bind to the pathogen. Subsequently, macrophages, neutrophils and dendritic cells ingest the pathogen by phagocytosis and destroy it within the cell. NK cells directly destroy the pathogen by cytolysis. Macrophages and dendritic cells are able to express pathogen components (antigens) bound to major histocompatibility complex (MHC) proteins (also known as human leukocyte antigens) on their surface and to serve as antigen-presenting cells (APCs), which can activate the acquired immune system. Circulating cells of the innate immune system also produce pro-inflammatory cytokines.

Acquired immune system

The acquired (or adaptive) immune system is a specific system, which develops after the first contact with a pathogen. It builds up a memory against the pathogen, which is responsible for a quick immune response following re-infection. The acquired immune system consists of a humoral arm (with B lymphocytes, mainly targeting extracellular pathogens) and a cell-mediated arm (with T lymphocytes, mainly targeting intracellular pathogens), which closely interact.

In the humoral arm, B lymphocytes are activated by APCs (cells of the innate immune system or T lymphocytes). Activated B lymphocytes develop into plasma cells and produce antibodies [(immunoglobulins (Igs)], which neutralize the antigen or directly destroy the pathogen. An antibody–antigen complex can also activate the complement system. Furthermore, B lymphocytes can serve as APCs for T lymphocytes.

In the cell-mediated arm, T lymphocytes are activated by APCs (cells of the innate immune system or B lymphocytes). Most T lymphocytes are T helper (Th) cells. Th cells produce pro-inflammatory cytokines. The Th1 subclass produces interleukin (IL)-12 and interferon γ, which support the cell-mediated system. The Th2 subclass produces IL-4, IL-5, IL-6 and IL-10, which support the humoral system. The relative contributions of the two respective subclasses of Th cells determine whether the cell-mediated or the humoral arm is predominant. Cytotoxic T cells (or killer cells) directly attack and destroy a pathogen and produce pro-inflammatory cytokines. Suppressor T cells provide a negative feedback mechanism to protect the host against an excessive immune response (i.e. hyperinflammation).

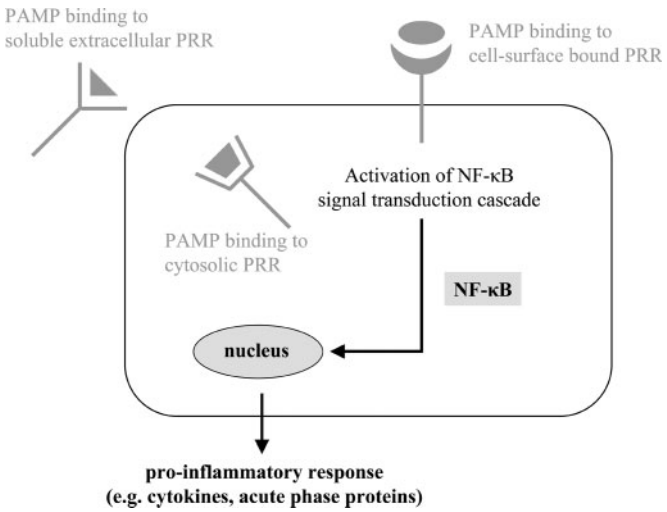

Figure 1. The initiation of the innate immune response starts by binding of pathogen-associated molecular patterns (PAMPs) to their pattern recognition receptors (PRRs) on or in circulating cells of the innate immune system (e.g. macrophages, neutrophils, dendritic cells, natural killer cells) or local epithelial cells or to soluble extracellular PRRs. This leads to activation of the nuclear factor (NF)-κB signal transduction cascade. Its end product NF-κB binds to specific DNA sequences in the nucleus, thereby enhancing the production of pro-inflammatory cytokines and acute phase proteins.

Complement system

The complement system consists of a group of over 20 proteins. Most of them are circulating in an inactive form (precursors). Once the complement system is activated, a cascade of reactions leads to active end products, which enhance the immune response or destroy the pathogen. Activation of the complement system can be induced by an antibody–antigen complex (classical pathway) or by membrane components of the pathogen (alternate pathway).

The course of a *C. trachomatis* infection: recognition of the pathogen

Clearance of a C. trachomatis infection

In most women, a normal immune response to a *C. trachomatis* infection will occur, resulting in an adequate clearance (Golden *et al*., 2000; Joyner *et al*., 2002; Morré *et al*., 2002; Molano *et al*., 2005). The host is exposed to the pathogen during a short period, leading to no or minimal tissue damage. A key element of a normal immune response to a *C. trachomatis* infection is an adequate recognition of the pathogen by PRRs on and in epithelial cells in the genital tract and initiation of the immune response. The role of PRRs of the TLR and NOD families in *C. trachomatis* recognition and an early initiation of the immune response will be discussed in this review and is summarized in Table I. The role of pro- and anti-inflammatory cytokines in the immune response will not be covered by this review.

TLRs

TLRs are cell-surface-bound or intracellular PRRs. So far, 11 different TLRs have been identified. The PAMPs of all TLRs, except TLR10, are known (Akira and Takeda, 2004). Binding of a TLR

Table I. Presence of toll-like receptors (TLRs) and nucleotide-binding oligomerization domains (NODs) in the genital tract, common genetic variations and their role in *Chlamydia trachomatis*-associated tubal factor subfertility

PRR	PAMP	Presence in genital tract				Common genetic variation	Association with tubal factor subfertility
		Human studies		Animal studies			
		In vivo	*In vitro*	*In vivo*	*In vitro*		
TLR2	Peptidoglycan	+	+	+	+	GT repeat in intron 2	NA
						Arg753Gln	NA
						–16933 T>A	NA
TLR4	LPS and hsp	+	+	+	+/–	+896 A>G (Asp299Gly)	Not significant, but trend
						+1196 Thr399Ile	NA
TLR9	Bacterial DNA	NA	+	NA	NA	–1237 T>C	Not significant, but trend
						+2848 G>A	Not significant, but trend
NOD1	Peptidoglycan	NA	NA	NA	+	Del T/Ins GG +32656	NA
NOD2	Peptidoglycan	NA	NA	NA	+	2023 C>T (SNP8, R675W)	NA
						2641 G>C (SNP12, G1881R)	NA
						2936insC (SNP13, Leu1007fsinsC, 980fs981X)	Not significant, but trend

hsp, heat shock protein; LPS, lipopolysaccharide; NA, not analysed; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor.
+ = Present in genital tract.
+/– = Present in genital tract in some, but not all, studies.

to its PAMP initiates the immune response by triggering the NF-κB signal transduction cascade. It is plausible that TLRs play a role in the host defence mechanism against *C. trachomatis* genital tract infections, because some TLRs are able to recognize *C. trachomatis* PAMPs and are expressed in epithelial cells in the human genital tract.

TLR2 is the PRR for the *C. trachomatis* component peptidoglycan (Schwandner *et al*., 1999; Yoshimura *et al*., 1999), and TLR4 is the PRR for the *C. trachomatis* components lipopolysaccharide (LPS) and heat shock protein (hsp) (Poltorak *et al*., 1998; Ohashi *et al*., 2000). TLR2 and TLR4 are expressed in the human female genital tract (Pioli *et al*., 2004; Fazeli *et al*., 2005) and in the human uterine epithelial cell line ECC-1 (Schaefer *et al*., 2004). TLR2 is also expressed in a cloned murine tubal epithelial cell line (Derbigny *et al*., 2005). Differential expression along the human genital tract has been observed for TLR2, mainly expressed in the tubes and cervix, and for TLR4, mainly expressed in the tubes and endometrium and weakly expressed or even absent in the ectocervix (Pioli *et al*., 2004; Fazeli *et al*., 2005). These differences in expression may be related to the different functions of the different parts of the genital tract: protection against sexually transmitted pathogens without disturbing the functional vaginal commensal flora and toleration of semen and embryonic implantation.

TLR9 recognizes bacterial DNA (Hemmi *et al*., 2000). So far, the expression of TLR9 has not been studied in the human female genital tract, although TLR9 expression has been found in the human uterine epithelial cell line ECC-1 (Schaefer *et al*., 2004). Its precise role in *C. trachomatis* female genital tract infections remains to be established.

TLR1, TLR3, TLR5 and TLR6 are also present in the human female genital tract (Pioli *et al*., 2004; Fazeli *et al*., 2005), but they do not recognize *C. trachomatis* PAMPs. This suggests that these TLRs may play a role in the host defence against non-*C. trachomatis* and/or polymicrobial genital tract infections.

Animal studies are able to provide information on the role of PRRs in *C. trachomatis* infections that cannot be obtained by human studies, although results of animal studies may not be freely translated to the human *in vivo* situation. Knockout (KO) mouse technology offers the opportunity to remove entire genes of interest from the genome, to compare the course and outcome of infectious diseases between KO mice and wild-type (WT) mice, which possess the gene of interest. Darville *et al*. (2003) have designed a KO mouse model to study the role of TLR2 and TLR4 in the course and outcome of a *C. muridarum* infection, which is the mouse variant of *C. trachomatis*. WT mice with normal *TLR2* and *TLR4* genes served as controls. The *in vitro* cytokine production of macrophages was down-regulated, but not totally inhibited, in macrophages derived from *TLR2* KO mice, whereas it was up-regulated in macrophages derived from *TLR4* KO mice. The *in vivo* resolution of a *C. muridarum* infection was equally efficient in KO and WT mice, indicating that the remaining and/or compensatory immune mechanisms seem to lead to sufficient clearance. Remarkably, *TLR2* KO mice developed less tubal pathology in comparison with WT mice, despite a down-regulated cytokine production. These findings suggest that *TLR2* genetic variations provide a balanced immune response leading to efficient clearance, rather than hypo- or hyperinflammation, and serve as protection against tissue damage (Darville *et al*., 2003).

NOD proteins

NOD proteins are intracellular PRRs. The family of NOD proteins contains at least 25 proteins, including NOD1 and NOD2 (Inohara and Nuñez, 2003). NOD1 and NOD2 are also referred to as caspase recruitment domain 4 (CARD4) and CARD15, respectively. NODs are able to recognize intracytoplasmatic bacterial PAMPs, such as LPS and peptidoglycan (Inohara *et al*., 2001; Girardin *et al*., 2003). Binding of a NOD to its PAMP activates the NF-κB signal transduction cascade, which initiates the immune response.

Because *C. trachomatis* is an intracellular pathogen containing LPS and peptidoglycan, a role of intracellular NODs in the recognition of *C. trachomatis* has been suggested. This is supported by findings of three recent studies (Derbigny *et al*., 2005; Opitz

et al., 2005; Welter-Stahl *et al*., 2006). Another *Chlamydia* species, *C. pneumoniae*, has been shown to induce a NOD-mediated pro-inflammatory immune response in endothelial cells *in vitro* (Opitz *et al*., 2005). Welter-Stahl *et al*. (2006) have found that *C. trachomatis* produces at least the rudimentary proteoglycan motif recognized by NOD1. The third study has proved that NOD1 and NOD2 are expressed in a cloned murine fallopian tube epithelial cell line (Derbigny *et al*., 2005).

Clinical aspects of clearance

Up to 70–80% of the *C. trachomatis* infections in women are asymptomatic, and therefore unrecognized and untreated (Rahm *et al*., 1988). A normal immune response (rather than antibiotic treatment) is essential to clear the pathogen and to protect women from ascendance of the infection to the upper genital tract and/or transmission to a sexual partner. Studies on the natural course of untreated *C. trachomatis* lower genital tract infections in women show spontaneous clearance rates of 30% in the first weeks to months, ~50% in 1 year, 80% in 2 years and 94% in 4 years (Golden *et al*., 2000; Joyner *et al*., 2002; Morré *et al*., 2002; Molano *et al*., 2005).

Although these studies indicate that most infected women seem to have an adequate local immune response, a subset of infected women will have a long-lasting *C. trachomatis* infection and thereby an increased risk of late sequelae. The above-mentioned studies on spontaneous clearance of *C. trachomatis* lower genital tract infections may even underestimate the percentage of women at risk of complications, because clearance from the lower genital tract does not necessarily mean that the infection has not already ascended to the upper genital tract. Given a worldwide prevalence of 50 million new *C. trachomatis* infections in women each year, a clinically significant group of infected women may be at risk of late sequelae (World Health Organization, 2001).

Persistence of a C. trachomatis infection

In some women, a *C. trachomatis* infection will not be cleared adequately, which may result in a persistent infection. There is no generally accepted definition of persistence. From a clinical point of view, persistence involves exposure of the host to the pathogen during a longer period, increasing the risk of ascendance to the upper genital tract, endosalpingeal tissue damage and tubal factor subfertility. However, no consensus exists on the length of this period. From a scientific point of view, persistence is assumed to be characterized by a chronic low-grade immune response and/or the presence of aberrant *C. trachomatis* particles. In this review, we use both the clinical and scientific description of persistence.

The course of a *C. trachomatis* infection (i.e. whether the infection will be cleared or persist) may be determined by virulence factors of the pathogen, environmental factors or host immune factors.

Virulence factors of the pathogen

Several studies have evaluated whether different serovars are associated with differences in clinical course of *C. trachomatis* infections, i.e. symptomatic versus asymptomatic infection, lower versus upper genital tract infection and clearance versus persistence (Ito *et al*., 1990; Persson and Osser, 1993; Dean *et al*., 2000; Morré *et al*., 2000; Geisler *et al*., 2003; Molano *et al*., 2005).

Serovars D, E and F account for most *C. trachomatis* infections (Persson and Osser, 1993; Morré *et al*., 2000; Geisler *et al*., 2003; Molano *et al*., 2005). Two studies reported a significant relationship between serovars and symptoms: serovar F and the less-common serovar K were associated with a symptomatic course (Morré *et al*., 2000; Geisler *et al*., 2003), whereas serovar Ia was found in asymptomatic women only (Morré *et al*., 2000). However, both studies could not confirm each other's findings (Morré *et al*., 2000; Geisler *et al*., 2003), and Persson and Osser (1993) could not find any relationship between serovars and symptoms.

In asymptomatic untreated patients, spontaneous clearance from the cervix occurred more often in women infected with the common serovars F and G, whereas persistent *C. trachomatis* infections were observed more frequently among serovars D and E and the less-common serovars B, H, I, J and K (Molano *et al*., 2005). Remarkably, despite antibiotic treatment, serovars H, I and J were able to persist for 2 or 3 years in the lower genital tract of women (Dean *et al*., 2000). In a mouse model, the duration of lower genital tract infection was longest with serovars D and E, and ascendance to the upper genital tract occurred more often in mice infected with serovar D as compared with that in mice infected with serovar H (Ito *et al*., 1990).

Studies on the association between different *C. trachomatis* serovars and clinical course and outcome of the disease are relevant not only in the fertility field but also in the oncology field. It has already been shown that *C. trachomatis* cervical infections are associated with cervical cancer by increasing the risk of persistence of the high-risk types of the oncogenic human papillomavirus (Samoff *et al*., 2005). Serovar studies have revealed that exposure to certain single *C. trachomatis* serovars (G, I and D) or to multiple *C. trachomatis* serovars is associated with the development of cervical squamous cell carcinoma (Anttila *et al*., 2001).

In brief, studies on the association between virulence of the most common serovars and the course of *C. trachomatis* infections did not yield consistent and clinically applicable results. A hypothesis which is currently under investigation is that genetic variations in the plasticity zone (i.e. a virulence region in the bacterial genome) may account for intra-serovar or strain differences in the course and outcome of *C. trachomatis* infections (Read *et al*., 2000; Read *et al*., 2003; Carlson *et al*., 2004).

Environmental factors

The risk of tubal pathology following pelvic inflammatory disease (PID) is dependent on the number of episodes and the severity of the disease (Weström, 1980; Weström *et al*., 1992) (Table II). In a large follow-up study in women with laparoscopically verified

Table II. The number of episodes and severity of laparoscopically verified pelvic inflammatory disease (PID) in relation to the risk of tubal factor subfertility (adapted from Weström *et al*., 1992)

Number of episodes of PID	Severity of PID	*n*	%	Risk of tubal factor subfertility (%)
One	Mild	312	25	0.6
	Moderate	450	36	6.2
	Severe	229	18	21.4
	All grades	991	80	8.0
Two		185	15	19.5
Three or more		65	5	40.0
Total		1241	100	11.4

PID diagnosed between 1960 and 1984, the risk of tubal factor subfertility was about 10% after one episode of PID, 20% after two episodes and 40% after three episodes (Table II). *C. trachomatis* accounted for ~40% of all PIDs in this study, although it should be noted that routine *C. trachomatis* testing was introduced in their clinic only in 1977 and was therefore not applied in all PID cases (Weström *et al*., 1992). The incidence of tubal factor subfertility increased significantly with the severity of PID at laparoscopy and was 0.6% after one mild episode, 6% after one moderately severe episode and 21% after one severe episode of PID (Weström *et al*., 1992) (Table II).

A large follow-up study (over 13 000 participants) has evaluated the risk of subfertility following a positive *C. trachomatis* test on samples obtained from the cervix and/or urethra (Andersen *et al*., 2005). Birth rates and time to birth were comparable between women tested positive and negative (Andersen *et al*., 2005). It should be noted that nearly all positive cases received antibiotic treatment. Therefore, the risk of subfertility following untreated *C. trachomatis* lower genital tract infections is assumed to be higher.

Although precise data are not available, it is suggested that the presence of multiple micro-organisms in the genital tract increases the risk of tubal pathology. In large community-based and school-based screening programmes in the UK and in the USA, 4–12% of all *C. trachomatis*-infected women had a co-infection, such as *Neisseria (N.) gonorrhoeae* (Harindra *et al*., 2002; Nsuami *et al*., 2004). Studies in women attending clinics for sexually transmitted diseases have shown a 13–28% rate of co-infections in *C. trachomatis*-infected women (Harindra *et al*., 2002; Creighton *et al*., 2003). *N. gonorrhoeae* infections are more often symptomatic as compared with *C. trachomatis* infections. However, Nsuami *et al*. (2004) have found that only 14% of women with both *C. trachomatis* and *N. gonorrhoeae* infections reported symptoms. This indicates that genital tract infections with these two micro-organisms remain unnoticed and untreated in most women, increasing the risk of late sequelae.

Host immune factors

Introduction to immunogenetics. Because at this time neither virulence factors of the pathogen nor environmental factors do seem to play a major role in the difference of the clinical course of *C. trachomatis* infections, host immune factors are considered more important determinants of the inter-patient variability in the course and outcome.

Immunogenetic studies evaluate the role of genetic variations in immunologically important host genes in the course and outcome of infectious diseases. Among these genetic variations are single-nucleotide polymorphisms (SNPs), in which one nucleotide has been substituted, inserted or deleted, and variations in the number of repetitive DNA sequences (variable number of tandem repeats). Carrying a genetic variation may have direct or indirect biological consequences. Potential direct biological consequences of carrying a genetic variation are translation of an aberrant protein or up- or down-regulation of the translation of a normal protein. If a genetic variation is not functional, i.e. it does not change the function of the gene studied, it may have indirect biological consequences when it is inherited together with another, sometimes unidentified, functional gene nearby (linkage).

During the past years, immunogenetic studies have provided more insight into the inter-patient variability of the course and outcome of infectious diseases. Several studies have found an association between carriage of genetic variations and infectious diseases, such as hepatitis, inflammatory bowel diseases, meningococcal infections and *Ureoplasma urealyticum* lower genital tract infections (Jeremias *et al*., 1999; Smirnova *et al*., 2003; Franchimont *et al*., 2004; Peeters *et al*., 2004; Frodsham, 2005). Regarding *C. trachomatis* ocular infections, a 40% genetic predisposition was noted in a Gambian twin study, supporting the relevance of genetics in *C. trachomatis* infections (Bailey *et al*., 1998).

As discussed previously, a normal immune response to a *C. trachomatis* infection is based on an adequate recognition of the pathogen by, amongst others, TLRs and NODs on epithelial cells in the genital tract. In the next paragraphs, the role of genetic variations in genes encoding TLRs and NODs as potential risk factors for persistent *C. trachomatis* infections is discussed (see also Table I).

Variations in TLR *genes.* It is likely that TLR2, TLR4 and TLR9 play a role in the recognition of *C. trachomatis* in the genital tract, because they are able to recognize *C. trachomatis* PAMPs and because they are expressed in the human female genital tract. It is assumed that genetic variations in *TLR* genes may result in aberrant receptor density on or in cells or in dysfunctional receptors, leading to an inadequate recognition of *C. trachomatis* and an increased risk of persistence. However, only a few human studies have tested this hypothesis.

Regarding *TLR4*, it is known that only homozygous carriage of the *TLR4* +896 A>G (also referred to as Asp299Gly) and Thr399Ile SNPs affects the LPS receptor function, whereas heterozygous carriage has no effect on the LPS receptor function (Erridge *et al*., 2003). Because almost all carriers of the common *TLR4* +896 A>G SNP are heterozygous, no significant association between this SNP and *C. trachomatis*-associated tubal pathology has been found in a cohort of 71 subfertile women (Morré *et al*., 2003). In a larger cohort, the same results were found for *TLR4* +896 A>G, as well as for *TLR9* –1237 T>C and *TLR9* +2848 G>A, although a trend was observed towards a higher risk of tubal pathology among carriers of these SNPs (den Hartog *et al*., submitted for publication). Also for the –260 C>T variation in the *CD14* gene, the LPS-sensing co-receptor of TLR4, no involvement in the development of *C. trachomatis*-associated tubal pathology was found (Ouburg *et al*., 2005). So far, the studies mentioned are the only human studies on the role of *TLR* genetic variations and susceptibility to *C. trachomatis* genital tract infections. This limited number of human studies may be because of difficulties in collecting adequate sample sizes, because patients who have undergone a *C. trachomatis* infection ánd have had evaluation of the tubal function ánd carry a single or multiple genetic variations are exceedingly rare. Multicentre trials might resolve this drawback. Although functional SNPs in the *TLR2* gene have been described in relation to infection and inflammation (Lorenz *et al*., 2000; Sutherland *et al*., 2005; Yim *et al*., 2006), no studies have been performed yet for *C. trachomatis* infections.

Further studies are needed to investigate the precise role of TLRs in *C. trachomatis* genital tract infections, in particular to determine whether *TLR* genetic variations act in a damaging way, as generally assumed, or in a protective way, as suggested by Darville *et al.* (2003) in their KO mouse model.

Variations in NOD *genes.* The precise role of *NOD* proteins in the intracellular recognition of *C. trachomatis* in the genital tract has not been established, although several studies suggest that NODs are involved in the immune response to *C. trachomatis*

genital tract infections (Inohara *et al*., 2001; Girardin *et al*., 2003; Derbigny *et al*., 2005; Opitz *et al*., 2005; Welter-Stahl *et al*., 2006). If this association could be confirmed, *NOD* genetic variations may be risk factors of inadequate recognition and persistence in *C. trachomatis* infections.

Several genetic variations in the *NOD2* genes have been associated with the susceptibility to inflammatory bowel disease (Hugot *et al*., 2001; Ogura *et al*., 2001; Hampe *et al*., 2002; Murillo *et al*., 2002; McGovern *et al*., 2005). Carrying a *NOD2* genetic variation seems to result in hyporesponsiveness to enteric bacteria, increasing the risk of chronic bowel inflammation. Hugot *et al*. (2001) have also identified a so-called gene-dosage effect: the higher the number of genetic variations in a patient, the higher the risk of Crohn's disease. As compared with patients without *NOD2* variations, the relative risk of Crohn's disease was three in heterozygous carriers of a single variation, 38 in homozygous carriers of a single variation and 44 in heterozygous carriers of two variations (Hugot *et al*., 2001).

NOD1 is also a ubiquitous cytosolic receptor for peptidoglycan from Gram-negative bacteria, and recent studies have suggested that *C. trachomatis* and *C. muridarum* do, in fact, produce at least the rudimentary proteoglycan motif recognized by NOD1. Nonetheless, NOD1 deficiency has no effect on the duration of infection, the intensity of cytokine secretion or the extent of pathology in vaginally infected mice, compared with WT controls (Welter-Stahl *et al*., 2006). Thus, *Chlamydia* may not produce sufficient peptidoglycan to stimulate NOD1-dependent pathways efficiently in infected animals, or other receptors of the innate immune system may compensate for the absence of NOD1 during *Chlamydia* infection *in vivo* as has been shown by Netea *et al*. (2005).

The studies mentioned encourage investigation of whether NODs play a role as PRRs for *C. trachomatis* and, if so, whether genetic variations increase the risk of an aberrant immune response and persistence.

Conclusive remarks on immunogenetics. Carriage of a single variation in a single host gene does not necessarily lead to late sequelae of infectious diseases, especially in the case of a polygenic multivariate infection such as *C. trachomatis*. The immune system is a complex and flexible system, and compensatory routes will, to a certain extent, provide alternative pathways to trigger the immune response. For instance, blockage of the NOD1 pathway can be partially overcome by functional TLRs (Netea *et al*., 2005), and not only PRRs but also the complement system are involved in pathogen recognition. Furthermore, heterozygous carriage of some genetic variations may not have a large effect on the function of the gene (Erridge *et al*., 2003). It is also hypothesized that the risk of late sequelae increases with the number of genetic variations, as found for *NOD* variations in Crohn's disease (Hugot *et al*., 2001) and *TLR* variations in meningococcal infections (Smirnova *et al*., 2003). We have studied whether carrying multiple genetic variations in four PRR genes plays a role in the development of *C. trachomatis*-associated tubal pathology. The results showed a higher risk of tubal pathology in carriers of at least two genetic variations (73%) as compared with carriers of less than two variations (33%) (den Hartog *et al*., submitted for publication). Although this cohort was too small to obtain significant differences and larger cohorts are needed to retest this hypothesis, it is tempting to suggest that carrying multiple genetic variations, rather than a single genetic variation, is a determinant of the risk of late sequelae such as tubal pathology following a *C. trachomatis* infection (den Hartog *et al*., submitted for publication).

In general, the main goal of immunogenetic studies is to provide more insight into the immunopathogenesis of infectious diseases. Regarding *C. trachomatis* female genital tract infections, the precise role of PRRs and their genetic variations remains to be elucidated. As long as this is not being clarified, there is no place for clinical application of immunogenetic analyses in screening for tubal pathology.

Clinical aspects of persistence

If a cervical *C. trachomatis* infection is not cleared adequately, the infection may ascend from the lower to the upper genital tract and/or may be transmitted to a sexual partner. Ascendance to the endometrium, tubes and pelvis may result in a (silent) PID and an increased risk of tubal factor subfertility. Histological evidence of endometritis has been found in 30–40% of women with cervicitis (Paavonen *et al*., 1985a; Wiesenfeld *et al*., 2002) and in 70% of women with suspected PID (Paavonen *et al*., 1985b). The microorganism itself has been isolated from the endometrium in one-third of women with a *C. trachomatis* cervicitis and/or urethritis (Jones *et al*., 1986). Salpingitis has been demonstrated in 10% of women with endometritis (Cates and Wasserheit, 1991). Tubal pathology accounts for 20–25% of the cases of subfertility in developed countries (Collins *et al*., 1995; Collins and Van Steirteghem, 2004) and up to 80% in developing countries (Collet *et al*., 1988).

The pathogenesis of *C. trachomatis*-associated tubal pathology is not yet fully understood. Two mechanisms are assumed to be responsible for the development of tubal damage following a persistent *C. trachomatis* infection. The first and probably most important mechanism is by a persistent infection causing a chronic low-grade immune response, which attacks and destroys the host cells (LaVerda *et al*., 1999). Secondly, *C. trachomatis* itself can damage the host tubal epithelial cells when its replication cycle has been completed and elementary bodies are released by cytolysis of the host cell. The latter mechanism does not appear to play a major role in persistent infections, because persistence is characterized by reduced replication of the dormant pathogen (AbdelRahman and Belland, 2005; Mpiga and Ravaoarinoro, 2006). These aberrant *Chlamydia* particles have been identified in the genital tract, whereas previously these aberrant forms were only visualized in cell culture under special conditions (Bragina *et al*., 2001). More studies are needed to elucidate the precise immunopathogenesis of *C. trachomatis* infections. The hypothesis that persistence and low-grade inflammation are associated with tubal pathology is presently the subject of investigation.

Screening for *C. trachomatis*-associated tubal factor subfertility

Because most *C. trachomatis* infections remain asymptomatic, a patients' history will usually not be helpful in assessing the risk of a previous *C. trachomatis* infection (Rahm *et al*., 1988; Logan *et al*., 2003). Several test methods to assess this risk are available.

The reference standard for diagnosing tubal pathology in subfertile women is laparoscopy with tubal testing, by which tubal patency and the presence of peri-adnexal adhesions can be assessed. However, a laparoscopy has several disadvantages. First,

it is an invasive and expensive procedure (in the Netherlands, about 1000 euros) (Fiddelers *et al*., 2005), requiring general anaesthesia. Operating facilities may not be easily available in every clinic. Furthermore, it holds a 1.5% risk of surgical complications (e.g. bleeding and infection) (Chapron *et al*., 1998). Owing to these disadvantages, laparoscopy with tubal testing is unsuitable to be applied as a screening procedure in subfertile women on a large scale. It would be preferable to estimate the risk of tubal pathology before laparoscopy, to select only high-risk patients for this procedure. Two frequently used screening methods to assess the risk of tubal pathology are hysterosalpingography (HSG) and serological testing.

HSG

Today, several methods are used to evaluate tubal patency in subfertile women, e.g. HSG, hysterosalpingo (contrast) sonography and transvaginal hydrolaparoscopy, of which HSG is most widely used and has been evaluated most extensively. Compared to laparoscopy with tubal testing, HSG is less expensive (in the Netherlands, about 150 euros) (Fiddelers *et al*., 2005) but also less accurate in diagnosing tubal pathology. HSG has a sensitivity of 58% and a specificity of 77% for diagnosing tuboperitoneal abnormalities (defined as at least unilateral tubal obstruction and/or hydrosalpinx and/or peri-adnexal adhesions) as compared with laparoscopy (Dabekausen *et al*., 1994). A meta-analysis has been performed to determine the accuracy of HSG in diagnosing tubal patency and adhesions separately (Swart *et al*., 1995). as compared with laparoscopy with tubal testing, HSG has a sensitivity of 65% and a specificity of 85% for diagnosing tubal patency, whereas HSG is unreliable for diagnosing peri-adnexal adhesions (Swart *et al*., 1995). The low sensitivity of HSG (tubal pathology at laparoscopy despite normal HSG findings) may be because of peri-adnexal adhesions not visualized during the procedure itself or at the abdominal X-ray after 24 h or of incorrect interpretation of the HSG results. The specificity of HSG is higher, but still ~20% of women without tubal pathology at laparoscopy have abnormal HSG findings. These false-positive HSG findings may be because of tubal spasms, dissimilar tubal filling pressure, too high viscosity of the contrast medium used or technical failure (Dabekausen *et al*., 1994). Another disadvantage of HSG is the risk of infection, which is up to 10% in patients with tubal pathology (Forsey *et al*., 1990). Furthermore, HSG is considered a painful test by patients.

Because HSG has a limited predictive value for tubal disease and holds a risk of febrile morbidity, it is questioned whether HSG is the best screening test in high-risk patients. Owing to the disadvantages of both laparoscopy and HSG, clinicians have tried to find an inexpensive and non-invasive test, which could accurately discern high-risk from low-risk patients for tubal factor subfertility. Ideally, on the basis of the results of such a screening test, one would subject high-risk patients to diagnostic testing (i.e. laparoscopy) and delay additional invasive and expensive testing in low-risk patients. For this purpose, serological screening tests have been developed.

Chlamydia antibody testing

Since the association between *C. trachomatis* IgG antibodies in serum and tubal pathology has been noted (Punnonen *et al*., 1979), serum *Chlamydia* IgG antibody testing (CAT) has been introduced as a screening test for tubal pathology in the fertility work-up. Following *C. trachomatis* infections, which mainly affect adolescents, a decade or more may pass until women present with subfertility. Serum IgG antibodies are known to remain detectable for many years (Gijsen *et al*., 2002), even after antibiotic treatment (Puolakkainen *et al*., 1986; Chaim *et al*., 1992; Piura *et al*., 1993; Henry-Suchet *et al*., 1994). Therefore, CAT is considered a useful tool in subfertile women to reflect a previous *C. trachomatis* infection which has mostly occurred more than a decade ago. The costs of CAT are low (in the Netherlands, about ten euros) (Fiddelers *et al*., 2005) and the patients' discomfort is negligible.

The negative predictive value (NPV) of CAT in subfertile women is 85–90% (Mouton *et al*., 2002; Veenemans and Van der Linden, 2002; Akande *et al*., 2003; Land *et al*., 2003; Logan *et al*., 2003), although NPVs around 75% have been reported (Eggert-Kruse *et al*., 1997; Tiitinen *et al*., in press). Because of the high NPV, the presence of tubal pathology in patients with a negative CAT is unlikely.

The positive predictive value (PPV) of CAT in subfertile women is lower than the NPV and ranges from 30 to 65% (Eggert-Kruse *et al*., 1997; Mouton *et al*., 2002; Veenemans and Van der Linden, 2002; Akande *et al*., 2003; Land *et al*., 2003; Logan *et al*., 2003). The results reported on the diagnostic accuracy of CAT are heterogenous because of differences in CAT tests, threshold levels for a positive test, reference standard and definition of tubal pathology used (Land *et al*., 1998; Land *et al*., 2003) (Table III). However, the main limitation of CAT is the number of false-positive results, i.e. positive CAT in the absence of tubal pathology, as reflected by the low PPV. A major concern of this high false-positive rate is that laparoscopies will be performed in women without tubal pathology. Unintended cross-reactivity with highly prevalent *C. pneumoniae* IgG antibodies has been suggested to account for false-positive test results in some CAT tests (Gijsen *et al*., 2001; Land *et al*., 2003). Probably an even more important cause of false-positive CAT results is that a positive CAT is a marker of a previous *C. trachomatis* infection but does not reflect the course of the infection and neither the extent of tubal damage. Therefore, CAT is not useful in discriminating between clearance and persistence of a *C. trachomatis* infection, whereas persistence is an important risk factor for tubal pathology.To screen for persistent *C. trachomatis* infections, the value of serological markers of persistence in identifying subfertile women at highest risk of tubal disease has been evaluated over the last few years. Part of the findings is summarized in Table IV.

Serological markers of persistence

High-sensitivity C-reactive protein

The acute phase protein C-reactive protein (CRP) is a general serological marker of inflammation. CRP levels are >10 mg/l in acute infections and <1 mg/l in the absence of an infection. CRP levels between 1 and 10 mg/l (so-called elevated levels within the normal range) are assumed to reflect a low-grade inflammation (Pearson *et al*., 2003) and can be detected using a high-sensitivity (hs) CRP test. The role of elevated hs-CRP levels as markers of an ongoing low-grade inflammation has been evaluated in studies on the relationship between persistent *C. pneumoniae* infections and cardiovascular diseases. These studies have shown that the known

Table III. Predictive value of different tests and threshold levels for tubal pathology (TP) in subfertile women (adapted from Land *et al*., 2003)

Chlamydia antibody test	Threshold	Number of patients with positive test	Number of patients with positive test and TP	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	OR	95% CI
MIF Biomerieux	8	231	45	88	30	19	93	3.1	1.2–9.9
	16	149	39	76	58	26	93	4.6	2.1–10.3
	32	132	37	73	64	28	92	4.7[a]	2.3–10.2
	64	104	36	71	74	35	93	6.9	3.3–14.9
	128	60	29	57	88	48	91	9.9	4.7–21.1
MIF AniLabsystems	8	91	32	61	77	34	91	5.3	2.6–10.8
	16	75	32	61	83	41	92	7.8	3.7–16.2
	32	52	30	59	92	58	92	15.7[b]	7.1–35.1
	64	37	24	47	95	65	90	17.2	7.1–42.4
	128	19	13	25	98	68	87	14.7	4.6–52.4
ELISA AniLabsystems	Equivocal	84	23	45	77	27	88	2.7	1.3–5.4
	Positive	53	19	37	87	36	88	4.0[c]	1.9–8.4
	Highly positive	26	12	24	95	46	87	5.5	2.0–14.4
pELISA Medac	Equivocal	74	28	55	83	38	90	5.8	2.8–11.8
	Positive	62	28	55	87	45	91	8.2[d]	3.9–17.3
ELISA Savyon	Equivocal	99	26	51	72	26	88	2.7	1.4–5.4
	Positive	87	25	49	77	29	89	3.1[e]	1.6–6.3

CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; MIF, micro-immunofluorescence; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value.
[b]versus [a,c,d,e] and [d]versus [c,e] $P < 0.05$.

Table IV. Predictive value of single tests as well as combinations of tests for tubal pathology (TP) in subfertile women (adapted from den Hartog *et al*., 2005)

Number of tests performed	CAT[a]	hs-CRP[b]	chsp60-IgG[c]	Ctr-IgA[d]	Number of patients with positive test	Number of patients with positive test and TP	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	OR	95% CI
One test	+				52	32	54	92	62	90	13.9[e]	7.0–27.5
		+			127	32	54	63	25	85	2.0[f]	1.1–3.5
			+		68	30	51	85	44	88	5.9[f]	3.2–10.9
				+	42	21	36	92	50	86	6.1[f]	3.1–12.3
Two tests[g]	+	+			22	19	32	99	86	86	39.7[f]	11.2–140.5
	+		+		41	28	47	95	68	89	16.7	7.9–35.7
	+			+	27	17	29	96	63	85	9.9	4.2–23.0

CAT, *chlamydia* antibody testing; chsp60, *chlamydia* hsp 60; CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; Ig, immunoglobulin; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value.
[a]MIF (AniLabsystems, Finland), threshold titre for a positive test 32.
[b]ELISA (DiaMed Eurogen, Belgium), threshold concentration for a positive test 1.0–10.0 mg/l.
[c]ELISA (Medac, Germany), threshold titre for a positive test 1.11.
[d]ELISA (AniLabsystems, Finland), threshold titre for a positive test 1.4.
[e]versus[f] $P < 0.05$.
[g]Combinations of two tests, not including CAT, performed poorer than the combinations shown and were removed from this table. The OR of the combination CAT/hs-CRP did not increase significantly by adding a third and fourth test, and these data were removed from this table.

association between *C. pneumoniae* and cardiovascular diseases is even stronger in the presence of slightly elevated hs-CRP levels (Roivainen *et al*., 2000; Gattone *et al*., 2001; Johnston *et al*., 2001).

The value of hs-CRP, in addition to CAT, in predicting the risk of tubal factor subfertility has recently been studied (den Hartog *et al*., 2005) (Table IV). This study showed that combining CAT, which has a PPV of 62%, an NPV of 90% and an odds ratio (OR) of 13.9, with hs-CRP significantly increases the predictive value for tubal pathology (PPV 86%, NPV 86%, and OR 39.7). CAT/hs-CRP seems a clinically important set of serological screening tests, which increases the low PPV of CAT without lowering the NPV (den Hartog *et al*., 2005). These results must be confirmed in larger studies.

IgG to Chlamydia hsp 60

Chlamydia hsp 60 (chsp60) is a *Chlamydia* genus-specific protein, serving as a strong antigenic target for the immune system (Morrison *et al*., 1989; Kaufmann, 1990). Antibodies to chsp60 have been suggested as markers of chronic inflammation (Kaufmann, 1990) and may therefore be good predictors for the risk of tubal pathology. Studies have shown a strong association between anti-chsp60 antibodies and tubal factor subfertility. Anti-chsp60 antibodies are significantly more prevalent in subfertile women with tubal

disease (45–75%) as compared with those without tubal disease or fertile controls (8–20%) (Freidank *et al*., 1995; Claman *et al*., 1997; Persson *et al*., 1999; den Hartog *et al.*, 2005). Among subfertile women with antibodies to *C. trachomatis*, anti-chsp60 antibodies are significantly more prevalent in women with tubal pathology (65–80%) as compared with those without tubal pathology (0–45%) (Toye *et al*., 1993; Arno *et al*., 1995; den Hartog *et al*., 2005) (Table IV). The PPV ranges from 45 to 60%, and the NPV is ~85% (den Hartog *et al*., 2005; Tiitinen *et al*., in press). Heterogeneity between the results of the different studies may be because of methodologic differences, such as the type of chsp60 IgG tests, threshold levels, reference standard and definition of tubal pathology used. In particular, cross-reaction with the highly prevalent and highly similar *C. pneumoniae* hsp60 IgG is assumed to account for false-positive results (den Hartog *et al*., 2005). As predictors of tubal factor subfertility, chsp60 IgG antibodies perform well, although not always superior to CAT (Persson *et al*., 1999; den Hartog *et al*., 2005; Tiitinen *et al*., 2006). Combining CAT with anti-chsp60 IgG does not lead to a significantly higher OR (16.7) than CAT alone (13.9) (den Hartog *et al*., 2005) (Table IV). It remains to be determined whether chsp60 IgG testing should be implemented in the fertility work-up as a screening method for *C. trachomatis*-associated tubal pathology.

IgA to C. trachomatis

IgA antibodies are assumed to reflect chronic inflammation. Previous studies have demonstrated an association between *C. pneumoniae* IgA antibodies and its chronic sequelae, e.g. respiratory and cardiovascular morbidity (Saikku, 1999; Falck *et al*., 2002; Wong *et al*., 2002).

Contradictory findings have been reported on the value of *C. trachomatis* IgA antibodies in screening for tubal factor subfertility. Mouton *et al*. (2002) have found that IgA antibodies are more useful than IgG antibodies in diagnosing tubal pathology, whereas other studies have reported that IgG antibodies are better predictors for tubal pathology than IgA antibodies (Paukku *et al*., 1998; den Hartog *et al*., 2005) (Table IV). These apparently contradictory findings may be because of methodological differences. Although the presence of serum IgA antibodies has been associated with chronic inflammation, the diagnostic accuracy does not seem superior to CAT or chsp60 IgG testing. Therefore, IgA antibody testing should not replace CAT in the fertility work-up.

Summary and future perspectives

Summary

C. trachomatis genital tract infections in women usually remain asymptomatic. Therefore, a normal immune response, rather than antibiotic treatment, is essential for clearing the pathogen. In most women with *C. trachomatis* infections, the immune response will be adequate and the pathogen will be cleared effectively. However, in some women, *C. trachomatis* infections will persist and ascend to the upper genital tract, increasing the risk of late sequelae such as tubal factor subfertility. PRRs of the TLR and NOD families play a substantial role in recognizing *C. trachomatis* and initiating the immune response. Virulence factors of the pathogen and environmental factors are suggested to be determinants of the risk of tubal pathology, but the role of host immune factors is considered of more importance. Genetic variations in *TLR* and *NOD* genes are presumed to affect receptor function, leading to an inadequate recognition of *C. trachomatis*, an inadequate immune response and subsequently an increased risk of persistence and late sequelae. However, the precise role of the PRR genetic variations in *C. trachomatis* female genital tract infections and tubal pathology remains to be elucidated.

To assess the risk of tubal pathology in subfertile women in an inexpensive and non-invasive way, CAT (measuring *C. trachomatis* IgG antibodies in serum) has been introduced in the fertility work-up and is nowadays commonly used in the Netherlands. The predictive value of CAT for tubal pathology is limited, because the presence of *C. trachomatis* IgG antibodies reflects a previous infection, but not a persistent infection. Therefore, CAT is not suitable to identify subfertile women with persistent *C. trachomatis* infections, who have the highest risk of tubal pathology. Serological markers of persistent *C. trachomatis* infections, such as hs-CRP, may help identify these women. The first study on the value of CAT/hs-CRP in predicting tubal factor subfertility has shown that the PPV of CAT (62%) increases to 86% if both CAT and hs-CRP are positive. Although several studies have reported encouraging findings, further evaluation is needed to decide whether markers of persistence deserve a place in the fertility work-up.

Future perspectives

Further immunogenetic studies may provide more insight into the immunopathogenesis of *C. trachomatis* female genital tract infections in general and into the role of PRRs and their genetic variations in particular. Owing to the expected low prevalence of subfertile women who have contracted a *C. trachomatis* infection ánd have had evaluation of tubal function ánd carry a single or multiple genetic variations, immunogenetic analyses are not expected to become clinically relevant as screening methods for tubal factor subfertility.

It is more likely that serum markers of persistence of the micro-organism will be added to CAT in the fertility work-up, because a non-invasive method to obtain samples of the upper genital tract for the detection of persistent *C. trachomatis* using nucleic acid amplification techniques does not exist. The combination of CAT and hs-CRP (reflecting a previous *C. trachomatis* infection and persistence of the micro-organism, respectively) appears to be a promising valuable set of serological tests to identify women at highest risk of tubal pathology. The role of chsp60 IgG as an additional marker of chronic inflammation is the promising topic of several studies. From a theoretical point of view, the ultimate goal of the fertility investigation would be to have a screening method available with 100% accuracy in ruling in and ruling out *C. trachomatis*-associated tubal pathology. This ultimate screening method is not available.

So far, many studies have measured serum antibodies, which are products of the humoral immune response, to estimate the risk of a previous *C. trachomatis* infection. However, clearance of intracellular pathogens such as *C. trachomatis* is known to depend also on the Th1 response of the cell-mediated arm (Bailey *et al*., 1995; Holland *et al*., 1996; Hawkins *et al*., 2002). A recent study has evaluated the role of measuring the cell-mediated immune response in predicting the risk of tubal factor subfertility and has shown that an *in vitro* lymphocyte response to *C. trachomatis* was

significantly more often detected in women with tubal factor subfertility as compared with subfertile controls (Tiitinen *et al*., 2006). In predicting tubal pathology, adding markers of the cell-mediated immune system to antibody testing improved the value of measuring markers of the humoral response alone (Tiitinen *et al*., 2006). If further studies can confirm these findings and test methods to evaluate the cell-mediated immune response become commercially available, measuring the cell-mediated immune response may be implemented in the fertility work-up as a screening method for tubal factor subfertility.

References

AbdelRahman YM and Belland RJ (2005) The chlamydial developmental cycle. FEMS Microbiol Rev 29,949–959.

Akande VA, Hunt LP, Cahill DJ, Caul EO, Ford WCL and Jenkins JM (2003) Tubal damage in infertile women: prediction using chlamydia serology. Hum Reprod 18,1841–1847.

Akira S and Takeda K (2004) Toll-like receptor signalling. Nat Rev Immunol 4,499–511.

Andersen B, Østergaard L, Puho E, Skriver MV and Schønheyder HC (2005) Ectopic pregnancies and reproductive capacity after *Chlamydia trachomatis* positive and negative test results: a historical follow-up study. Sex Transm Dis 32,377–381.

Anttila T, Saikku P, Koskela P, Bloigu A, Dillner J, Ikaheimo I, Jellum E, Lehtinen M, Lenner P, Hakulinen T *et al*. (2001) Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. JAMA 285,47–51.

Arno JN, Yuan Y, Cleary RE and Morrison RP (1995) Serologic responses of infertile women to the 60-kd chlamydial heat shock protein (hsp60). Fertil Steril 64,730–735.

Bailey RL, Holland MJ, Whittle HC and Mabey DCW (1995) Subjects recovering from human ocular chlamydial infection have enhanced lymphoproliferative responses to chlamydial antigens compared to those of persistently diseased controls. Infect Immun 63,389–392.

Bailey RL, Fowler A, Peeling R, Mabey D, Whittle H and Jepson A (1998) Lymphoproliferative responses to *C. trachomatis* EBS in a Gambian twin population; Estimating the role of host genetic factors. Proceedings of the Ninth International Symposium on Human Chlamydial Infections, Napa, CA, USA, June 21–26, 1998, pp. 474–477.

Bragina EY, Gomberg MA and Dmitriev GA (2001) Electron microscopic evidence of persistent chlamydial infection following treatment. J Eur Acad Dermatol Venereol 15,405–409.

Carlson JH, Hughes S, Hogan D, Cieplak G, Sturdevant DE, McClarty G, Caldwell HD and Belland RJ (2004) Polymorphisms in the *Chlamydia trachomatis* cytotoxin locus associated with ocular and genital isolates. Infect Immun 72,7063–7072.

Cates W and Wasserheit JN (1991) Genital chlamydial infections: epidemiology and reproductive sequelae. Am J Obstet Gynecol 164,1771–1781.

Chaim W, Edelstein Z, Sarov B and Sarov I (1992) The long-term follow-up of asymptomatic women with *Chlamydia trachomatis*. Arch Gynecol Obstet 251,159–164.

Chapron C, Querleu D, Bruhart MA, Madelenat P, Fernandez H, Pierre F and Dubuisson JB (1998) Surgical complications of diagnostic and operative gynaecological laparoscopy: a series of 29 966 cases. Hum Reprod 13,867–872.

Claman P, Honey L, Peeling RW, Jessamine P and Toye B (1997) The presence of serum antibody to the chlamydial heat shock protein (CHSP60) as a diagnostic test for tubal factor infertility. Fertil Steril 67,501–504.

Collet M, Reniers J, Frost E, Gass R, Yvert F, Leclerc A, Roth-Meyer C, Ivanoff B and Meheus A (1988) Infertility in Central Africa: infection is the cause. Int J Gynecol Obstet 26,423–428.

Collins JA and Van Steirteghem A (2004) Overall prognosis with current treatment of infertility. Hum Reprod Update 10,309–316.

Collins JA, Burrows EA and Willan AR (1995) The prognosis for live birth among untreated infertile couples. Fertil Steril 64,22–28.

Creighton S, Tenant-Flowers M, Taylor CB, Miller R and Low N (2003) Co-infection with gonorrhoea and chlamydia: how much is there and what does it mean? Int J STD AIDS 14,109–113.

Dabekausen YA, Evers JLH, Land JA and Stals FS (1994) Chlamydia trachomatis antibody testing is more accurate than hysterosalpingography in predicting tubal factor infertility. Fertil Steril 61,833–837.

Darville T, O'Neill JM, Andrews CW, Nagarajan UM, Stahl L and Ojcius DM (2003) Toll-like receptor-2, but not toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection. J Immunol 171,6187–6197.

Dean D, Suchland RJ and Stamm WE (2000) Evidence for long-term cervical persistence of *Chlamydia trachomatis* by *omp1* genotyping. J Infect Dis 182,909–916.

Derbigny WA, Kerr MS and Johnson RM (2005) Pattern recognition molecules activated by *Chlamydia muridarum* infection of cloned murine oviduct epithelial cell lines. J Immunol 175,6065–6075.

Eggert-Kruse W, Rohr G, Demirakca T, Rusu R, Näher H, Petzoldt D and Runnebaum B (1997) Chlamydial serology in 1303 asymptomatic subfertile couples. Hum Reprod 12,1464–1475.

Erridge C, Stewart J and Poxton IR (2003) Monocytes heterozygous for the Asp299Gly and Thr399Ile mutations in the toll-like receptor 4 gene show no deficit in lipopolysaccharide signalling. J Exp Med 197,1787–1791.

Falck G, Gnarpe J, Hansson LO, Svärdsudd K and Gnarpe H (2002) Comparison of individuals with and without specific IgA antibodies to *Chlamydia pneumoniae*. Respiratory morbidity and the metabolic syndrome. Chest 122,1587–1593.

Fazeli A, Bruce C and Anumba DO (2005) Characterization of Toll-like receptors in the female reproductive tract in humans. Hum Reprod 20,1372–1378.

Fiddelers AAA, Land JA, Voss G, Kessels AGH and Severens JL (2005) Cost-effectiveness of Chlamydia antibody tests in subfertile women. Hum Reprod 20,425–432.

Forsey J, Caul E, Paul ID and Hull MGR (1990) Chlamydia trachomatis, tubal disease and the incidence of symptomatic and asymptomatic infection following hysterosalpingography. Hum Reprod 5,444–447.

Franchimont D, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, Quertinmont E, Abramowicz M, Van Gossum A, Devière J *et al*. (2004) Deficient host–bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR) -4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. Gut 53,987–992.

Freidank HM, Clad A, Herr AS, Wiedmann-Al-Ahmad M and Jung B (1995) Immune response to *Chlamydia trachomatis* heat-shock protein in infertile female patients and influence of *Chlamydia pneumoniae* antibodies. Eur J Clin Microbiol Infect Dis 14,1063–1069.

Frodsham AJ (2005) Host genetics and the outcome of hepatitis B viral infection. Transpl Immunol 14,183–186.

Gattone M, Iacoviello L, Colombo M, Di Castelnuovo A, Soffiantino F, Gramoni A, Picco D, Benedetta M and Giannuzzi P (2001) *Chlamydia pneumoniae* and cytomegalovirus seropositivity, inflammatory markers, and the risk of myocardial infarction at a young age. Am Heart J 142,633–640.

Geisler WM, Suchland RJ, Whittington WLH and Stamm WE (2003) The relationship of serovar to clinical manifestations of urogenital *Chlamydia trachomatis* infection. Sex Transm Dis 30,160–165.

Gijsen AP, Land JA, Goossens VJ, Leffers P and Bruggeman CA (2001) *Chlamydia pneumoniae* and screening for tubal factor subfertility. Hum Reprod 16,487–491.

Gijsen AP, Land JA, Goossens VJ, Slobbe MEP and Bruggeman CA (2002) *Chlamydia* antibody testing in screening for tubal factor subfertility: the significance of IgG antibody decline over time. Hum Reprod 17,669–703.

Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ and Sansonetti PJ (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 278,8869–8872.

Golden MR, Schillinger JA, Markowitz L and St Louis ME (2000) Duration of untreated genital infections with *Chlamydia trachomatis*: a review of the literature. Sex Transm Dis 27,329–337.

Hampe J, Frenzel H, Mirza MM, Croucher PJP, Cuthbert A, Mascheretti S, Huse K, Platzer M, Bridger S, Meyer B *et al*. (2002) Evidence for a *NOD2*-independent susceptibility locus for inflammatory bowel disease on chromosome 16p. Proc Natl Acad Sci USA 99,321–326.

Harindra V, Tobin JM and Underhill G (2002) Opportunistic chlamydia screening; should positive patients be screened for co-infections? Int J STD AIDS 13,822–825.

den Hartog JE, Land JA, Stassen FRM, Kessels AGH and Bruggeman CA (2005) Serological markers of persistent *C. trachomatis* infections in women with tubal factor subfertility. Hum Reprod 20,986–990.

den Hartog JE, Ouburg S, Land JA, Lyons JM, Ito JI, Peña AS and Morré SA Do host genetic traits in the bacterial sensing system play a role in the development of *C. trachomatis*-associated tubal pathology in subfertile women? (submitted for publication).

Hawkins RA, Rank RG and Kelly KA (2002) A *Chlamydia trachomatis*-specific Th2 clone does not provide protection against a genital infection

and displays reduced trafficking to the infected genital mucosa. Infect Immun 70,5132–5139.

Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K *et al*. (2000) A toll-like receptor recognizes bacterial DNA. Nature 408,740–745.

Henry-Suchet J, Askienazy-Elbhar M, Thibon M, Revol C and Akue BA (1994) The post-therapeutic course of serum antibody titres in women with acute salpingitis and tubal infertility. Fertil Steril 62,296–304.

Holland MJ, Bailey RL, Conway DJ, Culley F, Miranpuri G, Byrne GI, Whittle HC and Mabey DCW (1996) T helper type-1 (Th1) /Th2 profiles of peripheral blood mononuclear cells (PBMC); response to antigens of *Chlamydia trachomatis* in subjects with severe trachomatous scarring. Clin Exp Immunol 105,429–435.

Hugot JP, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morrain CA, Gassull M *et al*. (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411,599–603.

Inohara N and Nuñez G (2003) NODs: intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol 3,371–382.

Inohara N, Ogura Y, Chen FF, Muto A and Nuñez G (2001) Human Nod1 confers responsiveness to bacterial lipopolysaccharides. J Biol Chem 276,2551–2554.

Ito JI, Lyons JM and Airo-Brown LP (1990) Variation in virulence among oculogenital serovars of *Chlamydia trachomatis* in experimental genital tract infection. Infect Immun 58,2021–2023.

Jeremias J, Giraldo P, Durrant S, Ribeiro-Filho A and Witkin SS (1999) Relationship between *Ureoplasma urealyticum* vaginal colonization and polymorphism in the interleukin-1 receptor antagonist gene. J Infect Dis 180,912–914.

Johnston SC, Messina LM, Browner WS, Lawton MT, Morris C and Dean D (2001) C-reactive protein levels and viable *Chlamydia pneumoniae* in carotid artery atherosclerosis. Stroke 32,2748–2752.

Jones RB, Mammel JB, Shepard MK and Fisher RR (1986) Recovery of *Chlamydia trachomatis* from the endomtrium of women at risk for chlamydial infection. Am J Obstet Gynecol 155,35–39.

Joyner JL, Douglas JM, Foster M and Judson FN (2002) Persistence of *Chlamydia trachomatis* infection detected by polymerase chain reaction in untreated patients. Sex Transm Dis 29,196–200.

Kaufmann SHE (1990) Heat shock proteins and the immune response. Immunol Today 11,129–136.

Land JA, Evers JLH and Goossens VJ (1998) How to use *Chlamydia* antibody testing in subfertility patients. Hum Reprod 13,1094–1098.

Land JA, Gijsen AP, Kessels AGH, Slobbe MEP and Bruggeman CA (2003) Performance of five serological chlamydia antibody tests in subfertile women. Hum Reprod 18,2621–2627.

LaVerda D, Kalayoglu MV and Byrne GI (1999) Chlamydial heat shock proteins and disease pathology: new paradigms for old problems? Infect Dis Obstet Gynecol 7,64–71.

Logan S, Gazvani R, McKenzie H, Templeton A and Bhattacharya S (2003) Can history, ultrasound, or ELISA chlamydial antibodies, alone or in combination, predict tubal factor infertility in subfertile women? Hum Reprod 18,2350–2356.

Lorenz E, Mira JP, Cornish KL, Arbour NC and Schwartz DA (2000) A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. Infect Immun 68,6398–6401.

McGovern DPB, Hysi P, Ahmad T, Van Heel DA, Moffat MF, Carey A, Cookson WOL and Jewell DP (2005) Association between a complex insertion/deletion polymorphism in *NOD1 (CARD4)* and susceptibility to inflammatory bowel disease. Hum Mol Genet 14,1245–1250.

Molano M, Meijer CJLM, Weiderpass E, Arslan A, Posso H, Franceschi S, Ronderos M, Muñoz N and Van den Brule AJC (2005) The natural course of *Chlamydia trachomatis* infection in asymptomatic Colombian women: a 5-year follow-up study. J Infect Dis 191,907–916.

Morré SA, Rozendaal L, Van Valkengoed IGM, Boeke AJP, Van Voorst Vader PC, Schirm J, De Blok S, Van den Hoek JAR, Van Doornum GJJ, Meijer CJLM *et al*. (2000) Urogenital *Chlamydia trachomatis* serovars in men and women with a symptomatic or asymptomatic infection: an association with clinical manifestations? J Clin Microbiol 38,2292–2296.

Morré SA, Van den Brule AJC, Rozendaal L, Boeke AJP, Voorhorst FJ, De Blok S and Meijer CJLM (2002) The natural course of asymptomatic Chlamydia trachomatis infections: 45% clearance and no development of clinical PID after one-year follow-up. Int J STD AIDS 13,12–18.

Morré SA, Murillo LS, Bruggeman CA and Peña AS (2003) The role that the functional Asp299Gly polymorphism in the toll-like receptor-4 gene plays in the susceptibility to *Chlamydia trachomatis*-associated tubal infertility. J Infect Dis 187,341–342.

Morrison RP, Lyng K and Caldwell HD (1989) Chlamydial disease pathogenesis. Ocular hypersensitivity elicited by a genus-specific 57-kD protein. J Exp Med 169,663–675.

Mouton JW, Peeters MF, Van Rijssort-Vos JH and Verkooyen RP (2002) Tubal factor pathology caused by *Chlamydia trachomatis*: the role of serology. Int J STD AIDS 13(Suppl 2),26–29.

Mpiga P and Ravaoarinoro M (2006) *Chlamydia trachomatis* persistence: an update. Microbiol Res 161,9–19.

Murillo L, Crusius JBA, Van Bodegraven AA, Alizadeh BZ and Peña AS (2002) *CARD15* gene and the classification of Crohn's disease. Immunogenetics 54,59–61.

Netea MG, Ferwerda G, De Jong DJ, Werts C, Boneca IG, Jéhanno M, Van der Meer JWM, Mengin-Lecreulx D, Sansonetti PJ, Philpott DJ *et al*. (2005) The frameshift mutation in *Nod2* results in unresponsiveness not only to Nod2- but also Nod1-activating peptidoglycan agonists. J Biol Chem 280,35859–35867.

Nsuami M, Cammarata CL, Brooks BN, Taylor SN and Martin DH (2004) Chlamydia and gonorrhea co-occurrence in a high-school population. Sex Transm Dis 31,424–427.

Ogura Y, Bonen DK, Inohara N, Nicolaes DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH *et al*. (2001) A frameshift mutation in *NOD2* associated with susceptibility to Crohn's disease. Nature 411,603–606.

Ohashi K, Burkart V, Flohé S and Kolb H (2000) Cutting edge: Heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. J Immunol 164,558–561.

Opitz B, Förster S, Hocke AC, Maass M, Schmeck B, Hippenstiel S, Suttorp N and Krüll M (2005) Nod1-mediated endothelial cell activation by *Chlamydophila pneumoniae*. Circ Res 96,319–326.

Ouburg S, Spaargaren J, den Hartog JE, Land JA, Fennema JSA, Pleijster J, Peña AS and Morré SA (2005) The *CD14* functional gene polymorphism –260 C>T is not involved in either the susceptibility to *Chlamydia trachomatis* infection or the development of tubal pathology. BMC Infect Dis 5,114.

Paavonen J, Kiviat N, Brunham RC, Stevens CE, Kuo CC, Stamm WE, Miettinen A, Soules M, Eschenbach DA and Holmes KK (1985a) Prevalence and manifestations of endometritis among women with cervicitis. Am J Obstet Gynecol 152,280–286.

Paavonen J, Aine R, Teisala K, Heinonen PK, Punnonen R, Lehtinen M, Miettinen A and Grönroos P (1985b) Chlamydial endometritis. J Clin Pathol 38,726–732.

Paukku M, Närvänen A, Puolakkainen M, Dreesbach K, Tiitinen A, Hao W, Anttila TI and Paavonen J (1998) Detection of *Chlamydia trachomatis* antibodies by 2 novel tests: rELISA and peptide EIA. Int J STD AIDS 9,604–607.

Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL *et al*. (2003) Markers of inflammation and cardiovascular disease. Application to clinical and public health practice. A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 107,499–511.

Peeters H, Vander Cruyssen B, Laukens D, Coucke P, Marichal D, Van Den Berghe M, Cuvelier C, Remaut E, Mielants H, De Keyser F *et al*. (2004) Radiological sacroiliitis, a hallmark of spondylitis, is linked with CARD15 gene polymorphisms in patients with Crohn's disease. Ann Rheum Dis 63,1131–1134.

Persson K and Osser S (1993) Lack of evidence of a relationship between genital symptoms, cervicitis and salpingitis and different serovars of *Chlamydia trachomatis*. Eur J Clin Microbiol Infect Dis 12,195–199.

Persson K, Osser S, Birkelund S, Christiansen G and Brade H (1999) Antibodies to *Chlamydia trachomatis* heat shock proteins in women with tubal factor infertility are associated with prior infection by *C. trachomatis* but not by *C. pneumoniae*. Hum Reprod 14,1969–1973.

Pioli PA, Amiel E, Schaefer TM, Connolly JE, Wira CR and Guyre PM (2004) Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. Infect Immun 72,5799–5806.

Piura B, Sarov B and Sarov I (1993) Persistence of antichlamydial antibodies after treatment of acute salpingitis with doxycycline. Eur J Obstet Gynecol Reprod Biol 48,117–121.

Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C *et al*. (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. Science 282,2085–2088.

Punnonen R, Terho P, Nikkanen V and Meurman O (1979) Chlamydial serology in infertile women by immunofluorescence. Fertil Steril 31,656–659.

Puolakkainen M, Vesterinen E, Purola E, Saikku P and Paavonen J (1986) Persistence of chlamydial antibodies after pelvic inflammatory disease. J Clin Microbiol 23,924–928.

Quayle AJ (2002) The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells. J Reprod Immunol 57,61–79.

Rahm VA, Gnarpe H and Odlind V (1988) Chlamydia trachomatis among sexually active teenage girls. Lack of correlation between chlamydial infection, history of the patient and clinical signs of infection. Br J Obstet Gynaecol 95,916–919.

Rasmussen SJ, Eckmann L, Quayle AJ, Shen L, Zhang YX, Anderson DJ, Fierer J, Stephens RS and Kagnoff MF (1997) Secretion of proinflammatory cytokines by epithelial cells in response to *Chlamydia* infection suggests a central role for epithelial cells in chlamydial pathogenesis. J Clin Invest 99,77–87.

Read TD, Brunham RC, Shen C, Gill SR, Heidelberg JF, White O, Hickey EK, Peterson J, Utterback T, Berry K *et al*. (2000) Genome sequences of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR39. Nucleic Acids Res 28,1397–1406.

Read TD, Myers GSA, Brunham RC, Nelson WC, Paulsen IT, Heidelberg J, Holtzapple E, Khouri H, Federova NB, Carty HA *et al*. (2003) Genome sequence of *Chlamydophila caviae* (*Chlamydia psittaci* GPIC): examining the role of niche-specific genes in the evolution of the Chlamydiaceae. Nucleic Acids Res 31,2134–2147.

Roivainen M, Viik-Kajander M, Palosuo T, Toivanen P, Leinonen M, Saikku P, Tenkanen L, Manninen V, Hovi T and Mänttäri M (2000) Infections, inflammation, and the risk of coronary heart disease. Circulation 101,252–257.

Saikku P (1999) Epidemiology of Chlamydia pneumoniae in atherosclerosis. Am Heart J 138,S500–S503.

Samoff E, Koumans EH, Markowitz LE, Sternberg M, Sawyer MK, Swan D, Papp JR, Black CM and Unger ER (2005) Association of *Chlamydia trachomatis* with persistence of high-risk types of human papillomavirus in a cohort of female adolescents. Am J Epidemiol 162,668–675.

Schaefer TM, Desouza K, Fahey JV, Beagley KW and Wira CR (2004) Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. Immunology 112,428–436.

Schwandner R, Dziarski R, Wesche H, Rothe M and Kirschning CJ (1999) Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. J Biol Chem 274,17406–17409.

Smirnova I, Mann N, Dols A, Derkx HH, Hibberd ML, Levin M and Beutler B (2003) Assay of locus-specific genetic load implicates rare Toll-like receptor 4 mutations in meningococcal susceptibility. Proc Natl Acad Sci USA 100,6075–6080.

Stephens RS (2003) The cellular paradigm of chlamydial pathogenesis. Trends Microbiol 11,44–51.

Sutherland AM, Walley KR and Russell JA (2005) Polymorphisms in CD14, mannose-binding lectin, and Toll-like receptor 2 are associated with increased prevalence of infection in critically ill adults. Crit Care Med 33,638–644.

Swart P, Mol BWJ, Van der Veen F, Van Beurden M, Redekop WK and Bossuyt PMM (1995) The accuracy of hysterosalpingography in the diagnosis of tubal pathology: a meta-analysis. Fertil Steril 64,486–491.

Tiitinen A, Surcel HM, Halttunen M, Birkelund S, Bloigu A, Christiansen G, Koskela P, Morrison SG, Morrison RP and Paavonen J (2006) *Chlamydia trachomatis* and chlamydial heat shock protein 60-specific antibody and cell-mediated responses predict tubal factor infertility. Hum Reprod 21,1533–1538.

Toye B, Laferrière C, Claman P, Jessamine P and Peeling R (1993) Association between antibody to the chlamydial heat-shock protein and tubal infertility. J Infect Dis 168,1236–1240.

Veenemans LMW and Van der Linden PJQ (2002) The value of *Chlamydia trachomatis* antibody testing in predicting tubal factor infertility. Hum Reprod 17,695–698.

Welter-Stahl L, Ojcius DM, Viala J, Girardin S, Liu W, Delarbre C, Philpott D, Kelly KA and Darville T (2006) Stimulation of the cytosolic receptor for peptidoglycan, Nod1, by infection with *Chlamydia trachomatis* or *Chlamydia muridarum*. Cell Microbiol 8,1047–1057.

Weström L (1980) Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. Am J Obstet Gynecol 138,880–892.

Weström L, Joesoef R, Reynolds G, Hagdu A and Thompson SE (1992) Pelvic inflammatory disease and fertility. A cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic results. Sex Transm Dis 19,185–192.

Wiesenfeld HC, Hillier SL, Krohn MA, Amortegui AJ, Heine RP, Landers DV and Sweet RL (2002) Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. Obstet Gynecol 100,456–463.

Wong BYL, Gnarpe J, Teo KK, Ohman EM, Prosser C, Gibler WB, Langer A, Chang WC and Armstrong PW (2002) Does chronic *Chlamydia pneumoniae* infection increase the risk of myocardial injury? Insights from patients with non-ST–elevation acute coronary syndromes. Am Heart J 144,987–994.

World Health Organization (2001) Global prevalence and incidence of selected curable sexually transmitted infections. Available at http://www.who.int/docstore/hiv/GRSTI.

Yim JJ, Lee HW, Lee HS, Kim YW, Han SK, Shim YS and Holland SM (2006) The association between microsatellite polymorphisms in intron II of the human Toll-like receptor 2 gene and tuberculosis among Koreans. Genes Immun 7,150–155.

Yoshimura A, Lien E, Ingalls RR, Tuomanen E, Dziarski R and Golenbock D (1999) Cutting edge: Recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via toll-like receptor 2. J Immunol 163,1–5.

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