Recognition and Treatment of Chlamydial Infections from Birth to Adolescence

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Abstract

The "silent epidemic" of *Chlamydia trachomatis* threatens to cause reproductive damage and infertility in many of the 50 million women who acquire it each year. Female reproductive tract infection has more recently been linked to stillbirth and premature delivery. Innate immune cells and mediators appear to be the primary players in pathogenesis, with neutrophils playing a prominent role in disease development. Although adaptive antibody and CD4 T cell responses appear primarily protective, these responses are inefficient. Infections are frequently chronic as a result, and when infection is diagnosed and treated with appropriate antibiotics, repeated infection is the rule. The lack of acute symptoms in many infected individuals contributes to the high prevalence of chlamydial infection. Although chronic sequelae are relatively rare in men, and many women sustain infection without developing pelvic inflammatory disease or chronic sequelae, the extremely high prevalence of chlamydial infection leads to significant morbidity and healthcare costs. A vaccine is urgently needed to prevent infection, but given the difficulties of inducing a CD4 T cell memory response that can home quickly to the genital tract, induction of sterilizing immunity may not be possible. A vaccine that prevents disease by lowering bacterial burden and dampening production of tissue-damaging responses may be possible. Until an efficacious vaccine is developed, screening and treatment programs appear to be the best method of disease prevention.

8.1 Introduction

Chlamydia trachomatis is an obligate, intracellular, nonmotile, Gram-negative bacterium recognized as one of the most common sexually transmitted agents in the world. Chlamydial genital infection primarily affects sexually

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active adolescents and young adults. Large-scale screening programs routinely detect infection rates of 5–10 % in young adults (19–25 years of age) [1, 2], and 10–20 % or greater in sexually active adolescents 15–19 years of age [3]. Most infected persons do not have symptoms, thus they do not seek medical care, and their infections go undetected. Consequently, screening is necessary to identify and treat this infection. The large reservoir of unrecognized infected individuals helps sustain transmission of this organism. Among men, urethritis is the most common illness resulting from *C. trachomatis* infection. Complications (e.g., epididymitis) affect a minority of infected men and rarely result in sequelae. Women bear the brunt of disease due to infection, for if left untreated, infection can ascend from the cervix to infect the uterus and Fallopian tubes to cause pelvic inflammatory disease (PID). Inflammation of the Fallopian tubes can lead to subsequent scar formation, and tubal occlusion. Tubal obstruction can lead to ectopic pregnancy, subfertility and infertility. In addition, an infected pregnant woman can transmit the organism to her newborn at the time of delivery, potentially resulting in neonatal conjunctivitis and/or afebrile pneumonia.

8.2 The Pathogen

Chlamydiae are obligate intracellular parasites that have been classified under the order Chlamydiales with their own family and genus (Chlamydiaceae, *Chlamydia*). Chlamydiae are small in size (0.25–0.8 μm in diameter) compared with typical bacteria such as *Escherichia coli* (1.0 μm) and have small chromosomes ranging from 1.0 to 1.2 megabases in size. They are Gram-negative in architecture and composition, with an outer membrane containing lipopolysaccharide (LPS), which is truncated, and not very endotoxic and a cytoplasmic membrane. While the classic bacterial cell wall component peptidoglycan has not been confirmed by isolation and identification, chlamydiae possess all the genes needed for its synthesis and are susceptible to β-lactam antibiotics [4]. Although chlamydiae contain DNA, RNA, and ribosomes, during growth and replication these obligate intracellular bacteria parasitize their host epithelial cell for nutrients and are auxotrophic for several amino acids and three of the four nucleoside triphosphates; the demand for host cell adenosine triphosphate (ATP) has led to their designation as an "energy parasite." *Chlamydia trachomatis* encodes an abundant protein called the major outer membrane protein (MOMP or OmpA) that is surface exposed and is the major determinant of serologic classification. Almost all strains of *C. trachomatis* harbor a plasmid, which confers the virulence properties of enhanced attachment/uptake and activation of the innate immune receptor, Toll-like receptor 2 (TLR2) [5].

8.2.1 Chlamydial Developmental Cycle

The biphasic developmental cycle of chlamydiae is unique among microorganisms and involves two highly specialized morphologic forms, both of which are required for infection and disease to occur: the infectious, extracellular form called an elementary body (EB) and the noninfectious but metabolically active intracellular form called a reticulate body (RB). The EB contains extensive disulfide cross-links both within and between outer membrane proteins giving it an almost spore like structure that is stable outside of the cell. The small infectious EB is inactive metabolically. Infection is initiated by attachment of EBs to the apical surfaces of epithelial cells of the conjunctiva, urogenital, or respiratory tracts, followed by receptor-mediated endocytosis. The EBs quickly modify their early endosomal membrane to exit the endosomal pathway, thereby avoiding fusion with lysosomes, and traffic on microtubules to the peri-Golgi/nuclear hof region. The EB-containing endosomes of *C. trachomatis* then fuse homotypically with one another to form their one nascent microcolony called an inclusion. The EBs then transform into RBs, the chromosome becomes relaxed and transcriptionally active, and metabolic growth and binary fission ensue to generate progeny. Chlamydiae-directed modification of their inclusion

membrane permits interception of trans-Golgi vesicles for transfer of sphingomyelin and glycerolphospholipids to the inclusion membrane, which can expand to accommodate some 200–1,000 progeny; this strategy of acquiring host cell markers for the inclusion also provides some degree of camouflage for the chlamydiae within. After 48–72 h, multiplication ceases and nucleoid condensation occurs as the RBs transform to new infectious EBs. The EBs are then released from the cell, allowing for infection of new host cells to occur.

The biphasic and relatively prolonged developmental cycle of chlamydiae are survival advantages. Antibiotic treatment or the host immune response must be able to kill both extracellular non-replicating infectious EBs as well as intracellular replicating RBs hidden within their protective vacuole if they are to rid the host of infection. Thus, antibiotic treatment requires multiple-dose regimens for 7–14 days. Single-dose azithromycin treats genital *C. trachomatis* infection effectively because it has a half-life in host cells of 5–7 days. The ability to cause prolonged, often subclinical infection is a major characteristic of chlamydiae.

8.2.2 Classification

Chlamydia trachomatis has been divided into subgroups based on antigenic variation in the major outer membrane proteins (serovars) and clinical expression. Microimmunofluorescence and monoclonal antibody testing have shown that there are more than 18 serovars of *C. trachomatis* with several distinctive clinical patterns of disease: trachoma is caused by serovars A, B, Ba, and C; oculogenital and neonatal disease by serovars B, Da, Ga, Ia, and D-K; and lymphogranuloma venereum (LGV) by serovars L1, L2, L2a, and L3. LGV infections are more invasive, as these serovars can replicate in macrophages, whereas replication of the other serovars of *C. trachomatis* is confined to mucosal epithelial cells.

8.3 Pathogenesis

8.3.1 Immunopathogenesis

In the realm of infectious diseases, it has often been observed that an overly aggressive inflammatory host response can be more problematic than the infection that initiated it. This is certainly true in the case of chlamydial infection, where the pathology that leads to the serious morbidities of chronic pelvic pain, ectopic pregnancy and infertility after female genital tract infection, is the result of the host inflammatory response. The *cellular paradigm* of chlamydia pathogenesis [6] states that the host response to chlamydiae is initiated and sustained by epithelial cells that are the primary targets of chlamydial infection. Infected host epithelial cells act as first responders, initiating and propagating immune responses through recognition of various chlamydial ligands via pathogen recognition receptors. They secrete chemokines that recruit inflammatory leukocytes to the site of infection, as well as cytokines that induce and augment the cellular inflammatory response and these mediators induce direct damage to the tissues. Unfortunately, this response is frequently ineffective at resolving infection, and ongoing stimulation of the host cells and bystander cells leads to continued release of tissue-damaging mediators. Since reinfection with chlamydiae is a frequent occurrence, repeated inflammatory responses may lead to repeated insult to the tissues, and promote further scarring. Since the host cell response to bacteria is the inciting inflammatory event, increased and prolonged bacterial burden correlates directly with disease development. Pathogen-specific and environmental factors that promote infection and bacterial survival lead to enhanced disease.

In vivo immunological studies in animal models and immune-epidemiological studies in humans indicate that resolution of infection can occur with minimal to no disease provided that the correct responses are induced in the right amount. Recognizing that the immune response to this organism leads to tissue damage, it is important to delineate the specific host

responses involved in disease promotion both for rational vaccine design and the discovery of biomarkers to monitor the effectiveness of candidate therapeutics and vaccines. Limited studies have been conducted in women investigating human genetic functional polymorphisms related to innate immune molecules. Investigation of polymorphisms in the gene for the innate immune receptor, TLR2 revealed single nucleotide polymorphisms associated with protection against tubal disease following *C. trachomatis* infection [7]. Interestingly, studies in the mouse model of female chlamydial genital tract infection have revealed activation of TLR2 is a key mechanism for induction of oviduct pathology [8]. Chlamydial induced activation of TLR2 leads to enhanced neutrophil influx, cytokine and protease production and prolongs neutrophil longevity [9]. Multiple reports indicate an important role for neutrophils and their products in oviduct tissue damage [10–13].

8.3.2 Immunoprotection

The adaptive immune response that occurs with chlamydial infection may lead to natural resolution of infection over time. However, the chronicity of infection in women indicates the suboptimal nature of the response. The natural course of *C. trachomatis* infection was described in a study of Columbian women followed for a 5-year period [14]. Eighty-two women found to be positive for *C. trachomatis* at the start of the study were studied at 6-month intervals. Most of the women (57.3 %) were >30 years of age (70.7 % were >25 years of age). Infection was classified as persistent if the same serotype was found at follow-up visits. Women who had taken antibiotics effective against *C. trachomatis* while infected were excluded. All study women reported 1–2 lifetime sex partners (82.9 % reported a single lifelong sex partner), thus the potential for repeated infection from an untreated male sex partner was high. Approximately 46 % of the infections were persistent at 1 year, 18 % at 2 years, and 6 % at 4 years of follow-up as determined by PCR of cervical scrape samples. Thus, in nearly half of this female cohort, an adaptive immune response effective in eradicating their infection or in preventing repeat infection did not develop for up to 1 year.

The adaptive response is also suboptimal with respect to protection from reinfection. The high frequency of repeat infections found in clinic-based studies has led some authors to recommend screening female adolescents for *Chlamydia* as frequently as every 6 months [15]. However, there is a strong inverse relationship between age and susceptibility to chlamydial infection even when corrected for frequency of sexual contact, suggesting effective adaptive immunity eventually develops. Lymphoproliferative responses, but not serum antibody titers increase with age [16]. Data from humans point to MHC Class II–restricted $CD4^+$ T cells of the Th1 phenotype as being critical to recovery from chlamydial infection as well as having a role in protection from disease [17, 18]. In a cohort of female commercial sex workers with HIV, susceptibility to chlamydial PID increased as numbers of $CD4^+$ T cells decreased. Furthermore, in a prospective cohort study of commercial sex workers in Nairobi at high risk of exposure, production of IFN-γ by peripheral blood mononuclear cells stimulated with chlamydial antigen strongly correlated with protection against incident *C. trachomatis* infection [19].

Data from the mouse model of genital tract infection reveal that chlamydia-specific B cells and antibody effectively lower the bacterial burden upon challenge, thereby partially protecting the oviduct from infection and disease [20, 21]. Surface proteins including MOMP are likely the principal targets of neutralizing antibodies. Anti-chlamydial antibodies are not sufficient to protect humans from reinfection. In female sex workers levels of chlamydial EB-specific IgA and IgG detected in endocervical mucus and plasma were not significantly associated with a decreased risk of infection [19]. Although antibody may not play a primary role in protection from reinfection, it may help control the shedding of organisms and protect against upper tract disease. One study reported the prevalence of mucosal IgA antibodies was inversely related to the quantity

of *C. trachomatis* shed from the human endocervix [22], and another found the presence of serum IgA and IgG antibodies reduced the risk for ascending infection among women undergoing therapeutic abortion [23].

8.4 Epidemiology

Chlamydia trachomatis is the most common bacterial sexually transmitted infection, with an estimated 92 million cases occurring globally each year, including more than four million in sexually active adolescents and adults in the United States [24]. In reports from other parts of the world, the prevalence ranges from 28.5 % among female sex workers in Dakar [25], to 5.7 % among pregnant women in Thailand [26], and 0.8 % overall among women seen in private gynecology practices in Paris and 5.2 % for those under the age of 21 years [27].

In the US, substantial racial/ethnic disparities are present in the prevalence of both chlamydial and gonococcal infections. One large study of US female military recruits found a chlamydial prevalence of 9 % that was maintained over four consecutive years [28]. Young age, black race, home-of-record from the south, more than one sex partner, a new sex partner, lack of condom use, and a history of having a sexually transmitted disease were correlates of chlamydia infection.

Urine screening for chlamydial infection in Louisiana public schools revealed the overall prevalence of *C. trachomatis* was 6.5 %, with rates among girls more than twice that of boys (9.7 % vs. 4.0 %). The highest prevalence for boys occurred in 12th grade (8.9 %), whereas the highest prevalence for girls occurred in 10th grade (15.8 %) [29]. The high prevalence rates in this cohort are in contrast to a rate of 0.9 % for a cohort of 1,114 patients aged 15–24 years in two pediatric private practices in suburban North Carolina. In sexually active participants, prevalence was 2.1 %; in sexually active females, 2.7 %; and in sexually active males, 0.9 %. Most participants were female (63 %), white (87 %), and from highly educated families (64 % of their mothers graduated from college) [30].

Identification of persons with the highest risk of infection should enhance cost-effectiveness of screening and treatment programs. However, in a recent study among 3,202 sexually active adolescent females attending middle school health centers in Baltimore, MD chlamydial infection was found in 771 first visits (24.1 %) and 299 repeat visits (13.9 %); 29.1 % had at least one positive test result [15]. Unfortunately, independent predictors of chlamydial infection—reason for clinic visit, clinic type, prior sexually transmitted diseases, multiple or new partners, or inconsistent condom use-failed to identify a subset of adolescent females with the majority of infections.

8.5 Clinical Manifestations

Most studies report that 25 % of men and 70 % of women infected with *C. trachomatis* are asymptomatic or minimally symptomatic. The National Longitudinal Study of Adolescent Health Study collected data prospectively from 14,322 US adolescents and followed them into adulthood [1]. Of the participants that tested positive for chlamydial infection, 95 % did not report symptoms in the 24 h preceding specimen collection. Among men with chlamydial infection, the prevalence of urethral discharge and dysuria were only 3.3 % and 1.9 %, respectively. Among women with chlamydial infection, the prevalence of vaginal discharge and dysuria were 0.3 % and 4.2 %, respectively. Among the small number of young men reporting urethral discharge ($n=17$), the prevalence of chlamydial infection was high (38.5 %), whereas the prevalence of chlamydial infection was only 0.9 % among women reporting vaginal discharge ($n=98$). Of note, 6.0 % of the women reporting dysuria ($n=232$) had chlamydial infection [1].

8.5.1 Infections in Males

When symptomatic, males frequently complain of dysuria or note a clear or mucopurulent urethral discharge at least 7–14 days following

contact with an infected partner [31]. The discharge may be so slight as to be demonstrable only after penile stripping and then only in the morning. Some patients may deny the presence of discharge but may note stained underwear in the morning resulting from scant discharge overnight. The primary complications of chlamydial urethritis in men are (1) epididymitis; (2) sexually reactive arthritis, including Reiter's syndrome; and (3) transmission to women. *Chlamydia trachomatis* and *N. gonorrhoeae* are the most frequent causes of epididymitis in men under age 35; urethritis also is usually present.

Although asymptomatic rectal carriage of *C. trachomatis* occurs in both infants [32] and adults, [33] *C. trachomatis* is a fairly common cause of proctitis and proctocolitis in men who have sex with men [34]. If the infection is due to a lymphogranuloma venereum strain, a severe proctocolitis can develop. Approximately 1 % of men with nongonococcal urethritis develop acute aseptic arthritis of presumed immune-mediated etiology [35]. One third of patients have the full complex of Reiter syndrome (arthritis, nonbacterial urethritis, and conjunctivitis); most such patients carry the histocompatibility antigen HLA-B27 [36].

8.5.2 Infections in Females

In women, chlamydial infections may cause PID, tubal infertility, chronic pelvic pain, and ectopic pregnancy. Chlamydial infection may also be linked to cervical cancer [37]. Chlamydial and gonococcal infections may increase susceptibility to and transmission of HIV in both men and women [38].

Symptoms in females include mild abdominal pain, intermittent bleeding, vaginal discharge, or dysuria-pyuria syndrome. The cervix can appear normal or exhibit edema, erythema, friability, or mucopurulent discharge. In prepubertal girls, vaginitis can occur secondary to infection of transitional cell epithelium by *C. trachomatis*. In contrast, the squamous epithelium of the adult vagina is not susceptible to chlamydiae, and vaginal discharge generally reflects endocervical infection.

Pelvic inflammatory disease is a sexually transmitted infection that ascends from the vagina and cervix to involve the uterus, ovaries, and peritoneal tissues as well as the fallopian tubes. Lower abdominal pain, usually bilateral, is the most common presenting symptom. Pain may be associated with an abnormal vaginal discharge, abnormal uterine bleeding, dysuria, dyspareunia, nausea, vomiting, fever, or other constitutional symptoms. It may also be present in a subclinical form that lacks the typical acute symptoms, but continues to lead to the associated long-term sequelae of infertility and ectopic pregnancy [39]. The most important causative organisms are *C. trachomatis* and *N. gonorrhoeae*; one or both of these agents cause well over half of cases. Other microorganisms implicated in PID include organisms found in the abnormal vaginal flora of women with bacterial vaginosis, such as bacteroides species, anaerobic cocci, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. *Escherichia coli* and other enteric organisms have also been found.

A recent review of 24 studies examined PID diagnosis and sequelae after untreated chlamydial infection [40]. In one study, eighteen of 109 (16.5 %) asymptomatic adolescent women infected with *C. trachomatis* followed for 2 months or more became symptomatic, but only 2 (1.8 %) developed clinical PID [41]. On average in high-risk settings, 2–5 % of untreated females developed PID within the ~2-week period between testing positive for *C. trachomatis* and returning for treatment [42, 43]. However, the rate of progression to PID in the general, asymptomatic population followed up for longer periods appeared to be low [44]. The best data regarding the risk of PID over a long period of time is probably from the prevention of pelvic infection (POPI) trial conducted in the UK. Women in the control group had their vaginal swab stored. When analyzed 12 months later, 7 of 75 (9.5 %) women positive for chlamydial infection at baseline had developed PID [45].

The spectrum of PID associated with *C. trachomatis* infection ranges from acute, severe disease with perihepatitis and ascites (Fitz-Hugh-Curtis syndrome), to asymptomatic or "silent" disease. When women with chlamydial salpingi-

tis are compared to women with gonococcal or with nongonococcal-nonchlamydial salpingitis, they are more likely to experience a chronic, subacute course with a longer duration of abdominal pain before seeking medical care. Yet, they have as much or more tubal inflammation at laparoscopy [46]. In several studies, repeated chlamydial infection was associated with PID and other reproductive sequelae, although it was difficult to determine whether the risk per infection increased with each recurrent episode [17, 47]. No prospective studies have directly assessed the risk of infertility after untreated *C. trachomatis* infection. However, according to the largest studies, after symptomatic PID of any cause has occurred, up to 18 % of women may develop infertility [48].

8.5.3 Infections in Neonates

Neonatal infection generally is acquired during passage through an infected birth canal. Prospective studies of infants born to women with a chlamydial infection of the cervix have shown a 50–75 % risk that the infant will acquire *C. trachomatis* infection at one or more anatomic sites [49–51]. In exposed infants, risk of conjunctivitis is 20–50 % and risk of pneumonia is 5–20 %.

Ophthalmia neonatorum is the major clinical manifestation of neonatal chlamydial infection [52]. The usual incubation period is 5–14 days after birth, but symptoms can occur earlier after premature rupture of membranes or as late as 6 weeks after birth [53]. Typically, the most common presenting symptom described is a watery ocular discharge which becomes progressively more purulent (95 %), followed by swelling of the eyelids (73 %) and conjunctival erythema (65 %) [52]. The majority of chlamydial conjunctivitis resolves spontaneously during the first few months of life. However, if the condition is untreated, a chronic conjunctivitis can develop and persist for weeks or months. Although conjunctivitis may be quite severe, corneal ulceration, scars and pannus formation are rare and recovery is usual without visual impairment. Mild or subclinical infection can persist in some cases for years [54]. The neovascularization of the cornea resulting from repeated infection in classic trachoma does not occur with neonatal disease.

Afebrile pneumonia caused by *C. trachomatis* in infancy occurs characteristically between 3 and 12 weeks of age, but may sometimes present later [55–57]. Characteristically, the infant has been symptomatic for three or more weeks before presentation. Most infants are only moderately ill and are afebrile. Symptoms of nasal obstruction and a pertussis-like, non-productive staccato cough gradually worsen over a week or more. Physical findings include tachypnea and rales but not wheezing. About 50 % of the affected infants have a history or evidence of conjunctivitis; a similar proportion has middle ear abnormalities [58]. Laboratory findings may include hyperinflation with symmetric interstitial infiltrates on chest radiography, peripheral eosinophilia (>400 cells/mm^3), and increased levels of serum immunoglobulins.

8.6 Laboratory Diagnosis

A positive laboratory test for *C. trachomatis* can be utilized for patient education and increases both compliance with drug therapy and the likelihood of referral of sexual partners. Although the development of tissue cell culture methods in the 1960s for detecting *C. trachomatis* was a major advance, the availability of nonculture tests has dramatically increased the availability and decreased the cost of laboratory detection. Definitive diagnosis of chlamydial infection, as would be required in a medicolegal setting (i.e., suspected sexual abuse or rape), requires isolation of *C. trachomatis* in cell culture or a positive nucleic acid amplification test (NAAT) confirmed by a second NAAT that targets a different sequence [59].

8.6.1 Diagnostic Specimens

Many screening tests for *C. trachomatis* require appropriately handled samples containing columnar epithelium from mucosal sites (e.g., endocervix, urethra, or conjunctiva) rather than

exudate; the adequacy of specimens should be verified by periodic cytologic evaluations. The discomfort caused by obtaining a urethral swab in males has precluded its widespread use in asymptomatic men. A dipstick test for leukocyte esterase performed on the first portion of a voided urine is a cost-effective and moderately sensitive screen (47–58 %) for detection of chlamydial infection in asymptomatic young males [60]. When feasible, urine NAAT provides a much more sensitive and equally noninvasive method of detecting *Chlamydia*.

8.6.2 Cell Culture

Use of chlamydial transport media containing antibiotics maximizes recovery and reduces the likelihood of culture overgrowth by other bacteria. Swabs used to obtain a specimen should have plastic or metal shafts, as soluble components from wooden shafts can have a toxic effect on cell cultures. Storage at −4 °C or maintenance at −70 °C is required if inoculation within 24 h is not possible. Cycloheximide-treated McCoy or HeLa cell lines are used most frequently to isolate *C. trachomatis.* Centrifugation techniques appear to enhance absorption of chlamydiae to cells. Intracytoplasmic inclusions can be detected at 48–72 h with species-specific immunofluorescent monoclonal antibodies for *C. trachomatis* and Giemsa or iodine stains. Generally, a higher isolation rate using cell culture is found in symptomatic patients than asymptomatic ones.

8.6.3 Nonculture Tests for *C. trachomatis*

NAATs amplify nucleic acid sequences that are specific for the organism being detected, and can detect as little as a single copy of target DNA or RNA. These tests have a higher sensitivity than all other tests, while retaining high specificity when cross contamination is being avoided [61]. NAATs have FDA approval for cervical swabs from women, urethral swabs from men, and urine from men and women [62, 63]. Similar to other nonculture tests, NAATs do not require viable organisms. NAATs detect *C. trachomatis* in urine or in self-administered vaginal swab specimens with sensitivity comparable to clinician obtained urogenital swab specimens, which makes noninvasive testing for chlamydial infections possible on individual as well as pooled specimens from a single patient [60, 64]. Multiple studies have determined that NAAT of self-obtained vaginal swabs are an acceptable, simple and sensitive diagnostic sample for the detection of *C. trachomatis,* as well as the sexually transmitted disease pathogens, *N. gonorrhoea* and *Trichomonas vaginalis* [65, 66]. Data suggest that NAATs are equivalent to or better than culture for the detection of *C. trachomatis* in the conjunctiva and nasopharynx of infants [67], and are currently being used in evaluation of newborns with conjunctivitis or pneumonia.

Enzyme immunoassays (EIAs) use enzyme-labeled chlamydial-specific antibodies to detect chlamydial LPS. The enzyme converts a colorless substrate into a colored product, which is detected by a spectrophotometer. EIAs are less sensitive than culture and NAATs; especially when using samples that contain few organisms (asymptomatic infections). EIAs lack specificity and will detect *C. pneumoniae* in respiratory specimens.

8.6.4 Serology

Antibodies to *Chlamydia spp.* are best detected with a microimmunofluorescent (MIF) assay, but these assays are not widely available. Serologic screening is of very little value in uncomplicated genital infections but may be useful for population studies. The MIF assay is species-specific and sensitive but is available only at a limited number of clinical laboratories.

8.7 Treatment

The most widely used treatments for uncomplicated oculogenital infections caused by *C. trachomatis* in nonpregnant adolescents and adults is doxycycline for 7 days or azithromycin in a

single dose [68]. In populations in which compliance with treatment is poor, azithromycin may be more cost-effective because it provides single-dose, directly observed therapy. Doxycycline costs less than azithromycin, and it has been used extensively for a longer period. Ofloxacin is similar in efficacy to doxycycline and azithromycin, but it is more expensive and offers no advantage with regard to dosage regimen. Erythromycin is less efficacious than both azithromycin and doxycycline, and gastrointestinal side effects discourage adherence to treatment.

Sex partners should be evaluated, tested, and treated if they had sexual contact with the patient during the 60 days preceding either diagnosis of *Chlamydia* or onset of symptoms in the patient. The most recent sex partner should be treated even if the time of the last sexual contact was >60 days before diagnosis of the index case. Patients do not need to be retested for *Chlamydia* after completing treatment with doxycycline or azithromycin unless symptoms persist or reinfection is suspected. A test of cure may be considered 3 weeks after completion of treatment with erythromycin. Testing at <3 weeks after completion of therapy to identify cases that did not respond to therapy may not be valid [69].

Azithromycin is currently recommended as first choice to treat *C. trachomatis* in pregnant women with amoxicillin as alternative [70]. Doxycycline and ofloxacin are contraindicated in pregnant women. Azithromycin is widely prescribed during pregnancy and lactation. Although it is excreted in breast milk, the dose delivered to the infant is quite low and not likely clinically significant [71]. Based on available data, the benefits of human milk feeding outweigh the risks of infant exposure to the small amounts of azithromycin transmitted through breastfeeding.

The optimal treatment for neonatal chlamydial conjunctivitis or pneumonia is uncertain. Erythromycin is recommended in many guidelines and has been the most widely-used antibiotic for neonatal chlamydial infection despite its association with infantile hypertrophic pyloric stenosis (particularly in the first 2 weeks of life). Azithromycin is an alternative but has not been well studied in this setting and it is also uncertain whether there is a decreased risk of infantile hypertrophic pyloric stenosis with this macrolide.

8.8 Complications and Sequelae

Chlamydia trachomatis has been implicated as a pathogen in 8–54 % of women who have PID and has been associated with the long-term consequences of tubal infertility (17 %), ectopic pregnancy (10 %), or chronic pelvic pain (17 %) [72–74]. *C. trachomatis* infection has been associated with spontaneous abortion, though not consistently, and stillbirth [75–77]. Additionally, *C. trachomatis* infection has been associated with chorioamnionitis, premature rupture of membranes and preterm delivery. A 2.6 and 3-fold increased risk of preterm delivery were determined in women with positive serology detected at 17 weeks gestation or diagnosed with cervical infection at 24 weeks gestation, respectively [76, 78]. Evidence grows with the use of NAATs that preterm delivery is associated with *C. trachomatis* infection [79–81].

In males, epididymitis, prostatitis, and reactive arthritis are the most common sequelae. Furthermore, untreated or incorrectly treated chlamydial conjunctivitis may result in chronic conjunctivitis that can develop alone or as part of Reiter syndrome. Given the high prevalence of chlamydial infections, complications due to this pathogen account for serious morbidity and economic cost.

8.9 Prevention

Because chlamydial infections usually are not associated with overt symptoms, prevention of infection and screening of asymptomatic high-risk patients is the most effective means of preventing disease and sequelae. Behavioral interventions (i.e., delaying intercourse, decreasing the number of sex partners, and use of barrier contraception) should be pursued aggressively. High-risk patients who should be routinely tested for *Chlamydia* include women with mucopurulent cervicitis, sexually active women less than

20 years old, and older women with more than one sex partner during the last 3 months or inconsistent use of barrier contraception while in a nonmonogamous relationship [70]. Because of the frequency of repeated chlamydial infections within the first several months following treatment of an initial infection, [15] more frequent (e.g., every 6 months) screening of asymptomatic sexually active adolescents may be necessary. Clinicians and health-care agencies should consider advising all women with chlamydial infection to be rescreened 3–4 months after treatment. Providers are also strongly encouraged to rescreen all women treated for chlamydial infection whenever they next present for care within the following 12 months, regardless of whether the patient believes that her sex partners were treated. Screening and treatment programs have resulted in reduced rates of complications [82, 83].

In a recent study conducted in the UK, seven of 74 women randomized to deferred chlamydial testing and treatment developed clinical PID over a 12 month follow-up period (9.5 %) compared to one of 63 (1.6 %) treated at the time of enrollment [45]. Although this difference was not significant ($p=0.07$) the annual incidence of PID (38 out of 2,377 women; 1.6 %) was less than the 3 % used in the sample size calculations, and thus the study was underpowered. In addition, participants were advised to be screened independently, and the one in five who acted on this advice had a high prevalence of chlamydial infection. Importantly, most cases of PID over 12 months occurred in women who were negative for chlamydia at baseline, indicating an importance for incident infection and the need to focus on testing those at higher risk, such as women with a new sexual partner.

In areas where screening and treatment programs have been established, rates of infection in screened populations have risen, causing speculation that early case identification and treatment interferes with development of immunity [84]. Others have insisted that the detection of increased rates of infection reflect a greater awareness of the infection, which has led to more testing being done, and still a larger number of positive tests [85]. Until a vaccine is developed to combat chlamydial disease, screening and treatment of infected persons remains the most logical mechanism of disease prevention.

To prevent maternal postnatal complications and chlamydial infections among infants, pregnant women should be screened for *Chlamydia* during the third trimester to permit completion of treatment before delivery. Ocular prophylaxis with topical erythromycin or tetracycline has reduced the incidence of gonococcal ophthalmia but does not appear to be effective against *C. trachomatis* [86]. Infants born to infected mothers are at high risk for infection; however, prophylatic antibiotic treatment is not indicated, and the efficacy of such treatment is unknown. Infants should be monitored and treated appropriately if symptoms develop.

8.10 Future Directions

During the extracellular EB stage, antibodies can act to inhibit infection. However, since the replicating RB form resides within the intracellular inclusion, bacterial killing at this stage requires a cell-mediated immune response with the primary effectors being IFN-γ secreting CD4 T cells. Thus, an ideal *C. trachomatis* vaccine should induce both local antibodies to prevent infection by EBs, and a strong Th1 response to limit infection once initiated. Efforts to develop a *C. trachomatis* vaccine have concentrated primarily on the use of recombinant *Chlamydia* antigens with immune adjuvants [87, 88]. The use of a purified native preparation of MOMP combined with Th1-inducing adjuvants induced significant resistance in mice, but sterilizing immunity was not achieved [89]. Stimulation of long-term mucosal immunity in the genital tract is a challenge; persons are susceptible to reinfection with *C. trachomatis* after a brief period of immunity because memory cells are not retained in the genital tract. It is unclear whether all genital infections could be prevented or whether only more invasive disease, such as salpingitis, might be preventable using vaccine technology. Markers for protection from upper genital tract infection and/or disease in the female will be nec-

essary if vaccine candidates are to be tested in humans.

Although current antibiotic treatment is highly successful when administered, most persons infected with *C. trachomatis* are asymptomatic and thus go undiagnosed and untreated. Although rates of infection have increased in certain areas where widespread screening and treatment programs have been in place [84], complications from infection have decreased [82, 83], indicating the utility of such programs. Public health officials should pursue such strategies in parallel with the ongoing research for effective vaccines.

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